Histology and Embryology for Dental Hygiene

HISTOLOGY AND EMBRYOLOGY FOR DENTAL HYGIENE

LAIRD SHELDAHL

CONTENTS

PREFACE

Figure 0.1: This home-made record player stand is used as a metaphor. Bonus points if you can name the two records visible.

Why study histology and embryology?

This home-built stand for a record player is going to serve as a metaphor. Measurements were made to ensure there would be enough space for records. It is built out of wooden dowels, and used some floor tiles to create a smooth surface. Why use those materials? Actually, those building materials were *re-used*, they were left over from previous projects. Sure, a trip to the hardware store could have yielded more appropriate materials, but would the hassle have been worth it? No. This is why you should study **embryology**, many instructions and materials are re-used during human development. As a result, some organs or tissues have a shape that matches their function, others do not. For example, why is enamel made by epithelial cells, when it *looks* like bone tissue? Why can a piece of pig tissue be used to surgically repair gingival tissue? What is the function of the philtrum? These are questions that can be better answered by studying where things come from (embryology) rather than studying what they look like (anatomy). Imagine not knowing that flies develop from maggots, you might believe in spontaneous generation after observing the appearance of maggots on rotting food.

Then why study **histology**? The answer to that is simple: embryos are tiny, you need a microscope to see what is going on. But with a basic understanding of oral histology you will

understand why a pocket depth over 3 mm is considered unhealthy. You can conceptualize what makes the linea alba appear white in some patients. You can explain what causes perikymata. You are on your own, however, on how to pronounce perikymata.

Because it is no possible to take tissue samples from human embryos, we occasionally look at the development of other species. These include rats, frogs (in space!), sea urchins and tunicate worms. We can learn a lot from distant relatives because humans re-use developmental processes for different purposes. Hopefully, you will gain an appreciation for the link between evolution and development. What can a headless, toothless sea creature teach you about dental hygiene?

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I am the lead author and illustrator of this textbook. I have a Ph.D. in physiology and pharmacology. I currently teach at Mt. Hood Community College in Gresham, Oregon. My Ph.D. thesis studied a morphogen (Wnt) involved in the formation of the head and neural crest cells. This morphogen is re-used during development of the teeth. To study this

process in frogs (their embryos are not inside uteruses, which makes them easier to study) I did a lot of microscopy. I do not have a background in dental hygiene, and am therefore very happy to have expert collaborators.

Reviewers

Raye Ann Yapp, RDH, MS

Raye Ann Yapp is a retired full time dental hygiene instructor and program director at Mt. Hood Community College in Gresham, Oregon. Currently, she teaches part time in periodontics and second year clinical courses. Prior to her teaching experience, she practiced dental hygiene for a periodontal specialist for twenty-two years. In addition to her A.S. Degree in Dental Hygiene from MHCC, she holds a B.A. Degree in Organizational Communication from Marylhurst University and an M.S. Degree in Education from Portland State University. Her favorite people are her dental hygiene students and fellow faculty, and when not studying material for her courses, she loves to cook, quilt, and walk with her husband and Edgar (the Pug).

Babiy, Ilya, RDH, MS, EPP

Ilya Babiy is a full-time dental hygiene instructor, first year clinical coordinator, and radiology lead at Mt. Hood

Community College. He also serves seniors and people with special needs with his mobile dental hygiene business, On-Site Oral Health Care, which he started in 2014. Ilya earned his A.S. in Dental Hygiene at MHCC, B.S. in Dental Hygiene at Oregon Institute of Technology, and M.S. in Education from Portland State. He enjoys inspiring students to fulfill their dreams and make a difference in our world. Ilya and his wife have three children. They love to travel, entertain family and friends, and explore the great outdoors.

Editor

Amen Mohammed

My name is Amen Mohammed. I am originally from Ethiopia. 🇪🇹. I am a mom and a wife. My career was journalism when I was back home. Here in USA I do any work to survive. I have worked in many places. I worked at The Home Depot, K-mart, Tri-met, Mentor and Uber driver and Lyft driver. But I wasn't happy working as a laborer, and I couldn't find the job love with my experience, that is what made to go back to school. I am at Portland State University right now. I want to be medical student and want to be a Gynecologist. I like cooking sometimes, reading history books, walking, and running ?♀? is what I like to do when I have time. I have preteen boy ? who makes me busy next to classes. He love basketball ? and football ?.

Choose your own adventure

Some links in the book take you to an external website, such as Wikipedia. These links should be a monospace font, such as this: external link. We don't have control over how they appear on your eReader. External links are for further reading or watching if you are interested, but are not required to understand the material we present. If your eReader or computer is not connected to the internet, these links will not work.

Definitions, such as **morphogen**, allow you to click on a word and read a short description. Their behavior may be missing in some eReaders. Definitions appear once per paragraph, and the description is limited to human biology. We do at times cover the development of sea urchins, mice, fish and frogs (in space, no less). Humans share aspects with all of these critters, for reasons that should become clear.

For important words and phrases defined in another chapter, such as **differentiation**←, you can read the definition by clicking on the word, or follow the ← icon to jump forward or back to the section where it is described in detail. Use the "back" button on your web browser to return to your spot. Some eReaders may not have a way for you to return to your spot easily (try testing it now). Unlike external links, definitions should work regardless of your internet connection, assuming your eReader supports them. We

encourage you to use ⇐ icons to pick your own non-linear path through this textbook.

The basic format of each chapter is as follows:

Chapter outline	Content:
Overview	Provides a road-map for what is covered, and why.
Physiology	Where histology and embryology are covered.
Clinical significance	Why histology or embryology is relevant to clinical practice.

Table 0.1: Basic format of this textbook

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histology and animation of areolar CT

Figure 0.2: Example of an animated image.

Animated images

The image in Fig. 0.2 is an animated .gif file, it should be

cycling through a series of changes. The authors wish to take advantage of things that can be done in an eBook, but not a print book. Unfortunately, animated gifs do no work in pdfs or on the Amazon Kindle (.mobi format). They cannot be slowed down or paused.

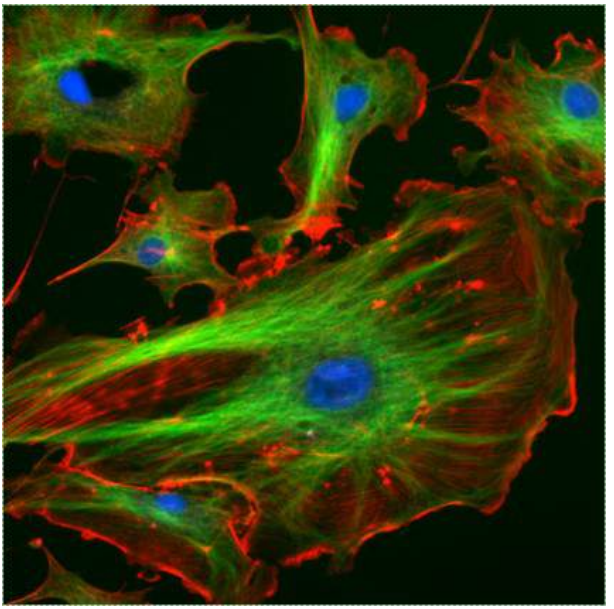


Figure 0.3 : Example of an image created by someone else. Image credit: "Fluorescent cells" by NIH image is in the Public Domain CC0

Other images

Images that we have not created have their sources listed in the figure legend, such as Fig. 0.3. This is to keep in line with the `Creative Commons` rules for using other people's work the way they have asked. This may be of use to teachers, but students can ignore this information.

Broken image

Fig. X: This image link is broken on purpose to show you what that looks like.

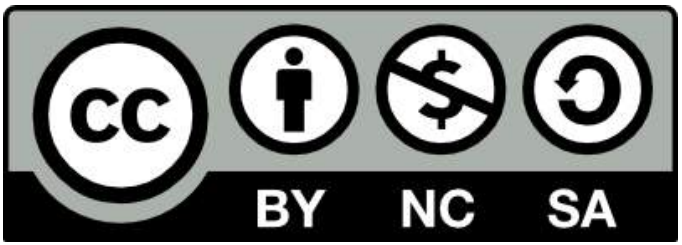
If you see something that looks like Fig. X, an image has failed to load. Try hitting the reload button if you are in a web browser (often located up in the menu bar, it probably looks like a circular arrow).

Course objectives

Course objective	Textbook chapter(s)
1. Identify core concepts of cell biology and general histology related to the face and oral cavity	1,2
2. Demonstrate an understanding of the role of epithelial tissues, underlying connective tissues and neural crest cells in the face and oral cavity	3,4,5
3. Identify the basic patterns of early human developmental biology related to formation of the face and oral cavity	6,7
4. Describe the major steps of amelogenesis and enamel structure	8, 9
5. Describe the major steps of odontogenesis and formation of the dentin-pulp complex	8, 10
6. Describe the major steps of development of tooth roots and the periodontium	8, 10, 11
7. Identify mesenchymal-epithelial relationships in tooth development and tooth eruption.	8, 9, 10, 11

Table 0.2: Course objective (for instructor use)

Copyright information



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navigation * Chapter 1 >

Chapter review questions

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https://openoregon.pressbooks.pub/histologyandembryology/?p=26

1.

CELL BIOLOGY REVIEW

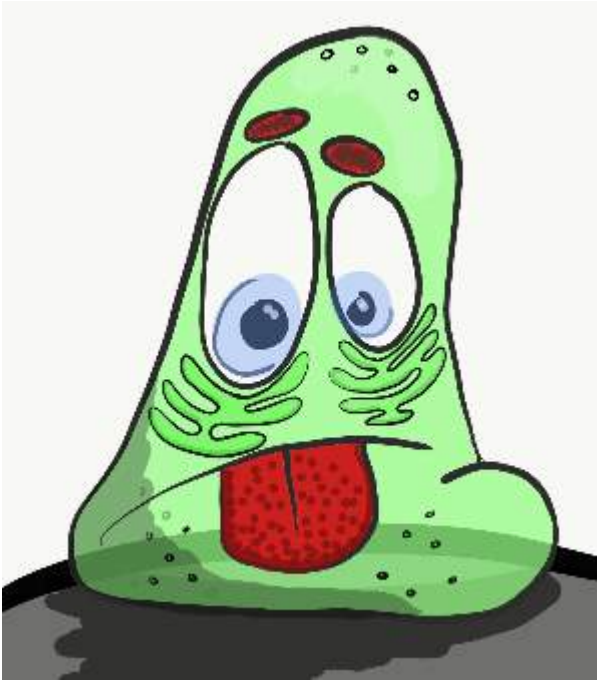

Figure 1.1: Cartoon of a cell.

Overview of cell biology

The cell is the basic unit of life. It is the smallest thing that we call living (without arguing about viruses), and the human body is made of 30 trillion of them. We started off as a single cell, and the purpose of this class to learn a little bit about how that one cell developed into the trillion-celled organism you are today. Nearly all of the instructions for making trillions of cells– how to make them, when to make them, and where to make them– are found within *every* single cell. That means cells in your teeth have the instructions for making toes. One important concept in this chapter is to see things in the

context of **nature** versus **nurture**. When we discuss how a cell's instructions influence the health of a patient, that is an example of *nature*.

Different cells have different functions, but every cell in one person has the same instructions (*with a few exceptions*). To become different from one another, cells use those instructions differently. This is an important process called **differentiation**. In a nutshell, this is no more complicated than the process of boring cells becoming different from one another. Differentiation is guided by a cell's environment, which we say is an example of *nurture*.

We give different names to cells as they make changes. We always start with some flavor of **stem cell**. If that stem cell is able to become, oh let's say a smurf, that stem cell would be named a Smurf Stem Cell. As the Smurf Stem Cell divides and differentiates into cells that build a smurf, those builders would be named Smurfo-**blasts**. When that smurf was finished, the cells inside the smurf would be named Smurfo-**cytes**. Would you prefer real names? The stem cells found in a tissue called **mesenchyme** are named **mesenchymal stem cells**, the cells that make dentin are named **odontoblasts**, and the cells in cementum are named **cementocytes** (so, stem to blast to cyte). Differentiation is not a single step, but a series of steps along a spectrum. We start from the least differentiated cells, to more differentiated cells, until we reach a **terminally differentiated** cell. The aim of this chapter is to review the parts of a cell that help

us to explain difficult concepts like differentiation and **development**, with the assumption you've been introduced to cell biology before.

If you need more than a quick refresher in cell biology, here are links from the National Institute of Health (NIH), who have a number of useful publications and videos (for free), including:

- 3D animations of the human cell
- Learn genetics (interactive website)
- Inside the Cell (pdf and epub downloads)

Two useful (also free) eBooks you may wish to download are from OpenStax:

- Biology 2ED
- Anatomy & Physiology

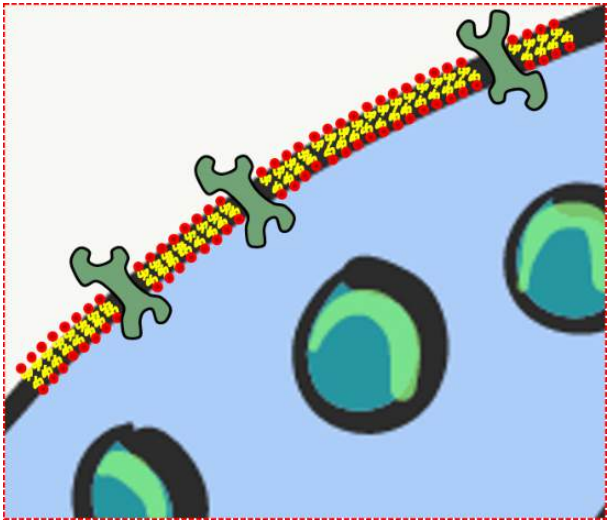

Figure 1.2: The plasma membrane, including the phospholipid bilayer (yellow and red) plus trans-membrane proteins (green).

Inside the cell

Cell membrane and cytoplasm

Every human cell is surrounded by the **plasma membrane**. In this book, we focus on materials that are on the *inside* of this membrane, on the *outside* of this membrane, and *in the membrane* itself. The plasma membrane separates the cell from its environment, and allows certain materials to enter or leave. Phospholipids and cholesterol form a lipid bilayer, creating a membrane. The double-sheet of lipid molecules acts as a barrier, separating the cell from the external environment. The other major component of the plasma membrane are proteins.

Trans-membrane proteins span the plasma membrane. Some trans-membrane proteins regulate what goes in or out of the cell. Other trans-membrane proteins receive signals from other cells or the environment, and relay that information to the inside of the cell. Still others trans-membrane proteins mediate attachment to structures outside of the cell. Not all membrane proteins span the entire width of the phospholipid bilayer, and are located on the outer or inner surface of the plasma membrane.

Many biology classes focus heavily on the nucleus. That's fine. But one of the first African Americans to earn a Ph.D., Dr. Ernest Everett Just, in his book Biology of the Cell Surface, wanted scientists to look more closely at the plasma membrane. Dr. Just was an embryologist, and in embryology the plasma membrane proteins, especially **receptors** and **cell adhesion molecules**, are very important. Receptor proteins can guide cells through the steps of differentiation that ultimately lead to the development of human **morphology**. Cell adhesion molecules adjust the integrity and permeability of a tissue, leading to relatively higher permability of **junctional epithelium**[←] compared to the **epidermis** of the skin.

Inside the plasma membrane is **cytoplasm**. Cytoplasm is the filling of a cell, sometimes referred to as Intra-Cellular Fluid (ICF). Cytoplasm includes nutrients and electrolytes absorbed from the the fluid surrounding cells. In addition, the cytoplasm includes numerous proteins and **glycoproteins**

synthesized by the cell. These molecules have other important functions, but they attract water from the **ECF**. The end result is cells have a gelatinous filling, rather than a watery one. This gelatinous filling is filled with a number of organelles, similar to the way grandmother's jello salad contains grapes, raisins and food-like substances that do not in any way turn jello into an actual salad.

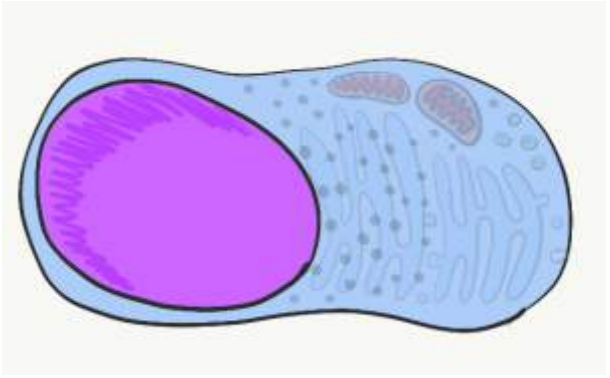

Fig. 1.3: The nucleus (highlighted)

The nucleus

The nucleus contains practically all of a cell's Deoxyribonucleic acid (**DNA**). DNA is the instructions for making a Ribonucleic Acid (**RNA**) copy, a process known as gene **transcription**. Most RNA is **translated** in the **cytoplasm** into proteins found inside and outside of a cell. In addition to instructing a cell *what* protein to make, DNA

instructs *when* and *where* to make proteins. For instance, epithelial cells of the **oral mucosa** do not make protein enzymes that secrete calcium and phosphate into **ECM**. On the other hand, epithelial cells that **differentiate**[←] into **ameloblasts** do make these proteins. They do so by **expressing** the DNA for these enzymes, after being told to do so by **neural crest cells**[←]. All cells in the human body have the same DNA (with a few exceptions), which means oral mucosa cells *could* secrete calcium and phosphate. However, different cells express different DNA at different times.

DNA can be divided into 2 basic regions. There are **genes**, each gene is more-or-less the instructions for a single protein. Genes are read in a linear fashion (like you are doing now). The rest of DNA folds up into 3-D shapes that provide instructions for when and where to express genes. These 3-D shapes are referred to as non-coding DNA. If geses are like pages of a book with words on them, non-coding DNA looks more like origami and acts more like a substrate for an active site (hopefully you are familiar with enzyme kinetics). To make a protein using the instructions in a gene, other proteins called **transcription factors** bind to non-coding regions of DNA. From there, they unzip the nearby gene, and recruit enzymes that transcribe one strand of DNA into messenger RNA (**mRNA**). The mRNA then leaves the nucleus to be translated into protein. Not all transcription factors *activate* gene transcription. Proteins called repressors inhibit the expression of genes, too.

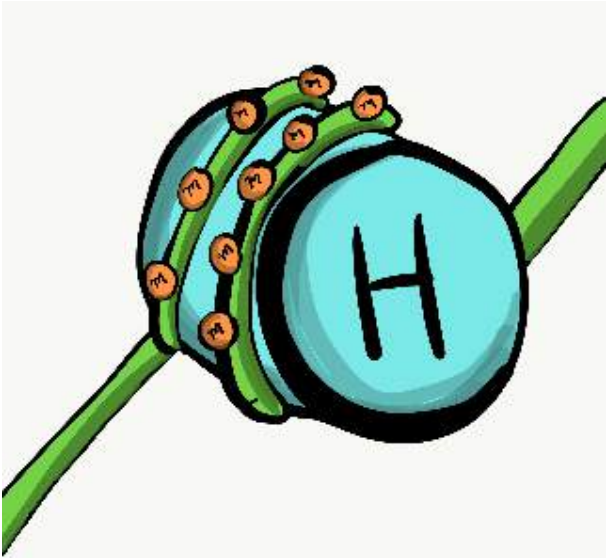

Figure 1.4: Methylated DNA wrapped around a histone.

Transcription factors turn **genes** on and off quickly. These on/off switches can respond to changes in a cell's environment. But when cells **differentiate**←, they shut down genes they won't need more permanently. Rather than allow transcription factors to act like on and off switches, unnecessary genes are **methylated** ($-CH_3$ groups are attached to the DNA), packed up around **histone** proteins, and kept in long-term storage. You will read about this repeatedly throughout this book: the pattern of DNA methylation and histone packaging is copied during **mitosis**. This means the pattern of genes that are available or packaged away is inherited by both daughter cells. Because this inheritance is not a difference in the DNA sequence, it is known as **epigenetic** inheritance. Epigenetic traits play an important role in cell

differentiation[←] and cell **fate**, covered in more detail in chapters 6 through 11 (further reading on epigenetic traits).

We have 46 molecules of DNA in the nucleus– they are very long molecules, but only 46 in number– 23 maternal, and 23 paternal. During **mitosis**, these 46 molecules are duplicated and packaged up tightly into 92 **chromosomes**. This packaging involves re-using histones along nearly the entire length of a DNA strand, and allows chromosomes to be seen under the light microscope. The rest of the time, DNA is mostly unwound (un-needed instructions are wound around histones, the rest wiggles around randomly, free to be transcribed if instructed). This unwound DNA fills up the nucleus in a way that doesn't look very exciting. We call that DNA **chromatin**, it is functionally it is the more exciting of the two forms.

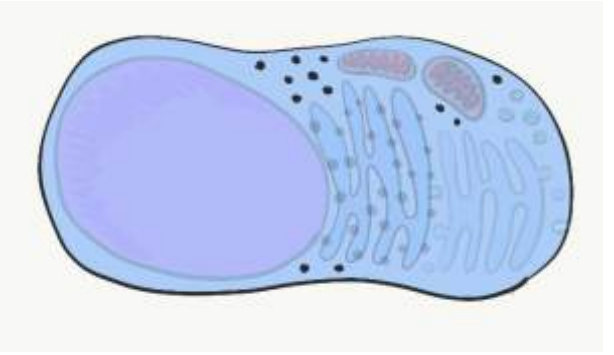

Figure 1.5: Free ribosomes in the cytoplasm.

Ribosomes

Visible throughout the **cytoplasm** are small specks made of protein and RNA called **ribosomes**. These structures **translate mRNA** instructions that come from the nucleus. mRNA instructions guide the linkage of amino acids into a long protein. Groups of three mRNA nucleotides, called codons, instruct the ribosome which amino acid to add next to the protein. Like regulatory regions of **DNA**, ribosomal RNA (rRNA) isn't read linearly but folds into a 3-D shape. That shape, along with ribosomal proteins, create active sites that catalyze the chemical reaction of mRNA translation. Free-floating ribosomes in the cytoplasm synthesize proteins that remain in the cytoplasm, such as **keratin** or enzymes that mediate **apoptosis**. If our description of **gene expression** left you wondering "if proteins convert DNA instructions into protein, where did *the first proteins* come from?" we have some reading material on RNA enzymes (and the beginning of life) here.

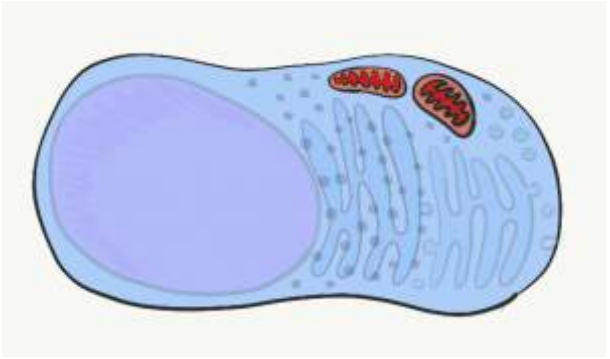

Fig. 1.6: Mitochondria have two phospholipid bilayers

Mitochondria

The **mitochondria** are where the majority of Adenosine Tri-Phosphate (**ATP**) is produced. ATP is made of Adenosine, plus three phosphate (PO_4^{3-}) groups– pay attention to the phosphate part, it is also a major component of bone, enamel, dentin and cementum. ATP powers almost all cellular processes, including the **transcription** and **translation** of **mucous** proteins within a salivary gland, the electrical signals sent by neurons in the tongue when food enters the oral cavity, and the contraction of **myo-epithelial** cells to cause salivation. Mitochondria burn glucose, using oxygen, and harness some of the energy released in the form of ATP. Mitochondria are different from other organelles in that they contain a little bit of their own **DNA**, which is inherited just from mother. Mitochondria contain two phospholipid bilayer membranes, versus one like other organelles. This extra membrane is used during ATP synthesis.

Perhaps you covered glycolysis and the citric acid cycle before. The part to remember is that mitochondria use a proton (H^+) gradient, which makes the inside of mitochondria acidic. It is toxic to the rest of the cell should mitochondrial membranes become damaged.

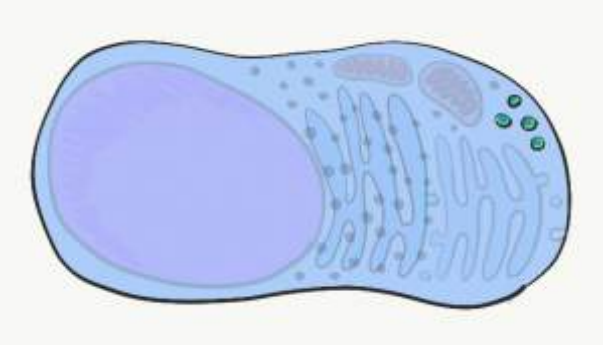

Figure 1.7: Lysosomes are capable of digesting materials.

Lysosomes

Lysosomes are small compartments surrounded by a phospholipid bilayer (a bilayer similar to **plasma membrane**). Inside lysosomes are acids and digestive enzymes. They are used to destroy stuff inside the cell when it wears out, or materials the cell phagocytosed from outside (e.g. debris, bacteria). When a cell dies and begins to break apart, neighboring cells are in danger of being damaged from the acids and enzymes found within lysosomes. In the oral cavity, epithelial cells only have a life-span of days before they wear

out, so it is very important for these cells to neutralize acids and enzymes in their lysosomes before dying (part of a process called **apoptosis**).

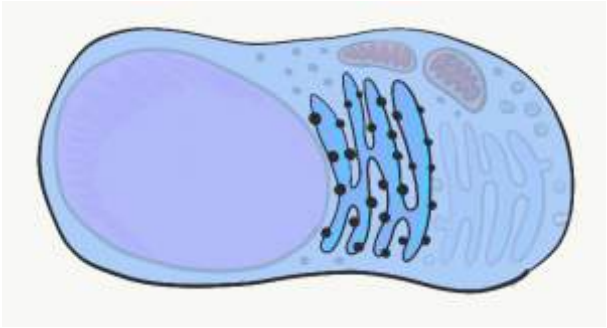

Figure 1.8: The rough Endoplasmic Reticulum (rER), with bound ribosomes.

Endoplasmic Reticulum

The Endoplasmic Reticulum (ER) is a series of interconnected tubes surrounded by a phospholipid bilayer– similar to **lysosomes**, only bigger, more tubular, and not full of acid. The smooth Endoplasmic Reticulum (**sER**) is where cells produce lipids and store calcium. The rough Endoplasmic Reticulum (**rER**) (Fig. 1.8) is covered in **ribosomes**. Proteins made by these ribosomes wind up inside the rER, travel to the **Golgi apparatus**, and are secreted (such as proteins in **mucus**)

or stay within the **plasma membrane** (such as cell-junction proteins, or **receptors** for **morphogen**[←] molecules).

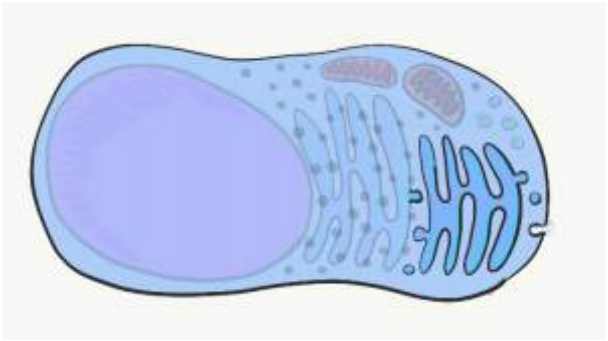

Figure 1.9: The Golgi apparatus is where proteins synthesized in or on the rER are taken next, via vesicles, before being transported to the plasma membrane.

Golgi apparatus

The **Golgi apparatus** is another set of tubes, similar to the rER. Small membrane-enclosed spheres called **vesicles** shuttle proteins made in the **rER** to the Golgi apparatus, where the

proteins are modified. Often, these proteins have sugars attached to them, making them **glycoproteins**. New vesicles take these proteins to the **plasma membrane**, where they are either secreted or become a part of the plasma membrane. An example of a secreted protein is **collagen**, found in cementum, dentin and the **PDL**. Other secreted proteins include the glycoprotein **fibronectin** and the **trans-membrane protein integrin**, which play important roles in the healing of damaged gingival tissue.

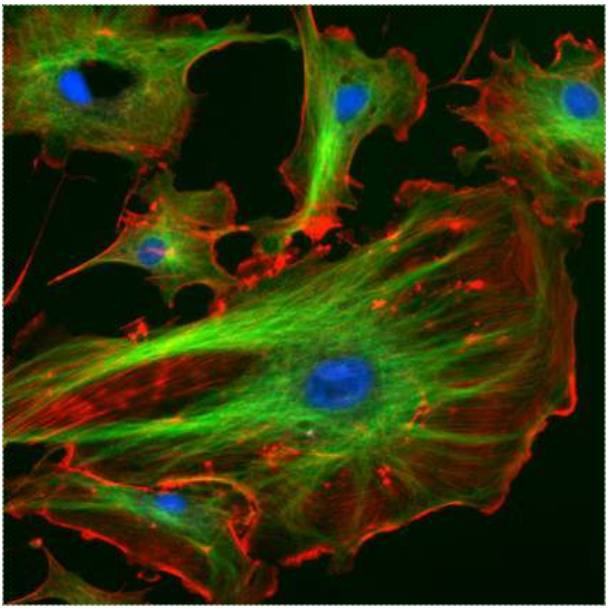

Figure 1.10: The cytoskeleton, viewed by confocal microscopy. Image credit: Fluorescent cells by NIH image is in the Public Domain CC0

Cytoskeleton

The **cytoskeleton** is a structural network within the **cytoplasm**. The cytoskeleton is composed of long structural protein fibers. Shown in Fig 1.10, cells have microtubules and actin filaments stained red and green, respectively. You won't see this on old-fashioned histology images– both proteins stain pink on a **H&E** stain, as do soluble proteins in the cytoplasm. Motor proteins, such as myosin and dynein, bind to cytoskeleton fibers and move. Depending on what the fiber and motor proteins are anchored to, this can cause the cell to change shape, to migrate, or move materials within the cell (such as **vesicles** or **chromosomes**). During **dentinogenesis**←, it is critical for **odontoblasts** to secrete enzymes that catalyze mineralization of dentin from only one side of the cell (from the **odontoblastic process**). Otherwise, odontoblasts could become cemented within their own secretions. This involves moving vesicles along the cytoskeleton, toward the **apical** surface of the cell. It is also important for **mesenchymal stem cells** to migrate over protein **scaffolding** during would repair in the **oral mucosa**. This involves changing the length of cytoskeleton proteins, which changes the cell's shape. Shortening the cell while extending the next pseudopod pulls mesenchymal stem cells along fibers in the **ECM**.

Outside the cell

Extra-cellular matrix:	Description:
Ground substance	The background color
Fibers	Lines over the background

Table 1.1: Two major components of ECM.

Extra-Cellular Matrix (**ECM**) includes all the material found outside of cells. It is usually divided into the two parts listed in Table 1.1.

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histology and animation of areolar CT

Fig. 1.11: Histology of areolar connective tissue, highlighting ECM components (fibers and ground substance)

Ground substance

Ground substance is the name for material outside of the cell that has no particular shape when viewed under traditional microscopy. One part of ground substance is Extra-Cellular Fluid (**ECF**), which is the water and nutrients surrounding cells. This fluid is plasma when it is inside of a blood vessel, but once this fluid exits the blood and surrounds cells, it is called

ECF. This fluid does not flow or drip, it is held in place by large solutes found in ground substance, including proteins, **glycoproteins**, and polysaccharides. Small solutes tend to diffuse away, down their concentration gradient. The large molecules of ground substance are stationary, and instead hold fluid in place, forming a gel. Ground substance doesn't look like much under a microscope, no more than if you looked really closely at jello. These proteins, glycoproteins and polysaccharides are made and secreted by cells (these cells have a lot of **rER** and **Golgi apparatus**).

One of the glycoproteins found in ground substance is **fibronectin**. Cells may recognize, bind to and move along fibronectin if they have the correct **integrin** (a **transmembrane protein**) spanning their **plasma membrane**. Fibronectin acts not only as a road along which cells travel, it is also a road *map*. Getting the right cells to the right place at the right time is very important both in wound healing and in **development**. In fact, what you learn about development is re-used (**recapitulated**) in wound healing.

Another important molecule found in ground substance is a large polysaccharide called **hyaluronic acid**. Like fibronectin, cells bind to and travel over hyaluronic acid (using a different type of plasma membrane protein). Hyaluronic acid has applications in dentistry, such as helping cells of the gingiva stick to a **dental implant** to form a bacteria-resistant seal. We can't see fibronectin or hyaluronic acid without using modern imaging tricks, which is why they

are listed as a part of ground substance, and not in the next section, **fibers**. Because cells migrate over ground substance proteins, we say that **ECM** proteins function as a **scaffold**. Without scaffolds, tissues grow only from their edges. This is fine if speed is not critical, such as when enamel and dentin are forming, while the embryo is safe inside a uterus. Following an injury, however, it is optimal for a wound to heal everywhere at once, not just from the edges. The body often puts down some form of scaffolding first, such as a scab. In dentistry, artificial scaffolds help the body heal faster, reducing the need for surgical replacements. Examples of artificial scaffolds include some **bone grafting**[←] materials and **periodontal membranes**[←].

In addition to their structural role guiding cells to new locations, ground substance molecules provide cells with **positional information**. This information tells cells where they are located, and what they should be doing. For instance, when a **stem cell** binds to fibronectin, fibronectin can instruct the stem cell to **express** different **genes** and **differentiate**[←] into a specific type of cell. This can mean the difference between a **stem cell** differentiating into an **odontoblast** which creates **reactionary dentin**, or a **fibroblast** which creates scar tissue. Getting cells to the correct location is nice, but they need to know what to do when they get there. When we cover tooth formation and tooth repair, remember these functions of fibronectin and hyaluronic acid.

As we learn more about how ground substance instructs

stem cells, we get better at helping teeth and **periodontal** tissues to repair themselves.

Fibers

Three stringy shapes were visible under a light microscope a century ago, and were grouped together as **fibers** of the **ECM**. Like **fibronectin** and other ground-substance molecules, fibers are secreted by cells called **fibroblasts**.

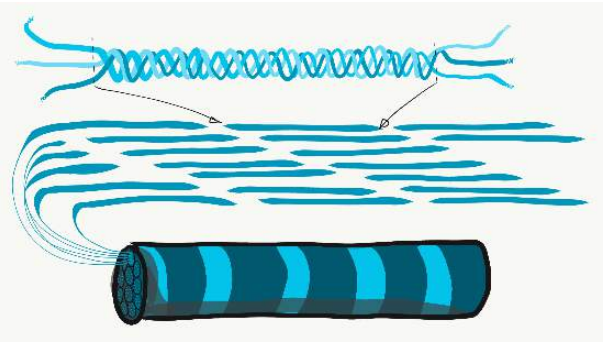

Figure 1.12: Collagen fibers are made of numerous individual proteins woven together, which are in turn woven together.

Collagen is the strongest of the three **fibers**. It is therefore referred to as a structural protein—it gives many organs their shape. Collagen is a large protein. Getting collagen from the **rER** (where it is **translated**) to the **Golgi apparatus** where it is modified, and finally secreted from the cell requires

extra-large vesicles for transport. Collagen *fiber*s are made of 3 coiled α-helices, that are in turn coiled and cross-linked together, making a very strong macromolecule with the same basic shape as a rope. It is very strong if you pull on it from the ends, but bends if you apply force from the side. For instance, an area of advanced caries on a tooth can be as soft as wet driftwood. It contains collagen fibers without the plentiful minerals that used to surround them. Collagen fibers are found in regions of the oral cavity where the ability to resist force is important, such as in dentin and the **PDL**. In fact, collagen is found throughout the human body, accounting for 25% of our protein content.

In addition to having a structural role, collagen acts as a **scaffold**. Similar to **fibronectin** and **hyaluronic acid**, cells use collagen fibers to migrate along. Like fibronectin, collagen also provides cells with **positional information**. This is possible because there are 28 different collagen genes which are assembled into slightly different versions in different locations (similarly, fibronectin comes in multiple isoforms thanks to alternative splicing). For example, **IEE** cells **differentiate** into **ameloblasts** after they come into contact with type IV collagen found in **pre-dentin**, rather than type I found in the **basement membrane**.

Reticular fibers, which are not shown in Fig. 1.11, look like a fine, spider web-like mesh under the microscope. Long after histologists first saw and named reticular fibers, molecular biologists discovered that reticular fibers are made of the

collagen protein. Nevertheless, they have their own name, and receive equal footing with collagen fibers on the list of **ECM** fibers. This web-like network of protein isn't as strong as collagen fibers, but provides enough of a frame for blood cells to rest in organs like the spleen and lymph nodes. Without reticular fibers, loose cells would sink to the bottom. To summarize: collagen *proteins* can form collagen *fibers*, or collagen *proteins* can form other shapes that aren't fibers, including reticular fibers. Got that? We apologize. Categorizing molecules as ground substance versus fibers is based on what things *look like* under a microscope. The collagen *protein* is a single molecule that is **transcribed** and **translated** from a **gene**. This won't be the last time where the **lineage** (in this case, genes) does not match the **morphology** (physical appearance). Be prepared to face this question repeatedly: which is more important, lineage or appearance?

Elastic fibers are thinner than collagen fibers, and look like fine hairs under the microscope. They are made from a different protein (elastin). As their name suggests, elastic fibers can be stretched and spring back to their original length. This is not something collagen does well. If you can't put your palms on the floor while keeping your legs straight, the collagen fibers of your hamstring and gastrocnemius tendons are limiting your range of motion. Regular stretching exercises activate **fibroblasts**, which in turn lengthen collagen fibers. Elastic fibers are found in higher quantity in regions of the oral

cavity that change shape during speech or swallowing, such as the **soft palate**.

Cell processes:

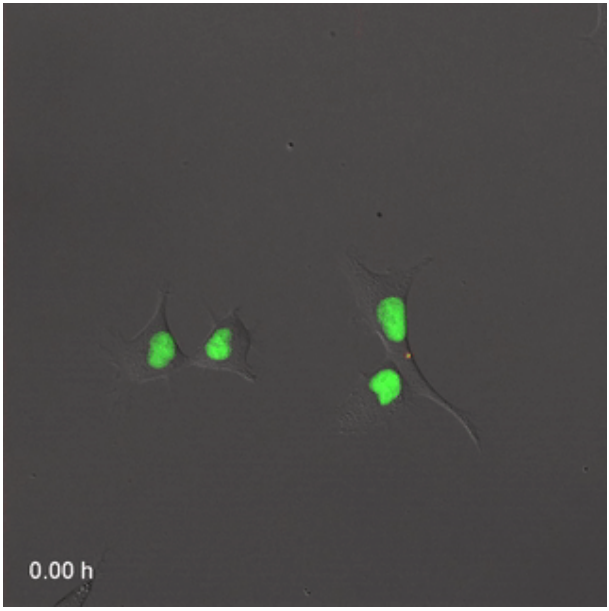

Figure 1.13: Mitosis. Image credit: "Far-Red & Near-infrared Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI") by Erin Rod is licensed under CC BY-SA 4.0 / *converted to gif*

Mitosis

Cell division, or **mitosis**, is the process by which one cell replaces itself with two copies. During mitosis, one cell divides into two identical daughter cells– there is no parent cell left after cell division. During **development**, we grow from a single cell into 30 trillion cells, so a lot of mitosis occurs. In the time it takes you to read this sentence, several billion cells in your body will go through mitosis to replace cells that just died. When a cell is not undergoing mitosis, it is said to be in interphase (in Fig. 1.15, interphase is G1, S and G2 combined). This is the time where a cell might be doing its job, such as secreting **fibers** or preparing for mitosis. Before mitosis can occur, a cell must have roughly double of everything. During mitosis, everything is divided in half between two new daughter cells.

Not all cells are capable of mitosis– in fact, most cells in an adult have **differentiated**. These cells are performing tasks, they are too busy to reproduce. We say these **terminally differentiated** cells have exited the cell cycle.

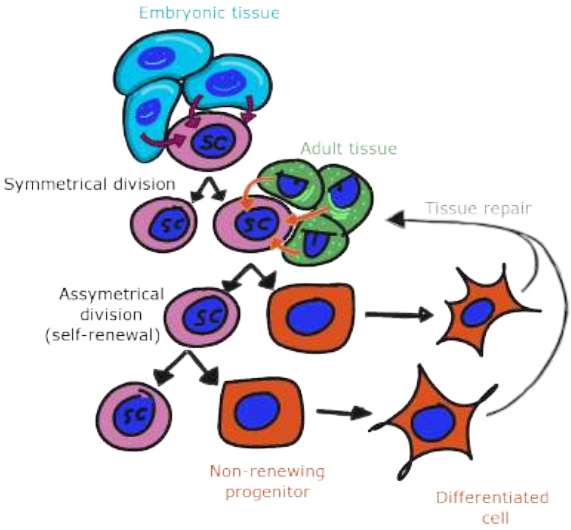

Figure 1.14: During early embryonic development, stem cells (SC) divide to produce two stem cells (symmetrical division). Later, stem cells typically undergo asymmetrical division, where mitosis produces one stem cell and one cell fated to become a terminally differentiated cell. Differentiated cells

can produce new tissue, or repair damaged tissue, but do not form new cells.

To repair damage, tissues have **stem cells**. A stem cells is an undifferentiated cells that is capable of dividing and **differentiating** into one or more different types of cells. When an **adult stem cell** divides into two daughter cells, one daughter typically remains a stem cell, and the other differentiates into something else. A tissue therefore keeps a steady supply of stem cells, as long as the stem cells don't die before they undergo mitosis. As we get older, our tissues don't heal as well because some stem cells have died before they replaced themselves. After early development, when a stem cell dies, it is gone. Another stem cell does not undergo mitosis to produce two stem cells to replace it.

Stem cells are named based on how many different types of cells they can potentially become. The uni-potent stem cells of the **oral epithelium** become **keratinocytes**, and only keratinocytes. The multi-potent **neuro-mesenchymal stem cells** differentiate into **odontoblasts**, **cementoblasts**, **fibroblasts** and **osteoblasts** (and might be referred to as partially differentiated). The omni-potent fertilized egg becomes every cell in a human, plus more.

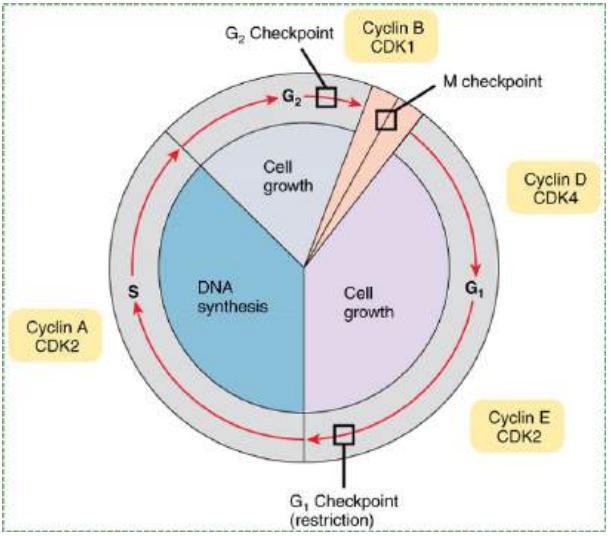

Figure 1.15: The cell cycle. Image credit: "Anatomy and Physiology Fig 18" by OpenStax is licensed under CC BY SA 4.0

To go through interphase and prepare for another round of **mitosis**, cells go through a series of **cell cycle checkpoints**. Checkpoints set up the base rate for cell division, which helps ensure that the correct amount of tissue growth occurs. This is like an hourglass, only instead of grains of sand accumulating in the base, it is phosphate residues accumulating on cyclin proteins. Cyclins are **transcription factors** that activate **genes** that allow progression through the checkpoint. Their activity is regulated by phosphorylation (carried out by enzymes called Cyclin-Dependent Kinases, or CDKs). The speed of this process can be sped up or slowed down by external signals, such as **growth factors**. Growth factors are

hormones that are secreted into the **ground substance** of a tissue. The density and stickiness of the ground substance influences how far the growth factor diffuses. If diffusion is limited, the growth factor only speeds up cell division in a localized area. This is important in the formation of new organs such as teeth, and for tissues such as bone and **PDL** fibers. Other growth factors might spread over a wide area, especially if they are secreted into the bloodstream. Secretion of Growth Hormone into the blood, for instance, allows different organs to grow at roughly the same speed.

Mutation in a gene for a growth factor, or the **receptor** for a growth factor, can lead to a **gain-of-function** (more reading here). If a mutation to a gene causes a cell to receive a constant go-signal for passage through the cell cycle, we call the mutated gene an oncogene. In this book, we cover the role of **Wnts** in head and tooth **development**, but Wnts were first discovered for because they cause cancer. It takes just one mutated copy of an oncogene to *gain* a function. Some mutations can stop proteins from functioning. If those proteins are necessary for passage through the cell cycle, we call those tumor suppressor genes. One example of a tumor suppressor gene is Retinoblastoma protein (pRb), which normally halts progression from G1 to S phase. For a cell to *lose* the ability to stop at a cell cycle checkpoint, both copies of a tumor suppressor gene alleles must be mutated. A metaphor might be helpful here. In your car, it takes *one* foot to step on the gas too hard, but

you would have to be missing *two* feet to not be able to hit the brakes (**loss-of-function**). Usually, cancers form when a single cell acquires mutations to multiple oncogenes and multiple *pairs* of tumor suppressor gene **alleles**. An allele is one of the two copies of a gene found in the same position on two **homologous chromosomes**. Even when all of these mutations happen in a single cell, there is another layer of protection covered in the next section.

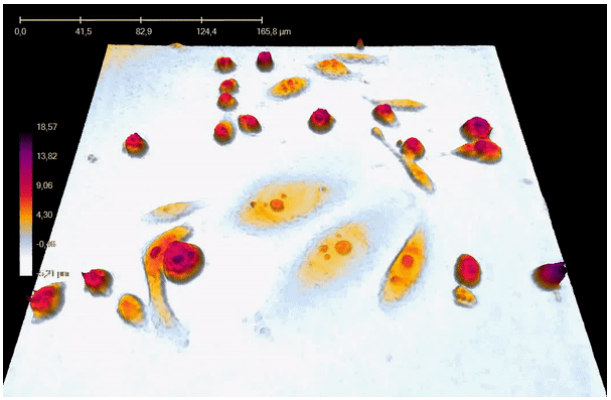

Figure 1.16: Cells undergoing apoptosis. Image credit: "43705" by Birgite Janicke is in the Public Domain CC0 / *converted to gif*

Apoptosis

All cells contain a group of cell-surface **receptor** proteins and intra-cellular enzymes that allow them to undergo programmed cell death, or **apoptosis**. Programmed cell death is critical to multi-cellular life. read that again, it is an odd thing to say. Without programmed cell death, our bodies would quickly fill up with old, non-functioning cells. Even worse, when cells die– the lifespan for epithelial and blood cells is only weeks– they release the contents of their **lysosomes** and **mitochondria**. The contents of these organelles are acidic and contain digestive enzymes, which damage or kill neighboring cells. If those neighboring cells die as a result, they too would release their lysosomal and mitochondrial contents, causing even more damage. When this happens in the human body, it is called tissue **necrosis**. That's not all, a dead cell spews out **DNA**, and DNA is long, stringy and sticky. This can trap other cells and prevent them from migrating properly. Spewing out DNA is great at trapping and killing bacteria, assuming the spewing cell doesn't mind dying in the process. But this is not something good to do to neighboring human cells on accident. This is related to how a periodontal infection can *lead to* destruction of the periodontium, but doesn't directly *cause* the destruction.

To avoid the postitive feedback loop of cell death leading to more cell death, cells can be instructed to undergo apoptosis. This occurs as cells reach the end of their lifespan, if the

immune system has determined cells to be infected or cancerous, or if cells aren't needed anymore. Apoptosis ensures that before a cell dies, it neutralizes the pH of its lysosomes and mitochondria, and chops up its DNA into safe, small bits.

Apoptosis is especially important during embryonic **development**. During development, more cells are produced than needed. Later, the extra cells are removed in an organized fashion. This is similar to the way construction of a large building involves building **scaffolding** first, and removing the scaffolding towards the end of the project. The process of wound repair also involves an over-production of cells followed by their organized removal. Hopefully, this will seem logical by the time you finish this book. During wound repair, DNA instructions are turned on again that were last used during embryonic development. If you want to sound fancy, and don't we all, you can say wound healing recapitulates embryonic development. To **recapitulate** means to state again (repeat).

The process of apoptosis begins either with an internal or an external signal. Internal signals include when a cell's **DNA** becomes too mutated, or if there are an odd number of **chromosomes** during **mitosis**. Alternatively, a cell can be instructed to undergo apoptosis from an extracellular signal (a **cytokine**) such as **RANKL** (a member of the Tumor Necrosis Factor (TNF) family of signaling molecules). RANKL activates a **transmembrane protein receptor**, which in turn activates a series

of enzymes called caspases, which leads to the neutralization of acids, destruction of DNA, and cause the cell to explode into numerous small bits (Fig. 1.15). These bits can be easily cleaned up by macrophages (which are stimulated by RANKL when other cells begin apoptosis).

Cell junctions

Types of cell junctions	Examples
Cell-to-cell	Desmosomes, tight junctions, gap junctions, Cell Adhesion Molecules.
Cell-to-ECM	Hemi-desmosomes, Cell Adhesion Molecules.

Table 1.2: Major types of cell junctions.

Junctions are specialized groups of proteins on or near the cell surface that make connections to other structures. These connections can be to other cells, or to the **ECM**, as listed in Table 1.2. Cell-to-ECM contacts are very important for cell migration. **Cell-to-cell contacts** allow **receptors** on the cell surface of one cell to bind to molecules on another cell, which is the basis for most cellular communication in very early **development**.

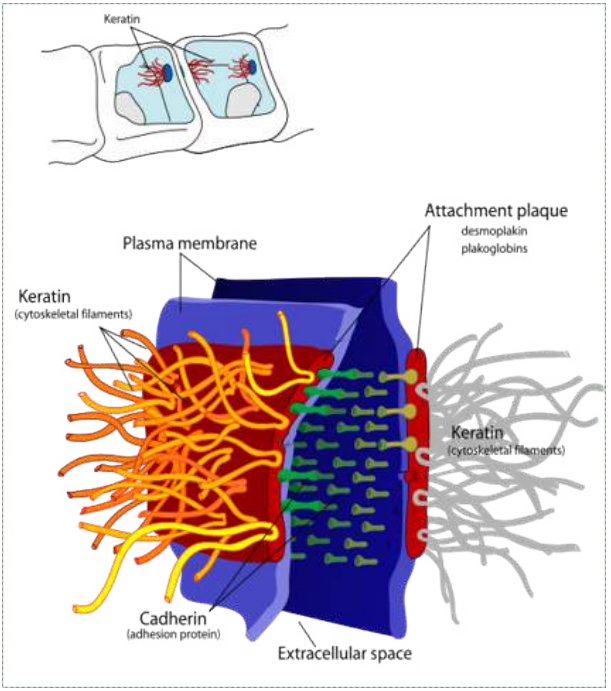

Figure 1.17: Desmosome (anchoring junction). Image credit: A desmosome, also known as macula adherens by Mariana Ruiz LadyofHats is in the Public Domain CC0

Desmosomes

Cell-to-cell junctions called **desmosomes** (anchoring junctions) are strong connections between two cells. Desmosome proteins pair up and anchor the **cytoskeleton**

of one cell to its neighbor. A large group of cells anchored together by these junctions are much stronger than the sum of the individuals.

"*A single twig breaks, but the bundle of twigs is strong*" —Tecumseh

Hemi-desmosomes are half of a desmosome anchored to the **ECM**, such as the seal between the **junctional epithelium** and the non-cellular surface of a tooth. One of the many proteins in a desmosome is an **integrin**. This protein recognizes and binds to proteins in the ECM such as **fibronectin**. When the integrin protein of a cell connects to fibronectin, this does two major things. First, it anchors the cell's **cytoskeleton** to the ECM and anchor the cell in place. Integrins also signal to the inside of the cell, allowing the nucleus to know what type of tissue the cell is located in.

Before a cell can migrate to a new location, it must first remove its desmosomes. During **development**, cells frequently migrate to new locations and form new structures. During wound healing, **stem cells** detach from their neighbors, migrate into the injured area, and undergo **mitosis** to create more cells to repair or replace damaged tissue.

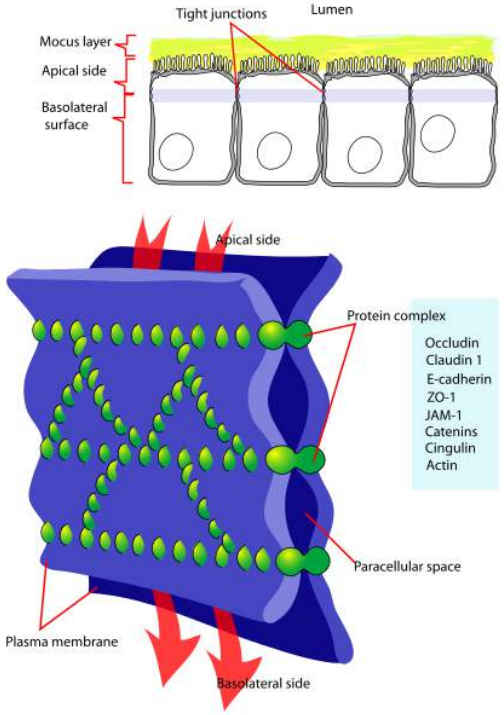

Figure 1.18: Tight junctions. Image credit: Diagram showing a tight junction by Mariana Ruiz LadyofHats is in the Public Domain CC0

Tight junctions

Tight junctions are smaller junctions between cells. A ring of tight junctions completely encircles a cell, and creates a water-tight seal between that cell and another cell. When lots of cells are joined in this fashion it creates barriers between one part of the body and another, allowing the cells to regulate what goes across and what does not. This also gives cells **apical-to-basolateral polarity**[←] (or a difference between top and

bottom), which is especially important to an epithelium. Proteins synthesized on the **rER** can be sent to either the **apical** or **basolateral** portion of the **plasma membrane**. Once there, **trans-membrane proteins** of the apical side of the cell cannot diffuse to the plasma membrane on the basolateral side, because the ring of tight junctions blocks their movement.

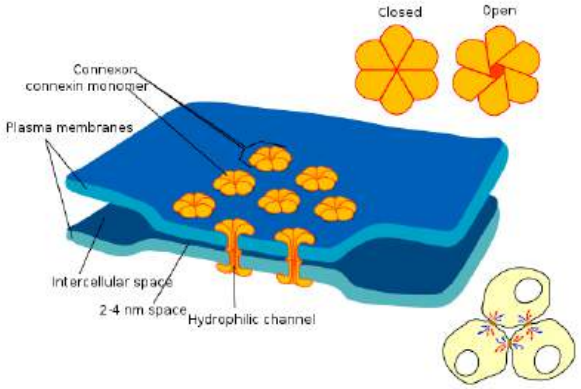

Figure 1.19: Gap junction. Image credit: the diagram shows a cell union called gap junction By Mariana Ruiz LadyofHats is in the Public Domain CC0

Gap junctions

Gap junctions (or connexons) are a group of proteins that form a small passage between cells, and can be opened or closed. This gap allows cells to communicate directly with one another. Because of the way epithelial cells are connected to

each other– in a sheet– this communication occurs across a plane. This is one way that cells know their position relative to one of the body's axes, and is a process called **planar cell polarity** (PCP) (further reading on PCP). Planar cell polarity is side-to-side polarity, while **apical-to-basolateral polarity**← is top-to-bottom. Planar cell polarity allows cells to know what direction they are facing in the body, ensuring that the structures they are forming are not only in the correct location, but in the correct orientation. For instance, planar cell polarity helps teeth to erupt straight into the oral cavity, rather than at an angle.

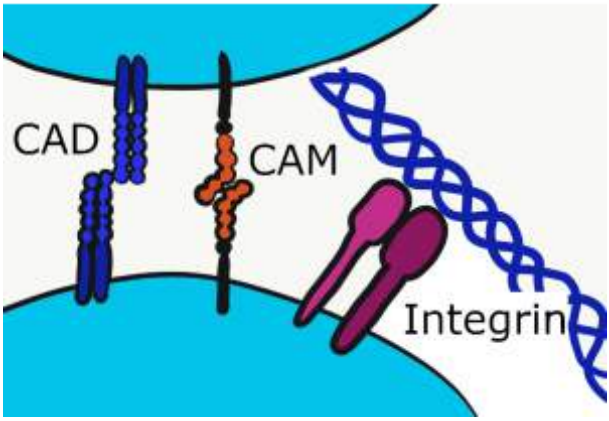

Fig 1.20: Illustration of some major Cell Adhesion Molecules, including Cadherins, CAMs and Integrins (binding collagen)

Cell Adhesion Molecules

Cells can stick to other cells (cells of the same type) using proteins not visible under the microscope. Collectively, these are Cell Adhesion Molecules (**CAMs**), which includes the **integrins**. These **trans-membrane proteins** adhere to **ECM** proteins, or to other CAMs of the same type. For instance, Neuronal CAM (NCAM) only binds to NCAM, not to Endothelial CAM (ECAM). These molecules not only adhere cells to the correct target, they signal to the nucleus when attachements are made, which can trigger **differentiation**←. Furthermore, it is just as important to let go at times as it is to stick. CAMs regulate the loss-of-attachment necessary for cell migration during important **developmental** processes like **gastrulation**←, or later in life during wound healing.

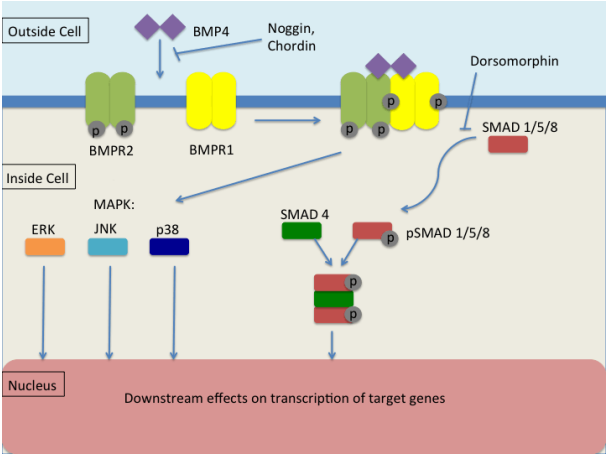

Figure 1.21. An example of a signal transduction pathway. Image credit: "BMP4 Signal Transduction Pathways" is licensed under CC BY-SA 3.0

Signal Transduction cascades

During **development**, there is a lot of communication between cells. For two or more cells to communicate, one cell must secrete a signal, such as a **growth factor** or **morphogen**[←] molecule. These signals are usually small molecules that diffuse through the **ground substance**, or larger molecules that become part of the ground substance. These signals bind to **trans-membrane proteins** called

receptors. The receptor next activate a series of intracellular signals, often enzymes (a **signal transduction cascade**). During development, these intracellular signals activate or inhibit **transcription factors** or alter **DNA methylation**, which in turn changes **gene transcription**, which in turn changes **cell fate**. Fig 1.20 illustrates how complex a signal transduction cascade can be. The important concept is that blocking any of the items in Fig 1.20 leads to the same result: the signal will not reach the nucleus, and **differentiation** will not occur (**loss-of-function**). Conversely, chemicals that mimic any of the items in Fig 1.20 trigger differentiation where is shouldn't be occurring (**gain-of-function**). We call these chemicals **teratogens** when they interfere with development.

Later in life, some cells become highly specialized at cell-to-cell communication. Examples include neurons which release neurotransmitters across synapses, endocrine glands which secrete **hormones** into the bloodstream, and immune cells which secrete **cytokines** into the bloodstream. You, however, will be reading about less-specialized forms of cell signaling. The signals may be basic, but work perfectly fine for small organisms like embryos. Plus, there needs to be signaling during embryonic development to guide the formation of synapses and the circulatory system.

Summary

Two big concepts introduced in this chapter are

differentiation, and **nature** versus **nurture**. Differences between two people may be caused by differences in **DNA** (*nature*). But not always,. People are different because of their environment as well (*nurture*). To explain why one patient is healthy and the other is unhealthy you must consider both nature and nurture. Nurture often plays a bigger role. For example, poverty has a much bigger impact on human health than genetics.

But what about when we discuss different cells in the same person? When we do, nature doesn't factor in at all (cells have the same **DNA**). So any differences between two cells must be due to nurture. We don't mean the environment the patient is in, such as wealth versus poverty. We mean the environment immediately surrounding a cell. We covered many *environments* in this chapter, including:

- what **ECM** surrounds the cell
- what signaling molecules (**growth factors** and **morphogens**) are in nearby **ground substance**
- what **receptors** and **CAMs** are on the **plasma membrane** of the cell
- which **genes** can be **expressed**, versus which ones are in storage using **methylation** and **histones**

These environmental factors guide the process of **differentiation**. Differentiation makes tissues look different in chapters 2 through 5. It makes body structures **develop**

differently in chaptes 6 through 11. But there are similarities as well. Those similarities can be due to cells having the same DNA (nature) or sharing a similar environment (nurture).

< Chapter 0 * navigation * Chapter 2 >

Review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:
https://openoregon.pressbooks.pub/histologyandembryology/?p=23

2.

HISTOLOGY REVIEW

- A short history of histology
- 4 main tissue types (by adult appearance)

    1. epithelia
    2. connective tissue
    3. muscle
    4. nervous

- 3 main tissue types (by embryonic lineage)
    - ectoderm
    - mesoderm
    - endoderm

histology and animation of areolar CT

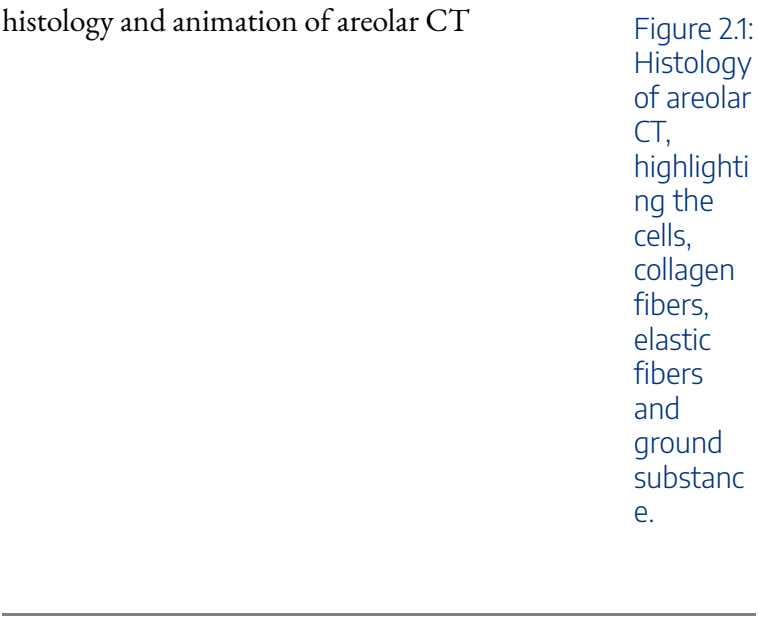

Figure 2.1: Histology of areolar CT, highlighting the cells, collagen fibers, elastic fibers and ground substance.

A short history of histology

The aim of this chapter is to review histology covered in Anatomy & Physiology classes– but only the tissues that appear in the head and neck, and only the parts relevant to dental hygiene. This is by no means a comprehensive review of histology. If you need an extra review on general histology, try these links:

- An interactive or media element has been excluded from this version of the text. You can view it online here: https://openoregon.pressbooks.pub/

histologyandembryology/?p=30

- An interactive histology flowchart (link provided if content above does not show in your eReader)
  - by Laird C. Sheldahl, Ph.D.
- The Internet Pathology Laboratory for Medical Education
  - at The University of Utah, Eccles Health Sciences Library.
- Histologyguide.com
  - by T. Clark Brelje, Ph.D. and Robert L. Sorenson, University of Minnesota.
- Histology Online Lab Manual
  - by Stephen Gallik, Ph. D.

Histology is the study of what tissues look like under a microscope. A **tissue** is a group of cells, all of the same type, working together to perform a function. Because most cells are transparent, microscopists use of a number of different stains to highlight different parts of cells. Fig 2.1 is an image of a very common staining technique using the dyes Hematoxylin and Eosin (**H&E).** These two stains turn molecules with negative charges blue, and positive charges pink. We don't actually care about charges, we need to make different parts of the cell different colors so we can see them. **DNA** and **RNA** take up both colors, which makes nucleuses purple, while most proteins turn pink. Unfortunately, most cells are full of

proteins and their cytoplasm turns pink, but the **ECM** is also full of protein, so it turns pink as well. This leaves us freueqntly trying to differentiate between similar-looking seas of pinks with purple spots. Sound familiar? On the plus side, if you purchase a histology coloring book, you only need two crayons.

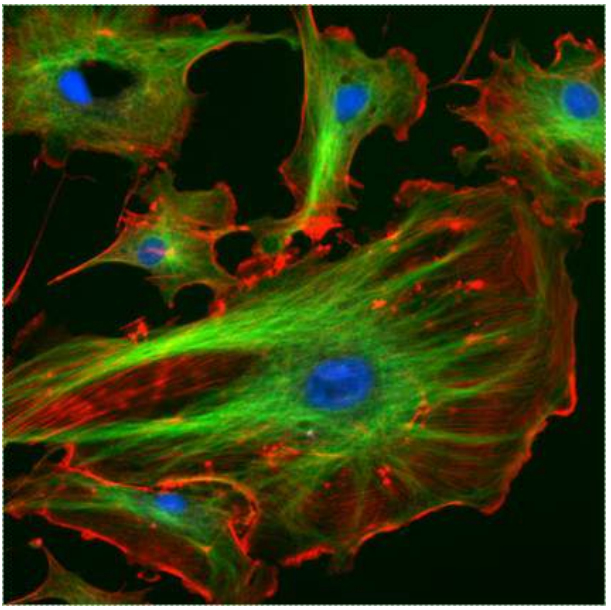

Figure 2.2: Example of a confocal microscope image. Image credit: "Fluorescent cells" by NIH image is in the Public Domain CC0

Imaging

Most of the images you see in histology textbooks use stains such as **H&E** stains– this type of technology dates back to the 1800s. Microscope technology has improved since then, such as the image in Fig. 2.2, which comes from a confocal microscope. The confocal microscope uses expensive lasers to generate very beautiful images, and uses stains that are much more specific than H&E. Sadly, most textbooks still use the old-fashioned (uglier) images. Since you should be using neither the newer nor the older microscopes in your line of work, we have illustrated most of the histology images in this book. This allows us to focus on the concepts, and spend less time developing the skills necessary to interpret pictures of pink blobs with purple spots. We presume that in the Anatomy and Physiology classes you took to get into a Dental Hygienist program, you were taught to spot the *differences* between different types of tissues. We review those differences in this chapter. That's great, especially in Anatomy, which focuses on classifying and naming things. You have a more important task now. If you didn't do so before, keep an eye out for *similarities* between different tissues (aside from most look like pinkish blobs with purple spots). Anothe way to say that is you should look for **patterns**[←]. If you jump ahead and read the embryology chapters, you'll have a better idea of what to look for. For now, keep that thought in the back of your head as we cover the major tissues of the head and neck.

Enough embryology to start learning about histology

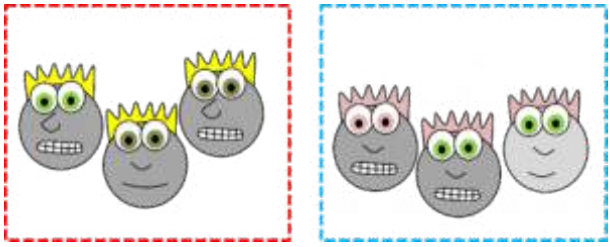

Figure 2.3: One possible way to group cartoon faces, based on physical characteristics.

Classification: the old way, and a better way.

Histology categorizes tissues based on what the cells look like in adulthood. If we determined people's families based off what they look like as adults, we might do an OK job of it, or we might make mistakes. For instance, we might lump the cartoon faces in Fig. 2.3 into two families based primarily on hair and eye color. That is how the four major tissues types are categorized. The problem with this sort of classification system is it is hard to tell what physical traits are due to **nature** (genetics, such as eye color) versus **nurture** (environmental factors, such as hair dye).

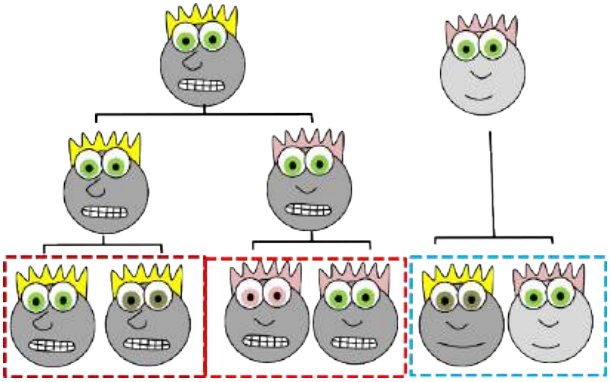

Figure 2.4: Another way to categorize cartoon faces, based off of lineage data.

When we learn about embryology, we see we can do better. We can categorize tissues using cell **lineage** instead, tracing who the adult cells are related to. Early embryologists stained individual cells and followed them through cell division after cell division to see what they became. Others, such as Hans Spemann, moved cells from one place to another and asked whether they still turned into what was expected, based on their new location. When transplanting just a few cells lead to embryos growing two complete heads, Spemann concluded that was unexpected (actually, that was the conclusion of his graduate student, Hilde Mangold, whose doctoral thesis became the basis for the work that won Spemann the Nobel Prize).

To get a better idea *how* that happens, science had to wait for the end of WWII. Suddenly, a large number of physicists no longer had jobs building deadly rockets and atomic bombs. Many turned their skills to studying genetics, creating the field of molecular biology. With new tools, developmental

biologists like Christiane Nüsslein-Volhard identified mechanisms by which a single embryonic cell becomes different types of cells in an adult. Her organism of choice was flies, and for that work she won the Nobel Prize. Unlike earlier histologists and embryologists who named everything after themselves, more recent embryologists gave their discoveries fun names like Bicoid, Dickkopf, Frizzled and Sonic Hedgehog.

So, rather than use the *appearance* of our cartoon people, if we identify their **lineage** (you might also see the fancier term **ontogeny**), we can classify them into more accurate families, with one sub-family (Fig. 2.4). When you see the word lineage, think *family tree*, where **differentiated**[←] cells are the youngest generation, and **stem cells**[←] are their parents or grandparents. Sadly, this is not how histology is taught, so we're going back to what adult cells look like and classify tissues based on physical characteristics. But as we do, look to see whether two tissues have distinct borders (likely different lineages) or blended borders (often the same lineage).

The 4 main tissue types

The 4 main tissue types
Epithelial
Connective Tissue
Muscle
Nervous

Table 2.1: The 4 main tissue types

Epithelia

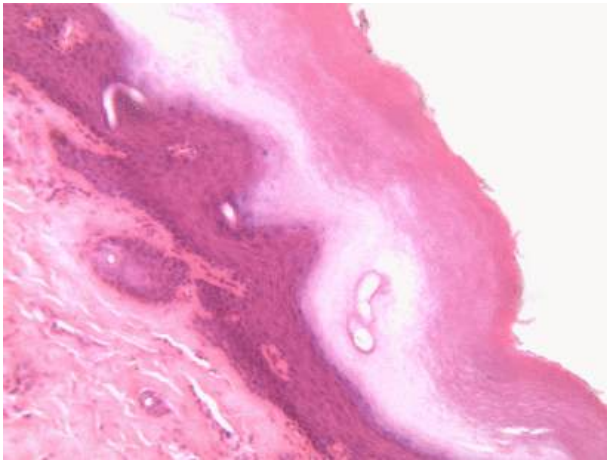

Figure 2.5: Animated illustration of an epithelium (with connective tissue below). Order of appearance: stratified squamous epithelium, connective tissue cells, ECM fibers, ground substance.

Location of epithelia

An epithelium is composed of epithelial cells, usually

connected to one another in a sheet. There is very little **ECM** in an epithelium. Epithelia are mostly cells, but they do make a small amount of ECM called the **basement membrane**. The basement membrane is located on the basal side (see below) and holds an epithelium in a single sheet. The cells are anchored to the basemement membrane by **hemi-desmosomes**, and to each other by **CAMs**← and **desmosomes**←. Desmosomes also hold an epithelium in a sheet. **Tight junctions**← ensure that the things that travel from one side of the epithelium to the other must pass *through* the epithelial cells. To cross an epithelium, molecules must either be lipid-soluble, or be recognized by **trans-membrane proteins**← on the **apical** and **basolateral plasma membrane**←. An epithelium therefore has **apical-to-basolateral polarity**. The apical surface faces the outside of the body, and the basolateral inward, and polarity means the two sides are different. The *outside* can mean the outer surface of the skin, or an inner surfaces, such as the **lumen** of the oral cavity, stomach, and bladder. Every surface of the human body is an epithelium. Therefore, under the microscope, if you see an empty space, an epithelium borders that space. The inner lining of a sweat duct is an epithelium. The inner lining of a blood vessel is an epithelium. All other tissues are between that epithelium and the epithelium on the outer surface of the body. Even enamel is made by an epithelium, so if anyone tells you enamel is a connective tissue because it *looks* similar to

bone tissue, they haven't studied embryology, and need to be educated. Use words like **ontogeny**.

Figure 2.6: Stem cells in the basal layer of a stratified epitheliu m.

Epithelia are thin

Epithelial tissue is good at healing because it contains numerous **stem cells** capable of undergoing **mitosis** (you can identify them when they have visible **chromosomes**, rather than **chromatin**). For thicker epithelia, stem cells reside in the **basal** layer. Epithelia cannot be terribly thick, however, because they are **avascular** (without their own blood vessels). Nutrients for an epithelium must diffuse from underlying connective tissue, which is why in Fig. 2.6, the epithelial cells on the **apical** side lack nucleusses. The cells are dead, but still connected to the rest of the epithelium by **desmosomes**.

The down-side of being good at mitosis is it means those cells are a few steps closer to becoming cancerous. Many people regularly expose themselves to high doses of carcinogens found in alcoholic beverages and tobacco, which can lead to oral cancers.

In addition to flat sheets, epithelial cells form glands. **Exocrine** glands, such as salivary glands, contain single layers of epithelial cells rolled up into tubes called ducts. These ducts bring exocrine secretions to a surface of the body. **Endocrine** glands are different. They are not arranged in a sheet, but are **amorphous** clusters of epithelial cells. Endocrine glands aren't **avascular**, either. Blood vessels grow into the epithelia of an endocrine gland. This is important because endocrine glands secrete **hormones** directly into the bloodstream.

Number of layers	Shape of cells
Simple	Squamous
Stratified	Cuboidal
	Columnar

Table 2.2: The basic categorization of most epithelia.

Epithelia are mostly classified based on two criteria. With a few exceptions, epithelia have a name from the first column plus a name from the second column of Table 2.2. **Simple epithelia** have just one layer of cells, while **stratified epithelia** have more than one. **Squamous** means the cells are flat, like fried eggs. **Cuboidal** means square-ish in appearance (cells are 3-D, but look 2-D under the microscope). **Columnar** means the cells are tall (like columns).

Figure 2.7: Simple squamous epithelia (endothelia) forming the walls of capillaries.

Simple squamous epithelia

If you look at the lining of a capillary, you *might* be able to see the **simple squamous epithelium** that lines the inner surface.

These cells, called endothelial cells, are functionally really cool cells, even if they are hard to see. The tissue that is composed of the endothelial cells is called an **endothelium**. The endothelium forms the barrier between the blood (or lymph) and surrounding tissues, but can become damaged, such as during probing, flossing, coughing, or from systemic inflammation due to an infection or auto-immune reaction. During an intraoral exam, damaged capillary walls may present as punctate bleeds called petechia (often on the palatal mucosa).

An intact endothelium regulates the permeability of materials in or out of a tissue. Furthermore, endothelia perform important functions in signal cell signaling by secreting hormones, including VEGF which regulates **angiogenesis** and von Willebrand factor which regulates blood clotting.

Another important simple squamous epithelium is the one that lines organs in the ventral body cavity (such as the lungs and intestines), called a mesothelium. The main function of a mesothelium is to secrete serous fluid, composed primarily of **glycoproteins** and the water they attract. A mesothelium also play important roles in cell signaling, including the inflammatory response and tissue growth.

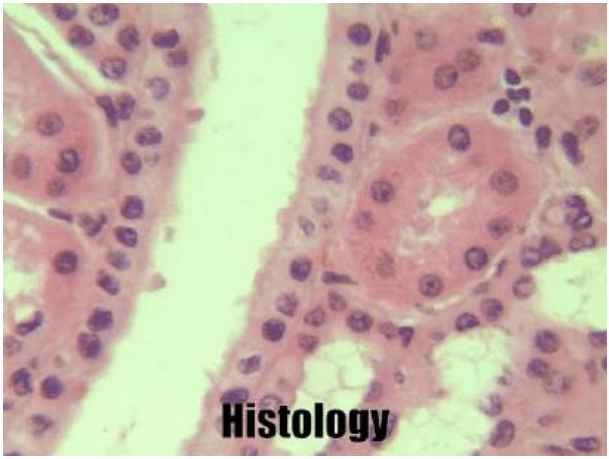

Figure 2.8: Several simple columnar epithelia (pink) and underlying connective tissue (ble).

Simple cuboidal epithelia

At one point in your life, you were a **simple cuboidal epithelium**. In adults, we find simple cuboidal epithelia lining most exocrine gland ducts, such as sweat and salivary glands. In Fig. 2.8, you should see two ducts coming out towards the camera, and one duct running up-and-down. The **lumen** of each duct is lined by a simple cuboidal epithelium. The cuboidal cells in the epithelium are connected to their neighbors on the sides by **CAMs**[←] and **desmosomes**[←]. Where two ducts are close together, there are two simple cuboidal epithelia, not one stratified epithelium. There are two separate layers of epithelia, with a little **ground substance**[←] between them. Stratified cuboidal epithelia exist, such as in parts of the parotid salivary gland, but they are rare enough that we don't need to look at them. The capillaries in Fig. 2.7

are lined by flat cells to allow for bulk flow of fluids (including nutrients) out of the capillary and waste products in. The salivary ducts in Fig. 2.8 have thicker cells to keep the flow of saliva down the tube, not leaking out of the tube creating a **ranula**.

Figure 2.9: A highly folded simple columnar epitheliu m (pink) containin g goblet cells (blue) plus underlyin g connectiv e tissue (light blue).

Simple columnar epithelia

Simple columnar epithelia are not found in the adult oral cavity. In an embryo, the columnar **IEE differentiates**[←] into

enamel-producing **ameloblasts** during the 6th week after fertilization. The shape isn't important other than it helps histologists identify which cells are **fated** to become ameloblasts in a developing tooth.

Figure 2.10: Lateral view of bed, showing a cat between the basolateral surface of the comforter and the apical surface of the thin bed sheet. Image credit: "photo" by unknown is in the Public Domain, CC0

If you are looking at the illustration in Fig. 2.9, you may wonder why there are fewer cartoon nucleuses than the histology picture. Did the illustrator mistakenly give you a picture of a stratified columnar epithelium and try to pass it off as simple? No. When we say an epithelium is simple, it means one cell thick from **apical**-to-**basolateral**. But when we cut a tissue sample to view under the microscope, the slice may be several cells thick from side-to-side. And when we lay the tissue sample on its side, the cells that were running side-to-side are now sitting on top of one another. We have illustrated what a simple epithelium *should* look like if we had the ideal one-cell-thick slice of tissue. This is a good time to bring up perspective. Epithelia form flat sheets or **membranes**, and in Fig. 2.9 we are looking at a (folded) sheet in cross section. Imagine slicing through your bed and looking at it from the side view. You could make out the individual, simple layers of your unfolded bed sheet and comforter. Furthermore, you might note that while each is a single layer, the bed sheet is thin (squamous) and the comforter is thicker (columnar). You could identify the **basolateral** surface of your bed sheet (the side that touches you when you sleep) and the **apical** surface (the side close to the comforter). In Fig. 2.10, if there was a second cat on top of the first cat, we could say the layers of cats is stratified. But if the bed sheet was covered in a single layer of cats, side-to-side and front-to-back, the layer of cats would be simple. Under the microscope, we're doing the same thing, but cells are semi-transparent (unlike cats, which you understand if you work at

home on a laptop). Histology gets messy. When we call Fig. 2.9 a simple epithelium, it means there is only one pink cell between the white space (the **lumen** of the intestines) and the very light-blue space (the connective tissue, where nutrients are absorbed into blood vessels). Folding a sheet doesn't turn it into multiple sheets. The cells in Fig. 2.9 are thicker than the cells in Fig. 2.8, but there are no columns on top of other columns.

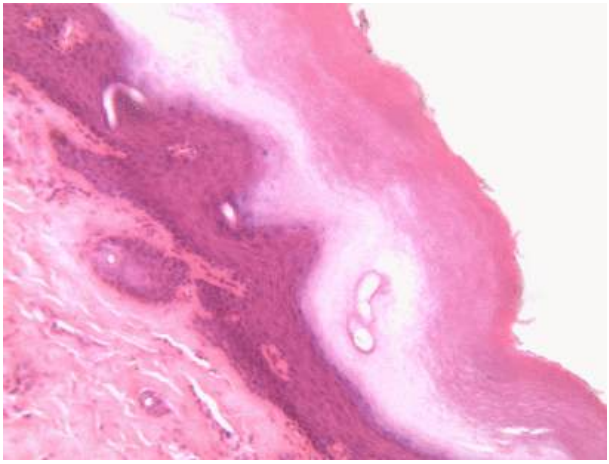

Figure 2.11: A stratified squamous epithelium (purple) and underlying connective tissue (lighter pink).

Stratified squamous epithelia

While we have different names for the skin and the **oral**

mucosa, if we pay attention to their **lineage** rather than their location or moistness, we note both contain a **stratified squamous epithelium** on their outer surface. Fig. 2.11 shows a **keratinized** stratified squamous epithelium, covered in more detail in Chapter 3. **Keratin** is a tough, water-resistant protein made by the main type of epithelial cell in this tissue, the **keratinocyte**. The stratified squamous epithelium of the skin changes at the **vermilion zone** to less-keratinized, and the stratified squamous epithelium of the oral mucosa is either less or **non- keratinized**. Based on that information, should we classify the vermilion zone as oral mucosa, skin, or it's own thing? Or is there a line in the vermilion zone that divides into a skin half and an oral mucosa half? After you learn about embryology, decide for yourself. If you change careers and go into proctology, use this same information down there.

Stratified cuboidal, stratified columnar and transitional epithelium

These tissues do not appear in this book.

histology and animation of pseudostratified epithelium

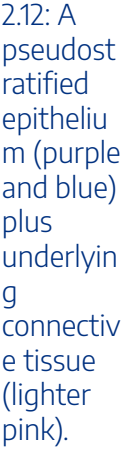

Figure 2.12: A pseudostratified epitheliu m (purple and blue) plus underlyin g connectiv e tissue (lighter pink).

Pseudo-stratified epithelia

Ciliated pseudo-stratified columnar epithelium, or as many people say, **pseudostratified epithelium**, is found in upper portions of the respiratory tract, such as lining the nasal cavity and the para-nasal sinuses. This tissue deserves a different name from the **stratified squamous epithelia** of the skin, **vermilion zone** and **oral mucosa**. However, it probably didn't need to break the naming rules outlined in Table 2.2. A pseudostratified epithelium has more than one layer, but it is hard to count how many. As a result, someone named this tissue *pseudo*-stratified (think of it as *sort-of-stratified*). The big blue cells in Fig. 2.12, which don't have cilia, are **goblet cells**. These cells produce **mucus**. Goblet cells synthesize **mucous** proteins within their **rER**[←] and secrete them, and when they

attract water, they become mucus. Mucus is very similar to **ground substance**. However, mucus is secreted out of the body, ground substance is secreted into the body. The blue coloring of the goblet cells tells us that mucus proteins do not carry the same ionic charges as most proteins found within the epithelial cells of the pseudo-stratified epithelium. Due to this difference in color, they are classified as a distinct cell– a unicellular gland found within the pseudo-stratified epithelium. But what does our friend the embryologist say? Well, the columnar cells can't become goblet cells, and the goblet cells can't become columnar cells, but they do share a common ancestor (or **stem cell**). We should consider the goblet cells part of the pseudo-stratified epithelium. Does it matter? No, but questions like this matter later in the book, it's good to start practicing now.

Connective tissue (CT)

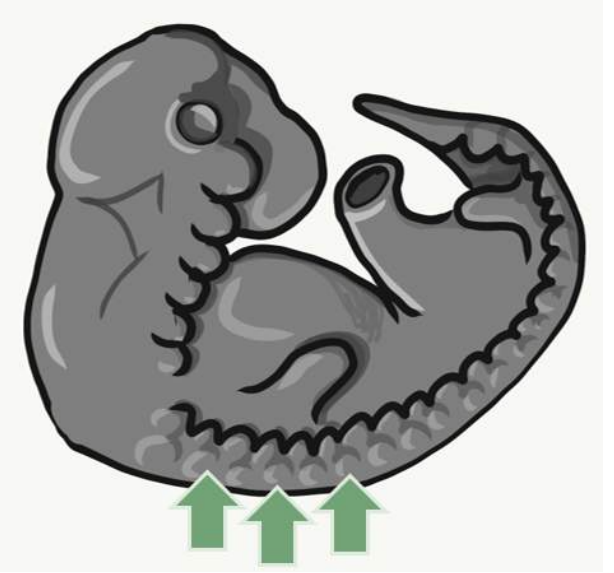

Figure 2.13: Somites of an early stage embryo, from which connective (and muscle) tissue develop

General characteristics of connective tissue

Many textbooks tell you connective tissue *connects* two other tissues. That sounds like a simple and straight-forward job. But that's like saying your smart-phone connects you to other people. It is technically true, but glosses over the fact these could be people you know, people you don't know, or people you don't want to know. It ignores the fact you could be communicating synchronously with people on the other side of the world, communicating asynchronously over email, accessing information from the near sum-of-all human

knowledge (the internet), or reading the sophomoric rant of some knuckle-dragger who, years ago, posted a rude comment on a YouTube video of a histology lecture (also the internet). So far, we've tried to skip information about histology that is not relevant to dental hygiene. But here we go into *more* detail than your average undergraduate histology class.

Many connective tissues come from segments in an embryo called **somites** (Fig. 2.13). Because of their shared **lineage**, connective tissues share a number of features. The average connective tissue has few cells, and is mostly composed of **ECM**. That matrix includes **glycoproteins** and polysaccharides made by the cells. These molecules attract water to form the gel matrix of **ground substance**. Some important ground substance molecules covered earlier are **fibronectin** and **hyaluronic acid**. ECM also includes visible fibers. The three visible fibers are **collagen**, **reticular** and **elastic** fibers. Connective tissues are typically highly **vascular**, meaning they contain blood vessels. There are a number of different cell types found within a connective tissue. The **stem cells** are **mesenchymal stem cells** (MSCs). Mesenchymal stem cells are extremely important cells (here is further reading). Since you can find them in adult tissues they are called a type of **adult stem cell**. These cells are capable of undergoing **mitosis** to produce more stem cells, which can **differentiate** into **fibroblasts**, **chondroblasts**, **osteoblasts**, hemocytoblasts, **myoblasts** , **adipocytes** and other cell types. Thus, they form most connective tissues,

including bone, cartilage, and blood (both red and white blood cells). Mesenchymal stem cells share **lineage** with muscle stem cells (myoblasts), and differentaite into muscle cells in the right environment. That's pretty much everything except epithelia and neural tissue, which mesenchymal stem cells can form *after* going through an **mesenchymal-to-epithelial transition**[←].

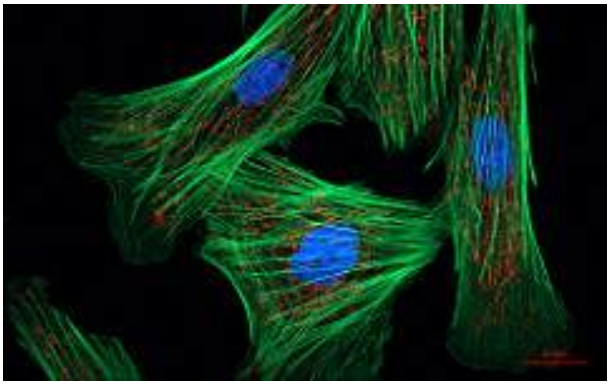

Figure 2.14: Fibroblasts, as seen under a confocal microscope. Image credit: Indian Muntjac fibroblast cells by Michael W. Davidson is licensed CC BY 2.0

In a basic connective tissue, **mesenchymal stem cells** divide and **differentiate** into **fibroblasts**. Fibroblasts are the cells that blast out **fibers** and **ground substance** (the **ECM**). There may be other cells found in a connective tissue, including adipocytes, red and white blood cells, or other cells that have emigrated from a different tissue. In a mature connective tissue, the fibroblasts are sometimes called fibrocytes, which fits with the nomenclature for a cell going through stages of differentiation (from stem to blast to cyte). More frequently, however, they are called fibroblasts and we don't worry about whether they are actively blasting out fibers or taking a rest.

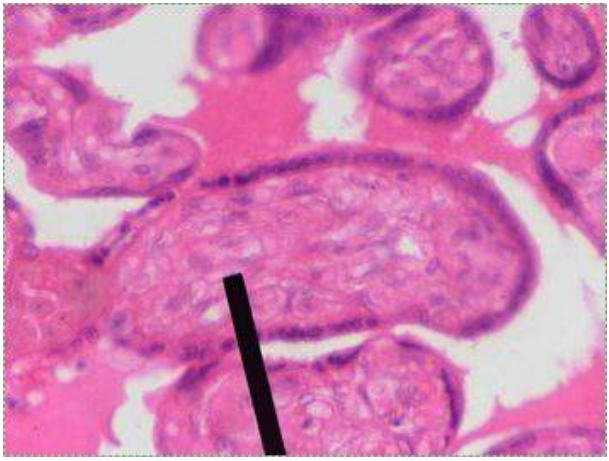

Figure 2.15: Mesenchyme, the generic embryonic connective tissue, doesn't have any specialization. Image credit: "Human Mesenchyme" By Jpogi at Wikipedia is in the Public Domain CC0

Mesenchyme

Mesenchyme is the first type of connective tissue an embryo makes. It **differentiates**[←] into other connective tissues. It is an embryonic tissue that has yet to decide what it will become when it grows up. It is composed mainly of **mesenchymal**

stem cells and **mucous ground substance**← (mucous is an adjective to describe loose **ECM**, while **mucus** is a substance secreted from the body). There is a special type of mesenchyme important to face and tooth development, called **neuro-mesenchyme**. The **lineage** of neuro-mesenchyme is not strictly from **mesoderm** like most connective tissues. Neuro-mesenchyme includes a special type of neuronal cell called a **neural crest cell**←. Neuro-mesenchyme forms connective tissues we have yet to mention: dentin, cementum, alveolar bone and the **PDL**.

---

histology and animation of areolar CT

Figure 2.16: Areolar connective tissue, histology and illustration.

Areolar connective tissue

Areolar connective tissue is the quintessential connective tissue, or the most boring depending on how you look at it. It contains a little of everything other connective tissues might have: cells, **ground substance**←, and all 3 **fibers**. Because it

has a good amount of ground substance, it is an ideal tissue to occupy places where blood vessels might need space to grow in the future. Hence areolar connective tissue is found in regions that are highly **vascular**. This is why you can find a small layer of areolar connective tissue directly underneath nearly every epithelium, including the **stratified squamous epithelium** of the skin and **oral mucosa**. Ground substance also absorbs water and swells in size. This can be caused by changes in blood flow, which is why the areolar connective tissue of the nasal cavity may also be referred to as erectile tissue. The swelling of areolar connective tissue in response to inflammation, on the other hand, is called **edema**.

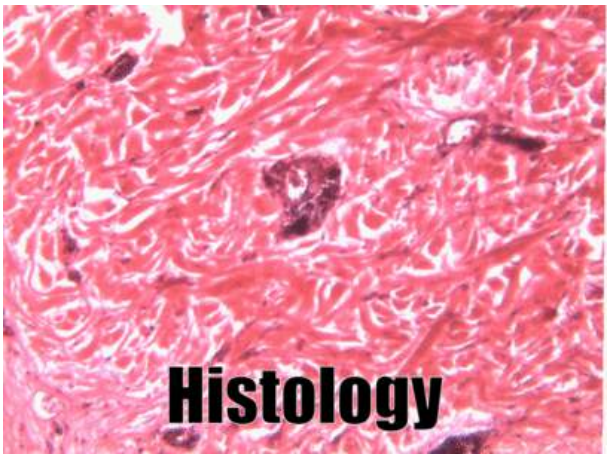

Figure 2.17: Dense irregular connective tissue, histology and illustration. Order of appearance: cells, collagen fibers, ground substance.

Dense irregular connective tissue

Underneath the areolar connective tissue of the **oral mucosa** is **dense irregular connective tissue**. **Fibroblasts** secrete the bulk of the tissue: **collagen** fibers, the strongest of the 3 fiber types. In a dense irregular connective tissue, the collagen fibers point in all directions. This makes this tissue strong in all directions. In Fig. 2.17, most of the pink is extracellular collagen fibers, and the white space **ground substance**. In addition to the **sub-mucosa** of the oral cavity, you find dense irregular connective tissue (along with its buddy, areolar

connective tissue) in the **dermis** of the skin. Why in both places, you ask? **Lineage**!

Figure 2.18: Animation of dense regular connective tissue.

Dense regular connective tissue

Like dense irregular connective tissue, **dense regular connective tissue** is mostly **collagen**[←] fibers, only the fibers run parallel to one another. That makes this tissue strong in one direction. You mostly find dense regular connective tissue between muscle and bone (tendons), or between bone and bone (ligaments). The place in this book where we find dense regular connective tissue is the **PDL**, between bone and tooth. Because dense regular connective tissue has very little **ground substance**, tendons and ligaments undergo very little **edema** when injured. They do experience pain, however.

You might be thinking the collagen fibers in Fig. 2.18 look wavy, not parallel. When someone sliced this section of a tendon, they sliced through the tissue using a thin blade. As a blade cuts softer tissue, the blade pushes fibers in one direction, then pulls fibers back, then pushes them again. For the best results, an expensive device that freezes or embeds soft tissues in wax can be used, such as a microtome, cryotome, or vibratome. We bring this up because many textbooks suggest histology is a nearly-perfect representation of what is found in the human body. In reality, there are **artifacts**, or errors, that histologists learn to ignore when we see them. We have chosen to illustrate many of the images in this book to avoid discussion of artifacts where possible, and focus on concepts relevant to dental hygiene.

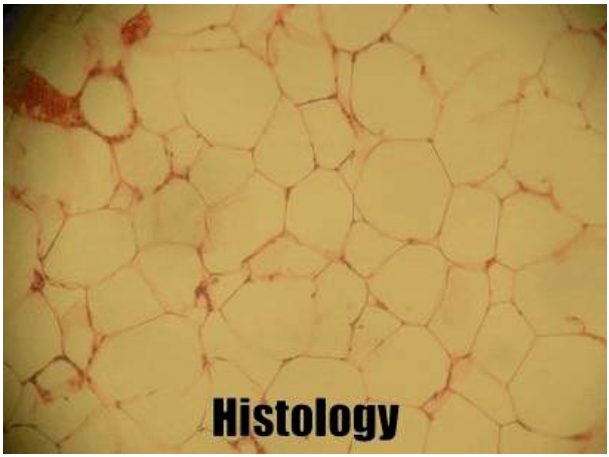

Figure 2.19: Adipose connective tissue.

Adipose connective tissue

Adipose tissue is a type of connective tissue. It is special in that is has very little **ECM**. It contains mostly **adipocytes**, the mature cells that store triglycerides. It also contains **mesenchymal stem cells**, so when a person needs more adipose tissue, these cells divide and **differentiate**[←] into more adipocytes. Technically, mesenchymal stem cells differentiate into a lipoblast first, but this is only important for the sake of staying consistent with a naming system. Maybe if someone had named the cells adipoblast and adipocyte, we'd use the -blast name more frequently, but someone messed that up. Many textbooks place adipose tissue next to areolar connective tissue and categorize them both "loose", on account of their low number of visible fibers and high **vascularity**. That's fine if you like categories based on adult appearances, but you don't need to call either of them "loose". Instead, we need to focus on embryonic **lineage**.

Histology and animation of cartilage

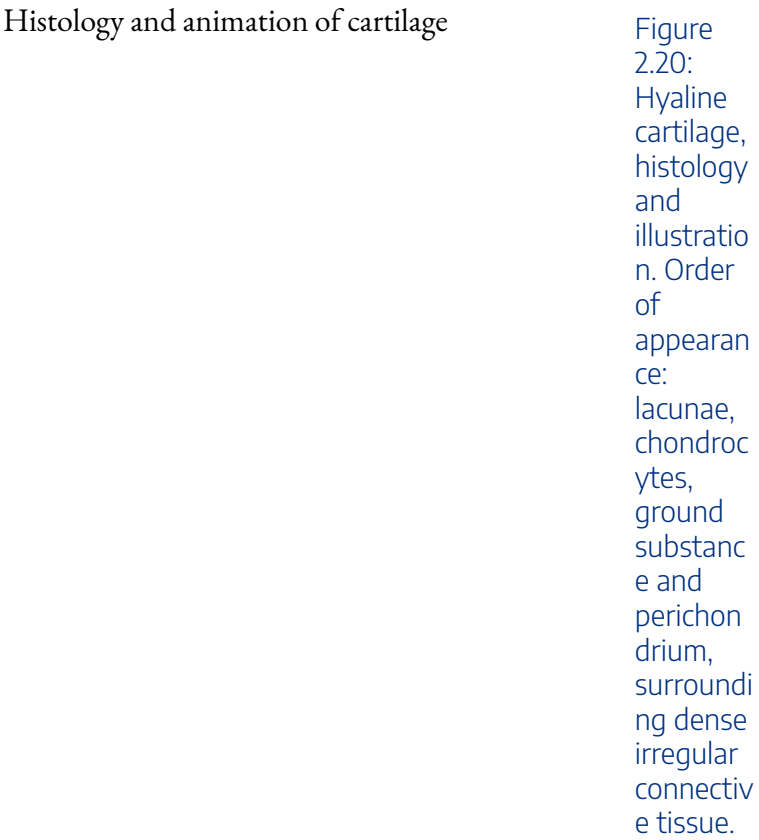

Figure 2.20: Hyaline cartilage, histology and illustration. Order of appearance: lacunae, chondrocytes, ground substance and perichondrium, surrounding dense irregular connective tissue.

Cartilage tissue

Cartilage, a connective tissue, comes in three basic flavors: hyaline cartilage, fibrocartilage and elastic cartilage. The hallmark feature of cartilage tissue is its large amount of **ground substance**[←]. **Chondroblasts** secrete glycosaminoglycans, **glycoproteins**, and **hyaluronic acid**, which attract water, forming a very dense but slippery

gel. The **ECM** of cartilage also contains **collagen**. There is more collagen in the fibrocartilage of the temporo-mandibular joint, whereas the elastic cartilage of the epiglottis has more **elastic fibers**. After secreting ECM, chondroblasts **differentiate** into **chondrocytes**, and reside within the tissue in spaces called **lacunae** (lakes).

At the outer edge of cartilage is a thin layer of dense collagen fibers known as the perichondrium. If you look at Fig 2.20, the perichondrium appears to be a layer of **dense regular connective tissue** (it is, perichondrium is its second name). Keep looking, and the edge of the perichondrium blends into **dense irregular connective tissue**. The reason the cartilage blends into dense regular connective tissue which blends into dense irregular connective tissue is they share the same **lineage**: chondroblasts and fibroblasts both differentiate from **mesenchymal stem cells**.

Unlike most connective tissues, cartilage is **avascular**. As a result, cartilage is mostly unable to undergo tissue repair following injury. Other connective tissues, like bone and the **sub-mucosa** in the oral cavity, can repair damage much more easily. It also means cartilage undergoes significant less **remodeling** throughout life.

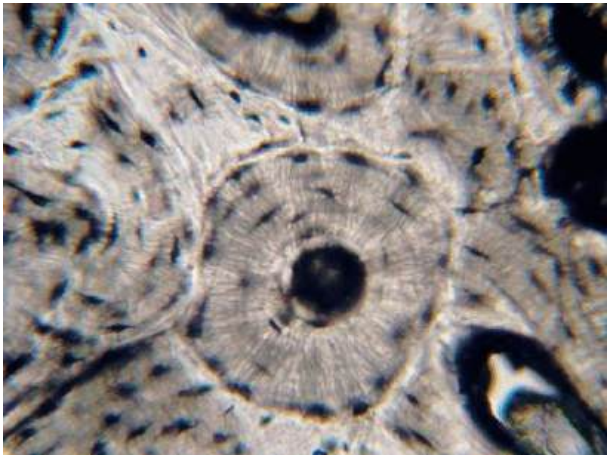

Figure 2.21: Bone connective tissue, histology and illustration highlighting osteocytes and lamellae of ECM.

Bone tissue

Bone tissue (or osseus tissue) has similarity to dentin, cementum and enamel. Therefore, we cover bone tissue in a fair amount of detail. Like any connective tissue, bone tissue starts as **mesenchymal stem cells**, which **differentiate**[←] into an intermediate **stem cell**[←] called an osteo-chondro-progenitor cell. This stem cell can in turn decide to adopt an **osteoblast** or a chondroblast **fate**. The choice depends on what signals it receives from its environment (**nurture**). A stem cell that has chosen the osteoblast fate (and denied the chondroblast fate) would be an **osteo-progenitor cell**. These osteo-progenitor cells are stem cells within the connective tissues surrounding bones, the **periosteum** (superficial) and

endosteum (deep). When stimulated, they undergo **mitosis**←, one daughter cell reamins an osteo-progenitor cell and the other daughter differentiates into an **osteoblast**. An osteoblast is the cell that secretes the **ECM** that makes up the bulk of bone tissue. Osteoblasts secrete **collagen**← fibers and **ground substance**←, which later mineralizes, trapping the osteoblasts inside of **lacunae**. Once trapped, osteoblasts differentiate into **osteocytes** and maintain the bone tissue. Next, a new set of osteoblasts lay down another layer of bone tissue around the previous one. This creates either the concentric layers of bone tissue found in the osteons of **compact bone**, or the thinner spiral layers of bone tissue found in the **trabeculae** of **spongy bone**.

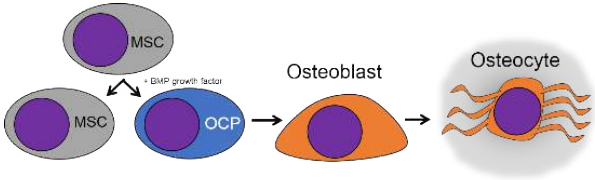

Figure 2.22: Three (of four) major cell types found in bone tissue and their lineage. MSC = mesenchymal stem cell, OCP = osteo-chondro progenitor cell.

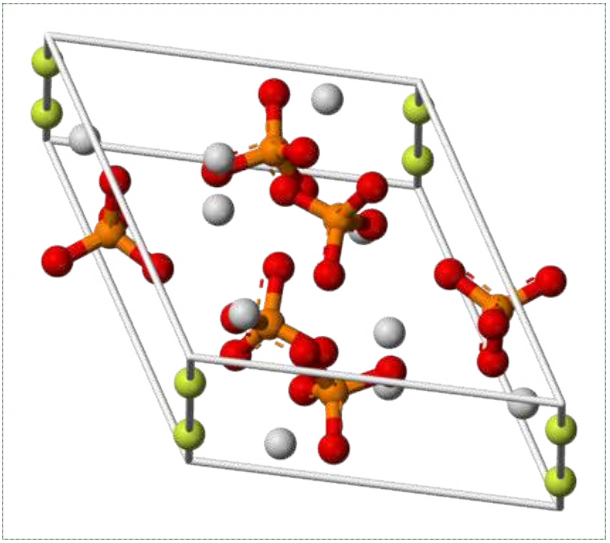

Figure 2.23: Calcium hydroxyapatite crystal structure of bone ECM. Legend: Red/orange = phosphate, white = calcium ions, yellow = hydroxide ions. Image credit: Fluorapatite by Von Benjah-bmm27 in the Public Domain CC0

The **ECM** of bone tissue is roughly 1/3rd **collagen**[←] fibers (organic protein) and 2/3^{rds} mineral (inorganic salts). The mineral component is a mixture of positive-charged Ca^{2+} ions,

which react with negative-charged phosphate ions (PO_4^{3-}) to form crystals. Because most cells are full of phosphate, Ca^{2+} levels in the cytoplasm must be kept very low. To store Ca^{2+} for secretion, an **osteoblast** does so within the **sER**. After secretion, $Ca^{2+}PO_4^{3-}$ reacts with water (or more specifically, hydroxide ions OH^-) and small amounts of fluoride (F^-) to form a harder crystal named **calcium hydroxyapatite**. Collagen fibers run parallel to each other within one layer of bone tissue, and 90° in the next layer. While the mineral component of bone and tooth tissues provides strength, the collagen provides flexibility, reducing the chances the tissue will shear off under stress. Collagen fibers, therefore, have a function similar to the rebar in reinforced concrete. Dentin, enamel and cementum have ECM very similar to bone tissue, with varying amounts of collagen versus mineral components. The higher percentage of minerals found in enamel makes enamel harder and more resistant to **dental caries** than dentin or cementum, but susceptible to fracture if enough force is applied.

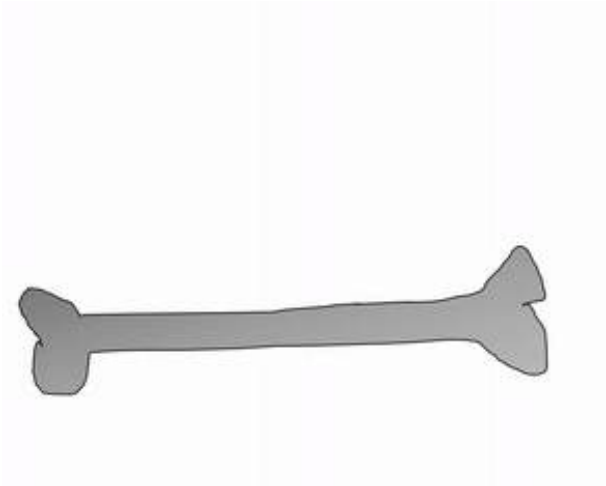

Figure 2.24: Collagen fibers of a tendon are continuous with the periosteum and Sharpey's fibers of bone tissue.

The **periosteum** is a continuation of (not separate from) the **dense regular connective tissue** of tendons and ligaments. In addition to collagen fibers and **fibroblasts**, the periosteum contains **osteo-progenitor cells**, **osteoblasts** and **osteoclasts** (coming up). In anatomy class, you learn about tendons and the periosteum in separate chapters. Sure, the perisoteum has some extra cells. But the options aren't binary. The perisoteum blends into a tendon. Lets apply that same idea to the other connection the periosteum makes: to bone. Where does the perisoteum end and bone tissue begin? Collagen fiber bundles from the periosteum named **Sharpey's fibers** penetrate the superficial layers of compact bone. This creates a strong connection between bones and tendons. Can you guess why these three tissues have such a tight connection? We hope you

didn't *guess*, but answered **lineage**. But maybe this raises the question: is the connection between muscle tissue and tendons as strong?

Bone remodeling

animated illustration of osteoclasts

Figure 2.25: Osteoclasts demineralize bone tissue.

Another important cell found in the **periosteum** and **endosteum** is the **osteoclast**. This cell is not derived from the **osteo-progenitor cell** that gives rise to **osteoblasts** and **osteocytes**. The **lineage** of an osteoclast traces to the bone marrow, from a **stem cell**[←] that also gives rise to red and white blood cells. An osteoclast is a closer relative of a white blood cell called a macrophage, more than it is to bone cells. Osteoclasts demineralize bone tissue, releasing Ca^{2+} into the bloodstream. This is important because muscle and nervous tissue cannot function without Ca^{2+}, which our diet cannot provide continuously. Hence, bone tissue can be thought of as a calcium storage organ.

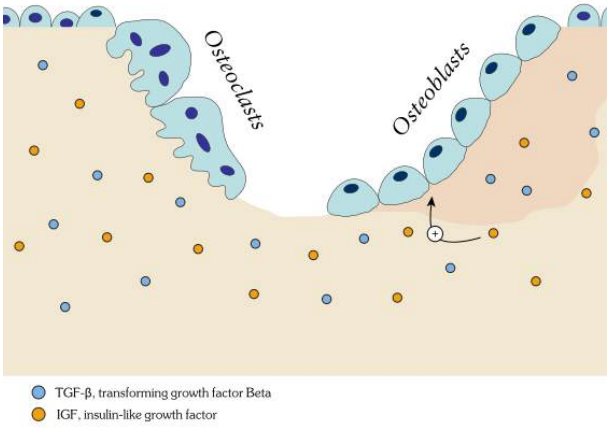

Figure 2.26: The bone remodeling unit: osteoblasts plus osteoclasts. Image credit: Own work By Shandristhe Azylean is licensed under CC BY-SA 3.0

Osteoclast activity is important during **tooth eruption**[←] and during the **exfoliation** of teeth. To help teeth move, bone tissue is removed to loosen the connection between bone and tooth. Osteoclasts activity is also key to the mechanism by which orthodontia works. Lastly, osteoclasts play an important role in maintaining bone health– this may seem counter-intuitive, because osteoclasts destroy bone tissue. But recall how we said programmed cell death (**apoptosis**) is essential to multicellular life. Bone tissue is constantly being repaired by a group of **osteoblasts** and osteoclasts working

together, known as a **remodeling unit**. Because compact bone tissue is dense, there is little room for cells to work. **Osteocytes** can repair small amounts of damage, but larger amounts of damage would build up over time. To prevent this, remodeling units constantly work to remove bone tissue and replace it with fresh bone tissue. The remodeling units cannot find damaged bone tissue, they simply keep removing and replacing bone tissue. In healthy bone tissue, the activity of osteoblasts and osteoclasts is equal. Therefore, healthy bone tissue is in dynamic equilibrium— it is constantly changing and staying the same. Physical tension on bone tissue causes osteoblasts to work harder, which leads to increased bone density. Lack of tension causes osteoblasts to work more slowly than osteoclasts, which lowers bone density and potentially leads to osteoporosis.

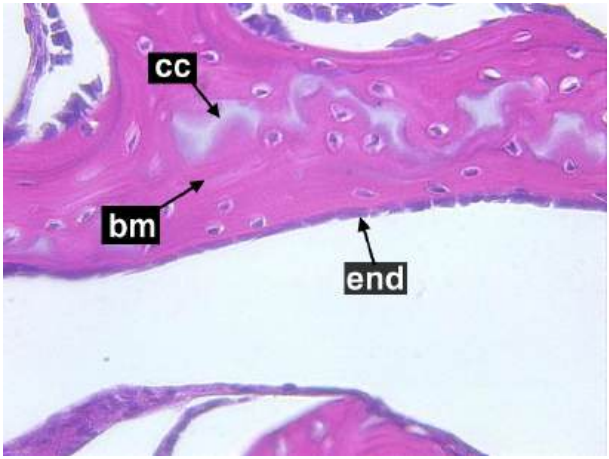

Fig. 2.27: Histology of spongy bone. Legend: cc = calcified cartilage, bm = bone matrix, end = endosteum (osteoblasts and osteoclasts). Image credit: "Cancellous Bone, Decalcified preparation, Human." by Steven Gallick, Ph.D. is licensed under CC

Fig. 2.21 shows the histology of compact bone, while Fig. 2.27 shows spongy bone. Because spongy bone has more surface area for remodeling units to work on, it undergoes more remodeling than compact bone. As a result, symptoms of osteoporosis often show up first in bones with high amounts of spongy bone, such as the mandible.

Figure 2.28: The basic steps of endochondral ossification.

To understand bone tissue well, we must understand its **lineage**. Bones form either by **endochondral ossification**, or by **intra-membranous ossification**. The two processes are similar, the major difference is that endochondral ossification begins with a **cartilage scaffold**, whereas intra-membranous ossification begins with a dense connective tissue scaffold.

Most of the skull develops by intra-membranous ossification, except the face, which uses both. Because of their similarity, we only cover endochondral ossification. Don't worry. After learning about endochondral ossification, if you replace the word *cartilage* with *dense connective tissue*, you understand intra-membranous ossification pretty well.

Figure 2.29: Blood vessels and nerves develop first, and are later surrounded by bone tissue. Image credit: "Dissected skull, Maison Tramond model, Paris, nineteenth century" by Centre for Research

Do sharks have a skeleton? Most of the human skeleton starts off as **cartilage**. Cartilage is typically **avascular**, but when **chondrocytes** receive the correct signals, they **induce** blood vessels to grow into their **ground substance**←. The growth of new blood vessels into a tissue is called **angiogenesis**. Angiogenesis provides the pathway that **mesenchymal stem cells** use to migrate into the central area, where they **differentiate**← into **osteoblasts**. After that, osteoblasts replace cartilage tissue with woven bone tissue (an immature form of bone tissue where collagen fibers are disorganized). This first site of ossification is named the *primary ossification center*. In long bones, it is located in the future shaft of the bone. Next, blood vessels grow into the epiphyses and begin the same process at what are named the *secondary ossification centers*. After producing woven bone, osteoblasts remodel the collagen fibers to produce lamellar bone (either compact bone or spongy bone), and differentiate into **osteocytes**. Ultimately, most of the cartilage is replaced, except in two important places. First, cartilage remains between the primary and secondary ossification centers, leaving growth plates (such as in bones of the arms and legs). Cartilage also remains at the ends, providing the articular cartilage cushions found in synovial joints. Intra-membranous ossification begins with a dense connective tissue. It leaves

fontanels (soft-spots) between bones until ossification is complete, rather than cartilaginous connections.

If you are wondering if we are going to answer the question about whether sharks have a skeleton, try a quick google search. Instead, we ask you a second question: why would we bring up such a distant relative of ours (speaking on an evolutionary scale) when describing the embryological **development** of bone tissue? It is because our evolutionary **lineage** helps us understand our developmental lineage. Sorry, but the job of shark dental hygienist has already been filled by the remora.

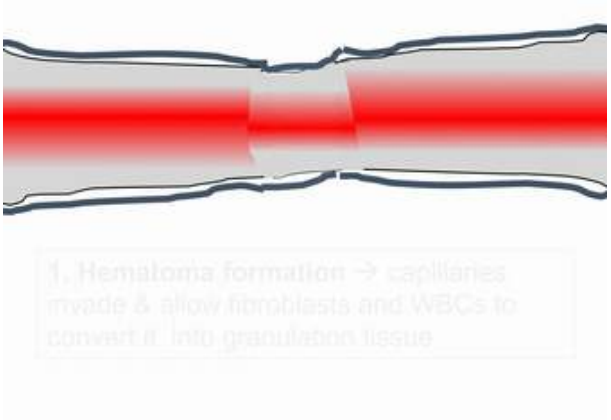

Figure 2.30: The basic steps of bone repair. Note the similarity to endochondral ossification.

When bone tissue is injured, it goes through a healing process similar to **endochondral ossification**. Perhaps you remember mention of how wound healing **recapitulates development**. Here is an example of an adult tissue *re-using* a mechanism

to heal that was used during embryogenesis to grow. Similar to an injury to the skin, bone tissue first goes through an inflammatory process, and damaged blood vessels form clots. An external blood clot is a scab, but inside the body it is named a hematoma. A blood clot contains **ECM** fibers named **fibrin** that act as a **scaffold**. This scaffold allows blood vessels to grow into the area, by **angiogenesis**. This in turn allows **mesenchymal stem cells** to leave the blood and migrate into the injured area along the scaffold, and **differentiate** into **chondroblasts**. The chondroblasts replace the fibrous hematoma with fibrocartilage. This cartilage step is the only step that is does not have a **homologous** step (a shared step) in wound repair in the skin. Injured skin uses the fibrin scaffold, but bone tissue needs a **cartilage** scaffold to grow. With a cartilage scaffold, more mesenchymal stem cells migrate and differentiate into **osteoblasts**, and replace the cartilage with woven bone tissue. Later, woven bone is **remodeled** so that it roughly matches the original **compact** and **spongy bone** layers. One difference can be seen afterwards, the former site of injury is a little bit thicker after it is repaired. This is similar to the way skin tissue is made stronger after an injury (**scar tissue**).

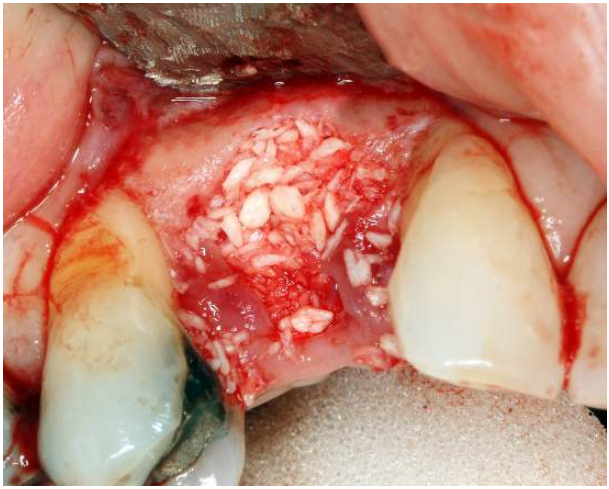

Figure 2.31: Artificial scaffolds help mesenchymal stem cells migrate into an injured area, differentiate into osteoblasts, and produce bone tissue. Image credit: Alloplastic particulate graft by Coronation Dental Specialty Group is licensed CC BY SA 3.0

Knowing the steps of **endochondral ossification** and bone fracture repair are important. Stimulating or mimicking the natural healing process can reduce the need for surgeries and implants. For instance, patients may lose a significant amount of **bone tissue** with prolonged **periodontitis** (for example, take a look at this periapical abcess now located at the Royal College of Surgeons of London). If enough bone tissue is lost, a surgeon may use a **bone graft** to replace it. Bone tissue from a cadaver can be ground up, destroying the cells but leaving the **collagen**[←] and minerals. Other sources may include a patient's own bone tissue (a full set of ribs or fibulas is less important than an intact set of jaw bones and teeth). The harvested **ECM** is spread into the injured site, where it acts as a **scaffold**[←], not as a replacement. The patient's **mesenchymal stem cells** migrate through the scaffold and begin the healing process, which can proceed quickly because plenty of raw materials are close by. Scaffolding does not have to come from actual bone tissue. It can be ground-up dentin, **fibrin**, or a synthetic polymer printed using a 3-D printer. The scaffolds, synthetic or biological, are ultimately replaced, which is why you may see this referred to as **Guided Tissue Regeneration**[←] (further reading here). A scaffold is just one part of the healing process, there are important signals that can be used to guide mesenchymal stem cell migration, **mitosis**[←] and **differentiation**[←]. These signals are **growth factors** and **morphogens**[←], which are covered inchapters 6-11. If you

wish to learn more, we have a short YouTube video that covers this new technology in more detail, aimed at 200-level Anatomy and Physiology students (the concept is essentail knowledge for your program periodontology course topics).

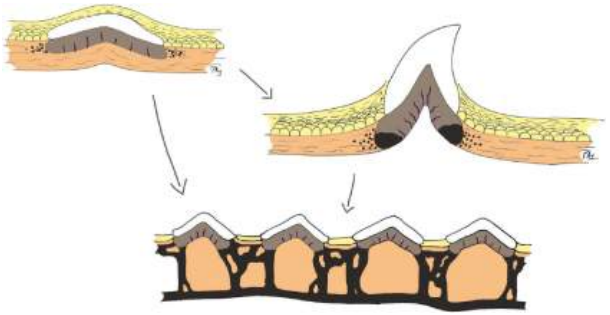

Figure 2.32: Mineralized tissues first evolved in the ectoderm (similar to enamel) and were later produced by mesoderm (such as bone). Image credit: "The origin of bone" by Darja Obradovic Wagner and Per Aspenberg is licensed under CC BY-NC-SA 4.0

Lastly, it is interesting to consider, from an evolutionary viewpoint (**phylogeny**), the lineage of the four mineralized tissues (bone, enamel, dentin and cementum) in humans and other vertebrates. Did one evolve first, and the others **develop** from the original? Thinking about the development of tissues in terms of their evolution (called evo-devo) tells us a lot about their similarities and differences. Fig. 2.32 provides a hint. We consider this question of **lineage** from a developmental viewpoint (**ontogeny**) in chapters 6 through 11.

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animated image of blood and reticular connecive tissue

Figure 2.33: Hypothetical comparison between blood connective tissue and reticular connective tissue.

Blood, Reticular, and Lymph Connective Tissue

There are other connective tissues covered in histology classes, here we cover them briefly. **Blood** is a type of connective tissue, composed of cells (red blood cells, mostly), liquid ground substance (blood plasma) and fibers (**fibrin**). **Lymph** is primarily white blood cells, plus liquid ground substance. **Reticular connective tissue** contains blood cells, liquid ground substance and **reticular fibers**. It can be found in lymphatic organs where blood cells tend to rest rather than flow, such as lymph nodes.

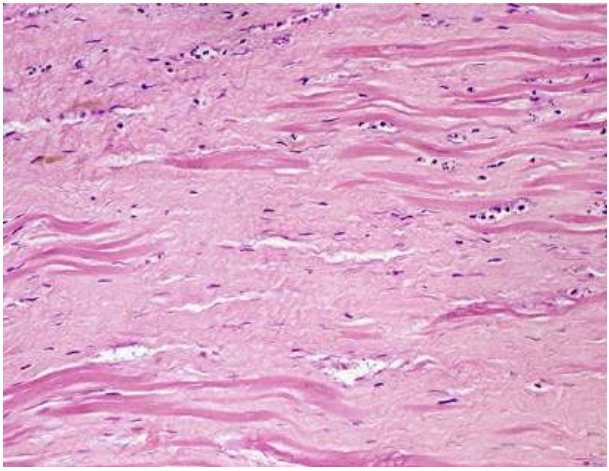


Figure 2.34: Scar tissue in the heart after a myocardial infarct. Image credit: "Histopathology of dense fibrous scar replacing myocyte loss in myocardial infarction" by Katarzyna Michaud et al is licensed under CC

There are other forms of connective tissue that aren't typically covered in 200-level histology classes, we cover one: **scar tissue** (or granulation tissue). Scar tissue is made by **fibroblasts** following an injury. If an injury occurs that is large enough the tissue cannot be replaced quickly by **stem cells**←, **fibroblasts** can fill in the area with **collagen**←. Scar tissue is very similar to **dense regular connective tissue**, except the collagen fiber moleculess in scar tissue are extensively cross-linked. This makes scar tissue very strong, but also reduces its mobility and capacity to be **remodeled**. Because scar tissue is difficult to remodel, it is more-or-less permanent.

After learning about the histology of the oral mucosa in Chapter 3, knowledge of the histology of scar tissue helps explain oberved changes to the gingiva. Repated periodontal infections and subsequent ifnlammation can trigger the production of scar tissue, creating firbotic or scarred gingival tissue. Chronic insults to the gingiva create thick, tough gums which don't function well to support and cover tooth roots. This can lead to exposed roots, which are not designed to endure continued exposure to the oral cavity environment.

Muscle tissue

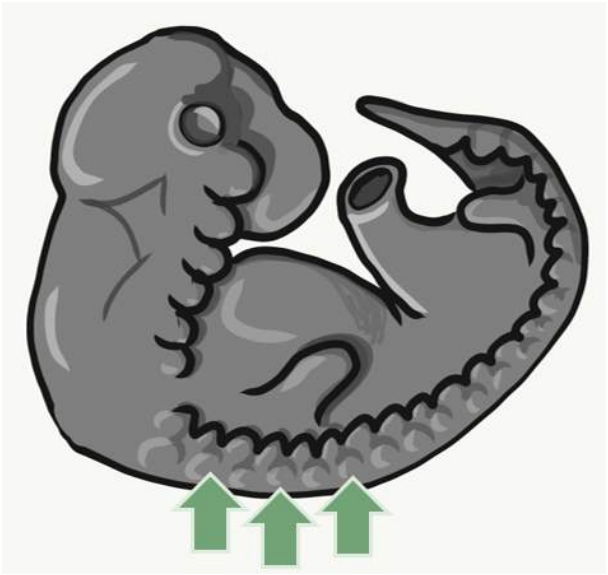


Figure 2.35: Somites, from which connective tissue and muscle tissue develop.

General characteristics of muscle tissue

Skeletal muscle tissue, like connective tissues, is derived from **mesoderm**. Fig. 2.35 shows regions of mesoderm known as **somites**. The **stem cell** that **differentiates** into one of the several types of muscle cells is called a **myo-satellite cell**. These cells resemble a **mesenchymal stem cell**, which is not unusual given their shared **lineage**. However, the **fate** of a myo-satellite cell is different. Given the correct extra-cellular signals, myo-satellite cells differentiate into small **myoblasts**. Hundreds of myoblasts **fuse** together and differentiate into

skeletal muscle cells. This occurs not only during **development**, but also late in life. Myo-satellite cell are responsible for the repair and growth of muscle tissue following excercise. Exercise can damage muscle cells and cause them to release inflammatory molecules, which (in part) trigger myosatellite cells to undergo **mitosis**←, differentiate into myoblasts and trigger tissue repair and growth. We can think of this as an example of how wound healing **recapitulates development**. Like healthy bone tissue, maintaining healthy muscle tissue requires dynamic equilibrium: ongoing destruction and re-growth of muscle cells.

Unlike **fibroblasts**, myoblasts create protein fibers that are mostly intra-cellular (such as actin thin filaments and myosin thick filaments). After fusing with other myoblasts, muscle cells are often confusingly referred to as muscle *fibers.* They are cells, not molecules like the protein and **glycoprotein fibers** found within **ECM**. They can also be called muscle cells or *myocytes*. We see skeletal muscle in the tongue and underneath regions of **oral mucosa**. We do not cover smooth or cardiac muscle tissue.

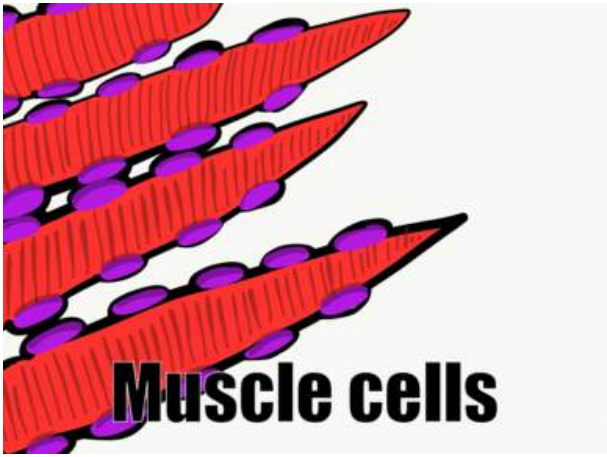


Figure 2.36: Muscles (organs) contain muscle tissue and dense regular connective tissue.

Muscle tissue is composed primarily of muscle cells and extra-cellular **collagen**[←] fibers. Recall how connective tissue cells move along collagen using **integrin**[←] **trans-membrane proteins** . Muscle cells anchor in place and move the collagen fibers. To do this, muscle cells express different proteins that bind collagen, including the protein dystrophin. For people studying musculo-skeletal diseases like muscular dystrophy, there is interest in creating myoblasts for patients. There are a larger number of **mesenchymal stem cells** in the bone marrow compared to **myo-satellite cells** in the muscle of a child or adult. Thanks to how closely related mesenchymal stem cells are to myo-satellite cells, it may be possible to trick mesenchymal stem cells into differentiating into myoblasts instead of fibroblasts. This could, in turn, reduce or reverse muscle tissue loss. If novel treatments result from this research, it could make dental hygiene

procedures for patients with muscular dystrophy more effective.

The layers of collagen are thin between muscle cells, and thicker on the outer surface of a muscle. These layers of collagen fibers ultimately become the **dense regular connective tissue** of a tendon. Under the microscope, it is difficult to determine where muscle tissue ends and dense regular connective tissue begins because of the collagen fibers in both tissues. By extension, it is also difficult to tell where the tendon ends and the **periosteum** begins. Some histologists draw lines between these tissues, but the junction is better described as a blending. This is because both muscle tissue and connective tissue have the same **lineage**, from **mesenchyme**.

Nervous tissue

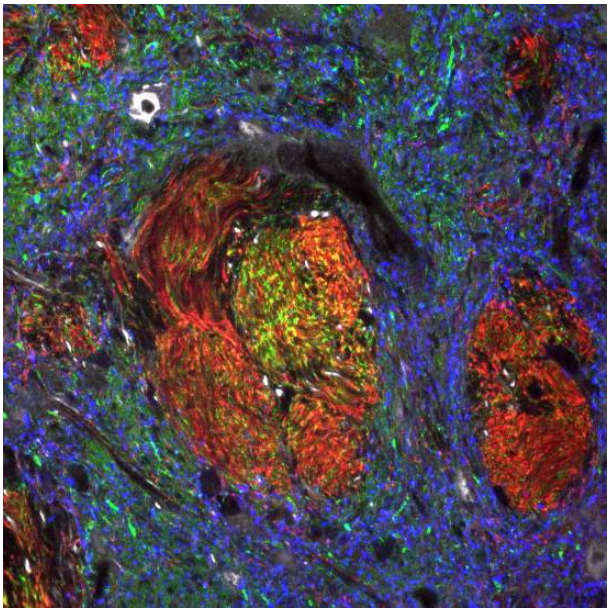


Figure 2.37: Confocal image of neural tissue, including neurons and glial cells. Image credit: Desert rose by Tarzerind is licensed under CC By SA 4.0

General characteristics of neural tissue

Fig. 2.37 is a confocal image of **neural tissue**. What is particularly nice about this image, and is often absent from images of neural tissue, is that this one shows *all* of the cells. The typical image of neural tissue uses stains to highlight **neurons**—the cells specialized at conducting electricity. When only neurons are stained, the **glia** stay invisible. Glia are majority of cells in the nervous system. While neurons act like

electrical wires, glia are responsible for everything else. This includes important tasks like guiding neuronal connections and changing the strength of those connections as we learn. You should see neural tissue is composed of cells, and has very little **ECM**. While the brain especially has a huge number of blood vessels inside of it, technically they do not run *within* the neural tissue. Instead, there is a thin blood-brain-barrier separating the brain from the blood vessels. Normally, we wouldn't call neural tissue **avascular**, we do so to make a point: neural tissue shares a lot in common with epithelial tissue. By now, we hope you have know why: neural and epithelial tissues share the same **lineage**.

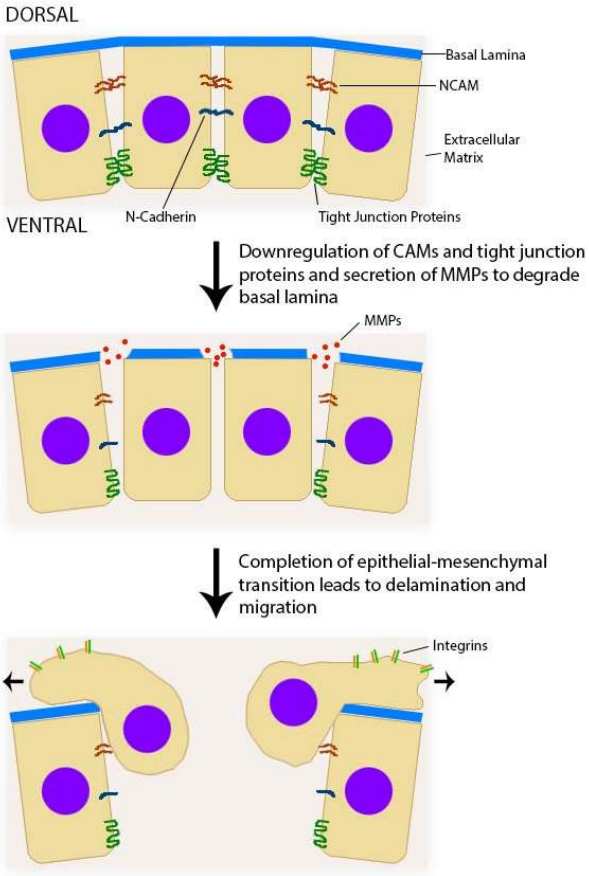


Figure 2.37: Migration of neural crest cells away from the neural tube involves several proteins covered in this book, including the removal of cell adhesion molecules and cadherins, expression of integrins, and the digestion of ECM by matrix metalloproteinase enzymes. Image credit: "Neural

We dont discuss neural tissue in this book. But we do discuss a number of tissues derived from **neural crest cells**. These are individual cells that arise during **neurulation**[←], migrate away from other neural tissue, and **differentiate** into lots of different things. They don't look like much under the microscope, and their existence is brief. Therefore, they shouldn't be included in a histology chapter. But you will identify many things they create, including **melanocytes**, craniofacial bones, the anterior pituitary, taste buds, salivary gland connective tissue, dentin, cementum, pulp, the **PDL** and alveolar bone. Don't memorize that whole list, maybe just the tooth and periodontium structures. Then remember that you would be a headless filter-feeder if it weren't for neural crest cells. In what is called the new head theory, the evolution of neural crest cells gave us vertebrates our paired sensory organs, powerful jaws and the ability to become predators.

3 major embryonic tissues

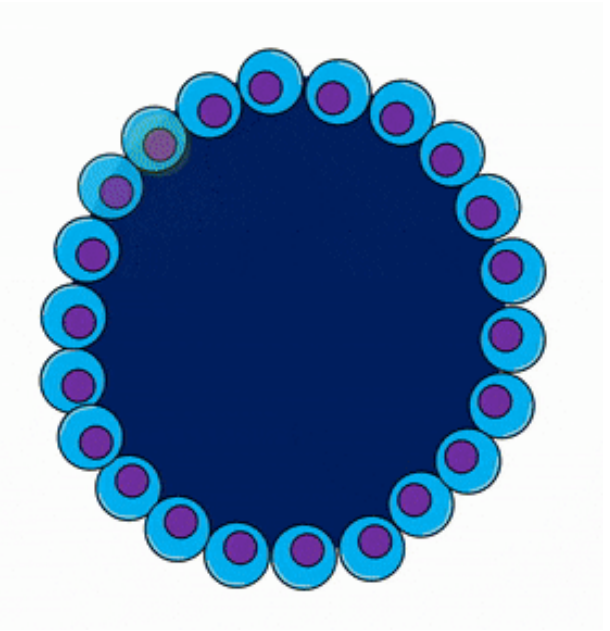


Figure 2.38: Animated illustration of gastrulation.

Gastrulation (a brief introduction)

We are done discussing tissues based on what they look like. Now, lets focus on where they come from. When an embryo implants in the uterus, it is a hollow ball of identical, omnipotent **stem cells**[←] that resemble a **simple cuboidal epithelium**. Soon, some epithelial cells migrate inwards, in an important process called **gastrulation**[←]. Now there are two different layers of cells. The layer on the outside becomes the epidermis, **oral epithelium** and the central nervous system. The layer one on the inside becomes the lining of the gut and

other hollow organs. Next, some cells from the outer layer change. They lose contact with neighboring cells, and migrate into the middle. As this happens, they stop looking like epithelial cells and begin to look like **mesenchymal stem cells**. This produces a third layer of cells in an embryo. These are the three **embryonic germ layers** are listed below, along with what they are **fated** to form in the adult:

3 embryonic layers	Cell fate
Ectoderm	Epithelium of skin and oral mucosa, neural tissue
Mesoderm	Connective and muscle tissue
Endoderm	Epithelial lining of hollow organs

Table 2.3: The three embryonic germ layers.

We have now sneakily introduced you to two important terms used in **embryology**. The first is cell **lineage**, which means *where a cell came from* (in the past). Now we add **cell fate**, which means what a cell *can become* (in the future). Can you think of when we would discuss cell ineage versus when we would discuss cell fate? Here is a hint: *timing* is everything. If we didn't think your license exam would include questions on the four major tissue types, we would stick with these three **embryonic germ layers**. Neural tissue would be a sub-family of **ectoderm** (you'll see why later). Epithelia would be divided between ectoderm and **endoderm**. We'd put connective and muscle tissue together in **mesoderm**, rather than separate them. We might even add **neural crest cells**[←] as a fourth tissue type. But we can see why academia hasn't changed the way it teaches histology. The confusion that comes with change would not be worth the benefits the average student would gain from grouping cells by lineage rather than appearance. You, however, need to know both the 4 major tissue types and the 3 embryonic germ layers.

< Chapter 1 * navigation * Chapter 3 >

Histology review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:
https://openoregon.pressbooks.pub/histologyandembryology/?p=30

3.

HISTOLOGY OF THE ORAL MUCOSA

Overview

We start this chapter reviewing the histology of the skin. The different regions of **oral mucosa** are similar to the skin because

they share the same **lineage**[←]: **ectoderm** and **mesoderm**. There are a few small differences. Unfortunately, the names we use for the skin are not the same names we use for oral mucosa. Otherwise, it is faster to focus primarily on the small differences, there is no need to cover the histology of the epidermis or dermis each time. In subsequent chapters on the teeth, pay attention to how the lineage of tooth tissues is different from that of oral mucosa, but their basic patterns are similar.

For more practice with histology of the oral mucosa (and more), websites worth visiting are:

- The oral histology lab
  - by Brian R. MacPherson, Ph.D. and James G. Tieman, Ed.D. at the University of Kentucky College of Medicine.
- The virtual oral histology lab
  - By Roger Shore at the School of Dentistry, University of Leeds

animation of the skin

Figure 3.1: The three major layers of the skin, and the major tissue types in each.

Histology of the skin – for comparison

The skin and the **oral mucosa** share a lot in common because of their shared **lineage**[←] from **ectoderm** and **mesoderm**. Both are composed of a **stratified squamous epithelium**[←], just deep to that **areolar connective tissue**[←], followed by **dense irregular connective tissue**[←]. Unfortunately, parts of the skin and oral mucosa receive different names and are classified differently based on their location. That means you have more names to memorize... Boo!

The **dermis** is the connective tissue of the skin. It is composed in part of a layer of dense irregular connective tissue, the old-fashioned name for which is the reticular layer of the dermis. The other part of the dermis is a layer of areolar connective tissue, which has an old-fashioned name, too: the papillary layer of the dermis. It received that name for having finger-like **dermal papillae** on the **apical** surface. In Fig. 3.1

and Fig. 3.7, upward-pointing dermal papillae meet downward-pointing **rete pegs** of the **epidermis**. The epidermis is stratified squamous epithelial tissue. The dermal papillae of the dermis meet the rete pegs of the epidermis like inter-meshed fingers from two hands, which makes for a strong connection between epidermis and dermis. Some regions of the oral cavity don't require such a strong connection, and the rete pegs and dermal papillae are smaller or absent. Note the border between epidermis and dermis is distinct, while the border between the reticular and papillary layers of the dermis is blended. That is because the epidermis is derived from the **ectoderm**, while the two layers of the dermis are derived from the **mesoderm**.

The epidermis is highly **keratinized**. The epithelial cells make a large protein called **keratin** (or more accurately, keratins, as there are over 50 genes for slightly different keratin molecules). Keratin is similar to **collagen**←, except keratin is not secreted. Keratins are long fibrous proteins which accumulate within the **cytoplasm** of **keratinocytes**, the principle cell of a **stratified squamous epithelium**←. Epithelial **stem cells**← in the **basal** layer of the epidermis give rise to new keratinocytes. As keratinocytes mature, they are pushed towards the **apical** surface. As the keratinocytes move superficially, they fill up with keratin, and receive fewer nutrients (an epithelium is **avascular**). Ultimately, the keratinocytes at the surface are dead and completely full of keratin. The keratin fibers are cross-linked to each other and

linked to **desmosomes**←. Desmosomes anchor dead cells together. These links and cross-links make a very tough and water-resistant barrier. The skin is highly keratinized everywhere. Keratinized regions of **oral epithelium**, on the other hand, are only found in locations where there is a lot of abrasion. In the rest of the oral cavity, moisture is beneficial, and there is less or no keratinization.

Skin color

Pigment	Color	Source	Location
Melanin	Red or brown/black	Melanocytes	Epidermis/oral mucosa
Carotene	Orange-yellow	Diet (plants)	Epidermis/oral mucosa
Hemoglobin	Red-maroon	Blood	Dermis/sub-mucosa

Table 3.1: The three major skin pigments.

There are 3 major pigments (molecules that absorbs certain frequencies of visible light) that contribute to skin color, listed in Table 3.1. Keratin is not listed because it has no color, but it can obscure the visibility of the deeper pigment hemoglobin. **Melanin** levels vary naturally in the skin. Everyone is born with roughly the same number of **melanocytes**, the cells that synthesize the melanin pigment. Melanocytes **differentiate** from from **neural crest cells** that migrate from the **neural tube** to the **basal** layer of the **epidermis**. The shape of melanocytes more closely resembles **neurons** or **glia** than they do epithelial cells. They are not anchored to **keratinocytes** by **desmosomes**, but they attach to keratinocytes by epithelial **CAMs**. Attachment to keratinocytes is important to melanocyte function. CAMs allow desmosomes to attach to epithelial cells, but also to let go and migrate away. Melanocytes can produce a lighter form of melanin (pheomelanin) or a darker form (eumelanin). After synthesizing melanin, melanocytes transfer it to keratinocytes inside of **vesicles** (melanosomes). Because melanocytes migrate, it doesn't take many melanocytes to distribute melanin to many keratinocytes. The amount and type of melanin produced has a baseline rate set at birth— this is not determined by **genes** we inherit from our parents, but by more complex **epigenetic** factors.

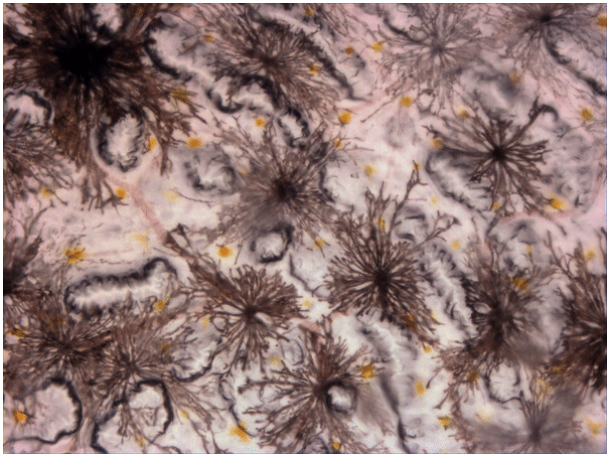


Figure 3.2: Melanocytes is some animals, such as these from a fish, move melanosomes to quickly change skin color (camouflage). Human melanocytes work more slowly, but similarly. Image credit: "7x speed timelapse video of fish

melanophores responding to 200uM adrenaline" by Zephyris is licensed under CC BY-SA 3.0 / converted to gif

Environmental changes trigger melanocytes to change melanin **expression**. These changes illustrate several functions of melanin. The most obvious function of melanin is to absorb UV-B light. When UV light causes **DNA** damage, it **induces** keratinocytes to secrete a **hormone** (melanocyte stimulating hormone, MSH). Because the epidermis is **avascular**, the hormone only travels a short distance. Nearby melanocytes are activated to produce more melanin. Extra melanin, when deposited into new **keratinocytes**, reduces damage to DNA and helps prevent skin cancer. Tan lines illustrate just how far MSH diffuses and melanocytes migrate– not very far on on the scale of the human body.

Melanin (in the epidermis) also protects folic acid levels in the blood (the dermis) from UV light exposure. For light-skinned people, one hour of sun exposure destroys about half of their folic acid. This is significant because folic acid is required for cell division. Low levels of folic acid during pregnancy lead to **congenital malformations** of the **neural tube**[←] (**spina bifida**).

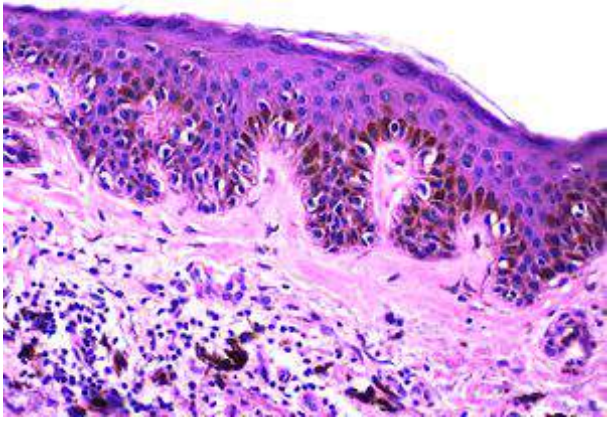


Figure 3.3: Histology of focal melanin in a melanocytic nevus (mole). Note the brown color of melanin is inherent, not added to the tissue like the pink and purple H&E stains. Image credit: "Lentigo simplex or simple lentigo or lentiginous

Melanin may be present within the oral cavity, despite very low levels of UV light exposure. This can be explained because there are two more important functions of the molecule melanin. Melanin protects tissues against abrasion. Pregnancy leads to an increase in the amount of melanin in the areola and labia minora, areas likely to suffer more abrasion during or after childbirth, not more UV damage. A third function of melanin is its ability to bind free radicals. This function requires a team effort between melanocytes and keratinocytes. Melanocytes make and transfer melanin to **keratinocytes**, where it binds toxic free radicals (often caused by UV damage). That alone would not protect the skin, it would trap free radicals in keratinocytes. But as keratinocytes are exfoliated, melanin helps remove free radicals from the body.

Two other major skin pigments are hemoglobin and carotene. Higher levels of the darker eumelanin may hide them. **Hemoglobin** allows red blood cells to pick up and dump off oxygen molecules. It undergoes a color change when it picks up oxygen, shifting from maroon to red. Healthy people of any skin color do not exhibit variation in hemoglobin. Keratin, however, can make skin appear *less* reddish. Because hemoglobin is in the dermis and keratin is in the epidermis, high levels of keratin hide hemoglobin.

Carotene is an orange pigment molecule made by plants. Unlike melanin, carotene is not made in the human body. We ingest carotene from our diet, and from there it accumulates in the epidermis and **oral epithelium**. Carotene is converted into Vitamin A, which is converted into the important **morphogen**[←] retinoic acid. Vitamin A is also a necessary co-factor in the **methylation** and **histone** modification of **DNA**. The amount of carotene that accumulates in the skin, along with **melanin**, contributes to diversity in skin tone. Unlike melanin, skin carotene levels usually do not change in response to environmental factors. The genetic or epigenetic factors that contribute to heritable skin carotene levels (yellow skin tones) are poorly understood.

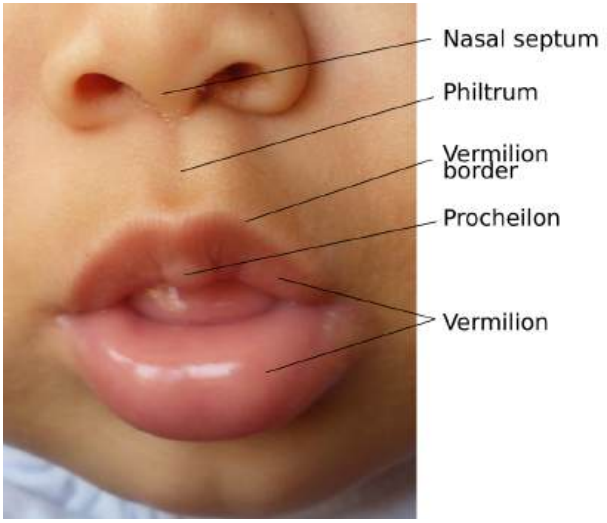


Figure 3.4. Anatomy of the lips. Image credit" "Anatomical features of the lips of a baby" by Occlusion is licensed under CC BY-SA 4.0

Vermilion zone

The **vermilion zone** (or red zone of the lips) is sometimes defined as part of the skin, or other times defined as part of the **oral mucosa**. It contains less **keratin**, no **hair follicles** and few if any **sebaceous glands**. Furthermore, its **keratinocytes** synthesize a clear protein (eleidin). This allows the reddish

color of **hemoglobin** in the capillaries of the **dermis** and muscle tissue to be more visible than in more highly **keratinized** regions of neighboring skin. After you finish reading about oral mucosa, decide for yourself whether the vermilion zone should be considered a part of the skin, oral mucosa, its own tissue, or create your own way of classifying the three.

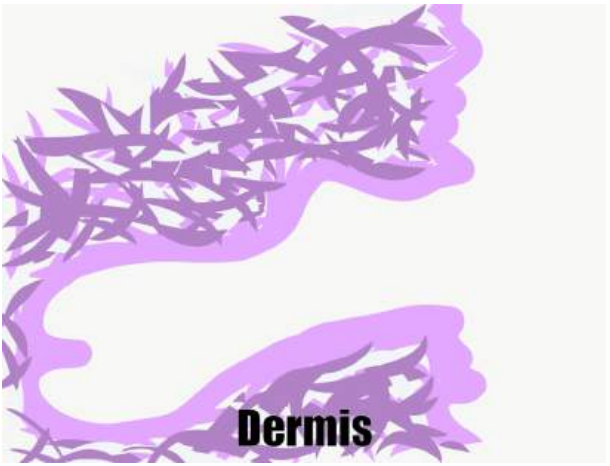


Figure 3.5: Basic components of a hair follicle.

Hair follicles

Hair follicles share **developmental** processes with teeth. That gives these two appendages of the skin and **oral mucosa** a similar pattern, only one makes **keratin** and the other **calcium hydroxyapatite**. A hair follicle is an **invagination** (an inward-

folding of a tissue, such as during **neurulation**) of the **epidermis**. The **stem cells** in the **basal** layer of the hair bulb divide, **differentiate** into **keratinocytes**, and die to form the hair itself. A hair, therefore, is an epithelial structure. Surrounding– or deep to– the hair follicle are the connective tissue layers of the **dermis**.

Where and when hair follicles invaginate from the surface epidermis is regulated by **planar cell polarity** signals. These signals ensure roughly-even spacing between follicles. The same signals govern the spacing of the teeth. Also similar to teeth, new hair follicles grow beneath old ones, pushing the old ones out in a process called **exfoliation**. Of course, hair follicles grow and exfoliate more times than a tooth. Nevertheless, it might be a good idea to keep scientific developments in the hair-loss treatment industry somewhere on your radar, as advances there may have applications in some future tooth-growth industry.

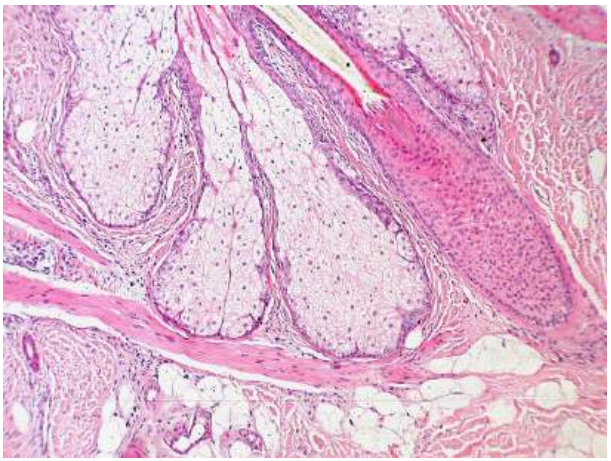


Figure 3.6: Sebaceous gland (H&E stain), associated with a hair follicle. Image credit: "Base of pilosebaceous unit" by Kilbad is in the Public Domain, CC0

Sebaceous glands

Sebaceous glands are oil-producing glands within the skin. They may discharge directly to the surface of the skin, but are mostly associated with **hair follicles**. The material they secrete, called sebum, is mostly lipids (triglyceride and others). Unlike proteins, these molecules are not synthesized on the

rER, modified in the **Golgi apparatus** nor secreted from **vesicles**. Instead, sebum accumulates in the **cytoplasm** and is secreted by destruction of the cell. New glandular cells are produced from **stem cells** in the **basal** portion of the gland. The **vermilion zone** and **oral mucosa** contain few or no sebaceous glands, but when sebaceous glands are present in these regions, they form benign whitish spots known as **Fordyce Spots**.

animation of the layers of skin

Fig. 3.7: Animation of the tissues lining the oral cavity. In this illustration, the dermal papillae are the upward-pointing projections of the areolar CT layer, while the rete pegs are the downward-pointing projections of the stratified squamous epithelial layer.

General histology of the oral mucosa

The **oral mucosa** is the mucous membrane that lines the oral cavity. It shares the same **lineage**← as the skin, and we therefore see the same tissue types in the same order. However, its layers get different names and are classified differently. Based on the **3 embryonic germ layers**, the layers of the skin are divided appropriately, but the oral mucosa is divided incorrectly.

First, **stratified squamous epithelium**← and underlying **areolar connective tissue**← are lumped together and called the **oral mucosa**. The stratified squamous epithelium may be referred to individually as the **oral epithelium**, and like the epidermis, is derived from the **ectoderm**. The layer of **areolar connective tissue**←, which is **homologous** to the papillary layer of the **dermis**, is called the **lamina propria**. It is produced by cells derived from **mesoderm**. Deep to the oral mucosa, the layer of **dense irregular connective tissue**← is called the **sub-mucosa**, and is the homologue of the reticular layer of the dermis. The sub-mucosa is also produced by cells derived from mesoderm.

Embryonic tissue	Skin		Oral mucosa	
Ectoderm	Epidermis	Stratified squamous epithelium	Stratified squamous epithelium	Oral mucosa
Mesoderm	Dermis	Areolar CT	Areolar CT	
		Dense irregular CT	Dense irregular CT	Sub-mucosa

Table 3.2: Summary of the layers of the skin compared to the layers of the oral mucosa. Note which grouping matches the embryonic lineage, and which does not.

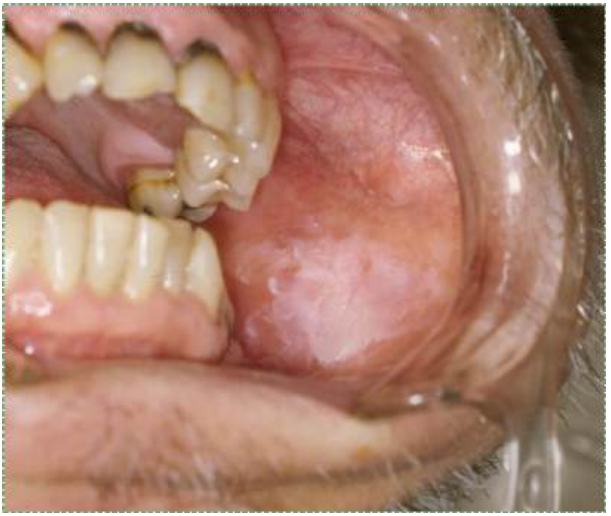


Figure 3.8: Increased amounts of keratin in the oral mucosa obscure underlying pigments, making regions appear more whitish. Image credit: leukoplakia by dozenist is licensed CC BY 3.0

The amount of **keratinization** of the **oral mucosa** reflects the amount of stress or abrasion that region experiences. This is similar to the formation of a callus on the hands or feet.

Higher-than-normal levels of keratinization are clinically relevant when they indicate bruxism, tobacco use, or other health-related issues. **Keratin** doesn't have a color, but higher levels of keratin in the epithelium obscures the maroon color of blood found within the **lamina propria** and **sub-mucosa**, hence **non-keratinized** mucosa look more reddish, while keratinized mucosa more whitish. Levels of keratinization are categorized into three or four groups listed in Table 3.3.

Table 3.3: Different levels of keratinization in the skin and oral mucosa.

Type of stratified squamous epithelium	Level of keratinization	Location
Keratinized	Full	Skin
Ortho-keratinized	Partial	Masticatory mucosa
Para-keratinized		
Non-keratinized	None	Lining mucosa

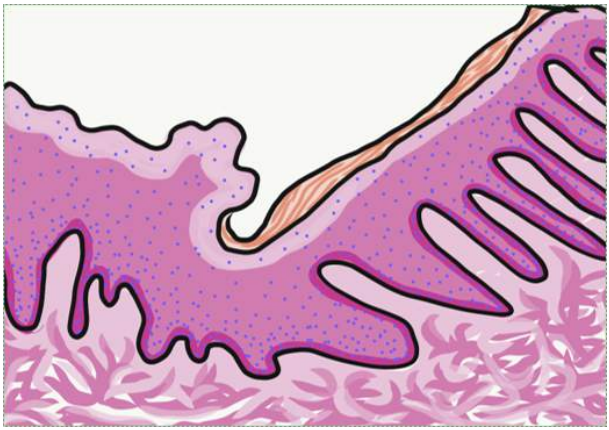


Figure 3.9: Lining mucosa (left half) is non-keratinized, masticatory mucosa (right half) is partially keratinized.

3 classes oral mucosa

Lining mucosa is found in most regions of the oral cavity, and is not involved significantly with mastication. These are regions more important for speech and swallowing. They are therefore mostly **non-keratinized**. They may have higher levels of **elastic fibers** within the **lamina propria**. Because lining mucosa does not get as much friction and abrasion, it has small or no visible **dermal papillae** and **rete pegs** between the epithelium and connective tissue layers. The left-half of Fig. 3.9 illustrates a lining mucosa.

Masticatory mucosa is found in regions of high abrasion caused by mastication, such as the **attached gingiva**. The epithelium is either be **ortho-keratinized** or **para-keratinized**, which are both partially **keratinized**. An ortho-keratinized epithelium contains **keratinocytes** with **keratin** and nucleuses, whereas the para-keratinized epithelium lacks nucleuses. Differentiating between ortho- and para-keratinized tissue is based on appearance, and has no clinical significance. For the rest of this book, we refer to them together as "ortho- or para-keratinized epithelium". Because this mucosa is generally under higher levels of stress, it has more pronounced **dermal papillae** and **rete pegs** than lining mucosa. The right-half of Fig. 3.9 illustrates a masticatory mucosa, whose **apical** surface contains a degree of keratinization. Where a lining mucosa and masticatory mucosa meet is a border referred to as the **mucogingival junction**.

Specialized mucosa is found on the **dorsal surface of the tongue**. More important than its level of **keratinization** is the precense of specialized structures, such as **lingual papillae** and taste buds.

Lining mucosa

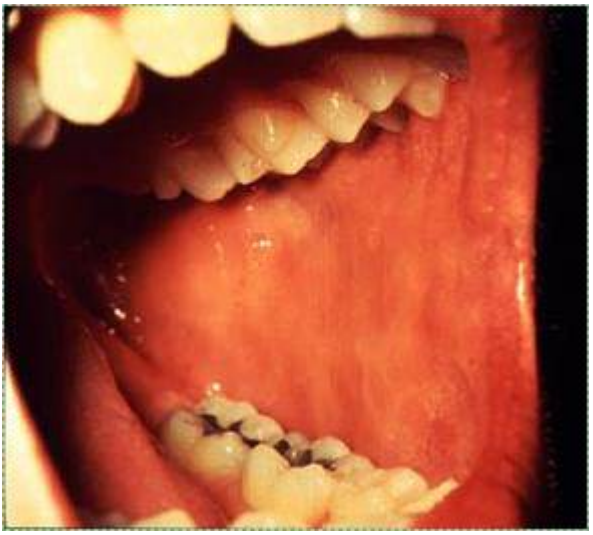


Figure 3.10: Buccal mucosa. Image credit: "buccal mucosa" by the NIH is in the Public Domain CC 0

Labial and buccal mucosa

Labial mucosa and **buccal mucosa** both have a **non-keratinized stratified squamous epithelial**[←] layer. This gives them a more reddish or pinkish *base* appearance (see alveolar mucosa pigmentation below). As with all **oral mucosa**, there are no hair follicles, but in places sebaceous glands may be present, forming **Fordyce spots**. As a lining mucosa, the epithelial layer is generally **non-keratinized**, but there can be regions of **keratinization** where stress occurs. Most likely, the **lnea alba** (or "white line") will be apparent,

running along the line in the buccal mucosa where the teeth meet.

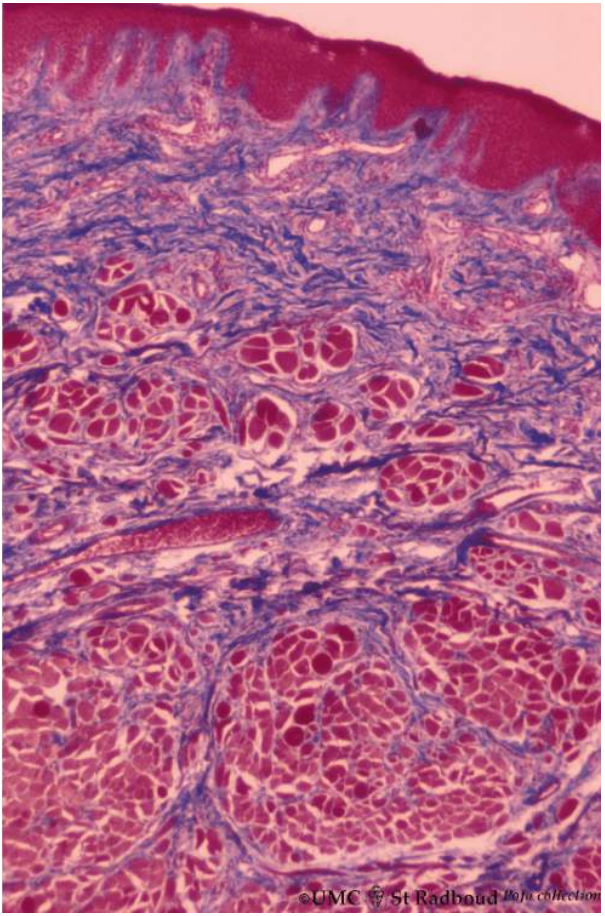


Figure 3.11: Histology of border between the labial mucosa (left side) and the vermilion zone (right side). Image credit: "Lip (human), region between red zone (vermilion border) and mucosa inner surface" by Poels,

Fig. 3.11 shows the histology of where the **vermilion zone** and labial mucosa meet. More prominent **rete pegs** and **dermal papillae** of the labial mucosa compared to the vermilion zone develop in response to higher levels of abrasion (**nurture**), and do not represent a **lineage**[←] difference between these two tissues (**nature**).

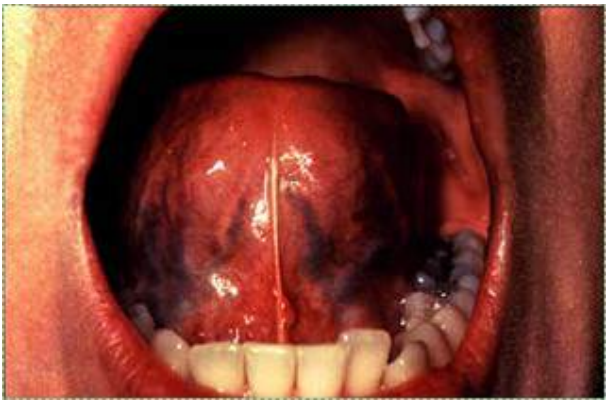


Figure 3.12: Ventral surface of the tongue. Image credit: Ventral tongue by the NIH is in the Public Domain CC 0

Ventral surface of the tongue and the floor of the mouth

The **ventral surface of the tongue** and the **floor of the mouth** both contain very thin, **non-keratinized stratified squamous epithelium**. The thinness gives these surfaces a more reddish-appearance than other lining mucosa. The thinness of the epithelium, coupled with the rich blood supply in the deeper **lamina propria** are also why some medications are given sublingually.

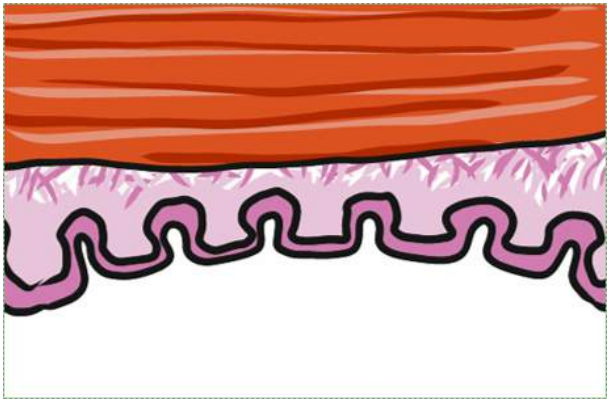


Figure 3.13: Illustration of the histology of the soft palate.

Soft palate

The **soft palate** is lined by a **non-keratinized stratified squamous epithelium**, with a very thin layer of **submucosa** deep to it. This gives the epithelium a firm attachment

to deeper muscle tissue, which is important for speech and swallowing.

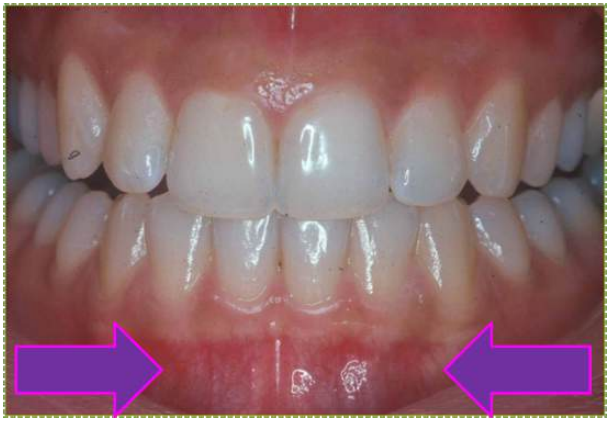


Figure 3.14: Alveolar mucosa. Image credit: "Gingiva of the human mouth" by John Crawford is licensed CC BY 3.0. / Arrows added

Alveolar mucosa

Alveolar mucosa is lined by a **non-keratinized stratified squamous epithelium**. It has a rich blood supply and numerous **elastic fibers** within the **lamina propria**, but

few **dermal papillae** and **rete pegs**. Because of the large blood supply and low levels of **keratin**, you may often hear healthy alveolar mucosa should appear pinkish, but this assumes the absence of **melanin**.

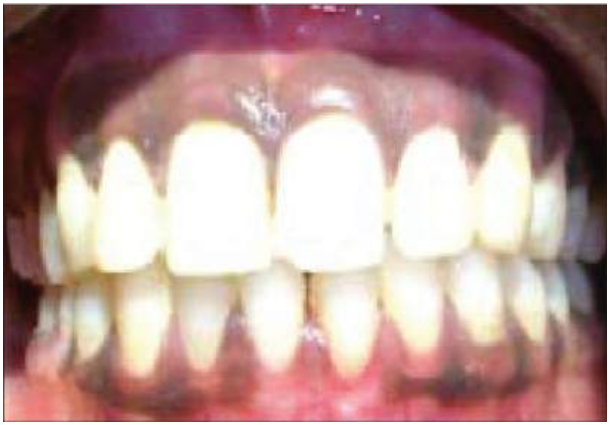


Figure 3.15: Example of healthy human gingival coloration. Image credit: "Preoperative picture of 25-year-old female complaining of black-colored gums" by Arthiie Thangavelu et al is licensed under CC

Clinical disorders may alter the coloration of the gingiva, but to assume all healthy gingiva are pinkish ignores healthy variation in skin color. The gingiva of darker-skinned patients may be darker and be completely healthy. **Melanin** can be deposited in alveolar mucosa, either uniformly (evenly) or focally (localized, such as a freckle or macule, a freckle on the lips). Tanning is a homeostatic change in **melanocyte** activity in response to UV damage to **keratinocytes** (**nurture**). The presence of melanin in the gingiva, however, rarely represents a homeostatic change (hence, not a pathological change). Instead, melanin levels in the **oral mucosa** are linked to inborn melanin level in the skin (**nature**), a concept that should be more obvious once we consider the shared **lineage**[←] of these two tissues.

Masticatory mucosa

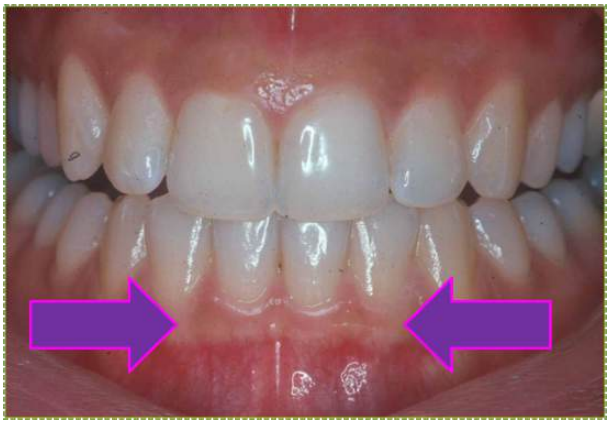


Figure 3.16: Attached gingiva. Image credit: "Gingiva of the human mouth" by John Crawford is licensed CC BY 3.0 / arrows added

Attached gingiva

Attached gingiva is a type of masticatory mucosa, lined with a **para-keratinized stratified squamous epithelium**[←]. The increased amount of keratin, compared to **alveolar mucosa**, obscures the underlying blood supply, creating a lighter appearance (which can be described as whitish in the absence of melanin). The attached gingiva is named for its firm

attachment to the tooth or to **alveolar bone** by groups of **gingival fibers**.

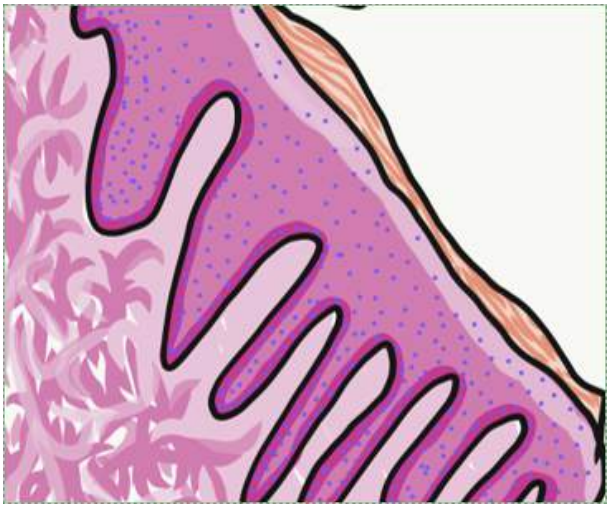


Figure 3.17: Illustration of a partially keratinized epithelium of the attached gingiva.

Large **dermal papillae** and **rete pegs** create the stippled (rough surface) appearance of the attached gingiva. The rough surface may also be described as orange-peel, which indicates the relative health of the attached gingiva due to sizeable rete pegs and dermal papillae between the **oral epithelium** and **lamina propria**.

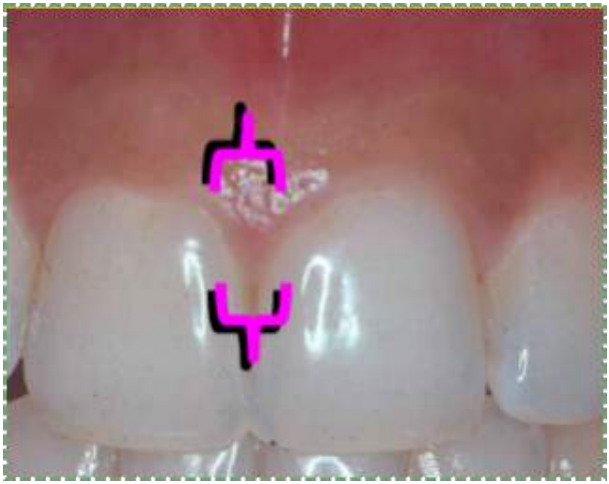


Figure 3.18: Interdental gingiva. Image credit: "Gingiva of the human mouth" by John Crawford is licensed CC BY 3.0 / cropped and brackets added

Interdental gingiva

Interdental gingiva (or the interdental papilla) is similar to the **attached gingiva**.

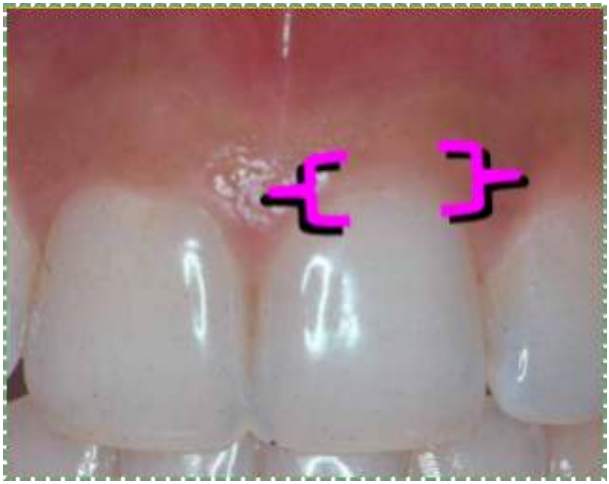


Figure 3.19: Marginal gingiva. Image credit: Figure 3.19: Marginal gingiva. Image credit: "Gingiva of the human mouth" by John Crawford is licensed CC BY 3.0 / *cropped and brackets added* by John Crawford is licensed CC BY 3.0 / cropped and brackets added

Marginal gingiva

Marginal gingiva epithelium is histologically similar to the **attached gingiva**— it has pronounced rete pegs and is partially keratinized. The gingival margin may be grouped together with junctional epithelium and sulcular epithelium (described below) and referred to as free gingiva. Unlike the attached gingiva, the sub-mucosa of free gingiva is not connected to bone tissue.

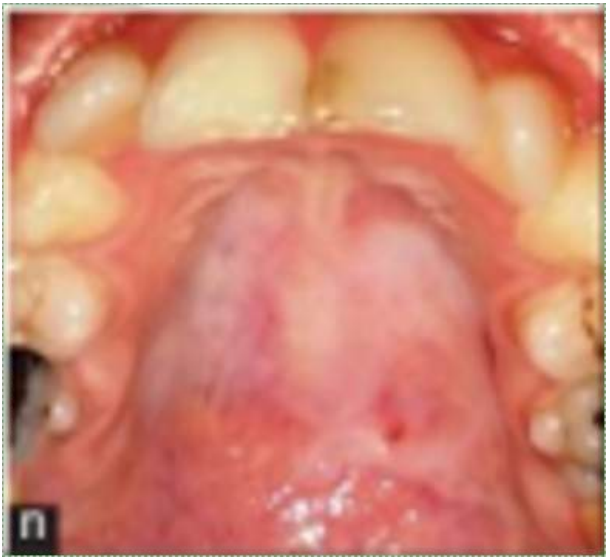


Figure 3.20: The hard palate. Image credit: "Pleomorphic adenoma of the left palate" by the NIH is in the Public Domain CC0

Hard palate

The **hard palate** is lined by an **ortho-keratinized stratified squamous epithelium**[←] and mostly lacks a **sub-mucosa**, making for a rigid connection to underlying **bone tissue**[←].

Mucosa of the Dento-Gingival junction

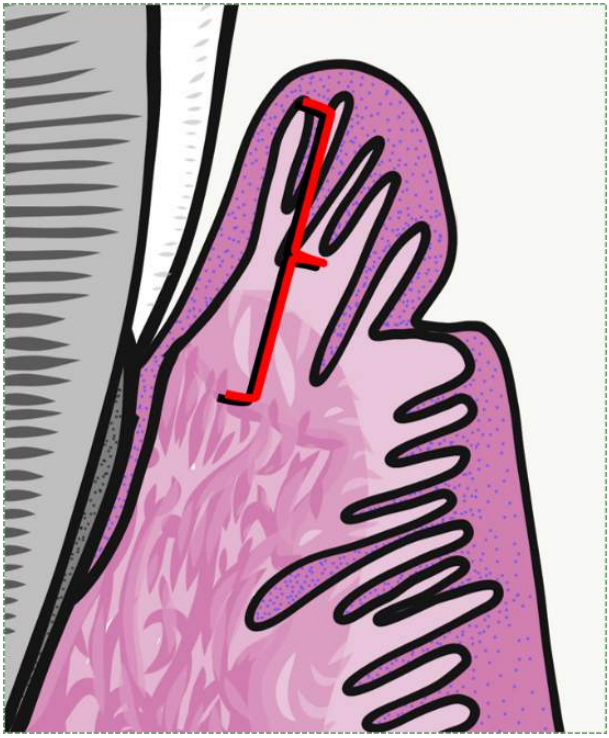


Figure 3.21: Sulcular epitheliu m (red bracket).

Sulcular epithelium

Sulcular epithelium (or crevicular epithelium) is lined by either a **non-keratinized** or **para-keratinized stratified squamous epithelium**. It creates a space between the gingiva and tooth, named the **gingival sulcus**. Sulcular epithelium is not attached to the surface of the tooth. Under

the microscope, the absence of **dermal papillae** and **rete pegs** indicates this tissue gets very little abrasion under healthy conditions. It is also more delicate and permeable, especially in the deeper regions closer to the junctional epithelium.

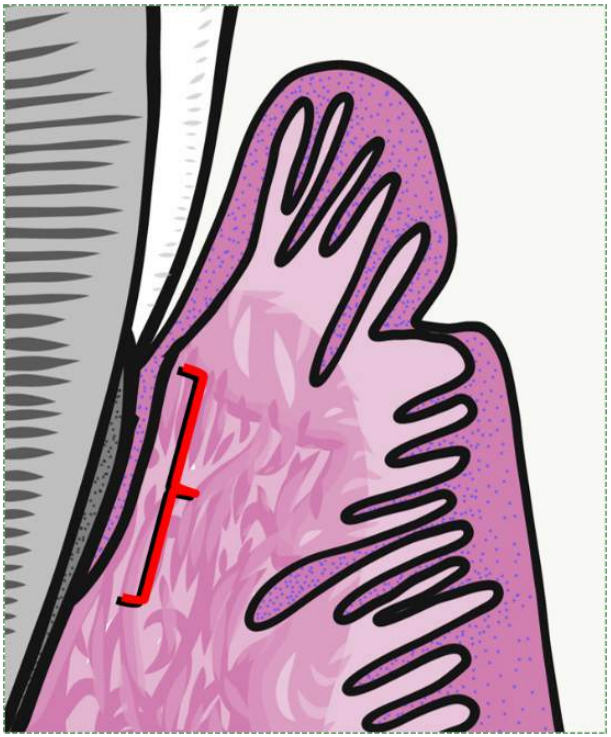


Figure 3.22: Junctional epitheliu m (red bracket).

Junctional epithelium

Junctional epithelium (JE) is a **non-keratinized stratified squamous epithelium**. It is special in that its **apical** surface

attaches to the tooth by way of **hemi-desmosomes**. Other epithelia attach to connective tissue only on their **basolateral** surface, their apical surface faces the external environment. This unique attachment to the tooth surface is referred to as the **epithelial attachment** (EA). This uniqueness is challenging to re-create with dental implants. In a subsequent chapter, we cover the **lineage** of the junctional epithelium that gives the adult tissue its uniqueness.

Junctional epithelium is thinner than other gingival mucosa, only five cells wide at the end (relative to the apical-to-basolateral direction of the epithelium, not the apical-to-coronal direction of the tooth). It is more permeable, having fewer **desmosomes** between cells. This allows white blood cells from the underlying **vascular sub-mucosa** to migrate through junctional epithelium and enter the gingival sulcus. But this also increases the potential for oral cavity bacteria to do the same in reverse, especially if the epithelial attachment is lost. This is an important factor in the study of periodontal infection and the immune response.

Specialized mucosa

Figure 3.23: Illustration of the histology of the tongue.

Tongue histology

The **dorsal surface of the tongue** contains multiple types of mucosa. This should make moe sense after we cover how the tongue develops from three different **pharyngeal arches**. The epithelial surface is mostly an **ortho-keratinized stratified squamous epithelium**, and can therefore be thought of as a masticatory mucosa. Scattered across the dorsal and lateral surfaces are four different shapes of bumps called **lingual papillae.** The most numerous are filiform papillae, which contain only keratinocytes, and may be ortho- or para keratinized. The other three lingual papillae also contain taste buds, which are not keratinocytes, and therefore not a masticatory or lining mucosa, but a specialized mucosa. These structures are appendages of the oral mucosa.

Anteriorly, deep to the oral mucosa and **sub-mucosa**, the tongue contains numerous bundles of skeletal **muscle cells**, and some **adipose tissue**. Posteriorly, the tongue contains more adipose and salivary gland tissue, and is covered by tonsillar tissue rather than an ortho-keratinized stratified squamous epithelium.

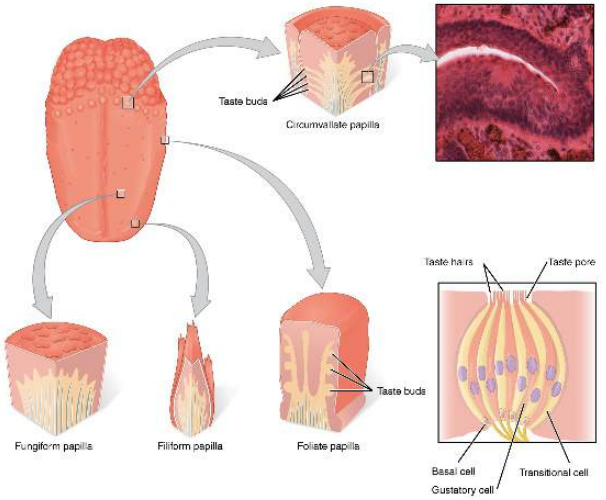


Figure 3.2: The4 types of papillae on the tongue. Image credit: "The Tongue" by the OpenStax is licensed CC BY 4.0

Filiform papillae are the majority of the tongue's dorsal surface, giving it avelvety appearance. They contain an **ortho-keratinized** or **para-keratinized stratified squamous epithelium**. These papillae function to provide friction only, their mucosa contain no taste buds.

Fungiform papillae are shaped like a mushroom (a type of fungus) and are dotted throughouot the dorsal surface of the tongue. They contain an **ortho-keratinized** or **para-**

keratinized stratified squamous epithelium[←] over a highly **vascular sub-mucosa**, giving these structures a more reddish-appearance than neighboring filiform papillae. The epithelial layer contains taste buds, which detect the sense of gustation, which is in turn a part of the perception of taste.

Foliate papillae are found on the lateral edges of the tongue. They contain an **ortho-keratinized** or **para-keratinized stratified squamous epithelium**[←] with taste buds.

Circumvallate papillae are found in a V-formation at the border between the anterior and posterior portion of the tongue, the **sulcus terminalis**. They contain an **ortho-keratinized** or **para-keratinized stratified squamous epithelium**[←] with taste buds and minor salivary glands.

Turnover time of epithelia

Epithelium	Turnover time (days)
Skin	27-38
Hard palate	24
Floor of mouth	20
Buccal and labial mucosa	14
Attached gingiva and taste buds	10
Junctional epithelium	5

Table 3.4: Turnover time of the various regions of oral mucosa and skin.

The time it takes to replace all of the cells within the epithelial layers of the skin and **oral mucosa** is shown in Table 3.4. As you should see, the **oral epithelium** grows quickly, which means it can regenerate quickly following injury. This is largely due to the presence of **growth factors** in saliva. This also means the lifespan of these cells is short, making oral cancers relatively rare in the absence of large doses of carcinogens (tobacco and alcohol). The epithelial cells of oral mucosa do not live long enough to easily acquire the multiple mutations to oncogenes and tumor-suppressor genes required to cause cancer.

Clinical applications

Hyper-keratosis

Hyper-keratosis is a homeostatic response of the oral mucosa to stress, either chemical or physical (e.g. smoking or denture friction). In response to stress, epithelial cells **express** more **keratin**, causing an increase in the degree of keratinization. Vitamin A-deficiency can lead to generalized hyper-keratosis.

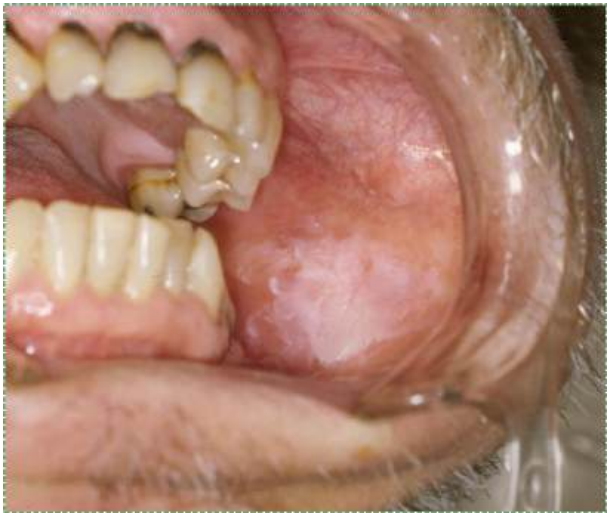


Figure 3.25: Leukoplakia, and example of hyper-keratosis. Image credit: "leukoplakia" by dozenist is licensed CC BY 3.0

Leukoplakia

If the increase in keratinization is localized, it is referred to as **leukoplakia**. Parafunctional habits can cause regions of the **buccal mucosa** to undergo hyper-keratosis. Bruxism may cause the **linea alba** to appear more whiteish. Chemical stress caused by use of smokeless tobacco products (snuff) cause leukoplakia at the site of use.

Beta-carotene supplements may be prescribed in the treatment of leukoplakia because **carotene** accumulates within the **oral epithelium** (and epidermis). There, it is converted into Vitamin A, which in turn is used to synthesize

the **morphogen**[←] Retinoic Acid (RA). RA **induces** the **differentiation**[←] of epithelial **stem cells**[←] into keratinocytes rather than undergoing **mitosis**[←]. After a week, this leads to fewer keratinocytes, reversing the severity of lesions caused by over-production of keratinocytes (psoriasis in skin) and keratin (leukoplakia in oral mucosa). However, beta-carotene supplementation is not effective at preventing the progession of leukoplakia into oral cancer in smokers.

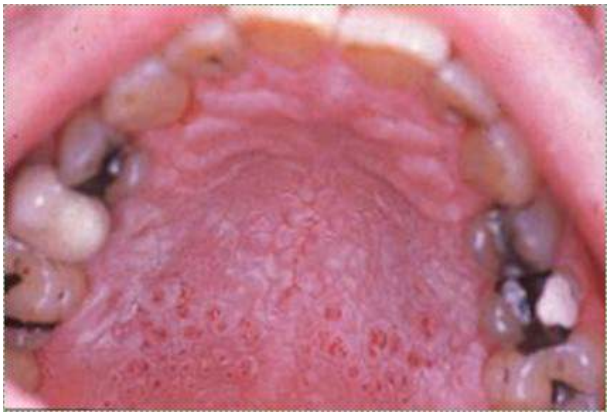


Figure 3.26: Nicotinic stomatitis, an example of hyper-keratosis. Image credit: "Nicotinic stomatitis" by DVIDS is in the Public Domain CC0

Nicotinic stomatitis

Hyper-keratosis can be caused by the chemical stress of cigarette smoke. It is not caused by nicotine, which is an addictive substance but mostly non-toxic to adult humans, despite what many otherwise reliable resources suggest. Nicotine is a **teratogen** to developing embryos. We cover adverse effects of nicotine with **PDL** loss in chapter 11. For the **oral mucosa** (and respiratory tract), the chemical stress from smoking is caused by benzene, dioxin, formaldehyde, poly-aromatic hydrocarbons, and other toxic chemicals produced by combustion (burning), not the tobacco plant. At the time we are writing this, there is no evidence that nicotine gum causes hyper-keratosis of the **oral mucosa**, and the link between vaping and hyper-keratosis is not strong, despite often containing nicotine. If your patients smoke, these two nicotine-delivery methods are considerably safer for both the lungs and oral cavity, and can be helpful tools in smoking-cessation (nicotine is the addictive component of tobacco products). This is **not** to be mistaken for an endorsement of taking up an e-cigarette habit, nor maintaining one, we are discussing *relative* risk.

Nicotinic stomatitis is a visible change to the hard palate. In response to chronic stress, the **ortho-keratinized** epithelium produces more keratin, leading to a more white-ish appearance. However, epithelial cells of the **minor salivary glands** do not respond to stress in this fashion, and remain

pinkish (the genes for keratin production are possibly **methylated** and packed around **histones** in glandular epithelial cells). This same pattern can also be caused by ingesting hot liquids, but is acute and temporary in comparison to nicotinic stomatitis. Other than visual change, nicotinic stomatitis is usually asymptomatic and may be unknown to the patient.

Clinical changes to the gingiva

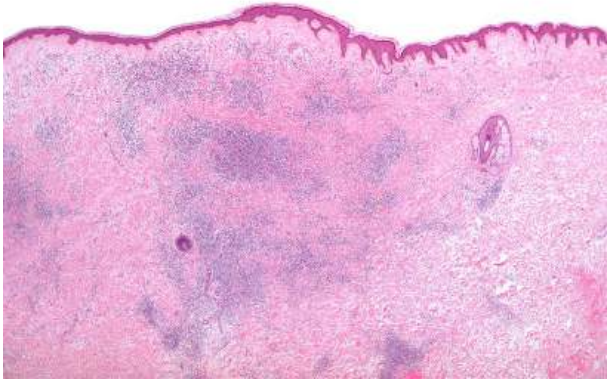


Figure 3.27: Migration into an injured area by leukocytes (identified as masses of purple nucleusses in the dermis) during an inflammatory response. Image credit: "Dermal perivascular lymphoeosinophilic infiltrate - very low

Inflammation of any issue is referred to as tissue-name-itis, hence **gingivitis** is inflammation of the gingiva, while **periodontitis** is inflammation to the **periodontium**. The redness, swelling, heat and pain symptoms indicate the body has likely suffered trauma, and is undergoing a response to that trauma. Capillaries exude more liquid into an area as a part of the inflammatory process, known as **edema**. This makes inflamed regions of **oral mucosa** thicker and paler. Edema may give gingiva a puffy or rolled appearance.

Ideally, an inflammatory response limits the spread of the initial damage. After inflammation has prepped the injured area, a tissue can undergo **regeneration**. First, **stem cells**[←] migrate into the affected area, undergo **mitosis**[←], and **differentiate**[←] into cells needed to repair the damage, such as **keratinocytes** or **fibroblasts**. Sometimes, these cells will produce an intermediate form of tissue first, such as granulation tissue, that will be **remodeled** afterwards.

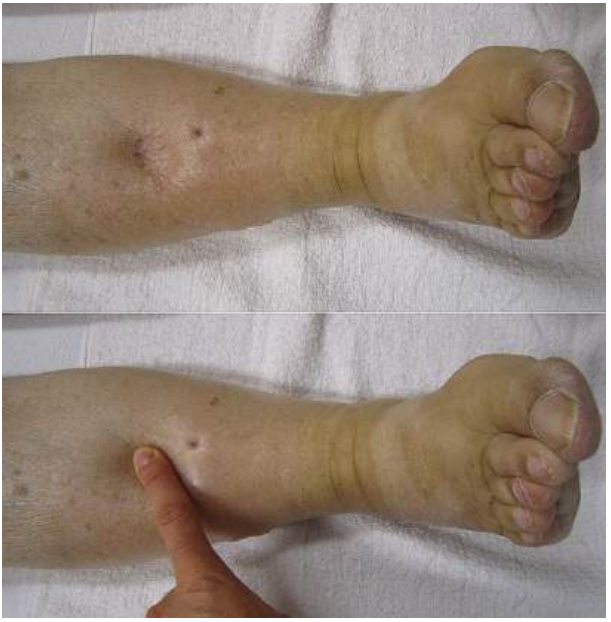


Figure 3.28: The fluids that cause edema in a tissue are easily compressible. Image credit: "Pitting edema" by James Heilman, MD is licensed under CC BY-SA 3.0

Chronic inflammation, on the other hand, often leads to cell death and the loss or recession of a tissue. This is because **stem cells**[←] generally halt progression through the cell cycle until the inflammatory process removes the source of the stress. Without stem cells generating new cells, chronic stress allows everyday wear on a tissue to accumulate.

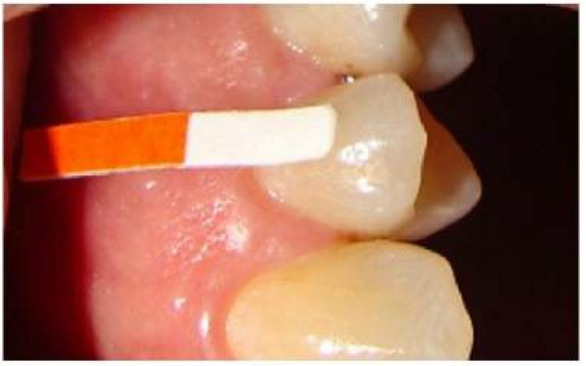


Figure 3.29: Collection of Gingival-Crevicular Fluid (GCF). Image credit: "Extracrevicular GCF collection" by Zeyad Nazar Majeed et al is licensed under CC BY 4.0

During an inflammatory response, the gingival sulcus fills with **gingival-crevicular fluid** (GCF, or gingival-sulcular fluid). GCF contains breakdown products of human cells undergoing necrosis or **apoptosis**[←], the breakdown products of bacteria being killed by white blood cells, bacterial toxins,

and inflammatory molecules released by human cells. Taking a sample of gingival fluid as a diagnostic tool for measuring gingival health should be considered.

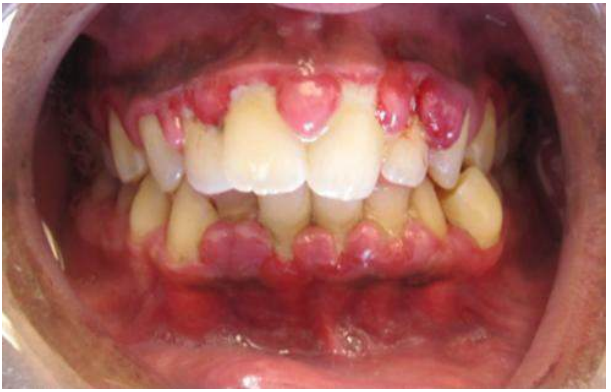


Figure 3.30: Really bad gingivitis. Image credit: "Really bad gingivitis" by D. Rosenbach, is licensed CC BY 3.0

Gingivitis

Gingivitis is any inflammation of the gums. It includes **edema** in the **ECM** of connective tissues in the **lamina propria** and **sub-mucosa**, as well as inside the epithelial cells of the **oral mucosa**. This causes the **marginal**, **attached** and **interdental gingiva** to become visibly swollen. When marginal gingiva is

swollen, it may produce a crescent-shaped edema known as a **McCall's festoon.** This inflammatory response generally causes no damage, but untreated gingivitis can progress to **periodontitis**, which in turn can lead to bone and tooth loss.

Research suggests gingivitis increases the risk of developing Alzheimer's Disease and heart disease, making it all the more important to intervene as early as possible. Currently, 70% of Americans over the age of 65, 50% over the age of 30 and 80% of school-aged children suffer from periodontal disease.

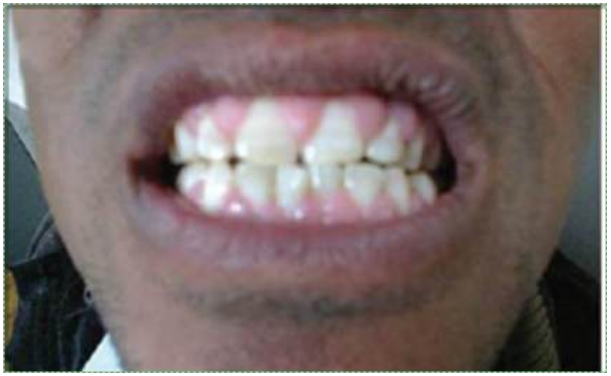


Figure 3.31: Gingival hyperplasia. Image credit: "Gingival enlargement due to S - amlodipine" by the NIH, is in the Public Domain CC0

Gingival hyperplasia

Hyperplasia means the increased growth of a tissue, which could mean an increased number of cells, an increased amount of **ECM** produced by cells, or both. **Gingival hyperplasia** is an abnormal growth of gingival tissue. It may look similar to **edema**, but the underlying cause (and therefore treatment) is different. A tissue that has undergone hyperplasia will be dense, not squishy as a tissue with edema. Hyperplasia can

be a side-effect of certain medications, such as phenytoin and cyclosporine. Other triggers exist, including pregnancy, hormonal disturbances, or it may even be a hereditary condition (Hereditary Gingival Fibromatosis). Like edema, gingival hyperplasia may be caused by poor oral hygiene. The first response of the immune system to oral microorganisms is inflammation (and therefore edema). Over time, the immune system may switch to releasing **growth factors** that trigger cell division of nearby **stem cells**←, and other signals (**morphogens**←) that trigger cell **differentiation**← and activity (such as the production of ECM proteins). This is a perfectly healthy response to stress in the palms of the hands or soles of the feet, generating a callus. It is also not bad way to shed parasites. Hoever, the body does not have separate mechanisms for reacting to physical stress and other types of stress, such as chemical stress caused by toxins produced by microorganisms. Increased growth of gingival tissue does not remove toxins or microorganisms.

Keep in mind some patients' response to poor oral hygiene is **gingival recession** (covered next), not hyperplasia, for reasons described under edema. This seemingly irreconcilable difference results from the complexity of the immune system. Both gingival recession and hyperplasia are triggered by signaling molecules from white blood cells. While blood cells are, in turn, a large group of different cells that respond to an even larger variety of environmental stimuli (most diseases,

physical trauma, allergens, tumors, parasites and more) by releasing a wide variety of chemical signals called cytokines. **Cytokines** produce a wide variety of different responses, including gingival hyperplasia in some patients and gingival recession in other patients. We encourage you to take a look at the list of different cytokines (Table 1) provided in the previous link *before* you come across the tables of different **morphogen**← signaling molecules we cover in chapters 6 through 11.

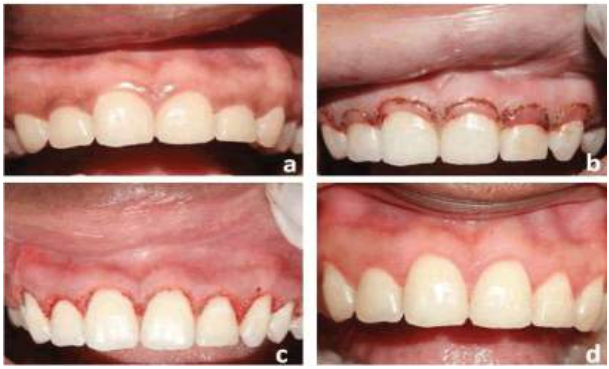


Figure 3.32: Cosmetic surgery for gummy smile. a) before, b) laser incisions, c) immediately after surgery, d) 2-week post-operative. Image credit: "Esthetic crown lengthening" by Shanmukha Srinivas Manikanta Kumar Tirumalasetty et al is licensed

Gingival hyperplasia should be addressed even when not caused by poor oral hygiene, as it may make maintaining oral hygiene difficult. In some cases, it may not be possible to remove the underlying cause (such as a hereditary condition, or a life-saving anti-seizure medication), in which case surgical removal of gingival tissue (gingivectomy) or gingival reshaping (gingivoplasty) may be warranted. Excess gingival tissue can be removed using a scalpel or a laser tool. The use of lasers can reduce bleeding and pain by cauterizing damaged blood vessels and ablating nerve endings.

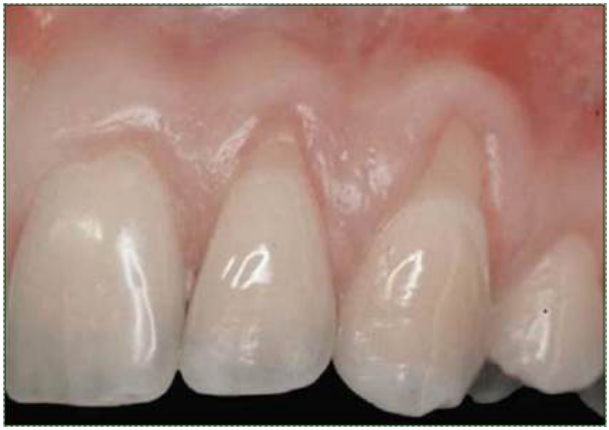


Figure 3.33: Gingival recession. Image credit: "Class II gingival recession on the left maxillary canine and lateral incisor" by Nitin Khuller, is licensed under CC BY 3.0

Gingival recession

Chronic inflammation of the gingiva can lead to **gingival recession** (receding gums), exposing deeper tissues of the tooth, which in turn may make teeth more susceptible to tooth decay. The most common cause of gingival recession is **periodontitis**. Gingival recession can also be caused by abrasion (improper tooth brushing), abfraction (bruxism), improper tooth position, and sometimes aging (depending on the tissue biotype, host response and genetics).

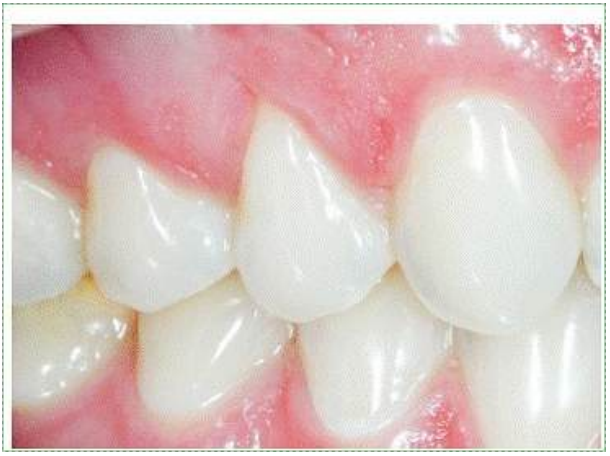


Figure 3.34: Stillman cleft. Image credit: "V-shaped gingival recession" by Ana Suzy Jati et al, is licensed CC BY 3.0

A **Stillman cleft** is a V-shaped region of gingival recession. It

is often caused by occlusal trauma. Other **etiologies** include gingival inflammation and improper tooth brushing and flossing techniques. Under the microscope, the gingival tissue found bordering the Stillman cleft will appear fibrous and full of white blood cells, the typical response of a connective tissue to inflammation (Fig 3.27).

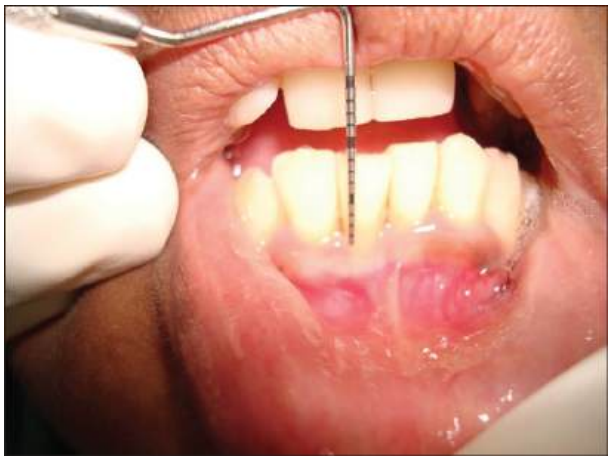


Figure 3.35: A tension test during an oral exam can identify whether the attached gingival mucosa has a firm attachment to the underlying sub-mucosa. Image credit: "Pre-operative photograph showing recession on teeth 31 and 41 along with insufficient attached gingiva

Tension test

During the intraoral exam, a tension tet may be performed by pulling the lip forward and moving it left and right. This helps to identify mucogingival defects. Mucogingival involvement are areas where there is gingival recession or loss of attachment of the gingiva. Loss of attachment causes the gingival margin to be freely moveable like the alveolar mucosa, not attached to underlying sub-mucosa and bone tissue. Healthy connections between **rete pegs** and **dermal papillae** prevent movement of the attached gingiva.

and high frenum attachment Figure 2: Pre-operative photograph showing recession on teeth 31 and 41 along with insufficient attached gingiva and high frenum attachment" by Gupta, V et al, Contemporary Clinical Dentistry is licensed under CC BY-SA 4.0

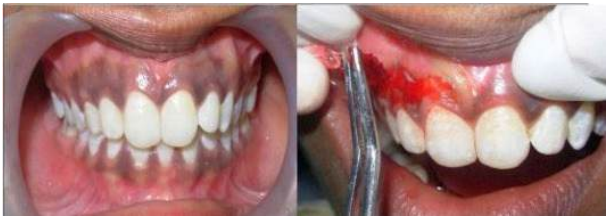


Figure 3.36: Gingival pigmentation, before and during pigment removal surgery. Image credit: "Pre-operative view and Maxillary pigmentation removal using scalpel surgical techni

Pigmentation of the gingiva

Similar to increased levels of **keratin** in the attached gingiva, increased levels of **melanin** may obscure underlying healthy level of **hemoglobin**. The presence of melanin alone should not be mistaken for unhealthy gingiva. Unlike keratin, melanin is a *pigment*, meaning it absorbs only certain wavelengths of light (has a color). Surgeries and treatments to remove gingival pigment exist, often advertising what is found in textbooks: *healthy gingiva should be pinkish or whitish in color* (try a google search for gingival hyperpigmentation if you are skeptical). It is worth noting skin whitening products exist because of systemic racism in the United States and other countries has led many people of color to feel displeasure with darker skin pigmentation, illustrated by experiments such as the Doll test by Drs Kenneth and Mamie Clark. Furthermore, the concept that pink is healthy and brown is unhealthy is not shared across cultures. For instance, dental offices in Ethiopia and surrounding areas may offer ethnobotanical tattooing of the maxillary gingiva to add a blue-ish or grey-ish coloration to the gingiva, masking pinkish regions.

Figure 3.37 Smokers melanosis. Image credit: "Smoker's melanosis in oral mucosa with brown black pigmentation" by Skinstudy is licensed under CC BY SA 3.0

A rapid, focal change in **melanin** production in the oral cavity may be a response to a medical condition. Most common among dark-skinned women between the ages of 30-50, increased melanin production in response to acute trauma or prolonged irritation (such as smoking) may arise, termed a

melanocanthoma (further reading can be found here). Despite the name this type of skin lesion has, it is not a tumor, but a homeostatic change in cell activity. Tobacco smoke creates numerous free radicals that damage epithelial cells. Melanin can bind to free radicals and prevent them from reacting with DNA or lipids, thus increased melanin production helps protect the **oral mucosa**.

Tetracycline antibiotics bind to melanin, which triggers melanocytes to up-regulate melanin synthesis. Tetracycline drugs are very common, found in over-the-counter first-aid creams, added to animal feeds, and were even in beers brewed in Sudan 2,000 years ago.

There are other pigments that cause color changes to the gingiva, and they are classified as either **endogenous** (produced by human cells) or **exogenous** (environmental). Endogenous pigments commonly found in the gingiva, besides melanin, include **hemoglobin**. A reddish lesion caused by hemoglobin or its breakdown products is a sign of damage to capillaries (bruising). An example of an exogenous pigment is jimsonweed (Datura stramonium), which can be used in the ethnobotanical gingival tattoos mentioned previously.

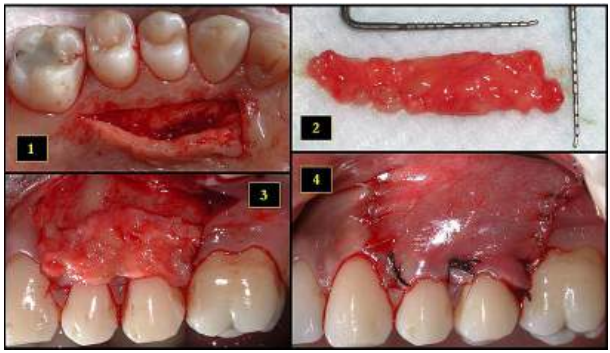
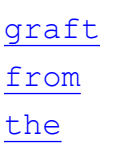


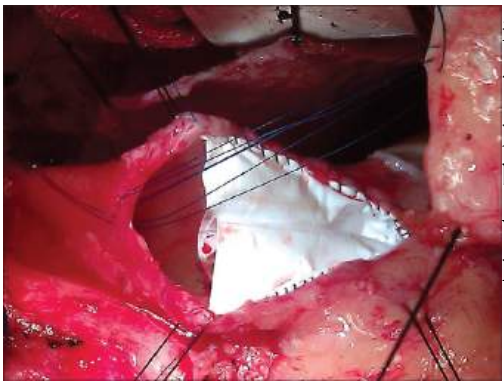
Figure 3.38: Sub-epithelial graft. Legend: 1) donor site (ipsilateral mucosa), 2) recovered connective tissue, 3) connective tissue added to recipient site, 4) sutured recipient site. Image credit: "Retrieval of the subepithelial connective

Gingival grafting



"tissue graft from the palate and placement at the recipient site" by DRosenbach is licensed under CC BY-SA 4.0

A **sub-epithelial connective tissue graft** (SECT graft) may be performed to repair **gingival recession**. Unlike a skin graft, which grafts epithelial plus some or all of the connective tissue from a donor, a sub-epithelial graft transplants only connective tissue from the **lamina propria** and/or **sub-mucosa**. The goal of a sub-epithelial graft is not to replace damaged tissue, but to provide a **scaffold**[←] that promotes healing of the patient's own tissues. The connective tissue contains **collagen**[←], **fibronectin**[←] and **hyaluronic acid** to which epithelial **stem cells**[←] are attracted to and migrate *over* (thanks to **integrins**[←] and other **trans-membrane protein receptors**). These stem cells undergo **mitosis**[←], producing more epithelial cells which **differentiate**[←] into **keratinocytes** and regenerate the **oral epithelium**. Similarly, the patient's **mesenchymal stem cells** migrate *through* the scaffold and replace the transplanted collagen, regenerating the lamina propria and sub-mucosa. Connective tissue grafts are relatively common in dentistry and maxillofacial surgery, but these technologies are starting to generate changes in tissue grafting below the neck, too.



in situ with transannular pericardial patch being sutured" width="779" height="587">

Figure 3.39: Use of a synthetic polymer scaffold in heart surgery. Image credit: Intraoperative view showing hand sewn polytetrafluoroethylene bicusp id

pulmonary valve in situ with transannular pericardial patch being sutured" by Prashant Ramdas Wankhade et al is licensed under CC BY-NC-SA 4.0

Sub-epithelial grafts splice connective tissue from nearby regions of healthy gingiva (such as from neighboring gingiva or hard palate). This leaves behind small wounds, which heal quickly. Nevertheless, damaging healthy tissue is not optimal. Another option is to use tissue from a human cadaver. Because the connective tissue used in such a procedure is mostly **collagen**[←] fibers, other options include the use of synthetic collagen-like polymers (Fig 3.38) or a **pericardial patch** procedure. Unlike human tissue, there is a large supply of cow and pig connective tissue. First, the connective tissue that surrounds a cow or pig heart (the pericardium) is harvested and stripped of any pig or cow cells. The acellular tissue that remains should not trigger tissue rejection. Collagen shares a very high degree of **homology** across vertebrate species, meaning our collagen is nearly identical to cow and pig collagen. Just like in SECT, collagen acts as a **scaffold**[←]. Ultimately, the patient's own epithelial **stem cells**[←] migrate *over* the scaffold to regenerate the **oral epithelium**. In addition, the patients **mesenchymal stem cells** from nearby healthy **lamina propria** and **submucosa** migrate *through* the scaffold, and replace cow or pig collagen with human connective tissue. This procedure is similar to types of **bone tissue grafts** and is not limited in use

to the gingiva—a video can be viewed here of a heart valve replacement using bovine pericardium. We hope you appreciate the amount of histology and cell biology required to understand how pieces of cow hearts can be used to repair damaged gingiva.

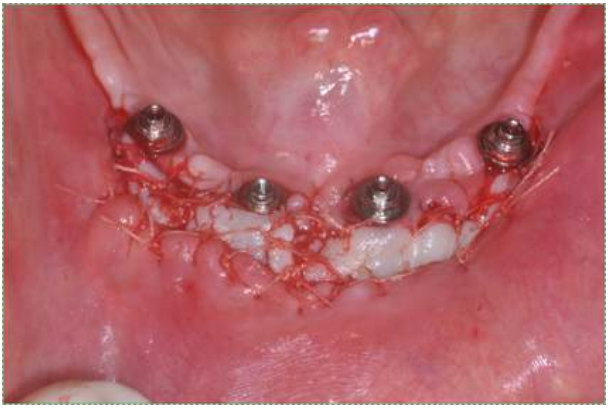


Figure 3.40: Free gingival graft. Image credit: "Free gingival graft placed around the implants" by Danny Omar Mendoza Marin, is licensed CC BY 3.0

A **gingival graft** (such as a free gigival graft or other related procedures) may be placed around **dental implants**[←], or used

to repair **gingival recession**. Similar to the sub-epithelial graft, a gingival graft harvests healthy tissue from the donor, only in this case epithelial cells from the **oral mucosa** are transplanted along with connective tissue. When placed around **dental implants**←, attached gingival tissue can adhere to the implant, similar to **junctional epithelium** which adheres to a tooth. The attachment may contain hemi-desmosomes, however it does not form a pocket with a thin epithelium– this difference in **morphology** will become more apparent after learning about the development of junctional epithelium during **tooth eruption**←. Coating a dental implant with **hyaluronic acid** helps grafted tissue adhere to the implant. Without adhesion to the implant, oral microorganisms bypass the **oral mucosa** and enter the **sub-mucosa**.

For further reading on dental biomaterials
FDA information on GINTUIT
Mucograft – acquired by Geistlich Biomaterials
Gengigel hyaluronic acid gel

Table 3.5: Further reading about currently available dental biomaterials

Clinical applications of dento-gingival junction histology

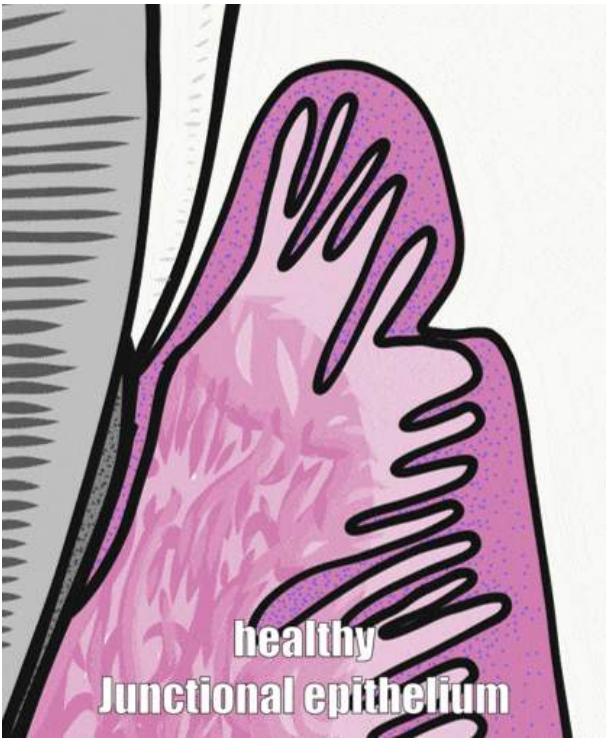


Figure 3.41: Illustration of periodontal pocket morphology (note: blood vessels are present deep to both JE and pocket epitheliu m, they do not suddenly appear).

Periodontal pockets

The depth of a **periodontal pocket** can be measured using a calibrated probe. In a healthy state, the distance from the **marginal gingiva** to the **epithelial attachment** should be between 1 to 3 mm. Pockets within this range typically have

an intact epithelial attachment, which prevents oral bacteria from entering the **sub-mucosa** and causing **gingivitis** or **periodontitis**. Poor oral hygiene, however, can lead to increased levels of oral bacteria within the periodontal pockets. Because **junctional epithelium** is more permeable than other regions of **oral mucosa**, white blood cells come into contact with this bacterial population and trigger inflammation. With chronic inflammation comes a loss of junctional epithelium, which can reduce the thickness of the junctional epithelium further, potentially causing the loss of the epithelial attachment. At this point, the pocket is said to be lined with **pocket epithelium.** Since it is no longer attached to the tooth, the probe can likely be inserted over 3 mm. The thinness of the pocket epithelium brings the probe closer to blood vessels in the lamina propria, making it more likely to damage these vessels, causing **bleeding on probing** (BoP).

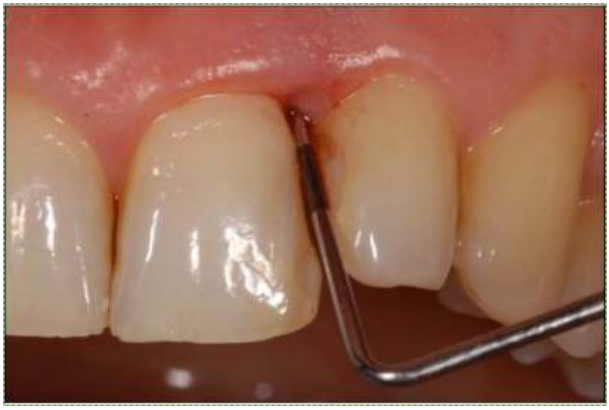


Figure 3.42: Bleeding on probing (BoP). Image credit:"Bleeding after probing" by Luigi Checchi et al, is licensed CC BY NC 3.0

The major risk with bleeding on probing involves the oral microbiome, which is a large collection of microorganisms. These microorganisms come into contact with the bloodstream with disruption to the epithelial barrier. Even so-called "good bacteria" trigger inflammation when they move inside the body. Until damage to the barrier is repaired, inflammation continues. Chronic inflammation within the pockets leads to damage to nearby tissue, such as alveolar bone. This leads to further damage to the oral cavity which we discuss later. A second risk with migration of the epithelial

attachment is that acid-producing bacteria now come into contact with softer cementum, leading to **root caries**.

It is possible for a periodontal pocket deeper than 3 mm to have **junctional epithelium** with an intact **epithelial attachment**. These exhibit minimal bleeding on probing, and can be considered **pseudopockets** rather than a clinical manifestation of **periodontitis**. Pseudopockets, which may also be called false pockets or gingival pockets, are caused by **gingival hyperplasia** or **edema**. Enlargement of the gingival margin, whether by **hyperplasia** or **edema**, deepens the measurement of a pocket, but does not share the same harmful loss of the epithelial attachment. The underlying cause of the pseudopocket may need to be addressed, especially if there is bleeding on probing.

Clinical applications of specialized mucosa histology

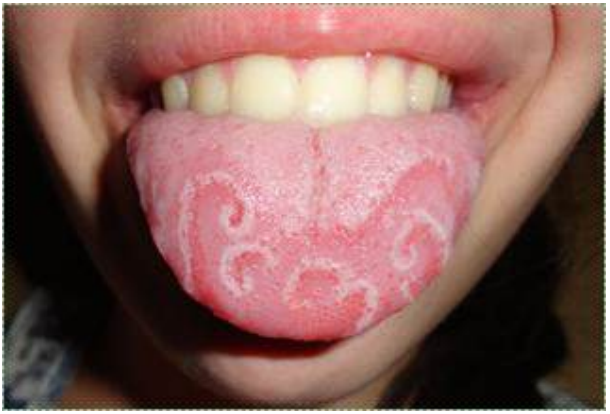


Figure 3.43: Geographic tongue. Image credit: "geographic tongue" by Jbarta, is licensed CC BY SA 3.0

Geographic tongue

Geographic tongue is a condition where **filiform papillae** on the **dorsal surface of the tongue** become non-uniformly **hyper-keratinized**, giving some filiform papillae a more white-ish appearance. Other papillae may be lost due to prolonged inflammation, leaving more reddish spots on the tongue. The pattern of keratinized versus partially-keratinized papillae changes over weeks. The result is almost always a cosmetic concern, although some patients may describe periods of heightened sensitivity to hot, acidic and/or spicy

foods. There are currently no treatments for geographic tongue. The underlying causes of geographic tongue are unknown.

Figure 3.44: Black hairy tongue. Image credit: "black hairy tongue" by Com4, is in the Public Domain CC 0

Black hairy tongue

Black hairy tongue occurs when filiform papillae **exfoliate** epithelial cells more slowly, thus papillae become enlarged

from the buildup of cells. This allows the papillae to pick up more stains from tobacco smoke, foods, or oral bacteria, creating thicker, darker bumps on the tongue. It is thought that this condition might be triggered by overgrowth of certain oral fungi, possibly following the loss of competition with the use of certain antibiotics. The reason the filiform papillae appear hair-like is that both filiform papillae and hairs are composed predominantly of dead **keratinized** epithelial cells. Patients are encouraged to brush their tongue when brushing their teeth.

< Chapter 2 * navigation * Chapter 4 >

Chapter review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:
https://openoregon.pressbooks.pub/histologyandembryology/?p=36

4.

HISTOLOGY OF TOOTH AND PERIODONTAL TISSUES

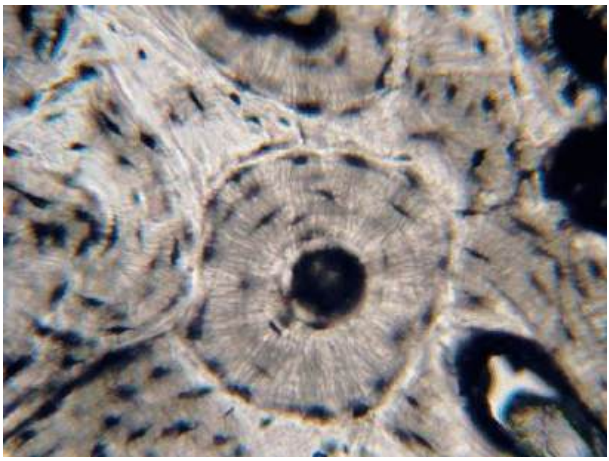


Figure 4.1: Histology and illustration of compact bone tissue, highlighting cells and layers of ECM.

Overview

This chapter briefly covers the histology of the hard tissues enamel, dentin, cementum and bone, as well as the soft tissues of pulp and the **PDL**. The definitions and clinical considerations of these tissues are covered in chapters on the development of these tissues (Chapters 8 through 11)

For more practice using histology images, we share these useful links

- The Oral histology lab
  - by Brian R. MacPherson, Ph.D. and James G. Tieman, Ed.D., at the University of Kentucky (*we linked to this in the previous chapter as well*)
- The Microanatomy Web Atlas

- by Gwen V. Childs, Ph.D., FAAA at the University of Arkansas for medical sciences. Jump to the digestive system/tooth unit.

Histology of bone tissue (short review)

Bone tissue← is deposited in layers by **osteoblasts**. These cells become trapped inside **lacunae** between layers of bone tissue, and **differentiate**← into **osteocytes**. Bone tissue can either be compact and made of osteons, or spongy bone made of **trabeculae**. Bone tissue is almost entirely **ECM**, summarized in Table 4.1. Bone tissue is highly **vascular**—compact bone contains central canals and perforating canals, whereas in spongy bone the space between bony trabeculae is filled with red bone marrow or highly vascular **adipose connective tissue**.

Components of bone tissue	
<1%	Osteocytes
66%	Mineral ECM: Calcium Hydroxyapatite
33%	Protein ECM: Collagen

Table 4.1: Components of bone tissue.

Histology of teeth

animation of enamel rods

Figure 4.2: The ECM of enamel is laid down in rods next to other rods, each rod is secreted by one cell. In contrast, bone tissue is deposited in a circular layer upon circular layer, each cell working only in one layer.

Histology of enamel

Enamel shares some mineral characteristics with bone tissue, but it is **acellular** and **avascular**. Enamel matrix is deposited in columns called **enamel rods** by cells called **ameloblasts**. Enamel is the strongest substance in the human body, due to its high mineral content, listed in Table 4.2. Like bone tissue, the **ECM** is mostly **calcium hydroxyapatite**, but instead of **collagen**[←] fibers, enamel contains proteins including **amelogenins** and **enamelins**.

Components of enamel	
0.0%	Cells (*there are no cells*)
96%	Mineral ECM: Calcium Hydroxyapatite
4%	Protein ECM: Amelogenins and Enamelin (*not* collagen) Water

Table 4.2: Components of enamel.

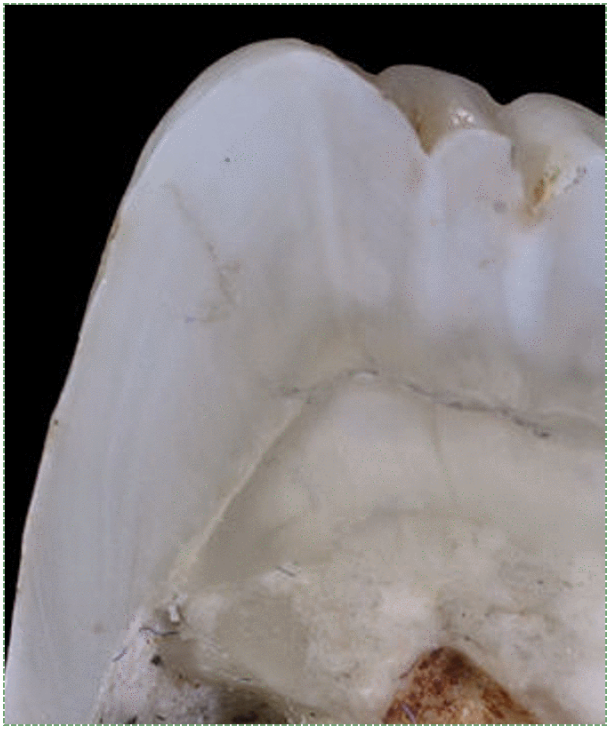


Figure 4.3: Cross-section of tooth enamel, highlighting the Striae of Retzius and Hunter-Schreger bands. Image credit: "Tooth of Parantropus robustus SKX 21841 from Swartkrans" by Didier Descouens, is licensed CC BY 3.0 / boxes and

animation added

During embryonic development, **ameloblasts** work on a circadian rhythm, depositing **ECM** at a regular pace for 4 days, then changing speed. The changes in speed lead to changes in enamel density, which can be seen as horizontal **Lines of Retzius** (or Striae of Retzius). Another pattern is visible because ameloblasts do not create an **enamel rod** in a perfectly straight line, but change direction slightly over days. This leads to the pattern known as **Hunter-Schreger bands**. The border between enamel and underlying dentin is a distinct line named the dentin-enamel junction (**DEJ**). Ameloblasts are lost during **tooth eruption**←, and are therefore not found in the adult tissue. As a result, enamel does not undergo **remodeling** the way the **remodeling unit** maintains bone tissue throughout life.

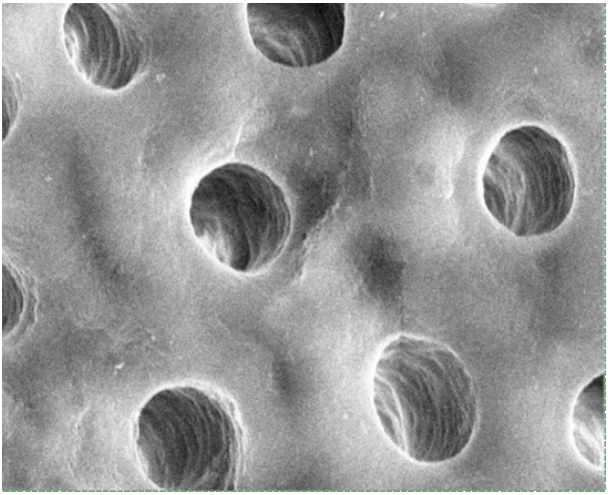


Figure 4.4: Surface view of dentin. Image credit: "Dentinal tubule occlusion of dentine discs after treatment" by Peiyan Yuan, is licensed CC BY SA 3.0 / *labels, arrows and animation added*

Histology of dentin

Dentin, like enamel, is **avascular**, but it should not be called **acellular**. Dentin matrix is deposited by cells called **odontoblasts**. These cells—or more accurately, their cell bodies– are found within the pulp cavity, immediately adjacent to the dentin. Each odontoblast has an arm-like protrusion called an **odontoblastic process** that extends nearly the entire thickness of the **ECM** that cell secreted. The odontoblastic process secretes more dentin (in a soft form that hardens later), as well as a fluid called **dentinal fluid** into the space known as a **dentinal tubule**. Dentin is not as strong as enamel, because of its lower mineral content, listed in Table 4.3. Dentin makes up the greatest bulk of teeth.

Components of dentin	
–	Cells (odontoblasts cell bodies in pulp, odontoblastic processes in dentin)
70%	Mineral ECM: Calcium Hydroxyapatite
30%	Protein ECM: Collagen, mostly Water

Table 4.3: Components of dentin.

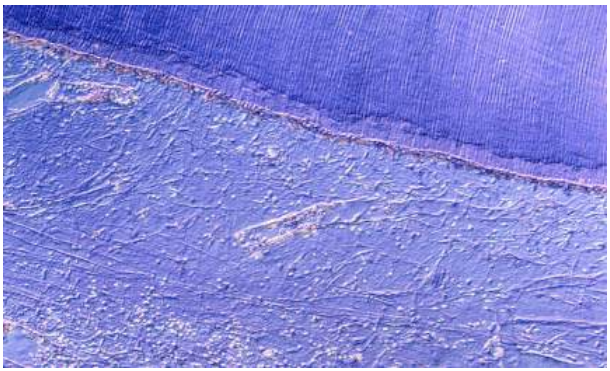


Figure 4.5: Histology of dentin, highlighting the cell bodies of odontoblasts and the imbrication lines of Von Ebner, Image credit: "histological section of tooth" by Doc. RNDr. Josef Reischig, CSc. is licensed under CC BY-SA 3.0 / *labels and animatio*

ns added Similar to the **Lines of Retzius**, dentin is deposited at different rates over days, which creates bands called the **Imbrication lines of von Ebner**. These lines run perpendicular to the **dentinal tubules**. Exceptionally-pronounced imbrication lines are named the **Contour lines of Owen**, and occur with changes in nutrition (such as during childbirth).

Figure 4.6: Illustration of Tomes' granular layer in root dentin (small dark spots)

Root dentin has a layer of speckles near the border with the cementum named **Tomes' granular layer**. It has no known function. It is a good landmark when looking at dentin under the microscope, its presence indicates you are viewing root dentin, not **mantle dentin**, and a thin layer of cementum should be close by.

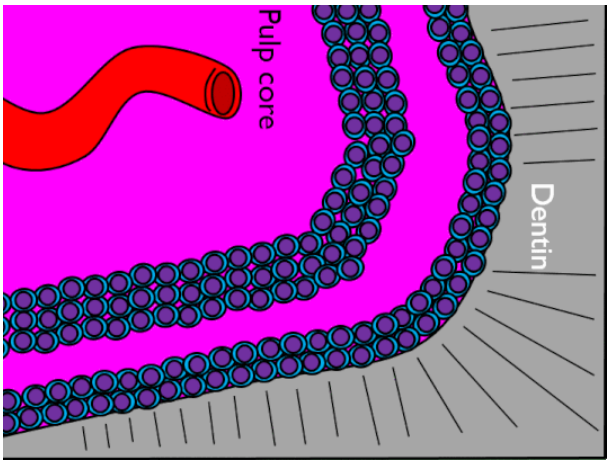


Figure 4.7: Illustration of the histology of the pulp chamber.

Histology of pulp

The pulp chamber is composed of an indeterminate type of connective tissue, although it most resembles **areolar connective tissue**. The most common cell types found within the pulp core are **fibroblasts** and **mesenchymal stem cells**. The pulp houses the blood, nerve and lymphatic supplies for each tooth. The pulp has 4 layers that appear distinct when using an **H&E** stain, listed in Table 4.4.

Layer	Predominant feature
Odontoblast layer	Odontoblast cell bodies
Cell-free zone	Not actually free of cells, cells aren't visible
Cell-rich zone	Cells are visible and densely packed
Pulp core	Location of capillaries, nerve endings and lymphatic vessels

Table 4.4: The four layers of the pulp chamber and their predominant features.

Histology of periodontal tissues

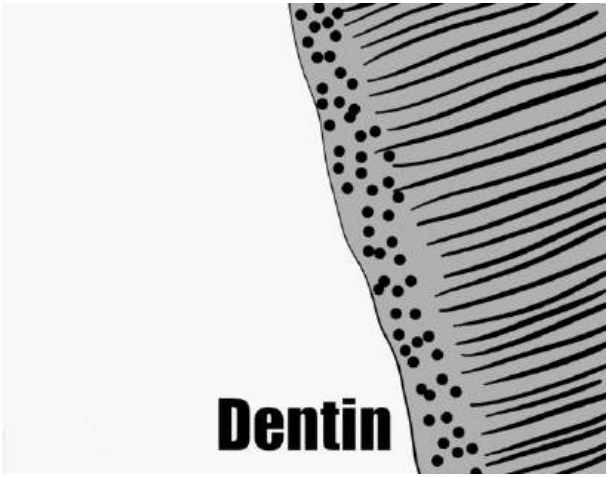


Figure 4.8: Illustration of cementum, and surrounding tissues.

Histology of cementum

Cementum forms a thin layer (between 50 to 200 μM, see below) over the surface of root dentin, which is roughly the width of a hair. The **ECM** of cementum differs significantly enough from enamel that the two can be differentiated by their color (cementum is yellowish in hue, enamel has no color). Both cementum and dentin are yellowish, making those two difficult to differentiate by color. The ECM of cementum is deposited by **cementoblasts**, which can be found on the surface of cementum throughout life. As a result, the thickness

of cementum can triple in thickness between the ages of 10 and 70 years of age. This is not the same as bone **remodeling**, as it does not involve the activity of **resorptive cells** in addition to cementoblasts. Cementum incorporates more fluoride than the other hard tissues.

Cementum, like dentin and enamel, is an **avascular** tissue, but it can contain cells. There are two types of cementum based on histological (visual) studies from the 1830s: **cellular cementum** and **acellular cementum**. More recent advances in molecular biology and genetics are yielding newer concepts, but the old classification system is still commonly used in clinical practice.

In **cellular cementum**, **cementoblasts** become trapped within the **ECM** and **differentiate**[←] into **cementocytes**. In the mature tissue, cementocytes are found within **lacunae**, similar to **bone tissue**[←]. However, **canaliculi** only extend in the direction of the (vascular) **PDL**. In the opposite direction is **avascular** dentin, which cannot provide cementocytes with nutrients. Cellular cementum is found in the apical half of the root.

Acellular cementum does not contain **cementocytes**, and can be found in the coronal half of the root as well as underneath cellular cementum in the apical portion (cementum is around 50 μM thick in the coronal region and 200 μM thick in the apical region).

The border between cementum and dentin, the Cementum-Dentin junction (**CDJ**), is less distinct than the

DEJ. Cementum is slightly less strong than dentin, and is composed of the materials listed in Table 4.5. Some publications list the mineral component of cementum to be 60 to 65% (further reading here). The difference between these numbers reflects whether a layer of mostly unmineralized **glycoproteins** at the CDJ is included or excluded. The glycoproteins are specific to cementum, therefore we have chosen to report measurements that include them.

Regardless of how we measure the mineral content of cementum, loss of bone, dentin and cementum in response to **periodontitis** and **orthodontia** is complex and requires discussion of **morphogens** and the **differentiation** of **resorptive cells**. Similarly, discussion of whether cementum should be categorized as a part of the tooth (based on its location and appearance) or a part of the **periodontium** is best left until we discuss the **lineage** of tooth and periodontal tissues.

Components of cementum	
–	Cellular cementum (apical portion) Acellular cementum (coronal portion)
50%	Mineral: Calcium Hydroxyapatite
50%	Protein: Collagen and glycoprotein mixture Water

Table 4.5: Components of cementum

Fibers in cementum

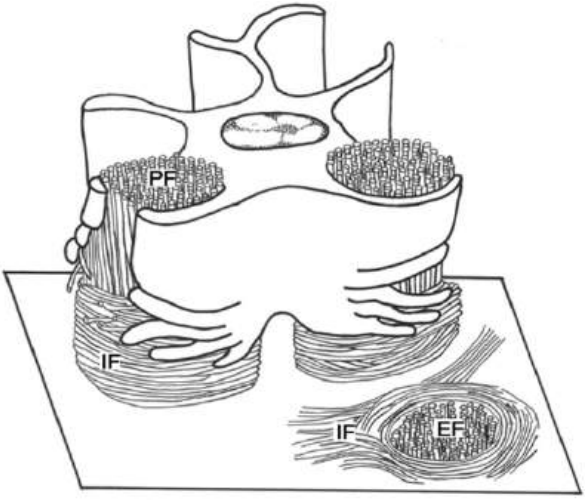


Fig. 4.9: Schematic of a cementoblast wrapping Principal Fibers (PF) with wing-like processes, then encircling those fibers with Intrinsic Fibers (IF) secreted by finger-like processes. Image credit: "Schematic diagram depicting how cement

oblasts produce intrinsic fibers around principal fibers on extrinsic fiber-rich CIFC." by Tsuneyuki Yamamoto et al, Jpn Dent Sci Rev is licensed under CC BY-NC-N

D 4.0 **Acellular cementum** contains **extrinsic collagen fibers** which travel perpendicular to the surface of the root, and are likely secreted by both **fibroblasts** and **cementoblasts**. These fibers connect all the way to alveolar bone, and represent the ends of the **principal fibers** of the **PDL** embedded within cementum (extrinsic fibers may be called **Sharpey's fibers**, the same as bone tissue).

Cellular cementum contains intrinsic fibers and possibly extrinsic fibers. **Intrinsic collagen fibers** are secreted by cementoblasts, and do not extend beyond cementum. They are oriented parallel to the root surface and are mainly involved in the repair of cementum (a **scaffold**←). More recent publications subdivide cellular cementum into Cellular Intrinsic Fiber Cementum and Cellular Mixed Stratified Cementum, based on the amount of extrinsic collagen fibers present. Cementum that does not contain extrinsic collagen fibers (closer to the cervical region) does not contribute to the attachment to bone tissue.

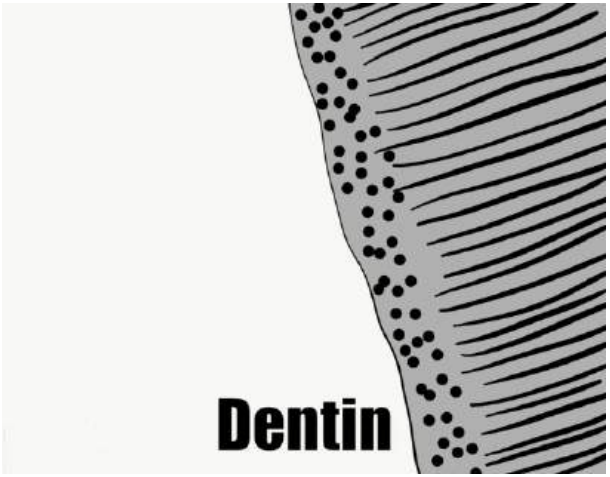


Figure 4.10: Illustration of the PDL, and surrounding tissues.

Histology of the Periodontal Ligament

The Periodontal Ligament (**PDL**) is a region of **dense regular connective tissue**[←] that connects the cementum to **alveolar bone**. **Sharpey's fibers** extend from one side of the PDL into cementum, and on the other side of the PDL into alveolar bone. Like the **CDJ**, the border between cementum and PDL is blurred due to their shared lineage. Clusters of rogue epithelial cells can be found within the PDL which are named the **epithelial rests of Malassez**. The PDL has a much higher degree of **vascularity** than other ligaments. It also has a more diverse and numerous population of cells (compare Fig. 4.14 to the link to dense regular connective tissue above).

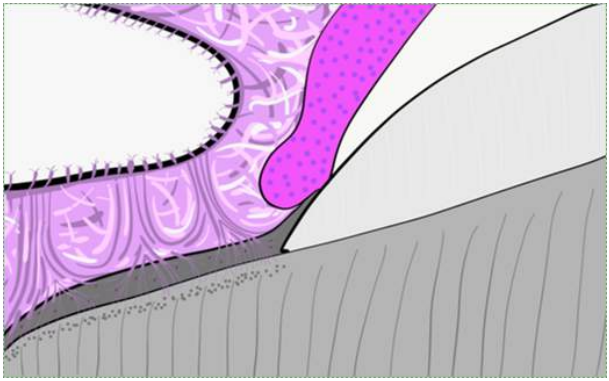


Figure 4.11: Illustration of alveolar bone, and nearby tissues. Look for Tomes' granular layer as a landmark, if needed.

Histology of alveolar bone

Alveolar bone contains compact bone tissue, with a semi-unique feature of many tiny holes through which **Sharpey's fibers** from the **PDL** insert, as well as larger holes for the nerves, blood and lymphatic vessels that exit bone tissue and enter the apical foramen of a tooth. This makes the surface of the alveolar sockets appear porous, which is *almost* unique for the surface of bones. The porous surface is called the **cribriform plate**, which is not to be confused with the other region of compact bone riddled with tiny holes, the cribriform plate of the ethmoid bone (whose holes are for olfactory nerve endings, connecting the nasal cavity to the brain).

Resorptive cells in hard tissues

The **periosteum** is a layer of **dense regular connective tissue**[←] that surrounds bone tissue. In addition to **collagen**[←] fibers, it contains **osteoblasts** and **osteoclasts** involved in **bone remodeling**[←]. Similar cells exist for cementum and dentin, but not enamel. **Cementoclasts** and **odontoclasts** are capable of demineralizing cementum and dentin, respectively. Like osteoclasts, these cells express genes involved in acid secretion as well as digestive enzymes, including members of the **matrix metalloproteinase** family. Unlike osteoclasts, they are not active continuously in a remodeling unit, they have no baseline activity and are typically only activated during tooth **exfoliation**.

Odontoclasts and **cementoclasts** likely **differentiate**[←] from the same bone marrow **stem cells**[←] that become **osteoclasts**. The signals that determine whether the multipotent stem cell differentiates into an osteoclast, odontoclast or cementoclast are currently not well understood. The body may regulate this process by long or medium-range **hormone** and **growth factor** signals. Regulation may also be very short-range: binding of the stem cell to proteins or **glycoproteins** within the **ECM** of each target tissue, providing the stem cell with **positional information**. While roughly identical to osteoclasts under the microscope, odontoclasts and cementoclasts have important physiological differences. For instance, the hormone calcitonin inhibits

osteoclasts but triggers odontoclast activity during root resorption. The significance of this detail is that root resorption does not always match bone resorption. For instance, in osteoporosis, it is bone resorption, not tooth root resorption, that leads to the loss of teeth. During tooth **exfoliation**, however, root dentin is resorbed but alveolar bone undergoes both resorption and deposition, to form a pathway through bone above the permanent tooth and fill in alveolar socket space below.

Some publications suggest **cementoclasts** are distinct cells found in the **PDL** and that they play a role in the maintenance of cementum throughout life. Other publications suggest cementoclasts are the same cell type as **odontoclasts** and **osteoclasts**. Without further study, it is hard to tell whether a cementoclast is a distinct cell type, or an osteoclast found near cementum. The difference may be semantic, like asking whether your teacher is still a *teacher* when you see them in the grocery store, or are they just another *shopper*? The mechanism of resorption is the same (acids and proteinases), but whether these cell are regulated the same is an important question during tooth **exfoliation** and **orthodontic movement**[←].

Summary of hard tissues

>

Hard tissue	Components	
Enamel	96%	Mineral: Calcium Hydroxyapatite
	1%	Protein: Amelogenins and Enamelins *(not collagen)*
	3%	water
Dentin	70%	Mineral: Calcium Hydroxyapatite
	20%	Protein: Collagen, mostly
	10%	water

Table 4.6: Summary of hard tissues. * = denotes significant variation in percentages found in the literature depending on whether cementum is measured by weight or volume, or whether the CDJ is included or not.

Cementum	50%*	Mineral: Calcium Hydroxyapatite
	50%*	Protein: Collagen and glycoprotein mixture Water
Bone	60%	Mineral: Calcium Hydroxyapatite
	25%	Protein: Collagen
	15%	water

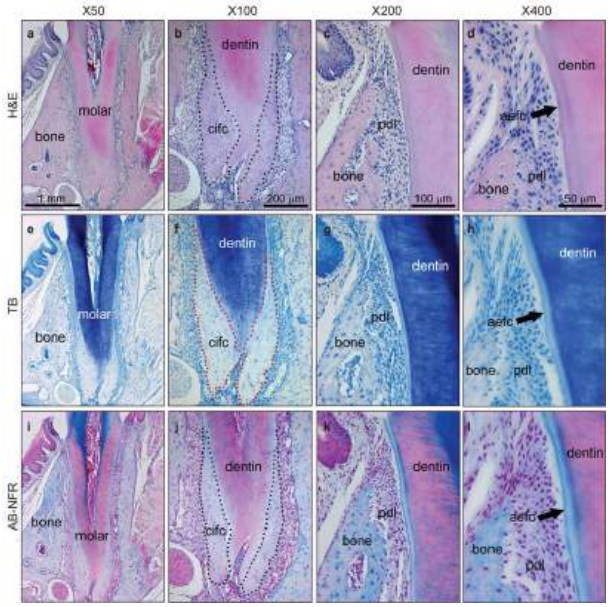


Figure 4.14: Summary of the histology of mouse tooth and periodontal tissues using various staining techniques. Legend: AEFC: acellular extrinsic fiber cementum, CIFC = cellular intrinsic fiber cementum, H&E, TB and AB-NFR: different tissue stains. Image credit: "Serial sectio

ns of the 44 dpn mouse mandibular first molar were stained with (a–d) H&E, (e–h) TB and (i–l) AB-NFR" by Brian L Foster is licensed under CC BY-NC-ND 4.0

< Chapter 3 *navigation * Chapter 5 >

Chapter review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:

https://openoregon.pressbooks.pub/histologyandembryology/?p=41

5.

HISTOLOGY OF GLANDS, LYMPHATICS AND SINUSES

- Basic anatomy
- Salivary glands
  - Components of saliva
  - Dental pellicle
  - Histology of salivary glands
  - Anatomy of salivary glands
  - Clinical applications of gland histology
- Lymphatic system
  - Lymph nodes
  - Tonsils
  - Clinical application of lymphatic histology
- Sinuses
  - Para-nasal sinus anatomy
  - Clinical application of para-nasal sinus histology

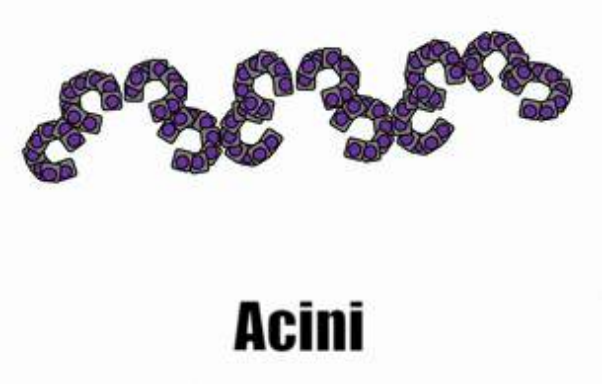


Figure 5.1: Basic anatomy of an exocrine gland. Order of appearance: acini, ducts, capsule, septa.

Basic anatomy of exocrine glands and lymphatic organs

The average **exocrine** gland contains **simple cuboidal epithelia**[←] that form grape-shaped or tube-shaped structures called **acini**. Liquids secreted by the cells of acini enter a duct, which can be made of a simple cuboidal epithelium or **simple columnar epithelium**[←]. It is possible for larger glands to have regions of stratified cuboidal epithelial cells as well. The outside of the gland is surrounded by a dense connective tissue **capsule** (not quite thick enough for us to worry about whether the **collagen**[←] fibers are regular or irregular). The connective tissue extends inwards forming trabeculae (or septa, but not to be confused with **trabeculae** of spongy bone tissue), which divide the organ into lobes.

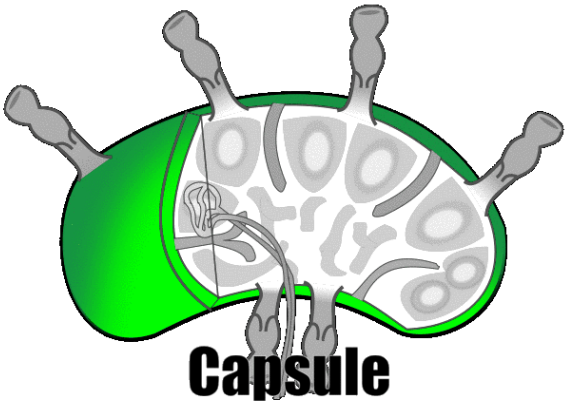


Figure 5.2: Anatomy of a lymph node.

The average lymphatic organ shares patterns with exocrine glands. A lymphatic organ is surrounded by a dense connective tissue **capsule**, and that connective tissue extends inwards forming trabeculae. Inside a lymphatic organ, clusters of white blood cells suspended within **reticular connective tissue** form **germinal centers** (or nodules) where white blood cells filter cellular debris and scan body fluids for pathogens. If they detect a pathogen, they multiply, release inflammatory signals, and migrate through the body's connective tissues looking for more pathogens.

Salivary glands

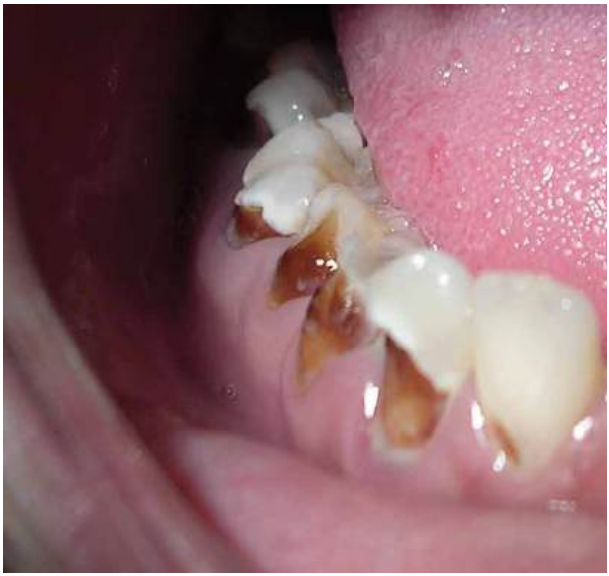


Figure 5.3: Accelerated tooth decay in meth mouth. Image credit: "Suspected meth mouth" by Dozenist, is licensed CC BY SA 3.0

Components of saliva

The major function of salivary glands is to produce **saliva**, which helps maintain the health of the **oral mucosa** and teeth, as well as assist in mastication. **Acinar** epithelial cells **transcribe** and **translate** long **mucous** proteins on the **rER**, modify them in the **Golgi apparatus** to form large **glycoproteins**, and secrete them. These cells also pump

electrolytes from their **cytoplasm** into the **lumen** of the acinus, which, along with the glycoproteins, attract water by osmosis. These components make saliva moist but sticky enough to adhere to all surfaces of the oral cavity, rather than sink to the floor of the mouth.

Other molecules are secreted and become a part of saliva. Buffers within saliva help to maintain a stable pH, despite the acidity or alkalinity of different foods, or the acidity of certain oral bacterial secretions. Buffers and electrolytes also help disrupt the formation of bacterial biofilms, which bacteria use to adhere to teeth. The watery nature of saliva helps to moisten food and the **oral mucosa**, assisting in mastication, speech and swallowing. Watery saliva also helps to limit the population of oral bacteria. People swallow saliva even when they are not eating. This flushes a percentage of oral microorganisms down to the stomach, where certain death awaits in the form of hydrochloric acid and the protein-destroying enzyme pepsin. The enzymes salivary amylase and lysozyme are produced by **acinar** cells to serve a similar purpose, breaking covalent bonds within the cell walls of viruses and bacteria, or their surface **glycoproteins** used to adhere to tooth surfaces. Without hours of mechanical digestion by the teeth and stomach, there is very little surface area for salivary amylase to digest carbohydrate molecules for nutritional purposes. Chemical digestion of carbohydrates for nutrition is carried out in the small intestines by the much more abundant pancreatic amylase. Bacteria,

however, do not form a bolus (a large solid mass), so their cell walls and surface glycoproteins are open to attack by enzymes without prior mechanical processing. A second function of salivary amylase is to cleave a small amount of small sugars from starch, allowing taste buds to detect them, so salivary amylase and other carbohydrases in saliva function in the sense of gustation as well as in limiting bacterial populations.

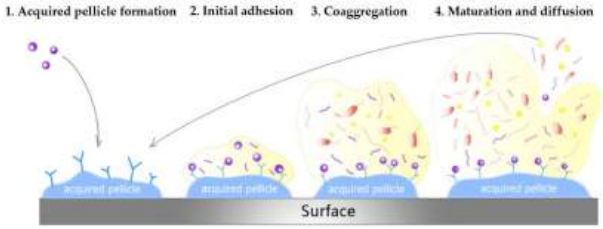


Figure 5.4: Stages of pellicle formation. Image credit: "The process of biofilm formation in the oral cavity is divided into four stages: 1. acquired pellicle formation; 2. initial adhesion; 3. coaggregation; 4. maturation and diffusion. " by Hao Y et al is licensed under CC BY-SA 4.0

Dental pellicle

The **dental pellicle** is a thin coating of **glycoproteins** from saliva on the surface of teeth. It prevents excess deposits of $Ca^{2+}PO_4^{3-}$ (calculus deposit minerals) and also prevents demineralization of enamel. Both depositing bumps or eroding crevices allows bacteria to adhere to teeth and avoid being washed into the stomach. Therefore, the dental pellicle can prevent adhesion of bacteria to the tooth surface. However, oral bacteria can adhere to the dental pellicle. Furthermore, saliva contains minerals that produce calculus, making the relationship between saliva, dental pellicle and the oral microbiome complicated. This illustrates the delicate balance that must be maintained in the oral cavity: too many bacteria can lead to **gingivitis**← and **dental caries**, while too few bacteria leave the oral cavity open to colonization by harmful bacteria, leading to gingivitis and dental caries.

Histology of salivary glands

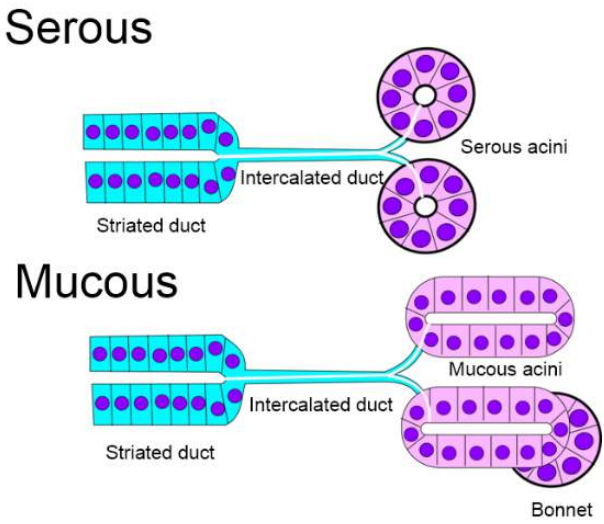


Figure 5.5: Illustration of the basic histology of serous versus mucous salivary glands.

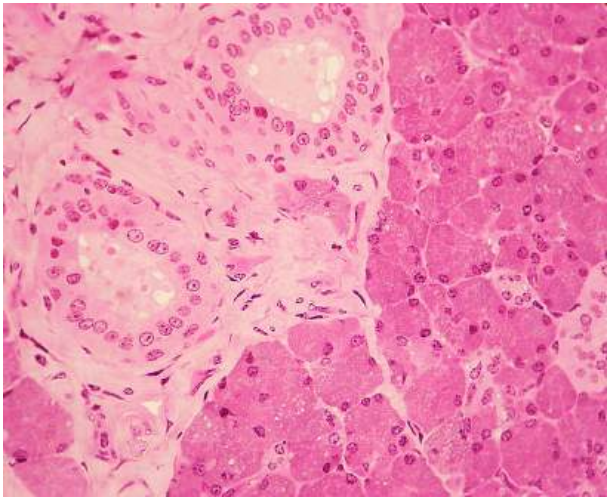


Figure 5.6: Histology of salivary gland ducts and serous acini. Image credit: "Stratified cuboidal/columnar epithelium is visible in a duct surrounded by connective tissue in the

parotid gland" by Wbensmith is licensed under CC BY 3.0

Serous acini produce more watery secretions. These are composed of **simple cuboidal epithelium**[←]. Serous acini tend to stain darker pink or purple using a traditional **H&E** stain.

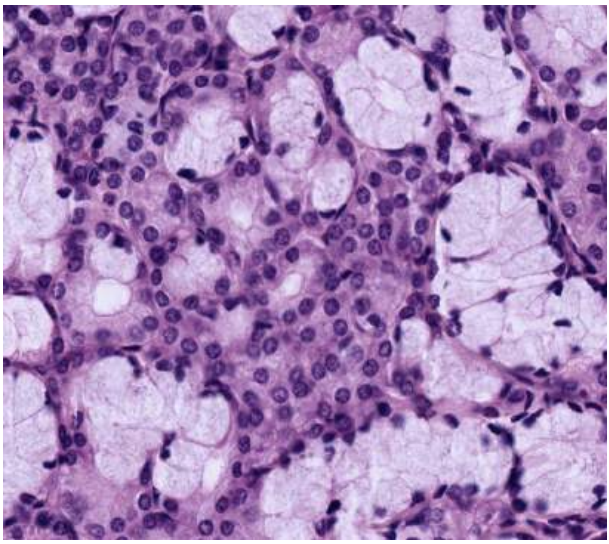


Figure 5.7. Histology of mucous acini (light areas). Image credit: "major salivary glands" by Dr. Patrice Spitalnik, Columbia University Center for Teaching and Learning is licensed under CC BY-SA 4.0

Mucous acini secrete more **glycoproteins**, making their secretion thicker and stickier (more **mucous**). Mucous acini are made of a **simple columnar epithelium**←. Mucous acini appear to have bubbles.

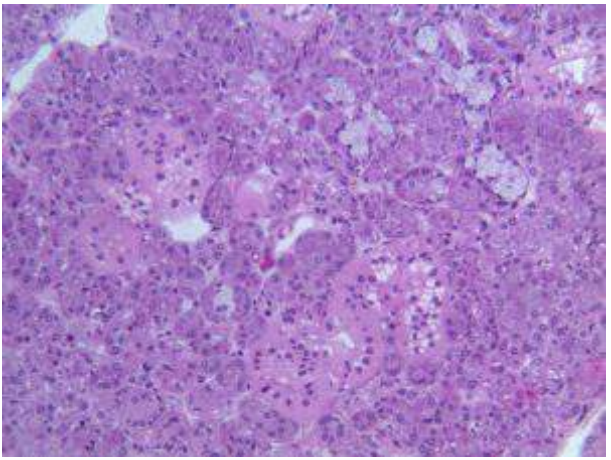


Figure 5.8: Histology of the sub-mandibular gland, with numerous purple-staining serous acini, plus mucous acini (whiter areas) and muco-serous acinini (pink columnar areas).

Muco-serous acini produce secretions halfway in-between the first two in consistency. These look like mucous acini under the microscope, with an additional bonnet (a hat) of **myo-epithelial cells**. These cells contract like smooth muscle cells. For most epithelial cells, the actin and myosin genes are found in the **cytoskeleton**← and allow for migration, which is especially important for **stem cells**← that move to help repair a wound. Myo-epithelial cells, on the other hand, use some of the **DNA** instructions for making larger amounts of actin and myosin and organizing them into specialized filaments similar to smooth muscle tissue. This makes myo-epithelial cells appear more like smooth muscle than an epithelium. However, these cells have an epithelial **lineage**—they **differentiate**← from **ectoderm**, not from a **mesenchymal stem cell** or **myoblast**.

All three secretions mix in the oral cavity to produce saliva. These secretions reach the oral cavity by way of different ducts, which can be identified under the microscope by their different types of epithelial cells. We know of no clinical significance to the types of ducts or their cells.

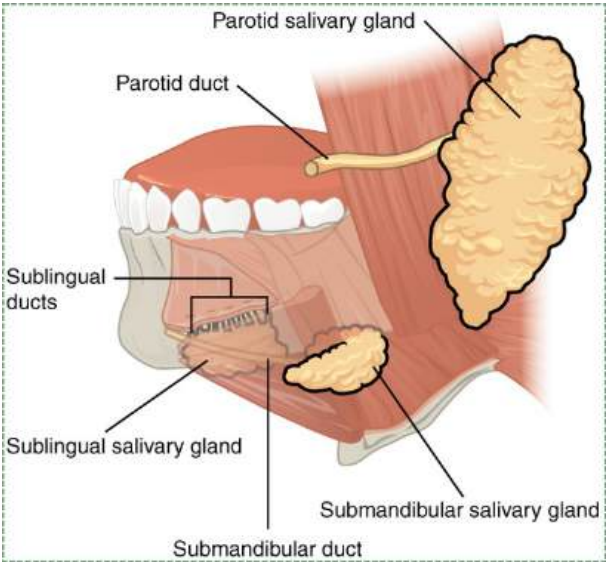


Figure 5.9: Salivary gland anatomy. Image credit: "Salivary glands" by OpenStax college, is licensed CC BY 3.0

Anatomy of exocrine glands

There are three major and numerous minor salivary glands. The three major salivary glands are listed in Table 5.1.

Major salivary gland	Details
Parotid salivary glands	Largest
	Serous (mostly)
	25% saliva by volume
Sub-mandibular salivary glands	2nd largest
	Muco-serous
	65% saliva by volume
Sub-lingual salivary gland	Mucous
	10% saliva by volume

Table 5.1: Summary of the three major salivary glands.

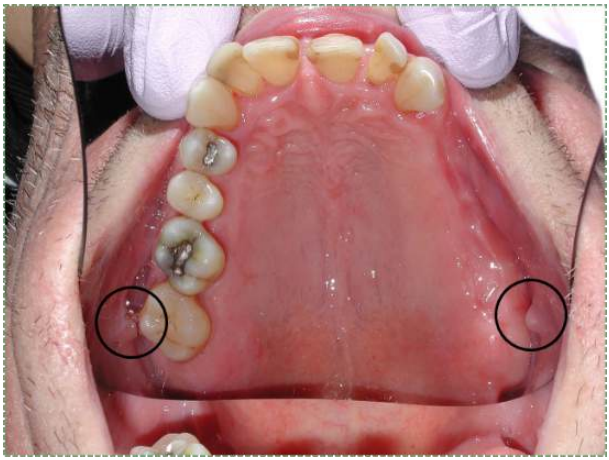


Figure 5.10: Parotid duct papillae. Image credit: "Parotid duct papillae" by D Rosenbach, is licensed CC BY SA 3.0

The **parotid glands** drain into the oral cavity by way of the parotid duct (or Stensen duct). The ducts travel through the masseter muscle and enters the oral cavity at a papilla on the buccal mucosa usually located lateral to the 2nd maxillary molar.

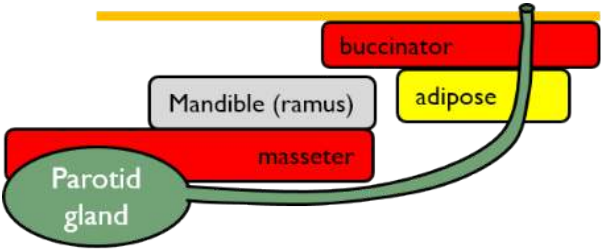


Figure 5.9: Illustrated representation of the path of the parotid duct.

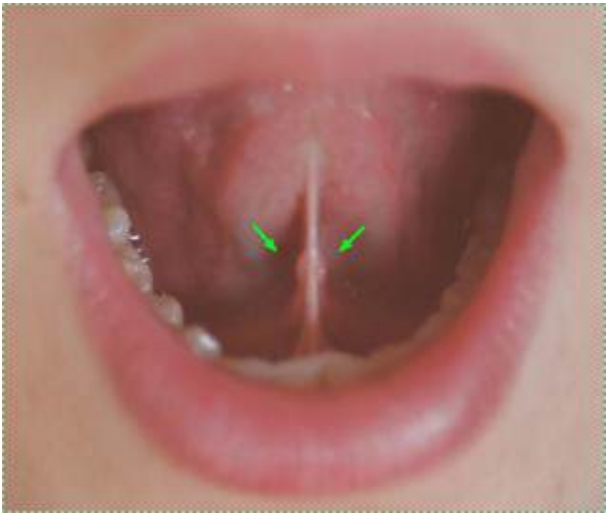


Figure 5.11: The sub-lingual caruncle. Image credit: "Caruncula sublingularis" by Hellerhoff, is licensed CC BY SA 3.0

The **sub-lingual glands** and **sub-mandibular glands** have

ducts that share an entrance on the floor of the mouth at the sub-lingual caruncle. The sub-lingual gland also has numerous smaller ducts that open onto the floor of the mouth.

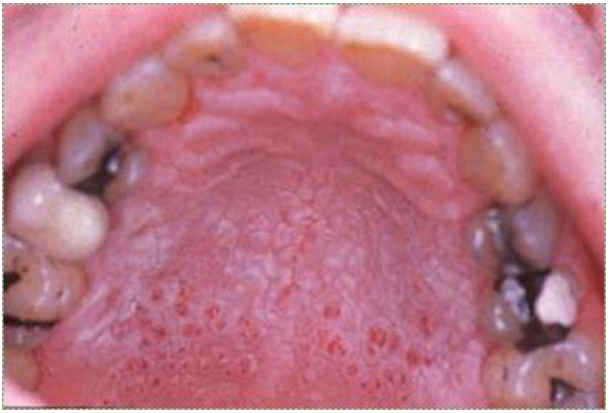


Figure 5.12: Minor salivary glands visible because of Nicotinic Stomatitis. Image credit: "Nicotinic stomatitis" by DVIDS is in the Public Domain CC0

Numerous **minor salivary glands** are located throughout the oral cavity. They are normally not visible, unless the oral mucosa is hyper-keratinized. Luckily, no one has bothered to

name these glands or their ducts, except for the **von Ebner salivary glands,** which are minor salivary glands associated with the circumvallate lingual papillae. The minor salivary glands are predominantly **mucous**, with exceptions, which helps their secretions to stick to the roof and sides of the oral cavity.

Clinical application of salivary gland histology

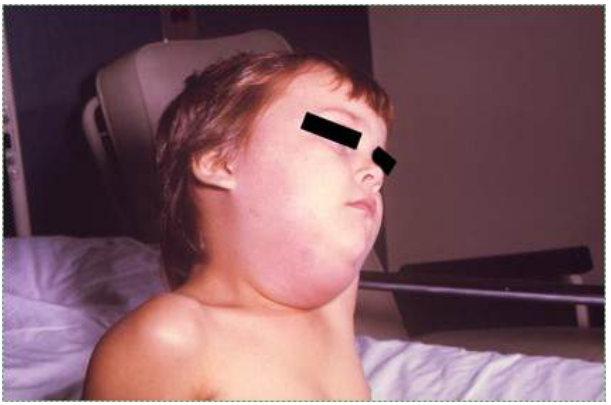


Figure 5.13: A patient with mumps. Image credit: "Mumps" by Photo CDC/NIP/ Barbara Rice, is in the Public Domain CC0

Mumps is a viral infection that causes swelling of the **parotid glands** and other organs. Vaccination has reduced mumps

cases in the US by over 99%. Vaccination against mumps works by triggering the primary immune response against mumps antigens. Unfortunately, mumps outbreaks still occur, often in high-density living areas (such as college dormitories) with a large percentage of unvaccinated individuals. It is difficult to counter beliefs in pseudoscience which seek to cast doubt on the safety or efficacy of vaccination, although some strategies work better than others.

Figure 5.14: Angular cheilitis, a sign of hyposalivation. Image credit: "Angular cheilitis" by Matthew Ferguson, is licensed CC BY SA 3.0

Hypo-salivation

Hypo-salivation, or decreased saliva production, can be caused by certain diseases, medications, cancer treatments, and aging. It may result in **xerostomia**, or dry mouth. Because saliva contains **growth factors**, reduced saliva decreases the healing ability of the **oral mucosa**, leading to sores. Reduced saliva also reduces limits to growth placed on the oral microbiome, leading to infections and caries. Reduced taste can also be a consequence, which patients might compensate for by flavoring their food with excessive levels of salt (NaCl). Using Monosodium Glutamate (MSG, or $NaC_5H_8NO_4$) may be used to enhance both flavor and saliva production, and is a significantly healthier option.

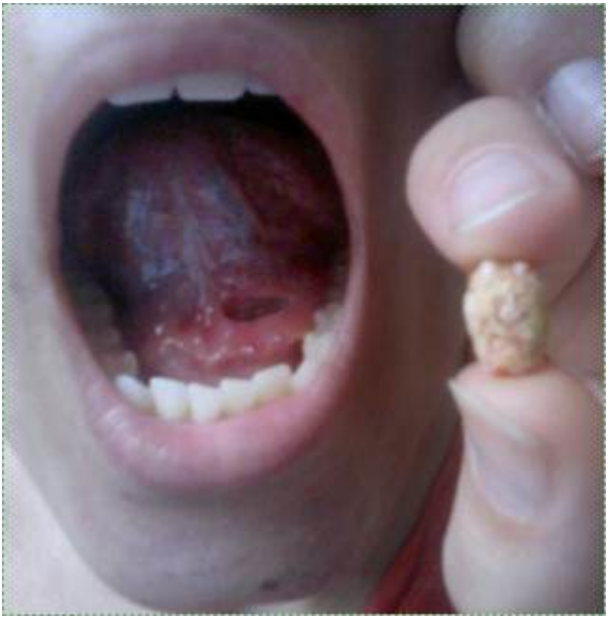


Figure 5.15: An impressive sialolith and its former location. Image credit: "Salivary gland stone and the operation mark" by Peter Nickson, is in the Public Domain CC0

Mineralized saliva (sialoliths)

Electrolytes in saliva can precipitate and form salivary gland stones, or **sialoliths**. Large sialoliths may block the duct of

one of the salivary glands, which stops the flow– but not the production– of saliva. As a result, saliva builds up in the gland, causing swelling and possibly inflammation. Blockage of a minor salivary gland produces a swelling within the **oral mucosa** known as a **mucocele** (or mucocoele) (Fig. 5.14), while blockage of a major salivary gland produces a **ranula** (Fig. 5.15). These are fairly common disorders. Inflammation of the **parotid gland** must be clearly distinguished from **mumps** before any treatment is given.

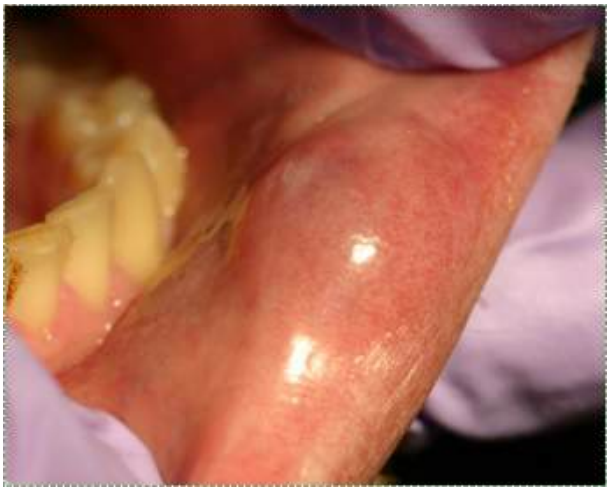


Figure 5.16: Example of a mucocele. Image credit: "Mucocoele" by Dozenist is licensed CC BY SA 3.0

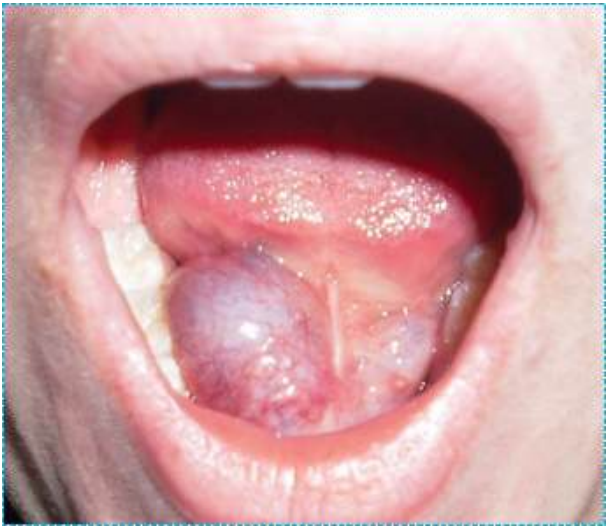


Figure 5.17: Example of a ranula. Image credit: "Ranula" by Ph0t0happy is licensed CC BY SA 3.0

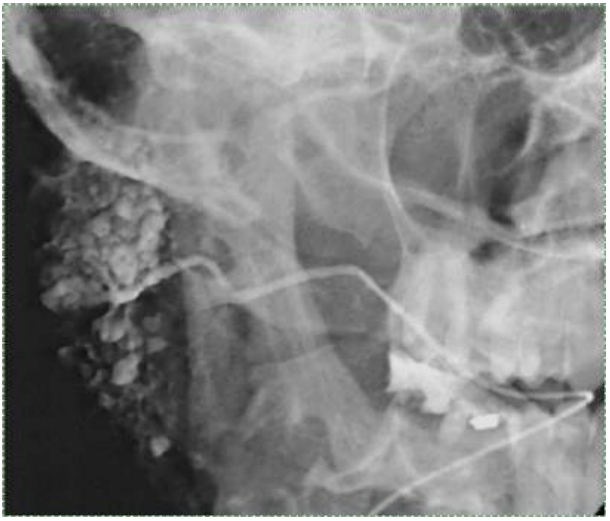


Figure 5.18: Sialograph. Image credit "Own work" by Hellerhoff, is in licensed CC BY SA 3.0

Sialography

Sialoliths may be palpated by clinicians. Alternately, an image of the blockage known as a **sialograph** can be taken by injecting a radiopaque dye as far into the salivary gland duct as it can go (until further flow is blocked by the sialolith). Usually, sialoliths are removed with minimal discomfort. Sialography should not be done in cases of acute infection, as the dye may push infectious exudate into deeper tissues, spreading the infection.

Lymphatic system

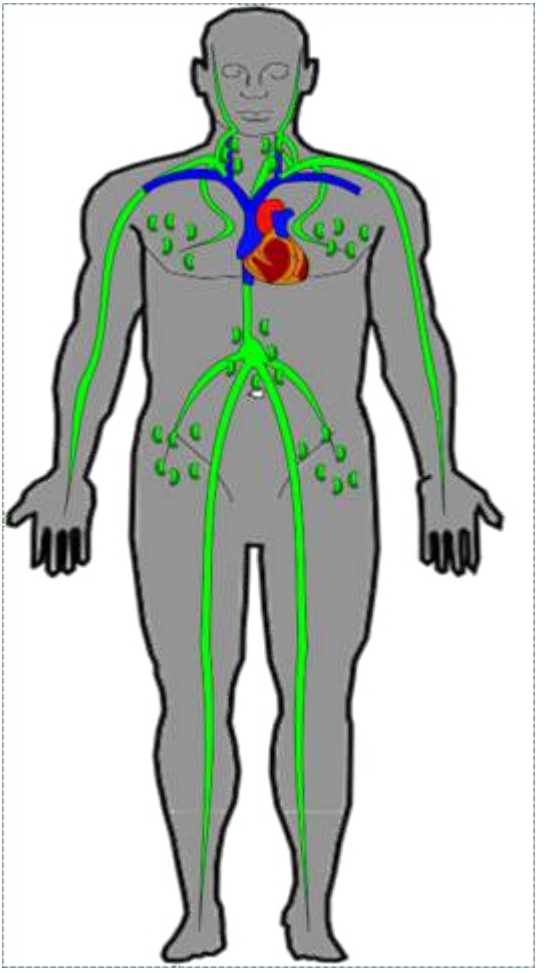


Figure 5.19: Basic anatomy of the lymphatic system.

Very basic overview of the lymphatic system

Lymphatic organ	Major function
Lymphatic vessels	Drain ECF off organs, including teeth
Lymph nodes	Filter debris and identify pathogens
Tonsillar tissue	Identify pathogens
Other lymphatic organs	Located below the neck

Table 5.2: Functions of the major lymphatic organs.

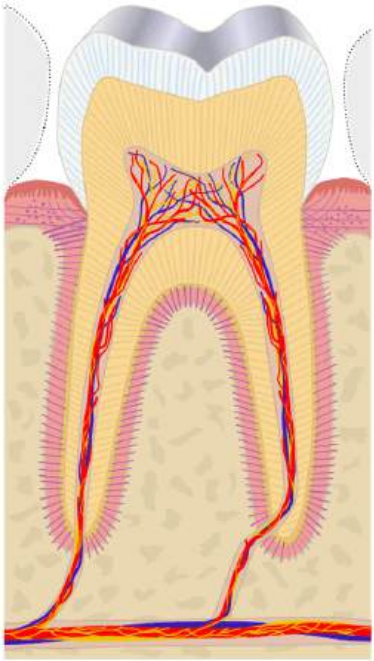


Figure 5.20: ECF production and drainage in a tooth. Legend: Red = blood, Blue = ECF, Green = lymph. Image credit: "Own work" by Goran tek-en, is licensed CC BY SA 3.0 / *Arrows and animation added*

Lymphatic drainage of the teeth

Fluid travels to the teeth via capillaries. As plasma exudes from the capillaries, it becomes **ECF**. Veins collect most of this fluid, the rest is collected by lymphatic vessels. ECF absorbed into a lymphatic vessel is called **lymph**, and it is returned to the circulatory system. Lymphatic vessels are more permeable than capillaries, having **mini-valves** on their outer edges. Mini-valves allow solids to enter the lymphatic vessels, including cellular debris, microorganisms, and cancer cells. The lymphatic vessels are connected to a number of **lymph nodes**, which clean up debris and mount an immune response against microorganisms and cancer cells. Knowing the anatomical connections of the lymphatic vessels helps locate areas of infection by the inflammation triggered in down-stream lymph nodes.

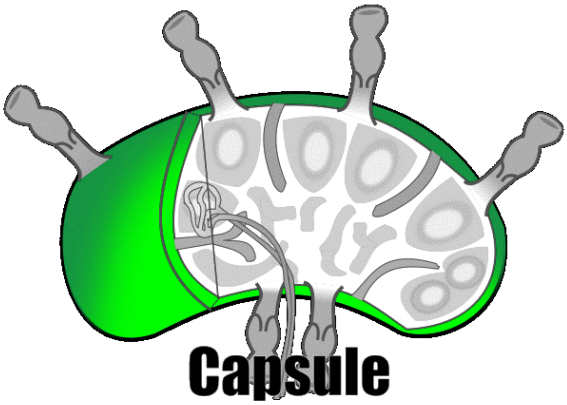


Figure 5.21: Illustration of lymph node histology.

Lymph nodes

Lymph nodes are found throughout the body. A large cluster of lymph nodes can be found in the neck, called the **cervical lymph nodes**, in addition to other locations. Lymph nodes are composed primarily of **reticular connective tissue**, which provides support for resident clusters of white blood cells. Afferent lymphatic vessels bring lymph to the node, while efferent vessels drain lymph towards the circulatory system.

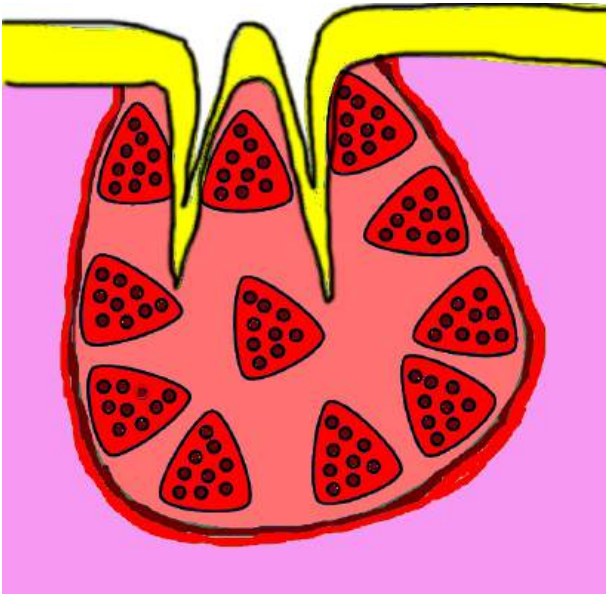


Figure 5.22: Illustration of the basic histology of a tonsil.

Tonsillar tissue

Tonsillar tissue is either a **pseudostratified epithelium** or a **non-keratinized stratified squamous epithelium**←, plus **reticular connective tissue** below. Tonsils are similar to lymph nodes, except there are no lymphatic vessels, and one end of the tonsil is un-encapsulated (the epithelial border). Clusters of white blood cells can be found in germinal centers, suspended within reticular connective tissue.

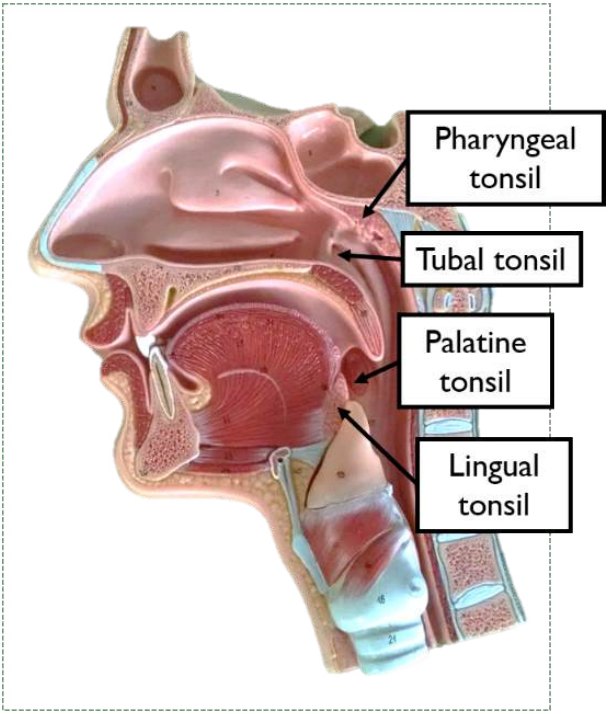


Figure 5.23: Location of tonsils on an anatomical model.

The tonsils

There are 4 major tonsils located the border of the oral cavity and pharynx: lingual, palatine, pharyngeal and tubal. These tonsils form a ring, called **Waldeyer's ring**, which serves to identify pathogens that are either ingested or inhaled, and begin an immune response before they reach deeper locations, such as the lungs or stomach. Because of their anatomy (no vessels), tonsils do not filter debris or microorganisms out of bodily fluids, they only identify pathogens. The un-

encapsulated side faces the **lumen** of the pharynx, coming into contact with many foreign molecules.

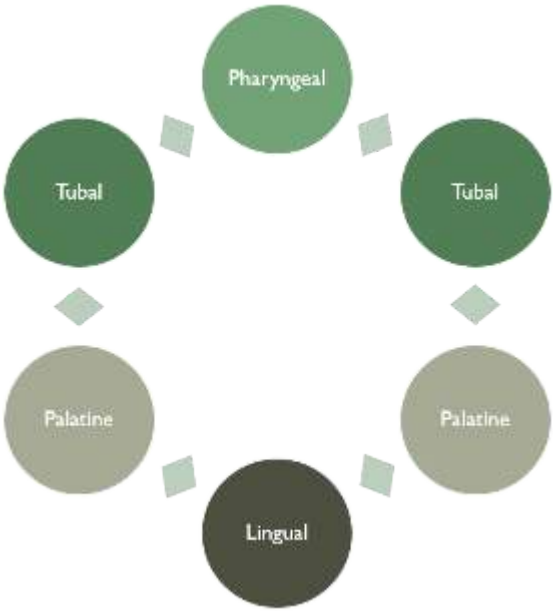


Figure 5.24: Schematic of Waldeyer's ring, as if looking at tonsils from the anterior view of the fauces. Tonsils in order, from anterior: pharyngeal, tubal, palatine, lingual.

Clinical applications of lymphatic system histology

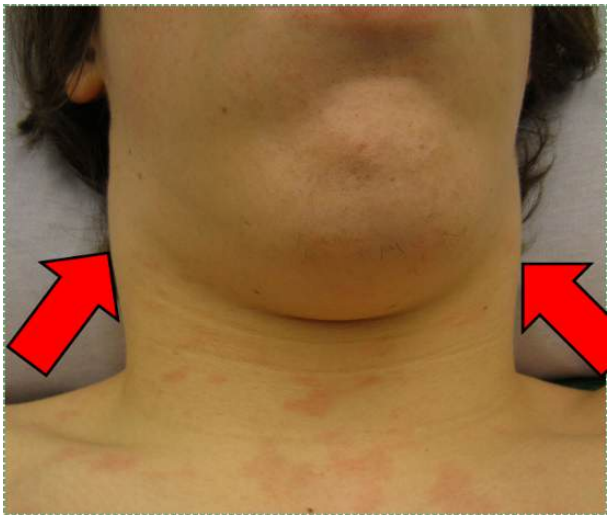


Figure 5.25: Lymphadenopathy. Image credit: "own work" by James Heilman MD, is licensed CC BY SA 3.0 / *arrows added*

Lymphadenopathy of lymph nodes

In the absence of infection, lymph nodes are soft and moveable. However, if white blood cells detect a pathogen, they undergo cell division and release inflammatory molecules. This leads to lymph nodes becoming palpable, possibly visibly swollen, and likely sensitive to touch. The swelling of lymphatic tissue is called **lymphadenopathy**, and is a non-specific sign of immune system activation.

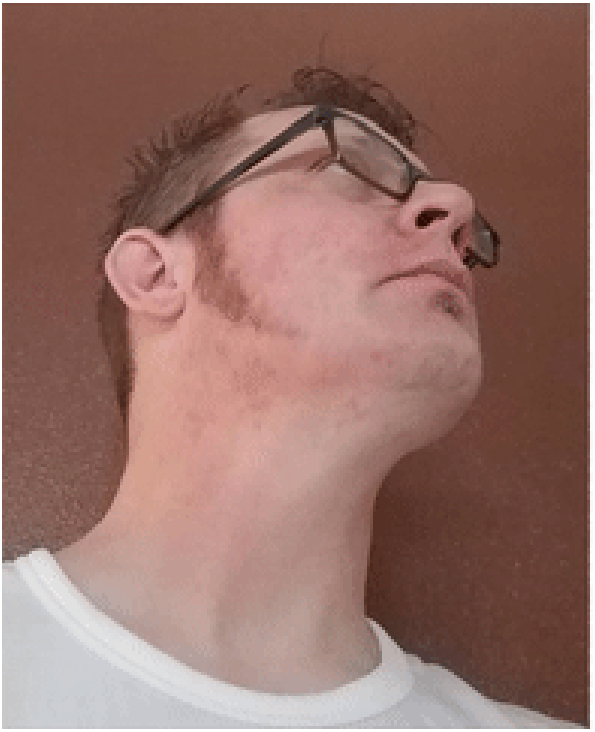


Figure 5.26: Pathway of the drainage of lymph from teeth into lymph node clusters

The mandibular incisors drain into **sub-mental lymph nodes**. The sub-mental lymph nodes and the rest of the teeth (other than maxillary 3rd molars) drain into **sub-mandibular lymph nodes**. The sub-mandibular lymph nodes then drain into **superior deep cervical nodes**. Swelling of some or all of these lymph nodes can indicate active periodontal disease.

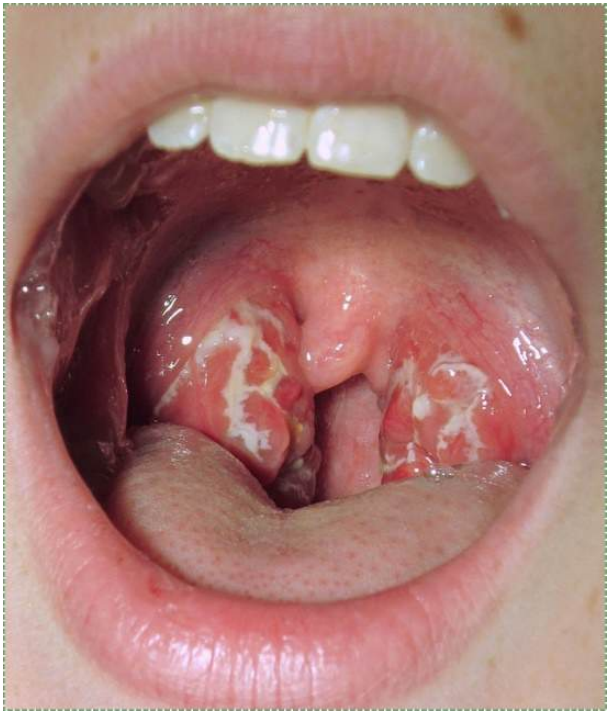


Figure 5.27: Lymphadenopathy of tonsillar tissue. Image credit: "own work" by James Heilman MD, is licensed CC BY SA 3.0

Lymphadenopathy of tonsillar tissue

Tonsillar tissue can become inflamed, and when it does it is usually called **tonsillitis**— when the increased number of white blood cells (living and dead) may be visible as white-ish patches within the inflamed tonsil(s). Tonsillitis is more common in children between pre-school and pre-teens because of the way the immune system develops immunocompetency and tolerance.

Para-nasal sinuses

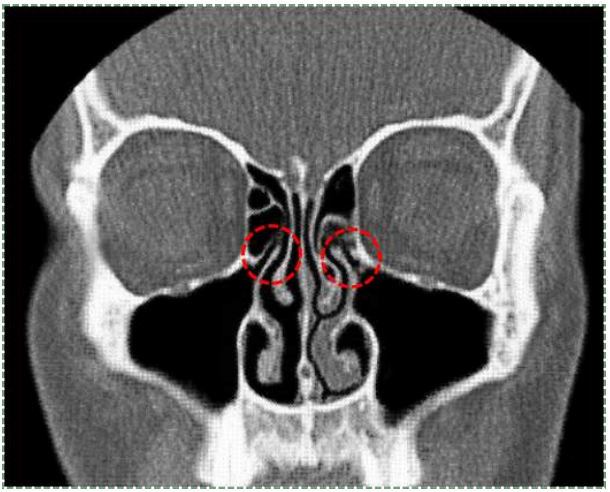


Figure 5.28: Para-nasal sinuses. Image credit: "conchae nasalis" by Simplicius, is licensed CC BY SA 3.0 / *red circles added*

Basic anatomy of the para-nasal sinuses

The **para-nasal sinuses** are spaces within the frontal, sphenoid, ethmoid and maxillary bones surrounding the nasal cavity. The maxillary, frontal and sphenoid sinus communicate with (connect to) the nasal cavity via small passageways. The para-nasal sinuses are lined with **pseudostratified epithelium**. This epithelium produces **mucus** which traps

pathogens and debris. With the aid of cilia, these harmful agents are removed from the body. Within the nasal cavity are 3 bumps called nasal conchae, which divide the nasal cavity into three winding spaces called the nasal meatuses. (for those who are counting, there is a fourth space above the superior nasal conchae called the spheno-ethmoid recess, it is not a meatus because it does not form a pathway from the nasal vestibule to the nasopharynx).

Clinical applications of para-nasal sinus histology

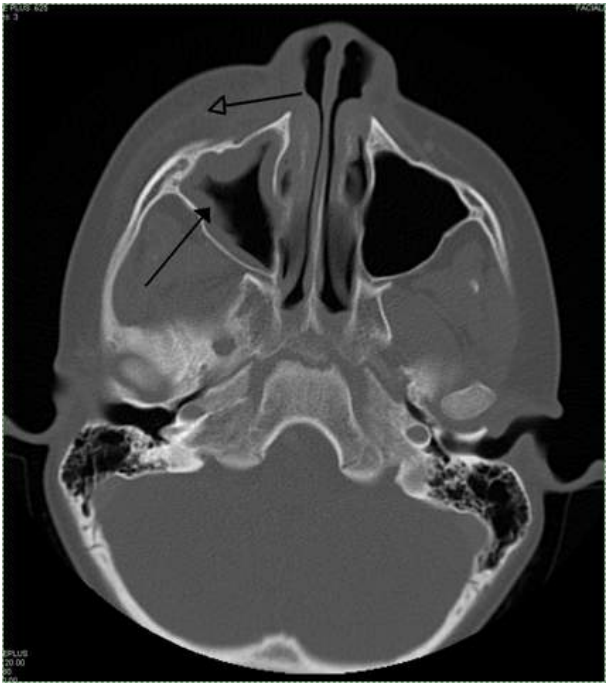


Figure 5.29: Inflammation of the left para-nasal sinus (black arrowhead indicates swollen mucosa). Image credit: "own work" by James Heilman MD, is licensed CC BY SA 3.0

Inflammation within the para-nasal sinuses

When **para-nasal sinuses** become inflamed (known as **sinusitis**), the small ducts leading to the nasal sinuses may

become obstructed. The increase in fluid that accompanies inflammation has nowhere to drain, leading to pressure within the sinuses. Furthermore, one response of **goblet cells** to inflammatory signals is to produce more **mucous** proteins, which again have nowhere to drain.

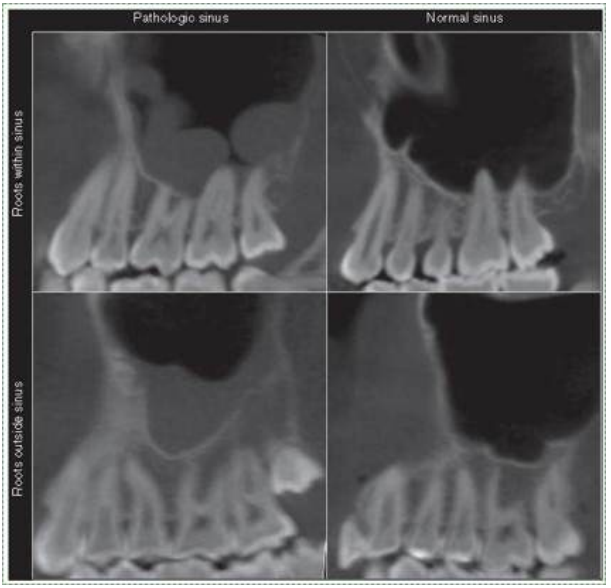


Figure 5.30: Tooth roots can extend into the maxillary sinus. Image credit: "Figure" by Gina Roque-Torres, is licensed CC BY 4.0

The posterior maxillary teeth lie close to, or sometimes partly within the maxillary sinuses. Therefore, if inflammation occurs within these sinuses, it can cause discomfort to the significantly more-sensitive teeth. Furthermore, sinus infections may spread to the posterior maxillary teeth.

< Chapter 4 * navigation * Chapter 6 >

Chapter review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:
https://openoregon.pressbooks.pub/histologyandembryology/?p=44

6.

EARLY DEVELOPMENT

- Overview of early development
- Patterning
- First trimester:
    - Pre-implantation
    - Gastrulation
    - Differentiation of the ectoderm
        - Neurulation
        - Neural crest cells
    - Differentiation of the mesoderm
        - Somite formation
        - Heart formation
    - Differentiation of the endoderm
        - Primitive foregut
- Clinical applications of early development

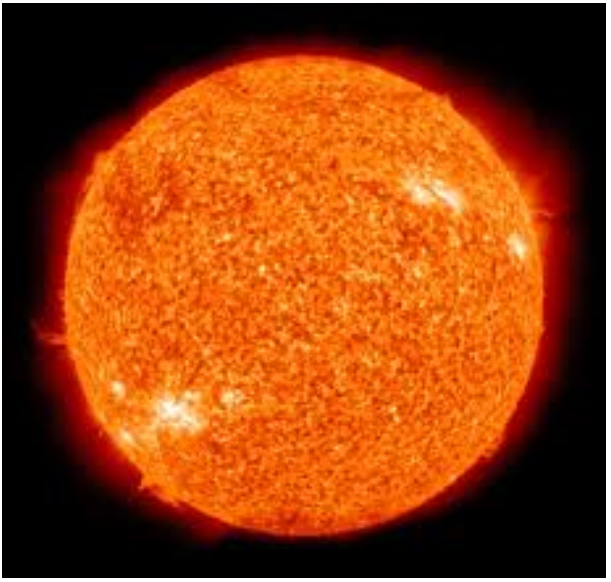


Figure 6.1: From this photo, the polarity of the Sun is probably not recognizable. Image credit: "The Sun photographed at 304 angstroms" is in the public domain CC0

Overview of early development

Human **development** is the process where a single cell, a visually unremarkable cell except for perhaps its size, slowly changes into thirty seven trillion different cells.

Embryology is the study of the phases of development, especially the early phases where most of the interesting stuff happens. A round cell, just a ball with no sides and no special parts, grows into an organism with different organs in different places. Fig. 6.1 is the Sun. You see it every day. Can you tell whether the photo is right-side up? Is there even a right-side up? We are not astronomers, but the Sun looks a bit like a fertilized egg, and in Fig. 6.1 there are no obvious landmarks to orient our view. An egg has a similar **amorphous** (without shape) appearance, but it **morphs** (smoothly changes shape) into the embryo shown in Fig. 6.2, which has a head-end and tail-end, and clear beginnings of arms and legs. The first part of this chapter focuses on *concepts* common to many developmental events seen in chapters 7 through 11. The second half of the chapter covers the details of early development.

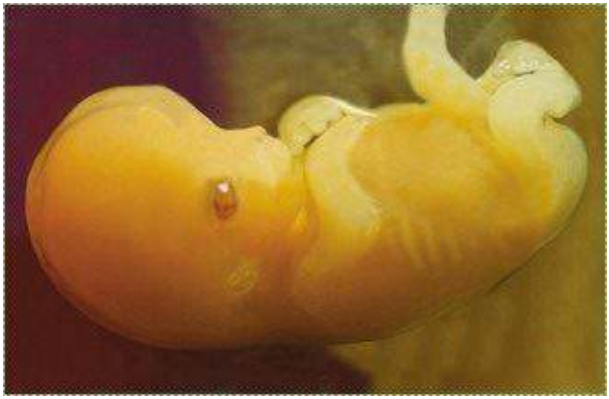


Figure 6.2: Seven-week-old human embryo. Image credit: "Human embryo 7 weeks", by GoldenBear is licensed CC BY SA 3.0

Pregnancy is divided into three equal time periods named **trimesters**. During the first two weeks of the first trimester, a single cell multiplies into a multicellular organism with no discernible shape (other than it is a round blob, not an icosohedron). Starting at week 2, the blob of cells develops into an **embryo** with different shapes. The embryo keeps developing new shapes until it has all of the basic human shapes, such as a head and arms and heart. At this time (week 8) it is a **fetus**, and the first trimester has ended. The next two trimesters are covered in much less detail.

In this book, we list all times in days, weeks, months or years

(e.g. Fig. 6.3). In a developmental biology class, times may be listed in carnegie stages, which are less useful to us (unless you want to discuss crown-rump lengths).

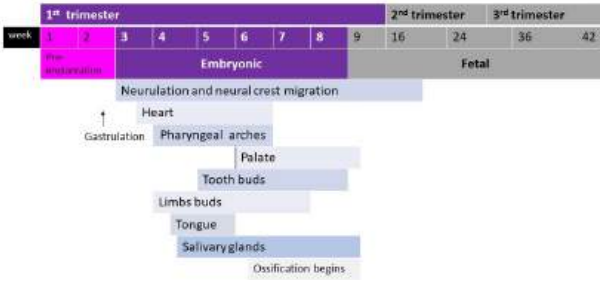


Fig. 6.3: A rough outline of a few major developmental landmarks (not to scale)

Figure 6.4: Director of the film NoBody's Perfect, a documentary on surviving the teratogenic effects of thalidomide. Image credit: "Filmmaker Niko von Glasow", is licensed CC BY SA 2.0

As human cells divide during the first **trimester**, they make decisions as to their **cell fate**, which ultimately determines

the number and location of arms and legs, head and heart. These decisions involve signals being sent between cells. Errors in these signals lead to **congenital disorders** (or congenital malformation**,** or birth defects (but see below)). These can happen without known cause. Or, chemicals may interfere with developmental signals, and we call these chemicals **teratogens**. Probably the most famous teratogen in the U. S. is the medication thalidomide. If taken by pregnant women at the wrong time, thalidomide interferes with the decision-making processes embryos use to decide where to grow limbs. This causes phocomelia, or malformation of the limbs. For many other countries, the teratogenic effects of agent orange (dioxin, which is also found in cigarette smoke) and atomic radiation (pdf download) are well known, but this information is notably absent in most American school classes (agent orange and atomic radiation also have adverse effects on adults, and the rate this information reaches the American public is subject to similar biases). We will cover a small number of signals involved in important decisions that are made during face and tooth development. 37 trillion decisions are made between the single-cell stage and birth, and errors are inevitable. Some errors are minor, changing the appearance but not the function of an organ; we might refer to this as a birth mark or human variation. Other mistakes are more severe, interfering with a person's health, and we refer to those as congenital disorders (or perhaps inborn errors of development, but preferably not birth *defects*,

because our choice of words influences the amount of health care people seek, it depends on the patient).

Much of what we cover in this chapter comes from studying the development of animals, some of which don't even have teeth, like sea urchins, tunicate worms and flies. These model organisms are useful to us for two major reasons. One, they develop quickly, in large numbers, externally (no uterus), and may even be see-though. Two, early developmental processes are highly conserved across metazoans (there have been few mutations to DNA instructions over millions of years). If you don't think you share much in common with a fly, take a look at *your larval stage* in Fig 7.1← in the next chapter. We do have significant differences. As a result, sea urchins are much less useful when we discuss traits like a head. Worms and flies are less useful when discussing traits unique to vertebrates, such as teeth and our "new head" (covered in chapter 7).

But for early development (this chapter), experiments on what those of us with *new heads* often call simpler organisms yields a wealth of useful information. Experimenting on other organisms allows researches to do **fate**-mapping. In essence, if you inject a **stem cell**← with a dye, you can follow it as it goes through **mitosis**← to produce a large number of **terminally differentiated** adult cells. Any adult cell that contains the dye must have come from that stem cell. This allows us to map the **lineage** of adult cells. Instead of using dyes, some developmental biologists use chimeras, a technique

invented by Nicole Marthe Le Douarin, to identify the diverse roles of **neural crest cells**. Others transplanted cells from one location to another to see if that altered the fate of those cells, or if those cells altered the fate of their new neighbors.

If you at any time feel like you need more information on **embryology**, these links are helpful:

- The Embryology Education page
  - by Dr. Mark Hill at the University of New South Wales, Sydney, Australia
- Embryo Images Normal and Abnormal Mammalian Development
  - by Dr. Kathleen K. Sulik and Dr. Peter R. Bream Jr. at the University of North Carolina School of Medicine.
- The 3D Atlas of Human Embryology
  - an open-source collection of 3D pdf files (readable using Adobe Acrobat reader) by over 70 different students at the Academic Medical Center, Netherlands.

Patterns

In arts and crafts, a *pattern* is a repeated form or design. The same is true in developmental biology. This section covers the basic patterns that occur during development (patterning, or

pattern formation). These patterns are repeated, often with slight changes (the way theme music is repeated with changes in different scenes of a film). Multiple patterns can overlap. However, it is useful to think about developmental patterns individually, the way you might focus on just the vocals– or cello, or accordion– in a song.

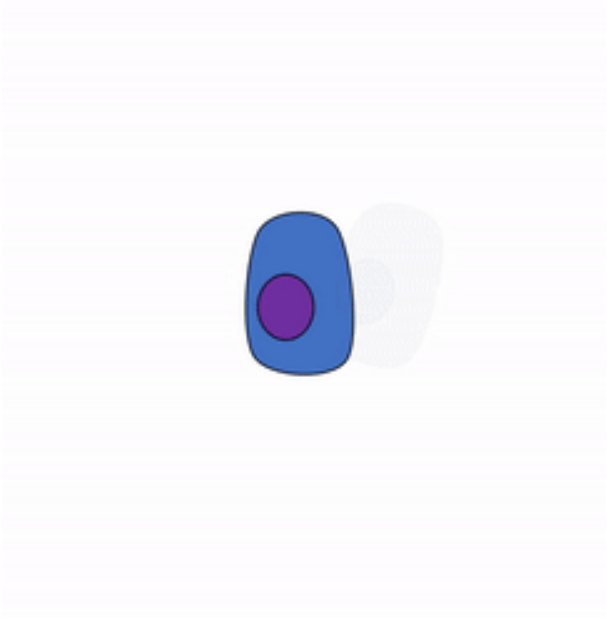


Figure 6.5: Illustration of cell cloning, followed by signaling.

Cloning

Mitosis[←] produces clones, or identical cells with identical **DNA**. However, cells **differentiate** and **express** different

genes to become different tissues and organs. In the first 2 weeks of **development**, cells divide and produce identical (but smaller) cells. Very soon, cells make decisions as to what they are going to become, and these decisions are coordinated with other cells. Without coordination, an embryo could wind up with two tails and no head.

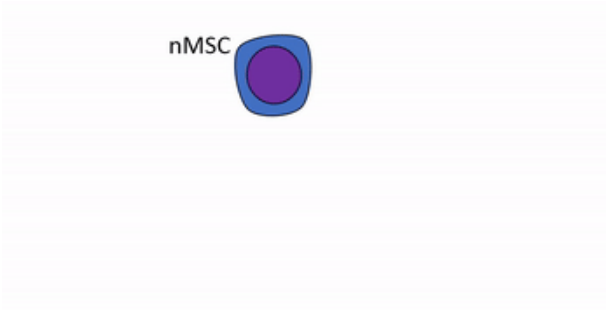


Figure 6.6: Differentiation is caused by changes in gene transcription that leads to cells looking (and behaving) different from other cells. These changes are usually triggered by morphogens.

Differentiation

Differentiation is the process where **stem cells**[←] begin to **express** different **genes**, causing them to appear and function differently from other cells. All cells in the human body have

the same **DNA**, because all cells copied their DNA from the zygote (a few exceptional cells change their DNA a bit). The zygote is a toti-potent stem cell, **transcription factors** can potentially bind to and activate any gene. As cells differentiate, they limit their **cell fate** by putting some genes into deep storage (not actually changing the genetic code, but changing what may or may not be accessible to RNA polymerase).

animation of differentiation

Figure 6.7: Differentiation alters cell fate, which is passed on to daughter cells in the form of conserved patterns of DNA methylation and histone packaging.

Differentiation is triggered upon receiving chemical signals.

These signals activate **signal transduction cascades**, which cause target cells to **methylate** the **DNA** of un-necessary **genes**, pack those DNA regions around **histones**, and permanently shut those genes off. For instance, cells **fated** to become ovaries never synthesize insulin, the **gene** for insulin is packaged away long before ovaries secrete estrogen or ovulate. DNA methylation and histone packaging *limits* the fate of these cells. The methylation pattern is copied during **mitosis** and passed to both daughter cells (and to their daughter cells). The significance of this is that causing just one cell to differentiate can affect the fate of millions of cells in the future.

Stem cells in the skin repair skin damage easily. Why can't we use those stem cells to repair damage from a heart attack or Alzheimer's Disease? That is the basic question we are addressing. In Fig. 6.7, imagine a signal instructs one cell to methylate green genes, leaving red genes available for **transcription**. Imagine a different signal does the opposite to the other cell, causing mehtylation of the red gene but leaving green genes open. As the red cell duplicates its DNA, it copies the methylation pattern, so that all of its daughter cells cannot express green genes. Conversely, none of the daughter cells of the green cell can express the red gene. We now have 4 red cells, who belong to one **lineage**, and 4 green cells who belong to a different lineage. All 8 cells have the same DNA (**nature**), but not the same DNA methylation pattern (**nurture**). Further **patterning** arises as cells transcribe un-methylated genes. In the cartoon, as red cells **transcribe** the red gene, the red

protein acts as a short-range signal which **induces** nearby green cells to express the dark-green gene. And if expression of the dark green gene made those cells look different, we would say those target cells **differentiated** further. This organism has become more complex, starting as 1 cell type but now having 3 different cell types. This continues until we have 200 or so different cell types. Generally speaking, as a cell becomes more specialized, its fate becomes more limited (more DNA methylation and histone packaging), possibly to the point of being limited to just one cell type.

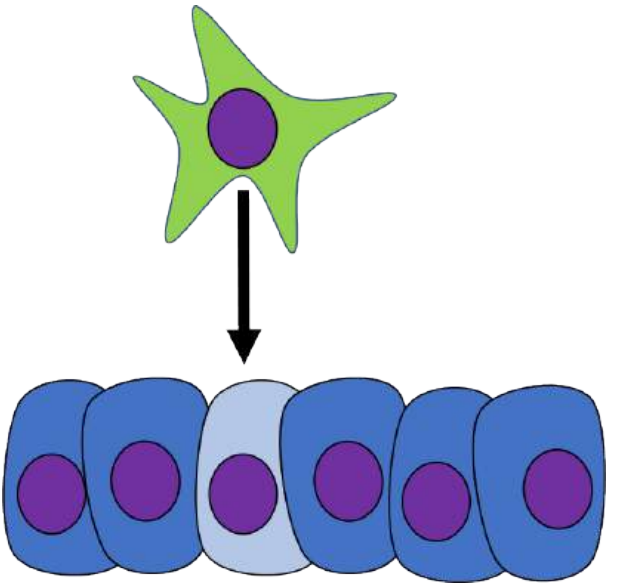


Figure 6.8: Green cell releasing a morphogen to induce the differentiation of a dark blue cell into a lighter blue cell.

Induction

One cell may signal to another cell and **induce** (instructs) that cell to **differentiate**. Chemicals that induce another cell to change its shape (to **morph**) and change **cell fate** are called **morphogens**. Some morphogens are referred to as **growth factors**, depending on how they were discovered. During early develoment, cells are both growing *and* differentiating. Morphogens are re-used during regenerating and healing in adulthood, if the chemical signals were discovered in this context, they are more likely referred to as a growth factor. Morphogens are similar to neurotransmitters or **hormones**, they often bind to cell-surface **receptor** proteins. Activation of morphogen receptors leads to activation of **signal transduction cascades**[←] in the cytoplasm, which in turn can either activate or inhibit specific **transcription factors** to alter the **expression** of different **genes**. Changes in gene expression cause the target cell to look and function differently. Morphogens also change the methylation of genes and their packaging around histones, which is a more permanent change to gene expression. This is passed down to daughter cells, which we learned in Chapter 1 is an example of **epigenetic** inheritance. By regulating gene expression through both transcription factor activity and methylation, morphogens change not only the **morphology** the target cell, but their cell fate as well.

There are often several closely-related molecules that belong

to a *family* of morphogens, each chemical having a name like morphogen-1, morphogen-2a, morphogen-2b, and morphogen-3. There are a large number of different morphogens. In this textbook we discuss 4 by name, ones directly related to tooth development, such as the Bone Morphogenetic Protein (**BMP**) family, the Wingless/Int-1 **Wnt** family, and the Fibroblast Growth Factor (**FGF**) family. Morphogens can act over different distances listed in Table 6.1. The names of specific morphogens are very important on embryology exams, much less so on dental hygiene exams.

Distance	Type	Examples
Direct	Cell-to-Cell	Epithelial-to-neuro-mesenchymal stem cell (Odontoblast differentiation)
	Cell-to-ECM	Integrin-to-collagen (Ameloblast differentiation)
Short-range	Diffusion through ground substance	Bone Morphogenetic Protein (BMP), Wnt, Fibroblast Growth Factor (FGF) (tooth bud induction)
Body-wide signals	Hormones	PTH, Calcitonin (tooth eruption)

Table 6.1: Examples of different type of signals seen during embryogenesis.

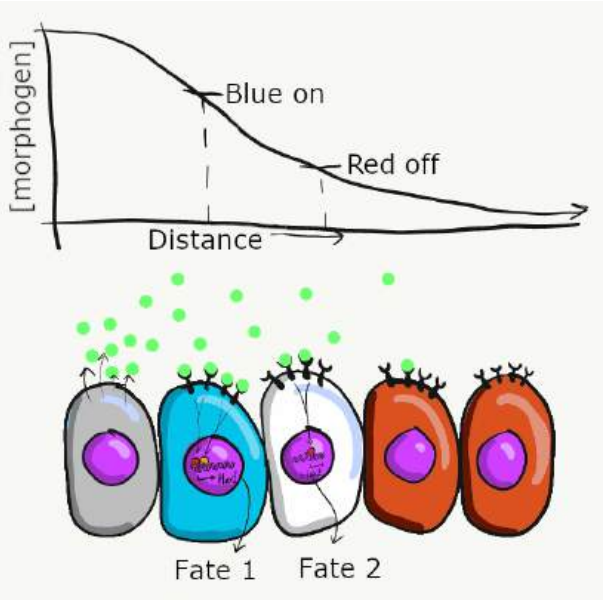


Figure 6.9: Illustration of a morphogen gradient having different effects on cell fate, dependent on the morphogen levels being above or below certain thresholds at different distances away from the source.

Morphogens are secreted into **ground substance**, creating a concentration gradient of morphogen levels as they diffuse slowly away from the source. The amount of morphogen molecules produced, the relative stickiness of the ground substance, the distance away from the source and the number

of receptor proteins on the target cell determine level of a signal cells receive. Sometimes, a morphogen produces visible effects that fall along a linear gradient (or spectrum), such as skin color (from lighter to darker) or body size (from shorter to taller). However, sometimes the effects of a morphogen are more discrete (tooth bud or no tooth bud, there are no half tooth buds). To help explain these observations requires a little knowledge of how **transcription factors** work. For example, in the French flag model (Fig 6.9), imagine receiving high levels of the morphogen signal triggers the transcription of a blue gene and inhibits a red gene, causing those cells to **differentiate** into blue cells. Moderate amounts of the morphogen signal may not be enough to recruit RNA polymerase to the blue gene, but enough to bring inhibitory transcription factors to the red gene, causing cells in regions of moderate morphogen levels to differentiate into white cells. Lastly, cells that receive little or no morphogen signal do not inhibit the red gene, causing them to differentiate into red cells. There are other models that help explain the link between induction and morphogenesis (such as a morphogenetic field). These models all share an important concept: chemical signals can communicate **positional information** in a developing embryo.

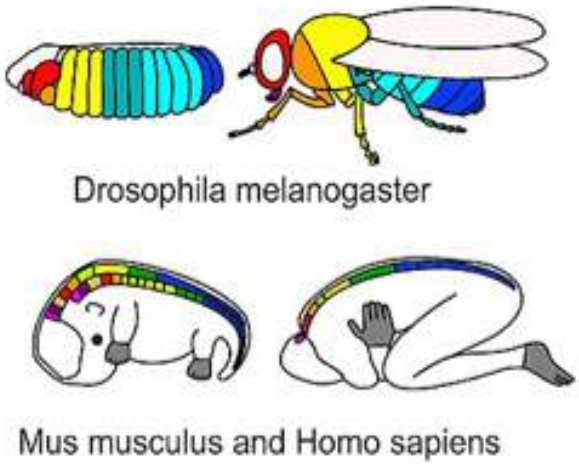


Figure 6.10: Hox (homeobox) gene expression in fruit flies, mice and humans. Image credit: "Hox genes in various species" by Stefanie D. Hueber, Georg F. Weiller, Michael A. Djordjevic, Tancred Frickey is licensed under CC BY 4.0 / *cropped*

During very early development, regional expression of **homeobox genes** controls **cell fate**, not **morphology** (further reading). Homeobox genes are **transcription factors** that specify which part of the body a particular cell is now a part of along the anterior-posterior axis, even if that area has no specific shape yet (Fig. 6.10, upper left). They do not encode the proteins which cause cells to become part of a specific organ. Instead, homeobox genes activate or inhibit a collection of other **genes** (a program), setting the stage for future changes. Such programs include morphogens like **FGF** and **BMP**, which activate genes that alter cell and tissues morphology (Fig. 6.10, upper-right).

Homeobox gene expression represents a very specific plan in embryology. It is highly conserved across animal species, and the pattern is very predictable in embryos. The pattern is also relatively simple and linear (Fig. 6.10). Think of homeobox genes like surveyors who determine the locations where houses will be built in a subdivision, while morphogens are the contractors who follow the **DNA** blueprints for each house, which are built by cells. We can extend this metaphor one step further: in a subdivision, houses are similar, derived from one basic blueprint, but given slight modifications with each re-use.

Lastly, it is important to note that in embryology, the anterior-posterior axis means head-to-tail, the same way it is used in all forms of animal biology *except* adult humans, where it means front-to-back. You may also hear the term rostral-

caudal applied to human embryos, but not human adults (except their brains).

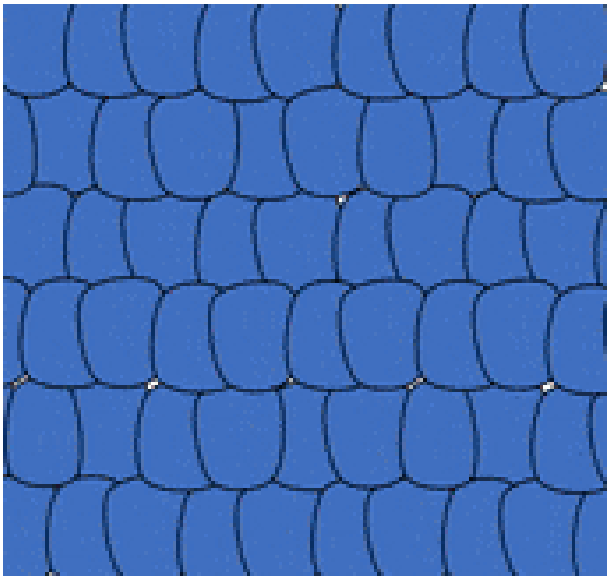


Figure 6.11: Illustration of a stable pattern arising in response to two antagonistic morphogens.

Self-organization

The ability of a tissue to create its own **patterns** is called self-organization (in contrast, maternal effect genes from mom's cells guide patterns for the embryo). The French-flag model describes how one morphogen can **induce** multiple cell fates. But what if you want a flag with repeating stripes? Self-organization involves multiple **morphogens**, initially expressed randomly.

Interactions between these morphogens leads to a stable pattern of **expression**, and a complex pattern of **differentiation**.

For instance, some morphogens block cells from producing the same morphogen. In Fig. 6.11, imagine a blue morphogen induces one cell to differentiate into a blue cell, but blocks neighboring cells from becoming blue cells. These neighbors would be free to differentiate into red cells. At the same time, a distant cell produces red morphogen, which induces it to differentiate into a red cell, but blocks its neighbors from doing the same. Its neighbors would be free to produce blue morphogen and differentiate into blue cells. The initial pattern that arises is alternating rings of red and blue cells. However, rings produce regions of red-next-to-red and blue-next-to-blue, which violates our make-believe rules, and this starts a battle. To minimize the number of red-next-to-red and blue-next-to-blue, cells change their **cell fate** until a stable pattern arises, in this case stripes. It would be hard to predict from the start which cells would be red versus blue, but you could predict the development of red and blue stripes. If these were pigment-producing cells in a zebra, we'd have a zebra with stripes (the best type of zebra). But this pattern could also be alternating regions of oral mucosa that grow a tooth bud or don't grow a tooth bud, ensuring each tooth bud has enough space to grow (these interactions can be modeled mathematically). When a morphogen blocks another signal, we call that **antagonism**, and antagonist

signals are just as common as induction signals. It is fundamentally just as important to grow one head as it is to not grow two heads.

---

animated illustration of growth types

Figure 6.12: Cell proliferation can occur appositionally or interstitially

Proliferation

Growth of a tissue is called **proliferation**. Proliferation occurs either by **mitosis**[←] (adding cells) or by adding **ECM** (synthesizing proteins or other molecules). When new growth happens next to a region of older growth, this is **appositional growth**, whereas if growth happens from within and pushes older tissue outwards, that is **interstitial growth**. Dense tissues, such as **bone**[←], enamel and dentin, undergo appositional growth. Softer tissues like **mesenchyme**[←] grow interstitially.

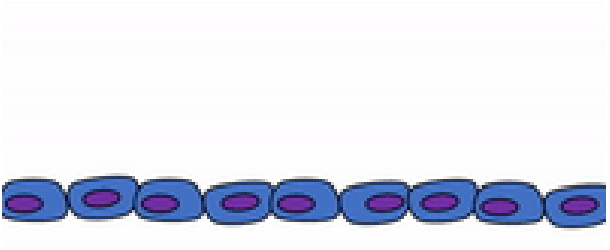


Figure 6.13: Interstitial growth in an epithelium can cause budding or invagination

The growth of an organ is regulated by hormones, such as Growth Hormone, which coordinates the rate of growth of tissues body-wide. On the other hand, local-acting **morphogens** cause small regions to grow faster than neighboring regions. To make room for new cells while still maintaining a single-layer of cells connected to neighbors by **desmosomes**←, **CAMs**← and **tight junctions**←, an epithelium bulges inwards or outwards if it can't grow wider. An outward growth is known as a **bud**, and an inwards growth is an **invagination**. Arms and legs grow from buds, while teeth, hairs and the brain grow from invaginations. These are both forms of interstitial growth.

When we learned about the histology of epithelia, we said epithelia are located on the outer or inner surface of the body, with the **apical** side facing a space and the **basolateral** side next to connective tissue (**junctional epithelium** being an exception). It is simpler to form a new epithelium by folding

an old one, as the process of folding creates a new space. Otherwise, if you tried to form a new epithelium in the middle of other stuff, that would either require re-programming the cells' **apical-to-basolateral polarity**, or first digesting a new space and then guiding epithelial cells to its location. Folding is simpler, it creates a space right where you need it (apical), and as a result you will see a lot of invaginations in the next three chapters.

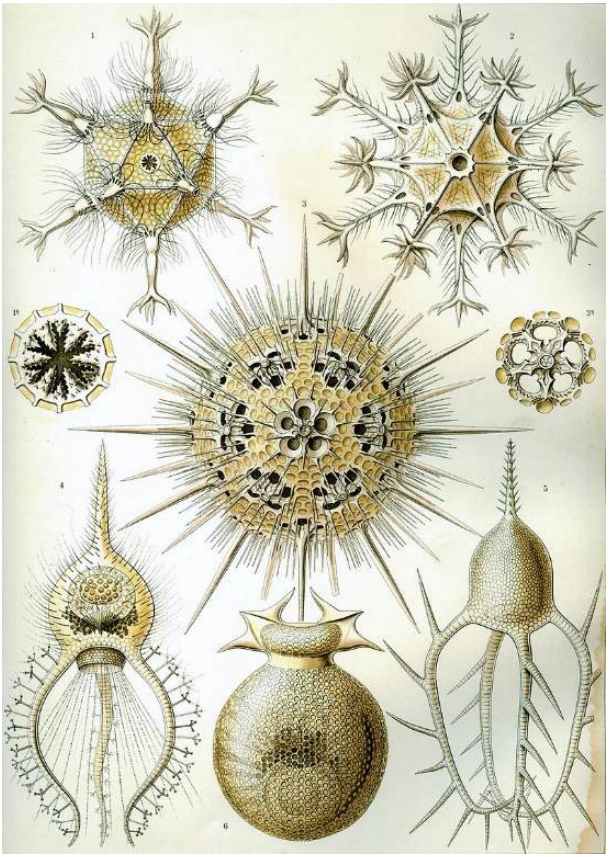


Figure 6.14: An example of different morphs (shapes) of the same basic pattern in dinoflagellates. Images credit: "Kunstformen der Natur (1904), plate 1: Phaeodaria", by Ernst Haeckel is in the Public Domain CC0

Morphogenesis

Morphogenesis is the appearance of new shapes in an organism. It means to go from no shape to some shape. The word **morphology** can mean the study of these shapes, or be used as an adjective to mean the shape of something (For example, a **cleft palate**[←] is a change to the healthy *morphology* of the palate). From the zygote to the blastula stage (sometimes referred to as the germinal stages) the ball of cells is **amorphous**. At the start of **gastrulation**, however, the embryo undergoes numerous morphological changes. New shapes arise in the developing embryo. It develops an inside and an outside, a tail end and a head end, a left and right, and a front and back. After that, a nervous system and a circulatory system become visible. How long do you think it takes before teeth develop? The answer may surprise you.

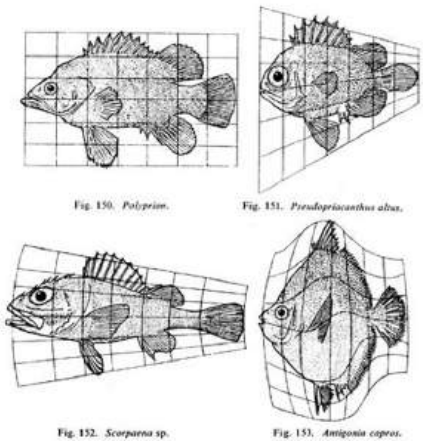


Figure 6.15: D'Arcy Wentworth Thompson proposed that what may look like different shapes in related species may be transformations of one basic pattern. Image credit: "Fig 150-153" by D'Arcy Wentworth Thompson, On Growth and Form is in the Public

Domain, CC0

Fig. 6.14 is a famous drawing of tiny sea creatures with mineralized exo-skeletons. This image was later analyzed by a mathematical biologist named D'Arcy Wentworth Thompson, who noticed different biological shapes can be different *transformations* of the same shape. Transformations, he proposed, occurred by different localized rates of growth– something our current knowledge of **morphogen** signaling supports. This is what he illustrated in Fig. 6.15. He is regarded as the first person to describe morphogenesis. When Thompson looked at the tiny sea creatures, he didn't see different shapes, he saw variations on one shape. These variations arose over *evolutionary* time, and led to different-looking sea creatures (who share **lineage**). In this book, we look at different body parts that share the same lineage but change over *developmental* time, such as hair follicles and tooth buds. Thompson's ideas were expanded upon by the mathematician Alan Turing, who coined the term morphogenesis. His other achievements include inventing computer science and breaking the Nazi code during WWII (with the help of many female mathematicians at Bletchley park).

Morphogenesis and genetics

To have a well-rounded understanding of how morphogen signaling works, it helps to understand some concepts of genetics. When we say *understand*, we don't mean *memorize*

the specific details, instead focus on the concepts. First, pay attention to when we use the word morphogen, because we likely mean the signaling molecule itself. We at times discuss the morphogen *signal*, and that extra word *signal* makes a big difference. Mutations that alter morphogen signals include mutations to the gene for the *extra-cellular* morphogen molecule itself (if it is a protein such as **FGF** or **BMP**) or enzymes that synthesize the morphogen (such as the conversion of **carotene** into Retinoic Acid), *cell-surface* **receptors** for the morphogens, *cytoplasmic* second messengers and *nuclear* **transcription factors**. Discussing morphogen *signaling levels* saves us from the complexity of having to mention the entire **signal transduction cascade**[←]. Next, some mutations might be **gain-of-function**, such as a mutation to a receptor that prevents it from shutting off, and these are often inherited in an autosomal dominant fashion (it only takes 1 faulty **allele** to create a signal where it shouldn't be). Alternatively, **loss-of-function** mutations prevent proteins from functioning properly, and are often inherited in an autosomal recessive pattern (it takes both copies to be faulty to lead to a loss of a signal where it should be, one good allele would be enough). Some mutations fully stop, or fully start, morphogen signals. In such cases we say theses mutation have complete penetrance. Other mutations might partially block or partially start morphogen signals, and have partial or incomplete

penetrance. The severity of the symptoms fall on a spectrum.

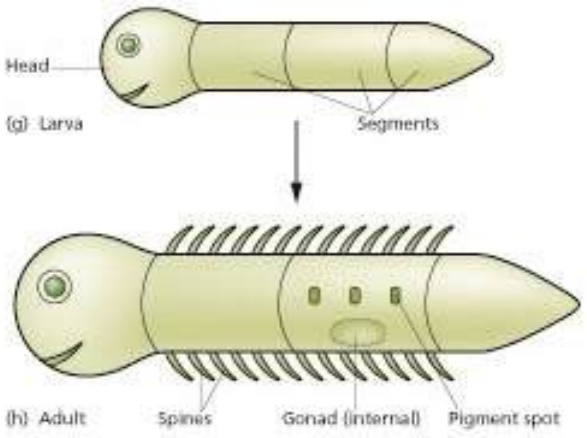


Figure 6.16: Maturation involves the changing of already present patterns. Image credit: "Generalized scheme of embryonic development" by JMW Slack is licensed CC BY SA 4.0

Maturation

Maturation is the process by which new shapes created by **morphogenesis** continue to **develop** and take on a more mature (adult-looking) form. By the end of week 9, all of the basic organ shapes have formed, and we say the **embryonic** period is over and the **fetal** stages begin. The fetus undergoes maturation, and maturation involves bits of the previous processes (another way of saying that is maturation **recapitulates** other developmental processes).

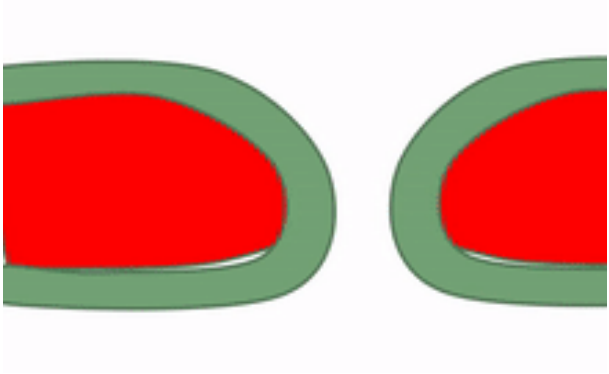


Figure 6.17: Illustration of the fusion of two separate structures and their tissues.

Fusion

Sometimes, two separate bits of the same type of tissue come into contact with one another and **fuse** into a single tissue.

For an epithelium, this requires the cells share the same type of **CAM**. For a connective tissue, this requires the correct **integrin** binding to the correct **fibronectin**. In Fig 6.17, the basic pattern of green being on the outside and red in the middle is initially disrupted by the fusion of two structures. There are three ways to remove green cells from the middle area. The first way is for cells to receive **guidance cues** and cause cells to migrate. Some guidance cues are repulsive, such as the lack of correct **cell-to-cell contacts**, or high concentrations of a repulsive **morphogen** (*if you are at a party and leave the living room because someone there smells bad, you are acting on a repulsive guidance cue*). Other guidance cues are attractive, such as the correct cell-to-cell contact or a positive guidance morphogen (*at the same time, you smell delicious hors d'oeuvres in the kitchen and head there, you are acting on an attractive guidance cue*). Whether a cell-to-cell connection or morphogen is attractive or repulsive depends on how the target cell has been programmed (what **receptor genes** it is **expressing**). The second way to remove cells from the wrong location is to induce them to undergo **apoptosis** (see below). Lastly, another way to re-organize cells is to induce them to **de-differentiate** (revert to a **stem cell**) and then **differentiate** into the correct cell type. This is called a transition; both early development and tooth development involve examples of **epithelial-to-mesenchymal transitions** and **mesenchymal-to-epithelial transitions** (discussed later in this chapter).

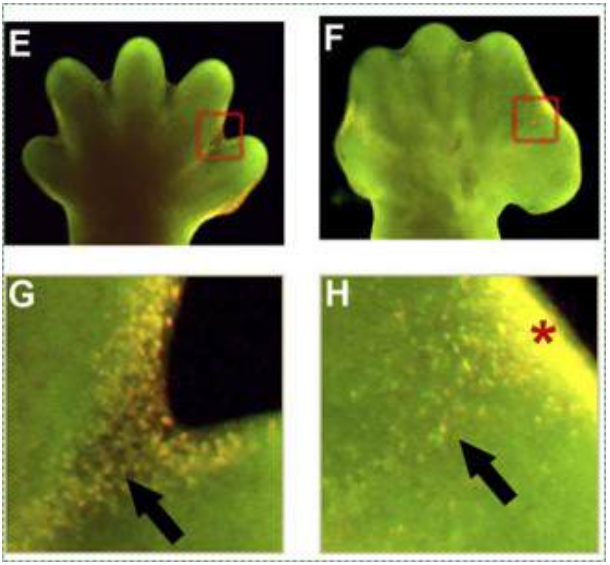


Figure 6.18: Growth of fingers occurs by apoptosis of regions of a hand plate, guided by morphogen gradients. Figure legend: E and G: adeauate apoptosis, F and H: reduced apoptosis. Image credit: "Depletion of BMP Signaling Causes Interdigital Syndac

tyly", by Bandyopadhyay A licensed CC BY SA 4.0

Apoptosis

Cell death is necessary for multicellular life. In the construction of tall buildings or large ships, **scaffolding**← is built first to allow construction workers to get where they need easily, and the scaffolding is removed after construction is complete. Similarly, during **development**, **apoptosis**← is an integral part of the construction of body parts. Many tissues grow more cells than are needed in the mature form. To undergo **maturation**, un-needed cells undergo **apoptosis**←. For instance, during the growth of **bone tissue**, **cartilage** tissue is made to act as a scaffold for **osteoblasts**. Shown in Fig. 6.18, the hands and feet start off as limb paddles, and only with apoptosis in alternating regions does the **morphogenesis** of fingers and toes occur. Without enough apoptosis, people are born with webbed fingers or toes, or suffer **ankyloglossia** (their tongue is tightly anchored to the floor of the mouth by a pronounced lingual frenulum).

The first trimester

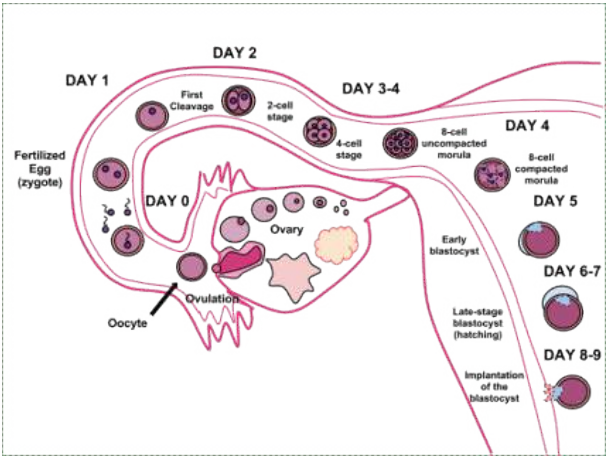


Figure 6.19: The per-implantation period generally occurs in the fallopian tube. Image credit: "Fertilization in humans", by Ttrue12 is licensed CC BY SA 3.0

Pre-implantation period

The **pre-implantation period** begins with fertilization, spans a week or so of development, and ends with an embryo implanting into the uterus. **Fertilization** is the union of one sperm cell and one egg– thousands of sperm are needed, but only one is allowed into the egg. This union joins 23 maternal

chromosomes with 23 matching paternal chromosomes, forming a **zygote**. Fertilization most often occurs towards the distal end of a fallopian tube. Over the next week the zygote undergoes **mitosis**[←] and increases in cell number, but does not grow in size. All of the amino acids and nucleotides for making new cells during this first week come from the **cytoplasm** of the zygote, which is why the egg is a very large cell. After **implantation**, the uterus provides the raw materials for an embryo to grow larger.

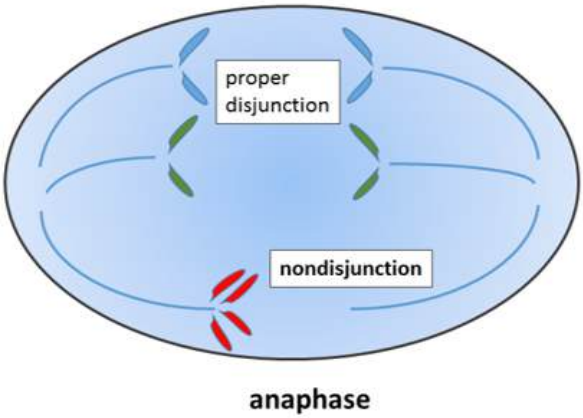


Figure 6.20: Errors separating chromosomes during mitosis generally triggers cell-cycle arrest. Image credit: "Non-disjunction of sister chromatids", by Wpeissner is licensed CC BY 3.0

During **mitosis**←, the 23 pairs of **chromosomes** are duplicated and divided between 2 daughter cells evenly. If mistakes are made and an odd number of chromosomes wind up in a daughter cell, this fails a **cell cycle checkpoint** and

triggers **apoptosis**[←]. If a chromosome breaks, it can lead to the same result.

Figure 6.21: Cells with an extra copy of chromosome 21 can survive and develop. Image credit: "Boy with Down Syndrome using cordless drill to assemble a book case" by Rob Kay is licensed CC BY SA 3.0

There are exceptions, however. A cell with an odd number of chromosome 21 or 23 can survive. If that cell is the egg, then the **zygote** and all subsequent cells will have an odd number of chromosomes. This leads to congenital disorders such as **Trisomy 21**. If the chromosomal segregation error happens at the 2-cell stage of development, then only half of the cells in the adult would have an extra chromosome, and the condition would be less severe. Extra chromosomes interfere with decision-making processes in **development**. People with Trisomy 21 frequently develop **microdontia**, and may suffer from hypo-salivation and bruxism. They therefore need to engage in extra care to maintain healthy oral hygiene.

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animation of earely divisions

Figure 6.22: The early cell divisions produce identical cells, from zygote to blastula stage.

Early divisions

During the first week of development, the zygote undergoes 3 **cleavage divisions,** where **mitosis**← occurs in a synchronized fashion. You can watch a cool video here, as you do see how the embryos are oriented in the same direction. Is the lower half of zygotes heavier? To answer that, frog astronauts helped NASA scientists study early development on the space shuttle. Both on earth and in space, cells duplicate along single planes, 3 times, producing an 8-cell embryo (suggesting density and gravity are *correlated* with early cell polarity but do not *cause* polarity). Synchronizing mitosis requires cell-to-cell communication between **gap junctions** to coordinate **cell cycle checkpoints**. Synchronized mitosis leads to the embryo developing symmetrically. As mitosis continues, the number of cells continues to double, but the cells do not divide along the same plane. This forms a solid ball of cells called a **morula**. As mitosis continues, cells continue to get smaller, until the solid ball of cells becomes a hollow ball of cells named a **blastula** (or blastocyst). From the zygote to the blastula stage, cells appear no different except in size. A fancier way to say this is that there are no changes to **morphology**. During the blastula stage, a group of cells named the **inner cell mass** move away from the others. The fate of the inner cell mass is to become the embryo, while the outer cells, known as the **trophoblast**, are fated to become extra-embryonic structures such as the amnion and

part of the placenta. Therefore, taking a DNA sample by amniocentisis allows for the analysis of fetal DNA without affecting the fetus itself. The blastula implants in the endometrium about a week after fertilization and continues developing.

Gastrulation

It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life.—Lewis Wolpert.

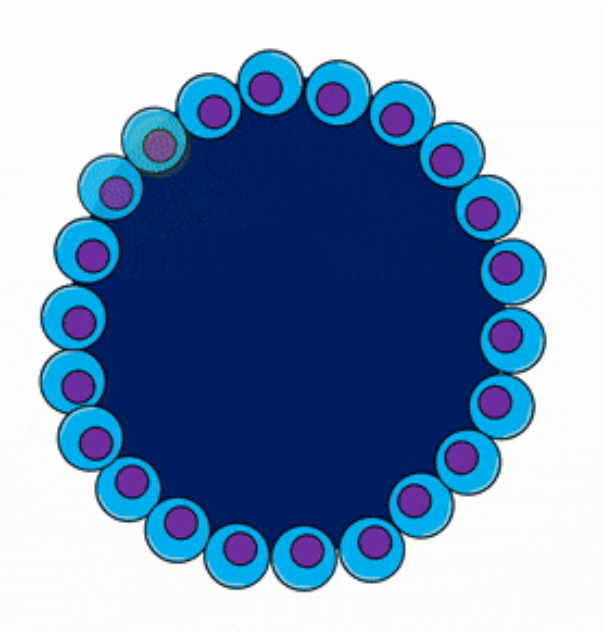

Figure 6.23: Illustration of gastrulation and differentiation of the three basic germ layers, in cross-section.

Gastrulation overview

During **gastrulation**, the embryo is reorganized from a single layer of undifferentiated cells into three layers of **differentiated** cells. Some of the cells from the single-layered **blastula invaginate**, forming a layer of cells on the inside and a layer of cells on the outside of the embryo. The significance of this is that this is the first instance where embryonic cells look different from one another. It is also the first instance where cells limit their **cell fate**. But, wait, if all the blastula cells are identical, why did only *some* of them migrate? **Mitosis**[←] generates two cells with identical **DNA**, but not necessarily identical **cytoplasm**. If an egg has **mRNA** for a **transcription factor** clustered into one spot in the cytoplasm, the first cleavage division produces two non-identical cells, one with the transcription factor mRNA and one without. Later, when the mRNA is **translated**, it could induce that cell to migrate inwards, while other cells stay on the outside. NASA's frog astronauts taught us that transcription factors don't sink in response to gravity to **induce** gastrulation. Instead the difference is a maternal effect. An mRNA for a transcription factor, not transcribed from the zygotes' DNA but made by mom's ovary cells, is deposited into a specific part of the egg's **cytoplasm**, and it induces some cells to invaginate during gastrulation. In fact, up through the blastula stage, the embryo hasn't used any of its own DNA. Because the embryo's DNA

is not guiding gastrulation, this is yet another example of an **epigenetic** trait.

Let us return to gastrulation: it first generates two layers of cells (outside and inside). Imagine holding a tennis ball and pushing your thumbs inwards, forcing some of the outer rubber to fold inwards. The tube that forms becomes the gastrointestinal tract, and the opening (can you guess?) becomes the anus. The embryo therefore now has a distinct tail direction and head direction. Fig. 6.23 looks more like the way a sea urchin undergoes gastrulation. Human embryos look different because it is only the **inner cell mass** that undergoes gastrulation, not the entire blastula. But the 2 layers are the same, human gastrulas simply look more squashed. Oh, and sea urchins don't form anus-first. We are sacrificing accuracy here to focus on important concepts shared across our species. If you are curious, watching movies of these processes in humans is not possible, but it is for organisms like xenpous laevis (frogs).

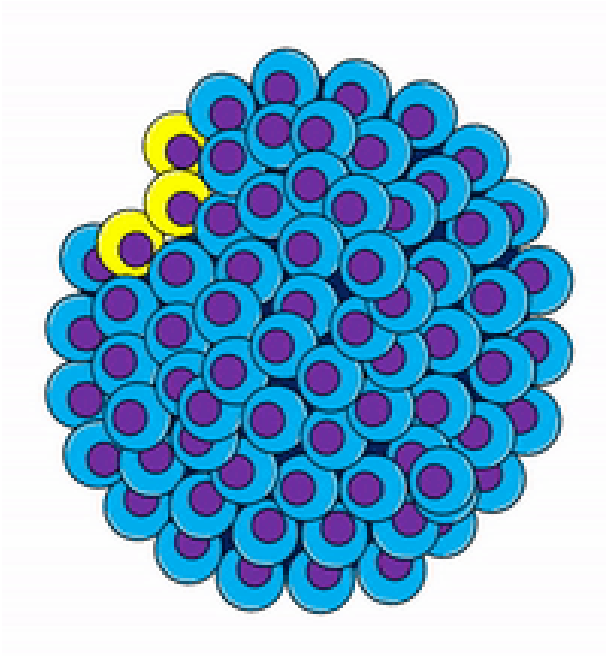

Figure 6.24: Illustration of gastrulation and differentiation of the three basic germ layers, superficial view.

During gastrulation, another important event occurs, cells from the outer layer migrate between the two layers that formed by **invagination**. This now gives the embryo 3 layers, which are the three **embryonic germ layers** (Fig. 6.23). The outer layer of **ectoderm** and the inner layer of **endoderm** remain epithelial in appearance, while the middle layer of cells (**mesoderm**) undergoes a **morphological** change, and becomes **mesenchyme**[←]. This migration begins at a single location called **Hensen's node** (or the Spemann organizer). which moves down the embryo forming the **primitive streak** behind it (Fig. 6.24). The side of the embryo with the node is fated to become the dorsal side, and the streak divides the

embryo into a left-half and a right-half. You have now witnessed, in cartoon form, how something (all the basic body directions) arose from nothing. Don't let the significance of this sneak past you. Scientists have a hard time explaining what there was before the universe formed, or how the Earth went from having no life to having life. Embryologists, by contrast, explain how embryos change from having no shape to having shape in great detail (we have the benefit of watching and manipulating this process).

3 embryonic layers	Cell fate
Ectoderm	Epithelium of skin and oral mucosa, neural tissue
Mesoderm	Connective & muscle tissue
Endoderm	Epithelial lining of hollow organs

Table 6.2: The three germ layers develop during gastrulation

animated illustration of epithelial to mesenchymal transition

Figure 6.25: During gastrulation, and epithelial-to-mesenchymal transition creates the mesoderm layer.

Epithelial-to-mesenchymal transition

The formation of **endoderm** from **ectoderm** was a simple matter of **invagination**. The process by which ectodermal cells quit being ectoderm and migrate to the middle to form **mesoderm** is called an **epithelial-to-mesenchymal transition** (EMT). The epithelial cells of the ectoderm first lose **cell-to-cell contacts**[←] with neighboring cells, which makes them less epithelial. They lose their polarity, **de-differentiate** into **stem cells**[←], then **differentiate** into **mesenchymal stem cells**. This process is not only required to form mesoderm during **gastrulation**, it occurs during **neural crest cell**[←] migration, wound healing, and cancer metastasis. There is a reverse process, called a **mesenchymal-to-epithelial transition** (MET), which also occurs during wound healing. EMT allows epithelial stem cells from a healthy region of **oral**

mucosa to turn into mesenchymal stem cells, migrate away from the healthy region into the damaged region. MET then allows the mesenchymal stem cells to turn back into epithelial stem cells, which divide and differentiate into new **keratinocytes**. Because this mimics what happens during gastrulation, we say "wound healing **recapitulates** development".

Differentiation of ectoderm

animation of neurulation 1

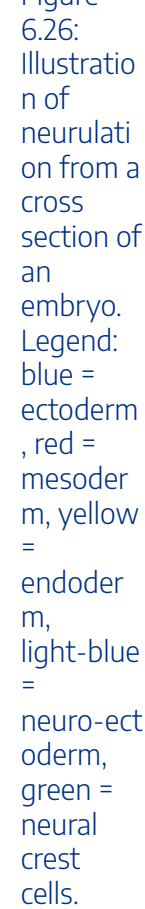

Figure 6.26: Illustration of neurulation from a cross section of an embryo. Legend: blue = ectoderm, red = mesoderm, yellow = endoderm, light-blue = neuro-ectoderm, green = neural crest cells.

Neurulation

Gastrulation gave the embryo its first polarity, as well as the beginning of the GI tract. One of the next organ systems to

develop is the central nervous system. The basic process used in **neurulation** is **recapitulated** when other hollow organs form (watch another cool video here). During the 4th week of development, underlying **mesoderm** known as the **notochord** releases **morphogens** which signal to nearby **ectodermal** cells. This induces the nearby region of ectoderm to **differentiate** into **neuro-ectoderm**, followed by local **proliferation**. Because these epithelial cells cannot easily spread side-to-side, they **invaginate**. Ultimately, invaginating neuro-ectodermal cells lose their contacts with the ectoderm and **fuse** to other neuro-ectodermal cells, creating a new structure called the **neural tube**. This tube develops into the brain and spine. If you are wondering about the notochord, its **cell fate** is to mostly undergo **apoptosis**←, although some remains as the nucleus pulposis of the vertebral discs. In headless fish-like chordates with no backbone called amphioxis, the notochord remains. This wont be the last time you see a human embryo make a structure only to remove it.

animation of neurlulation 2

Figure 6.27: A second illustration of neurulation, focusing on the anterior-to-posterior direction of neural tube folding. Legend: Blue = ectoderm, light-blue = neuro-ectoderm, red = mesoderm, yellow = endoderm.

The neural tube begins folding from the anterior portion of the embryo, and zips up in an anterior-to-posterior direction. The zippering is not perfect because the anterior end is growing wider as it is folding to form the primitive brain. For

almost all developmental processes, the anterior end is the region that develops first, followed by more posterior regions. Zipping the neural tube requires adequate levels of folic acid (which is needed for the **DNA methylation** and **differentiation**), so hopefully mom's **melanin** did an adequate job protecting it.

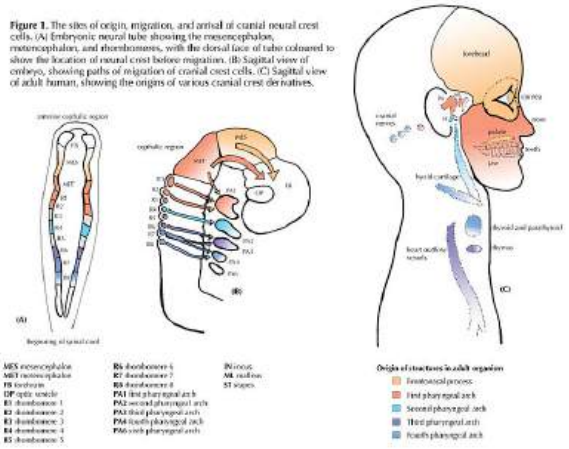

Figure 6.28: Origin of cranial neural crest cells and their target migratory locations. Images credit: "Cranial Neural Crest Cells - migration" by BiolCNC is licensed under CC BY-SA 3.0

Some **neuro-ectoderm** cells do not **fuse**. Instead, these cells undergo an **epithelial-to-mesenchymal** transition and migrate away from the neural tube. These cells are **neural crest cells,** they are **fated** to become a number of important

cells and tissues throughout the body including **melanocytes**, **odontoblasts**, dental pulp, **cementoblasts**, and the **neuromesenchyme** of the **pharyngeal arches**. These cells are sometimes referred to as the fourth embryonic tissue (in addition to the three **embryonic germ layers** that arise during **gastrulation**), which suggests some people think neural crest cells are important. Even after neural crest cells migrate to new and distant tissues, they often retain visible signs of their neural lineage, such as the dendrites on melanocytes or the **odontoblastic process** of odontoblasts. To make migration easier, neural crest cells **express** a **matrix metalloproteinase** enzyme. This enzyme digests proteins found in the **ECM**. By now we hope remember the phrase *wound healing* ***recapitulates*** *development*. Matrix metalloproteinase enzymes are re-used to heal certain types of tooth wounds, but can also be involved with the breakdown of the **PDL** and loss of alveolar bone tissue.

When neural crest cells reach the pharyngeal arches, they secrete **FGF** and **BMP morphogens**, which **antagonize** each other, producing a striped **pattern** along the **ectoderm**. Some regions are induced to form **tooth buds**, the in-between regions form **oral mucosa**.

Differentiation of mesoderm

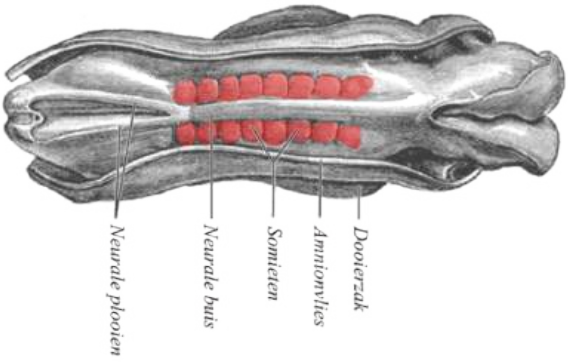

Figure 6.29: Somites in a neurula-stage embryo. Legend (from Dutch): dooierzak = yolk sac, amnionvlies = amniotic membrane, somieten = somites, neurale buid = neural tube, neurale plooien = neural folds. Image credit: "Plate 20" by Henrey Grey is in the Public

Somite formation

During the same time as **neurulation**, **mesoderm** undergoes **differentiation**. Mesoderm starts off as an **amorphous** layer of **mesenchyme**[←]. Then, regions of mesenchyme pinch off in repeating spherical structures called **somites**, forming segments along the anterior-to-posterior axis of the embryo. The **fate** of the somites is to become solid organs, either repeating units of connective tissue such as the ribs and vertebrae, or repeating units of muscle tissue such as the rectus abdominus and intercostal muscles. The formation of each somite involves a **mesenchymal-to-epithelial transition**, some **mesenchymal stem cells** differentiate into an epithelium that separates one somite from the next. Because they form in left/right pairs, the production of matching lateral structures is relatively simple (such as left and right biceps brachii muscles). On the other hand, formation of a single structure from somitic mesodem (such as one sternum) requires **fusion** of two bilateral structures.

There are other regions of mesoderm besides somites and **notochord**, such as splanchnic, lateral plate and paraxial mesoderm. Differentiating between all types of mesoderm is not essential for understanding clinical concepts in dental hygiene.

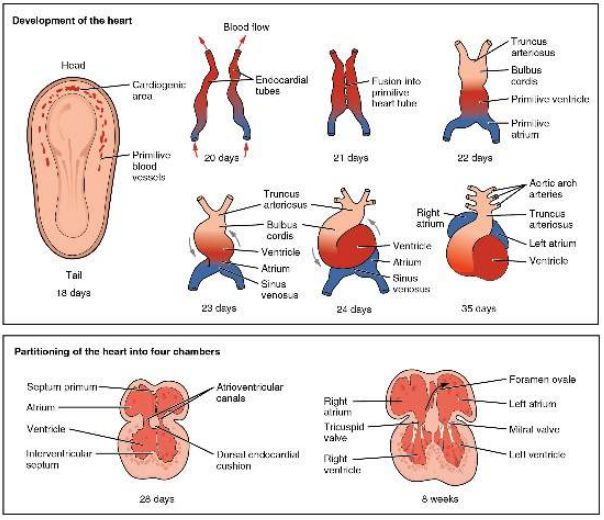

Figure 6.30: Development of the heart from two heart tubes. Image credit: "illustration" by OpenStax, is licensed CC BY 3.0

Formation of the heart

In the 3rd week of development, **mesoderm** begins to develop into blood, the heart and the circulatory system. **Mesenchymal stem cells** first differentiate into blood islands. Next, **angiogenesis** begins. Two large blood vessels fuse– with some help from **neural crest cells**— to form the primitive heart, which begins beating.

Differentiation of endoderm

Figure 6.31: Different embryonic stages highlighting the endoderm. Legend: yellow = endoderm, red = liver, green = gall bladder. Image credit: "Timeline of mouse liver development emphazing it" by Zorn, A.M., Liver

Formation of the pharynx

The **endoderm** that migrates interiorly during **gastrulation** extends its way towards the anterior end of the embryo. This forms the **primitive foregut**, which later becomes the pharynx. The term "primitive" is used because it has only one opening, there are no oral or nasal cavities. Those develop later from **ectoderm**. Formation of the face and pharynx are covered in detail in Chapter 7.

Figure 6.32: You at four weeks gestation .

4th week

We are now only up to the 4th week of development. A lot has happened! At this time, the embryo continues to undergo local regions of **proliferation**, **patterning**, **fusion** and the other processes covered at the beginning of the chapter. This leads to the formation of rudimentary (basic) structures. When a structure first becomes visible from the surface of an embryo, it is often called a **placode** (an **ectodermal** thickening). The cells

in a placode are **fated** to become an organ, but their overall shape is no more than a raised bump or shallow pit. By the 4th week, head-related organs listed in Table 6.3 begin to develop.

Structure	Fate
Optic placodes	primitive eyes
Otic placodes	primitive ears
Nasal placodes	primitive nose
Pharyngeal arches	so many things they get their own chapter
Oro-pharyngeal membrane	primitive mouth (not open yet)

Table 6.3: Primitive structures formed during the 4th week of development.

Clinical applications of early development

Disturbances in these early stages of development are usually so severe the embryo does not survive to become a fetus, let alone be born. However, clinical conditions exist related to these early stages and to oral health.

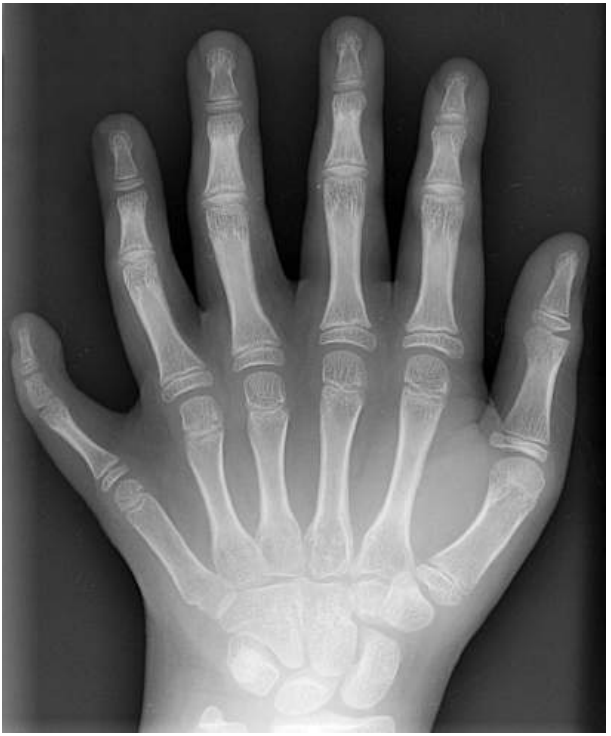

Figure 6.33: An example of body plan duplication. Image credit: "Polydactyly 01 Lhand AP" by Drgnu23 is licensed under CC BY-SA 3.0

Homeobox gene mutations

Mutations to **homeobox gene** can cause *entire structures* to be missing, or to develop in the wrong location. Early experiments on flies led to observations of legs where antennae should be located, or antennae where wings should be located. There are a few homeobox-related genetic disorders in humans. Homeobox-related disorders are rare because the mutations are usually lethal, but when they aren't the conditions are often severe. A small list is found in Table 6.4

Syndrome	Homeobox gene involved	Symptoms include
Axenfeld-Rieger Syndome	FOXC1, PITX2	Mostly eye-related, sometimes hypodontia and microdontia
Autosomal dominant syndactyly	HOXD13	Syndactyly (fused fingers or toes)
Guttmacher syndrome	HOXA13	Polydactyly (extra fingers or toes)
Ectodermal dysplasia	HOXC13	*Absent or under-developed hair follicles, teeth, nails and/or sweat glands*

Table 6.4: A few examples of homeobox mutations in humans.

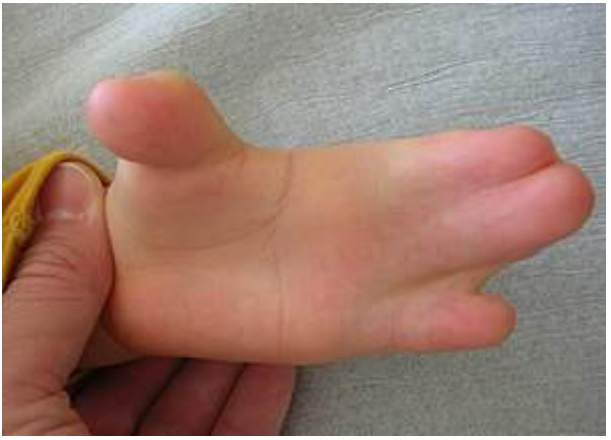

Figure 6.34: Lack of apoptosis leads to syndactyly. Image credit: "Hand of a child with Apert syndrome" by Gzzz is licensed under CC BY-SA 4.0

Apert syndrome

Apert syndrome is caused by a mutation in the **receptor** for the **morphogen FGF**. It is categorized by a wide range of symptoms, including cranial deformations and syndactyly (fusion of digits). FGF is involved in the formation of the

pharyngeal arches[←] (covered in the next chapter), which explains the craniofacial abnormalities. Relevant to this chapter is the ability of FGF (like many ***growth*** **factors**) to inhibit **apoptosis**[←]. Having a mutation that causes an FGF receptor to be on all the time inhibits apoptosis in the hand and foot paddles. Regions of apoptosis are required to produce fingers and toes, hence this mutation leads to syndactyly. Partial disturbances to FGF signals can lead to partial syndactyly, or webbing of the fingers or toes.

Otherwise there aren't many mutations to apoptosis **signal transduction cascade**[←] **genes** that lead to **congenital disorders**. Does this mean apoptosis isn't important to development? No, quite the opposite, it is essential. As a result, humans have redundancy when it comes to triggering apoptosis. We mentioned earlier that **loss-of-function** mutations require mutating both **alleles** for a physical change to occur, like only needing one of your two feet to operate the brakes on a car. Now imagine having 8 legs. Studing the roles of apoptosis in the development of mice shows that removing both alleles of the pro-death signal BAX has no effect, nor does removing both alleles of the pro-death signal BAK, but removing all 4 causes mice to die during the embryonic period after failing to form a functional nervous system and heart. Removing BOK, BAX and BAK is even more lethal (if you are wondering how something can be more lethal, here is further reading). The important concept here is that apoptosis is absolutely esential

to development, and as a result human cells are really good at doing it. And people who study apoptosis must be very good at tongue-twisters.

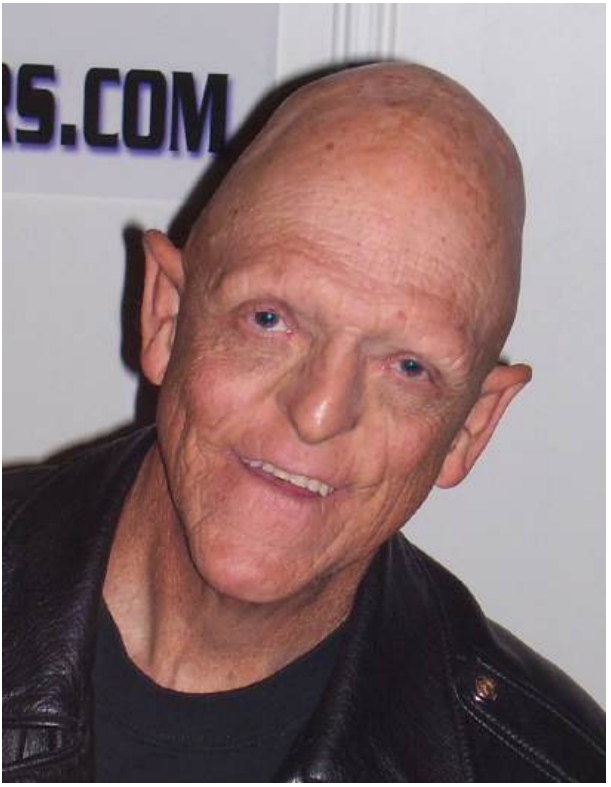

Figure 6.35: Image credit:: "Michael Berryman, actor" by Stefan Borggraefe is licensed under CC BY 4.0

Ectodermal Dysplasia (part 1 of 2)

Problems with the **induction** of **neural crest cells** during **neurulation** leads to disturbances in the formation of teeth,

hair follicles, salivary glands and other structures. What these have in common is they are all specialized structures of the **ectoderm**, induced by neural crest cells to **differentiate**. In a healthy embryo, ectodermal cells receive **morphogens** which activate or inactivate the correct **transcription factors** to trigger differentiation into neural crest cells. Neural crest cells migrate to distant regions of the body, determine their location by interacting with morphogens in the **ground substance**[←], and release other morphogens to induce regions of ectoderm to differentiate into sweat glands, salivary glands, tooth buds or hair follicles. Disruption of this process leads to a condition named **Ectodermal Dysplasia**.

This group of syndromes is rare, with only 7,000 cases worldwide, but there are at least 40 different genes implicated. Compare that to Sickle Cell Disease, which currently affects over 100,000 *Americans* (predominantly African Americans, Hispanic Americans, Greek Americans, Turkish Americans and Italian Americans), all due to mutations in a single **gene**, Hemoglobin-Beta. The point of this comparison is to highlight *when* these genes are **expressed**. The induction of ectodermal stem cells to **proliferate** and differentiate into different appendages is complex and occurs during embryogenesis. Mutation in the morphogens, the morphogen receptors, the second messengers or **transcription factors** and downstream genes that are activated to induce differentiation are all possible targets that cause Ectodermal Dysplasia. Mutations in these genes in a

neural crest cell leads to a disruption in any of the subsequent cells induced by this new cell type, similar to the way tackling the ball carrier in soccer (football) disrups the gameplay of any of his or her potential passing targets. By comparison, only red blood cells express hemoglobin-beta, and since they are **terminally differentiated** cells, they do not become any other cell type. Obviously red blood cells are an important cell type, one that develops early in embryogenesis, but the symptoms of Sickle Cell Disease present less of a spectrum than the types of diseases we have been discussing, such as Ectodermal Dysplasia.

We discuss Ectodermal Dysplasia further in Chapter 8 as we cover **tooth eruption**[←].

Figure 6.36: Gaten John Matarazzo III, actor and CCD activist. Image credit: "Gaten John Matarazzo III" by Gage Skidmore, is licensed CC BY SA 3.0

Cleido-cranial Dysostosis

Cleido-cranial dysostosis (CCD) is a **congenital disorder** caused by a mutation to a **transcription factor** required for the **differentiation** of **bone** and teeth. It is required to trigger osteo-chondro-progenitor cells to exit the cell cycle and differentiate into **osteoblasts**. It is also re-used to **induce** the differentiation of **odontoblasts**. Furthermore, after teeth have formed, this transcription factor is re-used to activate the

expression of a **matrix metalloproteinase** enzymes necessary for remodeling of the alveolar sockets. Without this enzyme, retention of deciduous teeth occurs. Dental implants or dentures (such as the ones the actor and CCD-philanthropist Gaten John Matarazzo III received in Fig. 6.36) are the preferred treatment. In addition, a person with CCD may have small clavicles and changes to shape of the skull– those are bones that form by **intra-membranous ossification**. This illustrates two major concepts in development. First, many structures form one way, but are **remodeled** later to serve a different function (teeth form by folding inwards, they later move outwards). Secondly, many different patterns in embryology are re-used (**recapitulated**), such as the removal of tissue during **neural crest cell** migration or the removal of tissue during **tooth eruption**[←].

Figure 6.37: Illustrations of Spina Bifida, which occurs due to incomplete closure of the neural tube. Image credit: "3D Medical Animation still shot of Spina bifida in an infant" by scientific

Spina bifida

Incomplete closure of the **neural tube**, or **spina bifida**, can result from a lack of adequate levels of folate during pregnancy. There are other less-common risk factors, such as taking certain anti-siezure medications or poorly managing diabetes during pregnancy. Folic acid is used in a wide variety of biological processes, but it is believed its role in **methylation of DNA** during **differentiation** is most important in spina bifida. Because **neurulation** occurs so early in development (week 4), waiting until a woman knows she is pregnant to prescribe folic acid supplements is often too late to be effective. Prescribing supplements to women who beleive they *could* get pregnant is better, or supplementing common foodstuffs like flour is even more effective for a populace as a whole.

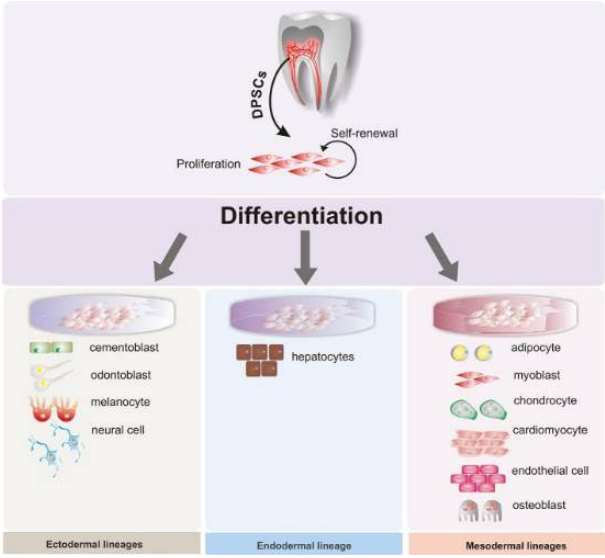

Figure 6.38: Differentiation of stem cells limits their cell fate (e.g. neurome senchymal stem cells of dental pulp can potentially differentiate into odontoblasts or heptatocytes, given the right morphogens, but odontoblasts can't become hepatocytes and hepatocytes can't become odontoblasts). Image

Stem cell therapies

credit: "Figure 3" by Beatriz A. Roldas-Junco et al, is licensed CC BY 3.0

When cells **terminally differentiate**, they permanently inactivate un-needed **genes** by **methylation** and storage around **histones**. Researchers are learning ways to reverse this process, and guide differentiated cells to revert to a **stem-cell**↩ state. This raises the possibility of promoting **regeneration** of tissues that do not otherwise regenerate, removing the need for tissue grafting or transplantation. Because some dental tissues do not regenerate well, there is potential for these technologies to be applied to the oral cavity, such as growing biological dental implants instead of using metals and ceramics. However, more interest has been placed on acquiring **mesenchymal stem cells** *from* dental tissues. For instance, stem cells isolated from maxillary third molars have been used in clinical trials to improve healing and reduce the need for transplanted tissue in maxillofacial surgery. Because of their potential to **differentiate** into a wide array of cells, great interest has been placed on collecting dental stem cells to treat diseases unrelated to the oral cavity. With the correct **morphogens** and plenty of dental stem cells, it may be possible to reverse the damage caused by disease such as Alzheimer's Disease (AD) and Parkinson's Disease (PD), spinal trauma, myocardial infarction (heart attack), and Muscular Dystrophy (MD). The

lineage of the cells that produce dentin, pulp, cementum and the periodontium helps explain the link from teeth to neurodegenerative disoders. What else will you do with those extracted third molars?

< Chapter 5 * navigation * Chapter 7 >

Chapter review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:
https://openoregon.pressbooks.pub/histologyandembryology/?p=49

7.

PHARYNGEAL ARCHES

- Overview of the pharyngeal arches
- Development of external structures
  - Fronto-nasal process
  - 1st pharyngeal arch
    - maxillary process
  - 2nd-6th pharyngeal arch
    - Pharyngeal grooves
    - Pharyngeal pouches
- Development of internal structures
  - Pituitary gland
  - Salivary glands
  - Palate
  - Tongue
  - Tonsils
- Clinical application of pharyngeal arch development
  - Cleft lip/palate

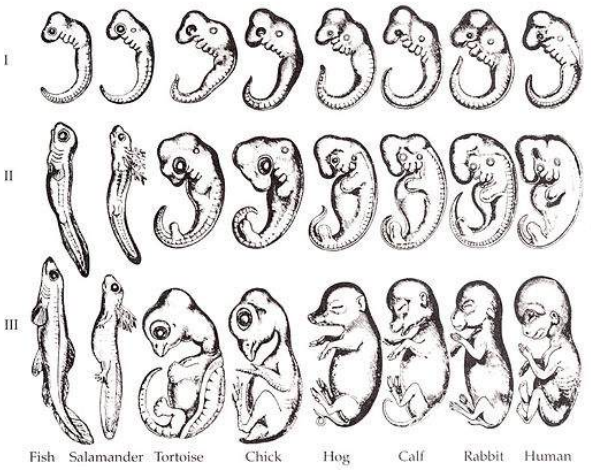

Figure 7.1: The mechanisms of early development are highly conserved, as seen in the similarity of these vertebrate embryos. Image credit: "Embryo drawings", by Ernst Haeckel, is in the Public Domain CC0

Overview of the pharyngeal arches

Ontogeny recapitulates phylogeny —Ernst Haeckel

Nothing in biology makes sense except in the light of evolution—Theodosius Dobzhansky

I suppose it is tempting, if the only tool you have is a hammer, to treat everything as if it were a nail— Abraham Harold Maslow

The purpose of this overview section is to conceptually prepare you for a complicated series of steps that go through unexpected transitions. To do so, we discuss the link between evolution and **development**. If the link seems complicated, you are correct, it **is** complicated. Evolution explains *why* transitions happen, your job is to learn *what* those transitions are.

The human embryo is taking shape. It might seem odd that we look a bit wormy at this early stage, and next we **morph** into something fishy in appearance. The phrase *ontogeny recapitulates phylogeny* is hallowed among developmental biologists. It means, more or less, our early embryonic stages look like our evolutionary **lineage**. That is what the famous drawing (Fig. 7.1) shows: trace our evolutionary lineage (over millions of years), it looks like our embryonic lineage (the 9 months before birth). In this chapter, we see human embryo grow what look like **gill arches**, then remodel those gill arches into other structures: a mandible and maxilla, ears, and salivary glands. Why not just grow a mandible and ears? Why go through the middle gill arch step? Why do we resemble worms, then fish, and only much later little people? The better question, from the perspective of an evolutionary biologist, is

why would we *stop* developing the way our ancient ancestors did. Would that help us avoid predation, have more offspring or have superior offspring? The answer is apparently no.

That's the first big concept: evolution is driving, you are a passenger. There may very well be a faster route from *there* to *here*, but it won't help pointing that route out now. The next big concept is we rarely see new structures arise in evolution *de novo* (from the beginning). Otherwise we'd have wheels, not inefficient legs. Instead, we observe structures changing slightly over time (*i.e. we see a lot of different types of legs*). This leads to different species having structures with similar *shapes* but different *functions*. We call such structures **homologues**. A bat's wing, a whale's flipper, a horse's leg and your arm have the same basic skeletal pattern: 1 bone, 2 bones, 4 bones (ignore the thumb and big toe, they were added later). All 4 of these limbs are homologous to one another. Their size, shape and purpose are different, but they share the same basic design. It is more efficient to morph a leg into a flipper than it is to design a flipper *de novo*. Some homologues have very different functions, such as human lungs and fish swim bladders. Conversely, similarity does not mean homology. A fly wing, chicken wing and bat wing, despite their similar function, are not homologous. Similarly, the panda's (6^{th}) thumb is not a homologue of the human thumb, either. This becomes more apparent when you study the **lineage** of different species and compare it to their **development**, a science called

evolutionary developmental biology (evo-devo). Can you summarize the difference between our first list of species with homolgous limbs versus the second list of species with non-homologous limbs? In this chapter, when you look at the **pharyngeal arches** in humans, you might ask why are they numbered 1, 2, 3, 4... 6? Who decided 6 comes after 4? The answer lies in observing homology across species.

Homologues also exist within a single organism. When we discuss homology between two human structures, instead of evolutionary **lineage**, we are discussing **developmental** lineage. That is why your arm and leg share the same skeletal pattern, they are homologues. That is also why there is so much similarity between the skin and **oral mucosa**. Evolution by gene duplication involves fewer steps than generating **DNA** instruction *de novo*. Small changes in duplicate DNA can lead to big changes in **morphology**. Therefore, it is both faster and easier to tweak a working design than create a whole new design *de-novo*. Human development is rife with examples of **recapitulation**, where a basic process is re-used with small changes.

Keep in mind humans did not evolve from modern-day worms or fish. Evolution is not a ladder humans have ascended. Modern day fish are as highly evolved as humans are. But the most recent relative a trout and humans share resembled a fish more than a human. The most recent relative a trout and a tapeworm share resembled a worm more than a fish. Now reverse that: our embryonic stages initially look strikingly like

some sort of parasitic larva, then more fishy, then kind of lizardy, and finally mammally. Another way to say that is **ontogeny recapitulates phylogeny**.

Figure 7.2: Brachial arches supported by cartilage. Image credit "Gill arches supporting the gills in a pike" by Uwe Gille is licensed under CC BY 3.0

Now is a good time to say this again: evolution explains *why* strange transitions happen. Your job is to learn *what* the transitions are. Before we finish this overview, we would like

to point out one more level of complexity. In this chapter you must differentiate between structures with similar names, including the pre-maxillary segment, inter-maxillary segment and maxillary processes, pharyngeal arches, pharyngeal grooves and pharyngeal pouches, and more. Furthermore, most of these structures have multiple names, such as pharyngeal arches, gill arches and branchial arches, which can make comparing this text to others tricky.

If you feel like you need more detail or a different description, here are good resources on embryology and evolution:

- The Embryology Education page
  - by Dr. Mark Hill at the University of New South Wales, Sydney, Australia
- Understanding Evolution
  - The University of California Museum of Paleontology, Berkeley

Development of the external structures

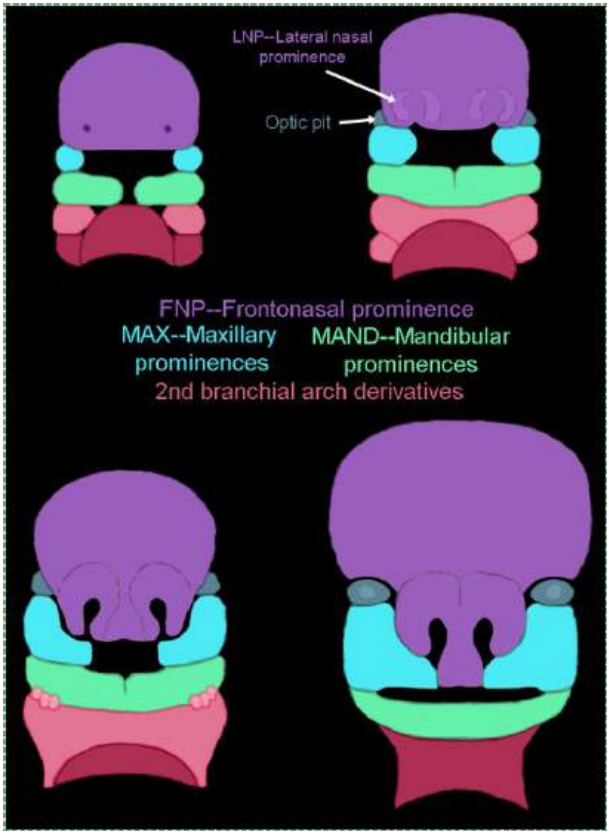

Figure 7.3: The pharyngeal arches, and the body parts into which they develop. Image credit: "Illustration of the seven facial prominences that give rise to specific regions of the face",

At 4 weeks, the face begins to develop. It is composed of several parts (or **prominences**), listed in Table 7.1. To use the word *prominence* means these are not necessarily the same type of thing, just some things that stand out. The prominences we focus on are **homologues** of **gill arches** (gill arches are in Fig. 7.2, their human homologues are in Fig. 7.3 upper left). They are quickly remodeled (Fig 7.3, lower right). These are the **pharyngeal arches** (or branchial arches, although technically the name branchial arches should only be used for vertebrates with gills). Pharyngeal arches are paired segmental bulges on the lateral borders of the primitive pharynx. The two halves of each arch grow medially across the face and neck (the ventral side) and **fuse** to form an arch. One pair of *processes* (not arches) tries to fuse, but run into a bump called the **inter-maxillary segment**, and fuse with it instead.

The facial prominences
2 halves of the Mandibular (1st) arch
2 Maxillary processes
Fronto-nasal process

Table 7.1: The 5 facial prominences include one pharyngeal arch.

In lampreys, seven arches become seven pairs of gill supports on the sides of the head. In bony fish, one arch is **remodeled** into a mandible and middle ear structures, while five become gill supports (only six form in the first place). Humans remodel the first arch the same way as bony fish, plus we remodel our other four (we have no gill supports). If you've looked at a lamprey recently, you might have this question: if humans remodel the first arch to produce jaws and teeth, but lamprey do not, what are lamprey teeth? They aren't enamel or dentin!

animation of embryo

Figure 7.4: Illustration of the pharyngeal arch apparatus at week 6, with a cutaway to show how they line the ventro-lateral walls of the pharynx. Note: the bumps around the pharynx are pharyngeal arches, and are not to be confused with the more caudal (posterior) bumps that are somites.

Formation of the pharyngeal arches

At the 4th week, the **primitive foregut** has not **fused** with **ectoderm**, so no oral or nasal cavities exist. There is visible as a depression in the ectoderm known as the **stomodeum** (the primitive mouth, Fig. 7.9). Think of this as a bowl-shaped **invagination**. A small part of that depression, the **oro-pharyngeal membrane** (or bucco-pharyngeal membrane), separates the stomodeum from the anterior end of the primitive foregut (which invaginated during **gastrulation**). The primitive foregut is lined by **endoderm**, and the stomodeum is ectoderm. Almost everywhere, there is **mesoderm** between endoderm and ectoderm. However the oro-pharyngeal membrane contains only endoderm and ectoderm. Some amphibians use the oro-pharyngeal membrane to breathe underwater. It is thin enough to allow gas exchange to occur, but prevents the lungs from filling up with water. Humans develop a stomodeum, then remove it.

The **pharyngeal arches** form under the instruction of **homeobox genes**. Activation of homeobox genes (in an anterior-to-posterior pattern) **induces** the **transcription** of a program of other **genes**. These other genes include **morphogens** which induce growth of all 3 **embryonic germ layers**. This causes outward **budding** on the lateral borders of the pharynx. 5 arches form, starting with arch number 1 (the most anterior arch) and ending with arch number 6 (the most posterior arch). Each arch grows medially

to fuse with its partner. Imagine drawing the letter **U** using two pens. Starting from each corner, bring both pens downwards and meet in the middle. You have drawn an arch. Now, instead of drawing a **U** on paper, draw on your head. Place both pen tips below your ears and draw across your face, meeting at your mandibular symphysis. You have drawn arch number 1. Now draw 4 more, each U below the next. There, you have 5 arches.

We now have 5 mounds on the outside of the embryo. The valleys between the mounds are called **pharyngeal grooves** (Fig. 7.6). At t same time as mounds are forming on the *outside* of the embryo, things are happening *inside* as well. Within the primitive pharynx, localized growths form **invaginations** known as the **pharyngeal pouches** (Fig. 7.7). If you remember **GAP**, this mnemonic may help you to remember the names of these structures from external to internal: Groove, Arch, Pouch. Or, if you prefer the name pharyngeal *cleft* rather than groove, the mnemonic becomes CAP. These structures appear one pair at a time, from anterior to posterior, and their **fate** is listed in Table 7.2. Because the arches are quickly remodeled into other structures, we say that they are *transient* structures. Their brief existence explains how and why the adult structures in Table 7.2 have the **morphology** that they do. Recall that in **embryology**, anterior-to-posterior means head-to-toe (rostral-to-caudal), not ventral-to-dorsal.

Arch #	Name	Ectoderm and neuro-ectoderm fate	Groove fate	Mesoderm and neuro-mesenchyme fate	Endoderm (pouch) fate
1st	Mandibular arch	Maxillary process –> upper lip epidermis	n/a (this is not an arch)	Dermis, maxilla, zygomatic, palatine, vomer	n/a (this is not an arch)
		Lower lip epidermis, Trigeminal nerve (CNV).	External acoustic meatus	Dermis, mandible, malleus, incus	Eustachian tube
2nd	Hyoid arch	Epidermis, Facial nerve (CNVII)	disappears	Dermis, most of the hyoid bone, stapes	Palatine tonsils
3rd		Epidermis, Glossopharyngeal nerve (CNIX)		Dermis, the rest of the hyoid bone	Thymus, Parathyroid glands
4th		Epidermis, Vagus nerve (CNX)		Dermis, Thyroid cartilage, epiglottis	Parathyroid, Thyroid glands

Table 7.2: The pharyngeal arches and their fate, separated by embryonic germ layers.

5^{th} never forms					
6^{th}		Epidermis, Vagus nerve (CNX)		Dermis, the other laryngeal cartilages	Larynx tissues

animation of pharyngeal arch formation

Figure 7.5: Growth of the arch pairs, as well as budding of the maxillary process off the mandibular arch, animated from a lateral view, early week 4 embryo. Legend: blue = ectoderm, red = mesoderm, yellow = endoderm, arrows = neural crest cell migration.

The 1st pharyngeal arch

During **neurulation**, **neural crest cells** undergo an **epithelial-to-mesenchymal transition**, migrate away from the **neural tube** and into the **mesoderm** of the **pharyngeal arches**. Once there, neural crest cells **differentiate** into **neuro-mesenchymal stem cells** (NMSCs). These cells guide **remodeling** of the pharyngeal arches. The first step is to begin forming an upper jaw off the lower jaw. The neuro-mesenchymal stem cells release **morphogens** which **induce** localized **proliferation** within the **mandibular arch** (the 1st pharyngeal arch), forming the **maxillary processes** (Fig.7.5). The rest of the mandibular arch grows medially to form the lower jaw. The maxillary processes also grow medially to form the upper jaw. You might expect there to be a mouth between the upper and lower jaw. Not yet, between them is **stomodeum**, which at this time is still not connected to the **primitive foregut**.

Tissue also grows on the medial and lateral side of each nasal placode (Fig. 7.6). The two **medial nasal processes** fuse to form the **inter-maxillary segment** (or globular process). Because some teeth develop from the inter-maxillary segment, we will discuss it more. The two **lateral nasal processes** develop into the alae of the nose. This is the last mention of the lateral nasal processes.

animation of pharyngeal arch formation, ventral view

Figure 7.6: Fusion of the pharyngeal arches. Growth occurs in a lateral-to-medial direction, while fusion occurs in an anterior-to-posterior (rostral-to-caudal) direction. Illustrated from the ventral view, early week 4. Legend: Blue = ectoderm, red = mesoderm or neuro-mesenchy

me, yellow = endoderm

Fusion of the 1st pharyngeal arch

The **mandibular arch** forms on the lateral edges of the embryo during the 4th week of **development** and grows medially. The two halves **fuse** by the end of the 4th week of development, creating a single structure that becomes the mandible, plus some nearby tissue. For the two halves of the mandibular arch to grow medially, **mesenchyme**← is removed. This requires **expression** of the enzyme **hyaluronidase**, which digests **hyaluronic acid**← found in **ground substance**←. This allows epithelial cells to **fuse** with epithelial cells from the other half of the arch. Fusion requires matching **CAMs**← and **desmosomes**←. The **mesoderm** of one arch also fuses with mesoderm of its partner, which requires matching the correct **integrin**← to **fibronectin**←.

Later, **neuro-mesenchyme** of the mandibular arch **differentiates**← into a cartilaginous structure known as **Meckel's cartilage**. Parts of Meckel's cartilage undergo **endochondral ossification** to become part of the mandible and middle ear bones, the rest undergoes **apoptosis**←.

Fusion of the maxillary processes and inter-maxillary segment

The pair of **maxillary processes** grow medially in the 4th week, but run into the **inter-maxillary segment** and **fuse**

with it by the 10th week of **development** (Fig. 7.6). The upper lip, therefore, is formed of three parts: the left maxillary processes, the right maxillary process, and the inter-maxillary segment (the lower lip is formed from two parts: the left and right halves of the **mandibular arch**). The **philtrum** is the middle section derived from the inter-maxillary segment. It does not serve a function in humans, it happens to be there because of how we develop (like the choice of wood used in the record player shelf mnetioned in the preface). Anatomy textbooks typically describe functions of organs based on their adult form (for example, read chapter 1 of the Openstax Anatomy and Physiology textbook). Some have tried to describe the function of the human philtrum based on its shape and location. If you study **development** (**ontogeny**) and evolution (**phylogeny**), anatomy can make more sense.

animation of pharyngeal pouches

Figure 7.7: Closer view of the fate of the pharyngeal grooves (exterior) and pharyngeal pouches (interior). Legend: Blue = ectoderm, red = mesoderm or neuro-mesenchyme, yellow = endoderm

Fate of the pharyngeal grooves and pharyngeal pouches

If you were a fish, most of the **pharyngeal grooves** would develop into gills. As creatures of the land, grooves are either filled in or develop into other useful structures. Between the **first** and **second arches**, the first pharyngeal groove

invaginates further and forms a tube that becomes the external acoustic meatus. It is lined by **ectoderm**. The other pharyngeal grooves disappear. One the opposite side, within the primitive pharynx, the **pharyngeal pouches** invaginate and grow towards the grooves. The first pharyngeal pouch elongates into a tube that is fated to become the Eustachian tube, connecting the pharynx to the middle ear (the internal acoustic meatus forms when bone tissue grows around cranial nerve VIII, connecting the inner ear to the brain). The Eustachian tube is lined by **endoderm**. The other pharyngeal pouches invaginate and form tonsillar and glandular tissue. Tonsils are covered by an endoderm-derived epithelium, but the white blood cells of the **germinal centers** migrate there from bone marrow (which, like most connective tissues, is derived from **mesoderm**). The mesoderm and **neuro-mesenchyme** between the first pharyngeal groove and pharyngeal pouch form middle ear structures, including the malleus, incus and stapes bones.

Development of the palate and other internal structures

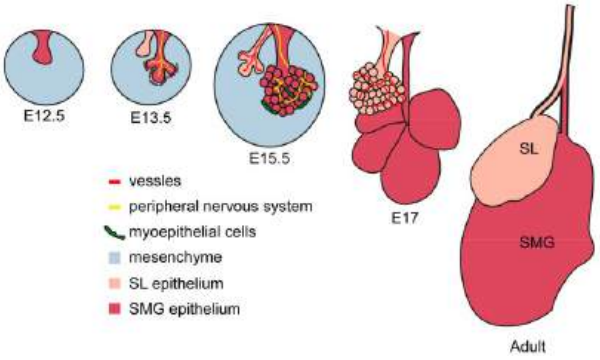

Figure 7.8: Development of sublingual (SL) and submandibular (SMG) salivary glands (in mice). Image credit: "Embryonic development of murine SMG and SL glands." by Cristina Porcheri and Thimios A. Mitsiadis is licensed under CC

Development of the salivary glands

The salivary glands develop in a process that begins similarly to **neurulation**←. **Placodes** form on the **ectoderm** starting between weeks 4 through 12. Notice this means salivary glands develop from the *outside* of the embryo, not from **pharyngeal pouches**. Growth of placodes is under the control of **morphogens**← including members of the **FGF** family. Salivary gland placodes grow and **invaginate** from there. Eventually, **stem cells**← **differentiate**← into a number of epithelial cell types. The ducts are mostly **simple cuboidal epithelia**←. These epithelial cells have different functions based on their distal-to-proximal location along the duct. The differentiation of salivary gland cells along a proximal-to-distal axis is guided by **planar cell polarity**← morphogens, including members of the **Wnt** family. Some cells in the **acini** differentiate into **myo-epithelial cells**. Myo-epithelial cells are **ectodermal** by **lineage**, and therefore epithelial, despite looking and acting like smooth muscle cells. Myo-epithelial cells **express genes** mostly used by muscle cells. For an epithelial cell to share this **morphology** it must reverse earlier decisions it made. **Histone** packing and **DNA methylation** is removed from genes shut down as ectodermal cells initially adopted an epithelial **fate**.

animation of pituitary formation

Figure 7.9: Lateral view of an embryo, week 4, showing the opening of the mouth, division of the oral and nasal cavities, and the invaginati on of the pituitary gland.

Formation of the pituitary and mouth

Inside the **stomodeum**, a single **invagination** of **ectoderm** forms, along the medial portion of the roof (so far, processes and pouches have been left/right pairs). This invagination is named **Rathke's pouch**. It grows and meets a downward budding of neuro-ectoderm. These two **fuse** to form the pituitary gland. The ectoderm forms the glandular half (adenohypophysis), and the **neuro-ectoderm** forms the

infundibulum and neural half (neurohypophysis) of the pituitary gland. Rathke's pouch fills in as the two halves of the pituitary fuse, but it is possible a small depression will remain.

At the same time, the **oro-pharyngeal membrane** undergoes **apoptosis**←. This connects the **primitive foregut** and **stomodeum**, forming the primitive **oro-nasal cavity**. Finally, the mouth and anus are connected! The **oral mucosa** therefore develops from **ectoderm** of the **stomodeum**. The lining of the pharynx is derived from the **endoderm** of the primitive foregut. The lining of the tongue is a mashup of the two.

Figure 7.10: Illustration of the fusion of the palate, inferior view. Legend: im: inter-maxillary segment, ps: palatal shelves, ns: nasal septum.

Formation of the palate

Shortly after the lips begin forming, the **palate** begins to form as well, dividing the primitive **oro-nasal cavity** into a more mature oral cavity and nasal cavities. The palate has 3 parts that **fuse** with each other, and with the nasal septum. The **primary palate** grows from the **inter-maxillary segment**, and two **palatal shelves** (or secondary palate) grow from the **maxillary processes**.

Structure	Lineage	Forms during	Fuses with
Primary palate (pre-maxillary segment)	inter-maxillary segment (globular process)	6^{th} week	Secondary palate: 9^{th} week
Secondary palate (Palatal shelves)	Maxillary process	7^{th} week	The other palatal shelf: 9^{th} week
			Primary palate: 9^{th} week
			Nasal septum: 12^{th} week

Table 7.3: Summary of the development of the palate.

animation of palate fusion

Figure 7.11: Fusion of the palatal shelves (purple) with the nasal septum, anterior cross-sectional view. Note the developing tongue moves out of the way before fusion of the palatal shelves occurs.

The first part of the palate to form is the **primary palate**, which develops from the **inter-maxillary segment**. When it forms, it partially divides the future oral and nasal cavities (Fig. 7.9). Next, two **palatal shelves** grow off of the **maxillary processes** (Fig. 7.10 and 7.11). The palatal shelves first grow inferiorly, then change direction and grow medially. At this

time, the developing tongue must move out of the way. This allows the palatal shelves to meet and **fuse** with the primary palate, as well as each other (forming the **secondary palate**). The fusion happens in an anterior-to-posterior direction. All of this growth is directed by **morphogens**, including **FGFs** and **BMPs**.

Maxillary incisors develop from the **primary palate**, while maxillary canines, pre-molars and molars develop from the **secondary palate**. At the 3-way corner where the primary palate and the two palatal shelves fuse, a small hole remains, the **incisive foramen.** The incisive foramen houses the nasopalatine artery and vein and a branch of the trigeminal nerve. The **oral mucosa** above this foramen has a bump named the incisive papilla**,** which shares more in common with olfactory epithelium than it does **oral epithelium** (it is the **homologue** of the vomeronasal organ found in many vertebrates). Where the two palatal shelves fuse leaves a ridge on the overlying oral mucosa called the (median) **palatine raphe**.

Keep in mind that we are referring to the entire palate. Much later, anterior portions of palate **mesoderm** undergo **endochondral ossification** and form the palatine bones and the palatine processes of the maxilla (the hard palate). The rest of palatal mesoderm differentiates into muscle tissue, forming the soft palate. Time out for spelling: this is the palate, not an artist's palette of colors, nor a pallet used in shipping, not even a plate on which we place a tasty dinner. Therefore, foodstuffs

shipped on a pallet, cooked by a chef with a harmonious palette, served to us on a plate, will be enjoyed for their flavor when they hit our palate because we have a refined palate (an appreciation for flavor). Got it? English is fun.

The nasal septum grows inferiorly at this time. It fuses with the completed palate around the 12th week of development. This creates paired nasal cavities. Initially, **mesoderm differentiates**← into the ethmovomerine cartilage, and then partially undergoes **endochondral ossification** to generate a bony portion (parts of the ethmoid and vomer) and leaving a cartilaginous portion. Ossification begins from a lateral pair of ossification centers, therefore the early septal bones develop as two layers (lamella) which fuse to form a single bony septum. The two layers are not the ethmoid and vomer (top-to-bottom) portions, but left and right. Why does a single septum develop from a left and right half? The same reason as the mandible: they are **induced** by **neural crest cells**←, which arise as distinct groups of cells on the left and right side of the **neural tube**. Taking another look at the illustrations of **neurulation**← may help.

animation of embryo

Figure 7.12: Illustration of the pharyngeal arch apparatus at week 6, with a cutaway to show the pharynx.

Development of the tongue

The tongue is a hybrid structure. It forms from multiple parts making its **development** complicated. Tongue development begins during the 4th week, after the **pharyngeal arches** fuse along the bottom of the **primitive foregut** and future oral cavity. The tongue develops from first four pharyngeal arches (although the contribution of the 2nd arch mostly disappears). Formation of the tongue involves **proliferation** and **fusion**, followed by **apoptosis**↩ to give the tongue mobility. The tongue is connected to four cranial nerves. That seems like a lot of nerves, doe it really need that many? The innervation of the tongue is easily explained by its development: four arches correspond to four cranial nerve connections. The **oral mucosa**, **sub-mucosa** and musculature of the tongue are more complicated.

animation of tongue development

Figure 7.13: Development of the tongue from 3 of the first 4 pharyngeal arches.

During the 4th week, the left half of each **pharyngeal arch fuses** with the right half along the floor of the future oral cavity and pharynx. A single triangular-shaped **tuberculum impar proliferates** off the **first pharyngeal arch**, followed by two **lateral lingual swellings**. Because they come from the first pharyngeal arch, their lining is not **endoderm** like the other arches, but **ectoderm** from the **stomodeum**. As these swellings grow, the 3rd and 4th arch develop a swelling named the **copula**, which grows over the 2nd arch. **Fusion** of these structures occurs during the 8th week. The **median lingual sulcus** forms where the left and right lateral lingual swellings fuse. The **sulcus terminalis** forms where the 1st and 3rd pharyngeal arches fuse. This border between the anterior and posterior portion of the tongue is obvious due to the difference in **lineage** on either side.

animation of tongue development

Figure 7.14: Apoptosis is important for the development of tongue mobility.

Apoptosis of tongue tissue on the ventral side leaves the tongue attached at the base, and freer to move around . Apoptosis does not remove all the tissue on the anterior portion. A small amount of mucous membrane remains, named the **lingual frenulum**. An **invagination** forms posterior to the **sulcus terminalis** and grows deeper, forming the thyroid gland. This process is similar to the way the anterior pituitary or the **neural tube** form. It leaves behind a small depression named **foramen cecum**, which is a confusing name because foramen means hole, but this foramen fills in most of the way, making it more of a pouch. Similar to **Rathke's pouch**, it serves no purpose in humans, it's a remnant of epithelial tissue **proliferation**.

The **oral mucosa** of the tongue is complicated. The outer surface is a **stratified squamous epithelium** with two separate **lineages**. Because the anterior 2/3^{rds} of the **dorsal surface of the tongue** develops from the **mandibular arch**, it shares lineage with the surface of the **stomodeum**, which

is **ectodermal**. The **ventral surface of the tongue** also develops from the mandibular arch. However, the ectoderm undergoes apoptosis, allowing **endoderm** from the **primitive foregut** to cover the ventral surface. As a result, the epithelium of the anterior 2/3^{rds} of the dorsal surface is thicker, and more closely resembles the rest of the oral mucosa. The ventral surface has a thinner epithelial lining, and more closely resembles the lining of the pharynx. The dorsal surface of the posterior 1/3rd of the tongue, coming from the 3rd and 4th arch, is also endodermal.

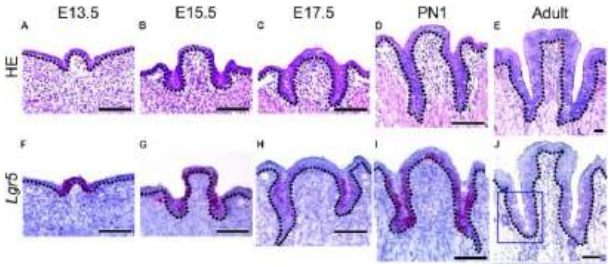

Figure 7.15: Invagination of lingual papillae. Image credit: "Morphology of developing CVP and expression patterns of Lgr5 and FGF10 during CVP development" by Sushan Zhang et al is

Development of the lingual papillae

Like the **oral mucosa** of the tongue, the **lineage** of the lingual papillae is either **ectoderm** or **endoderm**. The filiform and fungiform papillae develop from **invaginations** of the ectoderm, while the foliate and circumvallate from invaginations of endoderm. They form by a process similar to **neurulation**←. The growth and **differentiation**← of the papillae is guided by **morphogens**← secreted by underlying **neuro-mesenchyme**, including members of the **FGF** and **Wnt** families. **Keratinocytes** develop from an ectodermal precursor, while taste buds (including those in the soft palate and pharynx) are **induced** to develop from ectodermal or endoderm precursors starting the 8th week of development. Older evidence suggests taste bud differentiation depends on neural connections, but newer evidence suggests taste buds develop in response to the Sonic Hedgehog morphogen. By adulthood, both **keratinocytes** and taste bud cells continue to develop from a shared epithelial **stem cell**←, and both are replenished throughout life. Whether the lineage of this stem cell is endodermal, ectodermal or both is not known.

The connective tissue (**lamina propria**, **sub-mucosa**, and vasculature) of the tongue is derived from **neuro-mesenchyme**. The skeletal muscle tissue is derived from

somite mesoderm, guided by **morphogens**[←] secreted from the neuro-mesenchyme.

Clinical applications of pharyngeal arch development

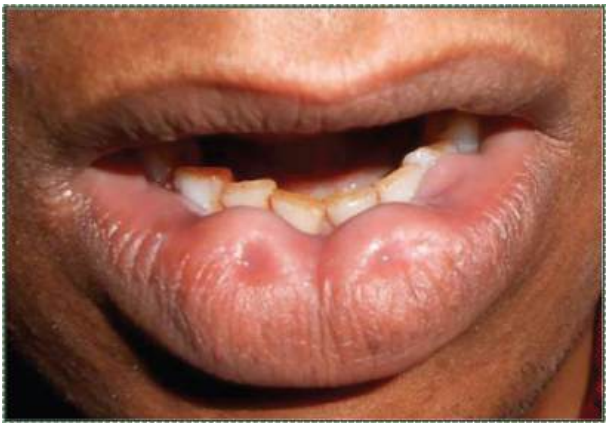

Figure 7.16: Example of commissural lip pits. Image credit: "Bilateral congenital lower lip" by Vela Desai is licensed CC BY-SA 4.0

Lip pits

Incomplete **fusion** of the **pharyngeal arches** leads to a

number of conditions, some more severe than others. Two **benign** conditions include a **lower labial pit**, which forms when the two halves of the **mandibular arch** fail to **fuse** completely.

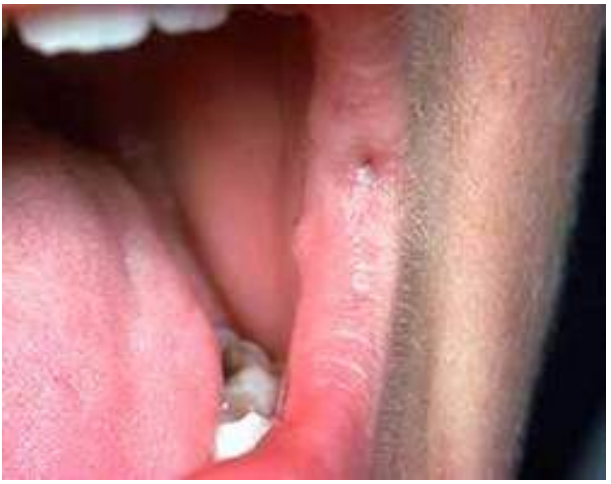

Figure 7.17: Commissural lip pit. Image credit: "Commissural Pit" by the National Human Genome Research Institute is in the Public Domain, CC0

Commissural lip pits (or congenital lip pits) may form

between the **maxillary processes** and **mandibular arch**. These are examples of cosmetic variations rather than **congenital malformations**.

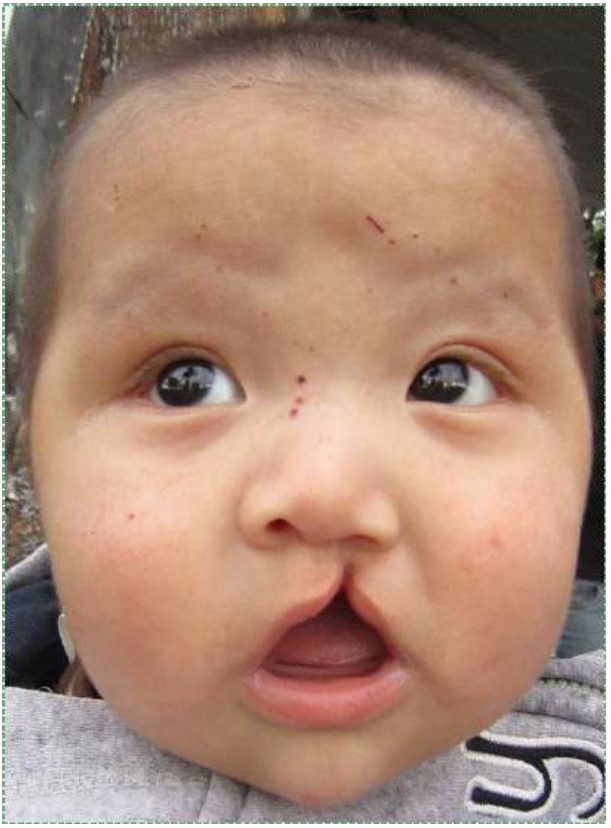

Figure 7.18: Left unilateral cleft of the lip. Image credit: "own work" by James Heilman, MD is licensed CC BY-SA 4.0

Cleft lip

Incomplete **fusion** of either **maxillary process** with the

inter-maxillary segment leads to the formation of a **cleft lip**. This can occur either on the left, right (**unilateral**) or both (**bilateral**) borders of the philtrum, although a left unilateral cleft lip is the most common. Cleft lips are more common and more severe in male children. A cleft lip may be accompanied by a **cleft palate**.

Cleft lip and palate occur in about 1 in 1000 births, making them a relatively common **congenital malformation**. Risk factors include older mothers, mothers who smoke during pregnancy or who take certain medications (e.g. some anti-convulsants). There are many genetic risk factors for cleft lip and palate, some examples are listed in Table 7.4.

Gene name	Class of gene	Function
IRF6	**Transcription factor**	Induced during development of mesoderm.
MSX1	**Homeobox transcription factor**↩	Limb patterning
BMP4	**Morphogen**↩	Induction and patterning of bone tissue, teeth and limbs
FGF10	Morphogen	Induction and patterning of connective tissue
Hyal2	Digestive enzyme	Digests hyaluronic acid prior to fusion of the lip or palate
p63	Transcription factor	Controls desmosome protein expression during fusion of the lip or palate

Table 7.4: A partial list of genes that, when mutated, contribute to the formation of cleft lip/palate.

Epithelial Cadherin 1	**Cell adhesion molecule**[←]	Allows epithelial cells to connect during fusion of the lip or palate

A cleft lip can cause difficulty with nursing, as it hinders the formation of a good seal around a nipple. With proper instruction, babies with cleft lip can be breast-fed or bottle-fed using a regular bottle. A cleft lip may cause problems with learning speech. Learning to speak requires sound mimicry, and because a cleft lip alters vocal sounds, it interferes with successful mimicry. Speech and hearing therapy help alleviate these problems. An increased risk of oro-nasal infections is also a concern. The preferred treatment for cleft lip is to seal the gap with surgery at 10 weeks of age. Surgery can leave behind a scar, but otherwise is highly successful.

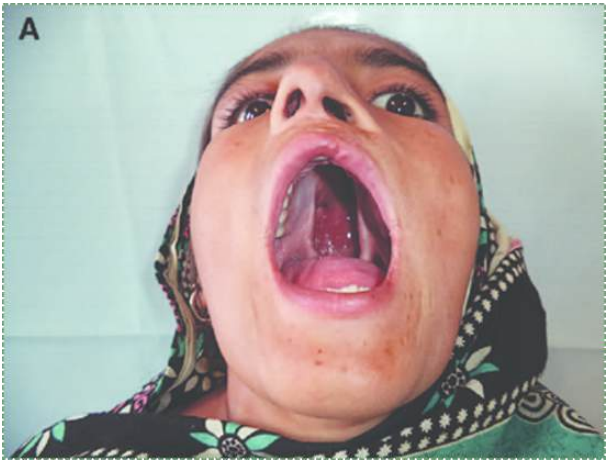

Figure 7.19: Example of a cleft palate. Image credit: "A 16 year old girl with unilateral complete cleft palate" by Ghulam Fayyaz is licensed CC BY-SA 4.0

Cleft palate

Incomplete **fusion** of the **primary palate** and/or the **palatal shelves** leads to a **cleft palate**. A cleft palate may or may not

be accompanied by a **cleft lip**. Cleft palate is more common in females.

Cleft palate causes difficulty with nursing, because a child cannot create suction with an opening from the oral cavity into the nasal cavity. There are a number of specialty bottles that help babies with cleft palate bottle feed. Similar to cleft lip, a cleft palate can lead to difficulty learning speech. Disruption of palate formation may also lead to shape changes in the Eustachian tubes. The Eustachian tubes develop from the first **pharyngeal pouches**— close to where the **palatal shelves** bulge off the **maxillary processes**. Changes in the shape of the Eustachian tube alter its ability to regulate middle ear pressure, which leads to an increased risk of hearing loss.

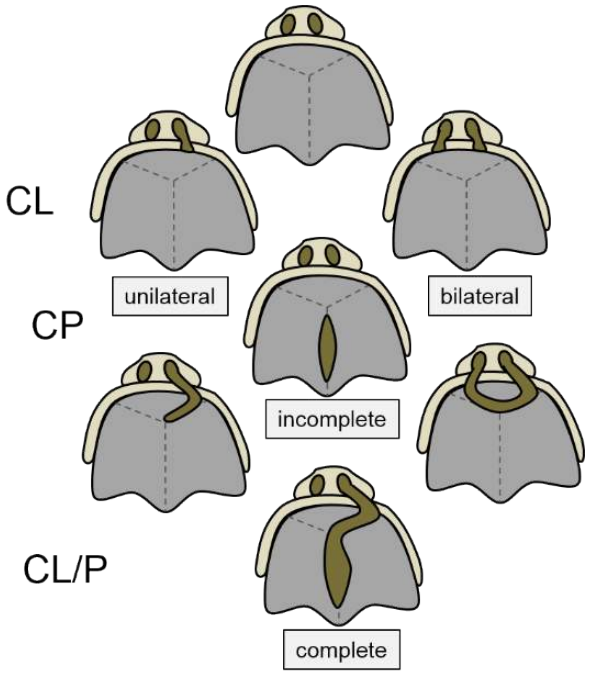

Figure 7.20: Summary of the different varieities of cleft lip/palate. Legend: CL = cleft lip, CP = cleft palate, CL/P = cleft lip and palate.

Orofacial clefts are categorized first as being a cleft lip (CL), a cleft palate (CP), or a cleft lip and palate (CL/P). A **unilateral** cleft lip or palate affects just the left or right side, while a **bilateral** cleft affects both sides. An **incomplete cleft** palate involves incomplete fusion between the **primary palate** and a **palatal shelf**, while a **complete cleft** also involves incomplete fusion between the two palatal shelves.

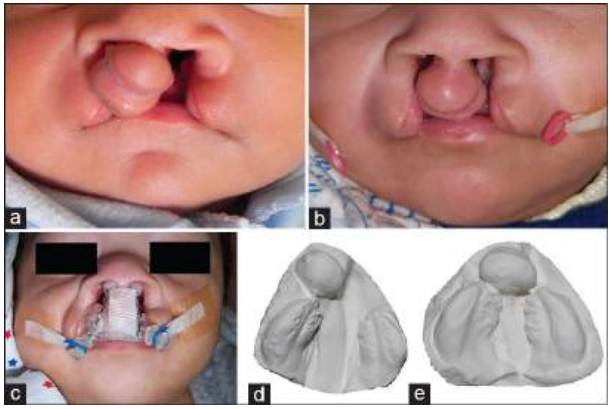

Fig. 7.21: An example of Nasoalveolar molding (NAM). Legend: a: pre-treatment photo, b: post-treatment photo, c: NAM taping, d: pre-treatment dental model, e: post-treatment dental model. Image credit: "Pretreatment extraoral photos

The preferred treatments for cleft palate include Naso-Alveolar Molding (NAM), followed by several surgeries. NAM involves screwing or taping an appliance to the maxilla at around 10 months of age. The appliance slowly pulls the regions of the upper lip derived from the **maxillary processes** in an anterio-medial direction, towards the **inter-maxillary segment**. Using such an appliance reduces the amount of surgery required to correct the cleft, relying more on guided growth of the child's tissues. The appliance is adjusted by an orthodontist every two weeks for about a year. This can get tissues closer together, but they won't **fuse**. Multiple surgeries follow in the treatment of cleft palate– it is a complicated region, made all the more complex by the fact the child is growing fast. It is not ideal to wait for a child to stop growing for the same reason early intervention for **cleft lip** was important: orofacial clefts hinder speech development.

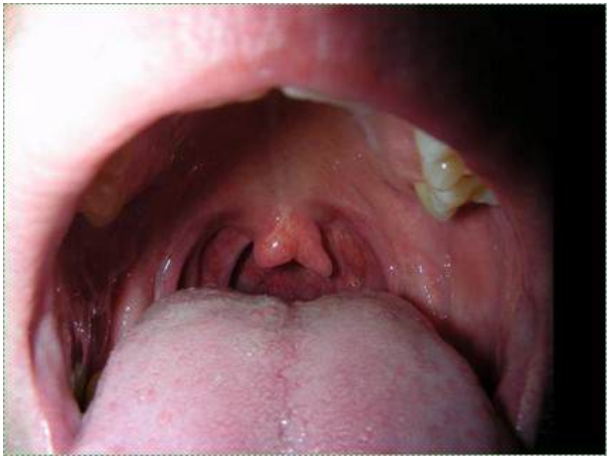

Figure 7.22: Example of a cleft uvula. Image credit: "Own work" by Adam6611 is in the Public Domain CC0

Cleft uvula

A cleft uvula is the least complicated form of **cleft palate**, and should be considered a cosmetic variation rather than a **congenital malformation**. A cleft uvula still closes off the nasopharynx during swallowing.

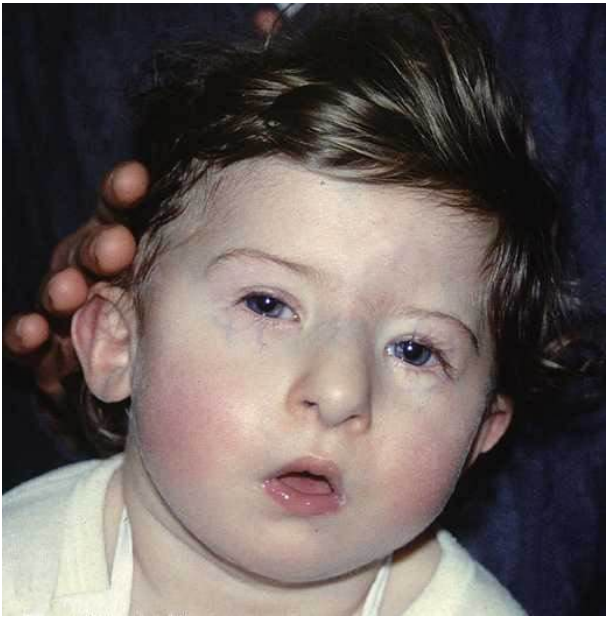

Figure 7.23: Photo of a patient with DiGeorge syndrome. Image credit: "DiGeorge syndrome1" by Prof Victor Grech is licensed under CC BY-SA 3.0

DiGeorge syndrome

DiGeorge syndrome (or 22q11.2 deletion syndrome) is caused by a spontaneous (not inherited) deletion to a part of **chromosome** 22. The deletion removes many **genes**. One lost gene is TBX1, a **transcription factor** that activates **FGF** in the **pharyngeal arches**. Without FGF, **neural crest cells** that migrate to the pharyngeal arches die after arrival. This leads to a wide variety of craniofacial abnormalities, including cleft lip/palate, multiple distubances in tooth

development (covered in chapters 8, 9 and 10), immune system dysfunction caused by malfomation of the thymus from **pharyngeal pouch** 3, and dangerous defects to the aorta (whos **development** is also guided by neural crest cells). Despite severly impacting both the immune system and cardiovascular system, patients with DiGeorge syndrome can have a normal life expectancy with proper and timely surgical interventions.

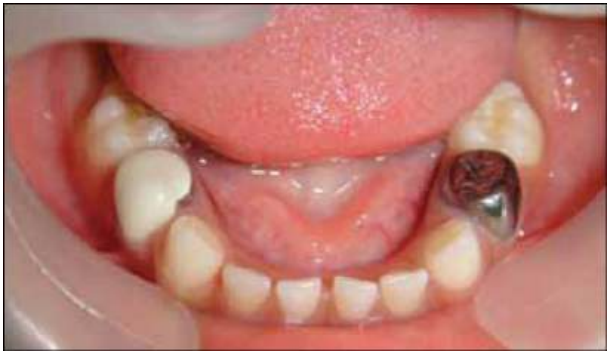

Figure 7.24: A Preformed Metal Crown (PMC) in a pediatric patient. Image credit: "Stainless steel and preveneered crowns after cementation" by Waleed M Bin AlShaibah, et al is licensed under CC BY-NC-SA 4.0

Becasue of the many roles **neural crest cells** play in formation

of the pharyngeal arches and teeth, management of dental issues in pateints with DiGeorge syndrome can be quite complicated. In the next three chapters, think about why loss of neural crest cells inhibits the formation of dentin and enamel. For now, it is enough to know that patients with DiGeorge syndrome often benefit from crowns. The preferred material in pediatrics and for patients with mental and physical disabilities like DiGeorge syndrome are Preformed Metal Crowns (PMCs, stainless steel crowns). PMCs are more durable than (white) composites or amalgams, making them optimal for patients who have difficulty controlling muscles to limit occlusal forces (pdf download on the history of PMCs).

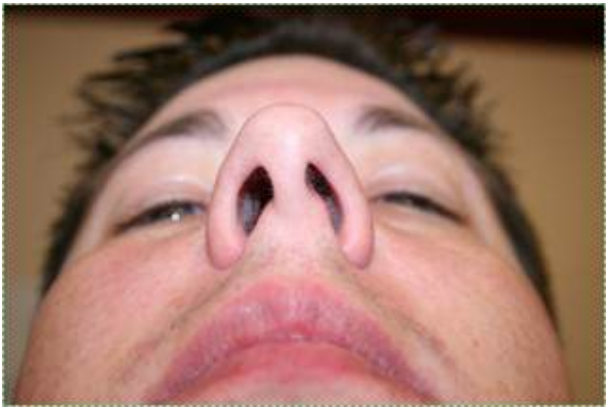

Figure 7.25: Example of a deviated septum. Image credit: "Nostrils before" by Jeff and Mandy G is licensed CC BY SA 2.0

Deviated nasal septum

If the nasal septum grows at an angle as it is developing, it leads to a **deviated septum**. In fact, it is rare for the septum to develop in a symmetrical fashion. 80% of people have some nasal septum deviation, usually without symptoms. Complications can arise because of nasal cavity physiology. The paired nasal cavities contain erectile tissue (**areolar connective tissue**) below the nasal mucosa. This tissue undergoes a nasal cycle, alternating between one side swelling shut and the other remaining open for breathing.

This prevents the nasal cavities from drying out from constant use. But for someone with a significant nasal septum deviation, it leads to difficulty breathing when the larger cavity swells shut. A relatively simple surgery called **septoplasty** can be done to increase the size of the smaller nasal cavity. Septoplasty is not the same as the plastic surgery procedure **rhinoplasty**, where the shape of the nose is altered.

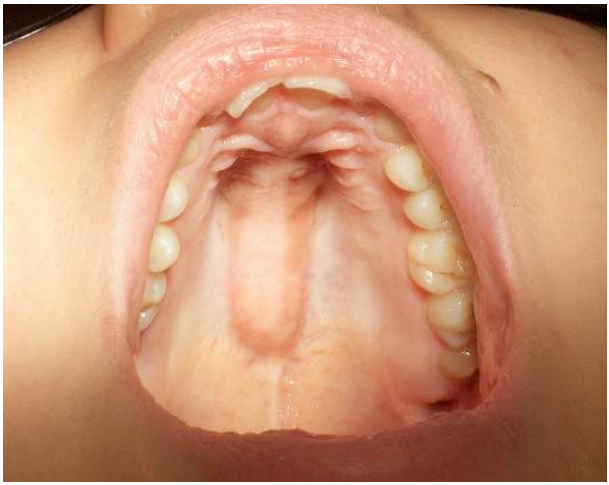

Figure 7.26: Example of a palatal torus. Image credit: "Photo" by Kozlovsk is licensed CC BY SA 3.0

Palatal torus

Excessive growth of the **palatal shelves** can create a **palatal torus (**or torus palatinus), another example of a cosmetic

variation. A palatal torus requires no treatment, as it usually does not cause any health-related issues, aside from complicating the fit to dentures. About 20-30% of the population has some degree of palatal torus. A **mandibular torus**, on the other hand can develop later in life, often as a response of bone tissue to bruxism (**nurture**). However, because mandibular tori develop more frequently in Asian and Inuit populations, this suggests there may be **genes** involved as well (**nature**).

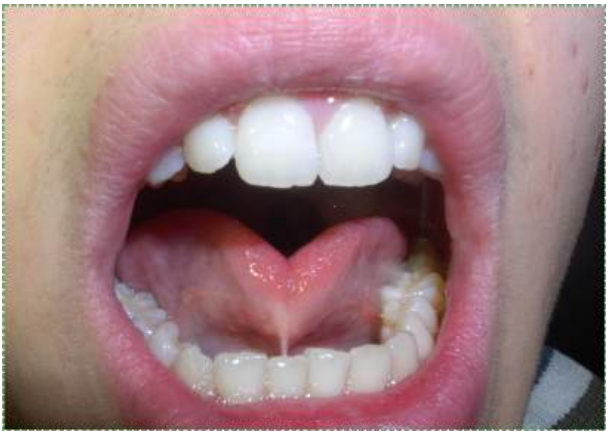

Figure 7.27: Example of ankyloglossia. Image credit: "Photo" by Klaus D. Peter is licensed CC BY 2.0

Ankyloglossia

Ankyloglossia (tongue tie) is the persistence of tissue

anchoring the tongue to the floor of the mouth. Most of the ventral side of the **mandibular arch** should undergo **apoptosis**←, leaving behind a small **lingual frenulum**. However, with inadequate apoptosis, a pronounced lingual frenulum results, limiting the mobility of the tongue. This causes problems with breastfeeding and learning speech, but it is correctable with a minor surgery (a lingual frenectomy). Ankyloglossia is a common **congenital malformation**, although there is significant amount of disagreement as to how prevalent it is. Estimates ranging widely, from 1% to 25% of births (potentially a lot of surgical bills). Similarly, there is disagreement as to how severe the **congenital disorder** must be before surgical intervention becomes necessary, and this disagreement has been going on for over 75 years.

Figure 7.28: Example of a pharyngeal cleft cyst. Image credit: "Patient with large right Pharyngeal Cleft Cyst protruding from neck, prior to excision" by BigBill58 is licensed CC BY SA 4.0

Branchial cleft cyst

Branchial cleft cysts form when incomplete **fusion** of neighboring **pharyngeal arches** leaves the remnant of a **pharyngeal groove**. These usually form a painless mass in the neck, until an infection occurs. They may be left untreated, or may be removed by surgery. This involves removing the extraneous **ectodermal** (epithelial) tissue trapped deeper in the neck. Whether surgery is or isn't performed may depend on how close the cyst is to the carotid artery, internal jugular vein or facial nerve.

Rathke's cleft cyst

Similar to **branchial cleft cysts**, a cyst may form from incomplete obliteration of **Rathke's pouch** during formation of the pituitary gland. This leads to a mucus-filled cyst near the anterior pituitary. Due to its location, it may put pressure on the optic chiasm, leading to visual disturbances, otherwise it is asymptomatic. Drainage is the preferred treatment over removal, owing to how close it is to the pituitary gland.

< Chapter 6 * navigation * Chapter 8 >

Chapter review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:

https://openoregon.pressbooks.pub/histologyandembryology/?p=51

8.

TOOTH DEVELOPMENT

animation of embryo

Figure 8.1: Teeth begin to form during week 5 to 6, shortly after the oro-pharyngeal membrane disintegrates and the pharyngeal arches develop.

Overview of tooth formation

In this chapter we cover major concepts in tooth development, saving many details for later chapters. The first big concept is the timing: so far everything has been measured in weeks, but in this chapter we measure time in weeks, months and years. Both the primary and succedaneous teeth begin developing early in embryonic development—around week 5. To put this in perspective, that is only shortly after the mouth forms– the **oro-pharyngeal membrane** ruptures in week 4. The process of tooth development begins around week 5. When tooth development ends is more complicated, as listed in Table 8.1

(*note: any timeline you see most likely reports an average or most common age, there is significant variation in the timeline between individuals).*

Table 8.1: Development times of the parts of a tooth and periodontium

Tissue:	Develops during:
crown enamel and dentin – primary	embryonic week 5 to 8
crown enamel and dentin – succedaneous	embryonic week 8 to after birth (5 years)
root dentin and cementum – primary	embryonic to after birth (1 – 3 years)
root dentin and cementum- succedaneous	after birth (6 – 25 years)
PDL, alveolar bone, **junctional epithelium**[←]	tooth eruption

The next big concept is the importance of the very first stage (**placode**, or **induction**). Small regions of epithelial cells in the **oral mucosa** are induced to speed up **mitosis**←. This happens because of secretion of **morphogens**← from **neural crest cells**← below. You can't see this under the microscope (not without more specialized tools than the **H&E** stain). As a result, this early phase is often skipped in histology books. That is a shame, because getting the correct number of teeth to form requires complex communication. Not only do the correct number need to form, the spacing between *succedaneous* teeth depends on where *primary* teeth develop. To put that another way, the embryo is making plans 5 weeks after fertilization for events that happen 5 to 10 years later. To put it yet another way, when the embryo is about 1.5 mm in length (about $1/16^{th}$ of an inch), it is making plans for the spacing of teeth in an adult skull that will be 150 mm across (about 6 inches). We think this early communication is really cool. There are, however, more names associated with later stages (usually named after long-dead European men), and those names appear on licensing exams, so we cover those in detail as well. Lucky you.

The third big concept is the **lineage** of the different parts of a tooth and periodontium. Enamel is produced by cells derived from the **ectoderm**, whereas dentin, cementum, pulp, **PDL** and alveolar bone are derived from **neuro-mesenchyme**. While the hard tissues enamel, dentin and cementum share

physical characteristics with **bone tissue**←, enamel has notable differences because of its different lineage. Perhaps the biggest difference is that people cannot *grow* new enamel after teeth erupt. Enamel can passively re-mineralize under the right conditions, but this does not involve the activity of human cells, **scaffolds**← or enzymes (which are more efficient). The enamel-producing cells, including the scaffolds and enzymes they secrete, are lost during tooth eruption. In contrast, dentin, pulp, cementum and the **PDL** contain cells throughout the life of a tooth, and have a much higher capacity to undergo **remodeling** and **regenerate** following trauma.

The fourth big concept is that the **mesenchyme**← is worthy of attention. We did not say **mesoderm** (one of the three **embryonic germ layers**), but mesenchyme (the tissue type)—the difference is important. Most mesenchyme is derived from mesoderm, but tooth connective tissues are derived from **neural crest cells**← plus mesoderm. We give this mesenchyme a unique name: **neuro-mesenchyme**. This leads to similarities in the behavior of tooth and brain cells, which makes less sense to people who haven't learned embryology.

The fifth big concept is that enamel and dentin-producing cells do not form separately. They induce one another in a **reciprocal** fashion. After their team-effort, the cells move apart from one another and do their own thing. This reduces the chances that enamel or dentin will be produced in the wrong place or wrong time. Production of these tissues requires a special set of conditions, one that occurs only briefly

during early embryonic **development**. Unfortunately, this makes it harder for medical professionals to re-create these conditions later in life should enamel or dentin production be beneficial.

The sixth and final big concept is that the process of tooth eruption is complicated. There are multiple steps which are poorly understood, and scientists disagree on which steps are important. We cover what is known, but we can let the experts keep arguing. What is important to us is that to learn about the **development** of the **PDL**, alveolar bone and **junctional epithelium**←, we must cover tooth eruption. Tooth eruption should be considered a developmental process even though it happens long after the embryonic and fetal stages.

Crown development

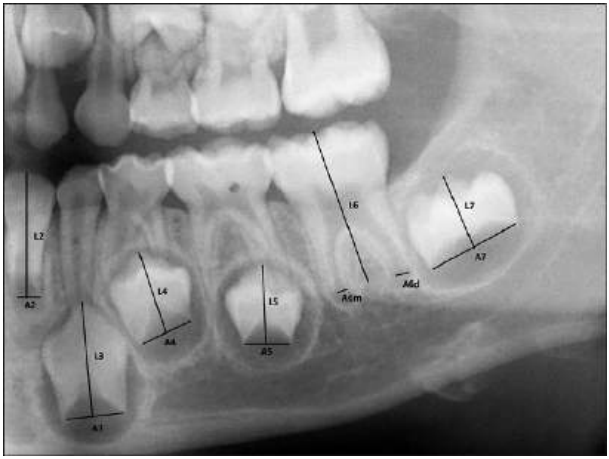

Figure 8.2: The crowns of most teeth (primary and succedaneous) form at roughly the same time, but the crown and root(s) of a single tooth develop at very different times. Image credit: "Camerie's method of measurement for the teeth

Tooth formation, or **odontogenesis**, starts with primary teeth around weeks 5 to 6, and succedaneous teeth around weeks 9 to 10. Odontogenesis is similar to **neurulation**, teeth arise from **invaginations** of epithelium, derived from **ectoderm**. Hair follicles share this pattern as well, but instead of being called a tooth *follicle*, teeth grow from **tooth buds**. At week 5, the oral cavity is lined by ectoderm-derived epithelium, and deep to that is **mesenchyme**. For the skin and most of the oral cavity, the layer of mesenchyme is derived from **mesoderm**. Teeth, however, grow from ridges called the **dental lamina**, found on the **mandibular arch** and the **maxillary processes**, along the occlusal border. Here, mesenchyme contains clusters of **neural crest cells** that migrated from the **neural tube**. Hence, we say the connective tissues of the teeth are derived from **neuro-mesenchyme** (or ecto-mesenchyme). Remember, because neural crest cells come from the neural tube, and the neural tube invaginates from the ectoderm, these cells are ectodermal by lineage. They go through an **epithelial-to-mesenchymal transition** and *resemble* mesenchyme derived from mesoderm under the microscope. If you remember how cells express **matrix metalloproteinase** enzymes during this transition, you shouldn't be surprised to

see these enzymes re-used during **odontogenesis**, and re-used yet again during **tooth eruption**.

By 8 weeks (before the palate **fuses**), there are 10 **tooth buds** in each **dental lamina** (the regions fated to become the maxillary and mandibular alveolar ridges). The tooth buds of succedaneous teeth begin forming around this time, before the primary buds have made any enamel or dentin. Both primary and permanent buds develop into crowns and become surrounded by the maxilla and mandible (Fig. 8.2).

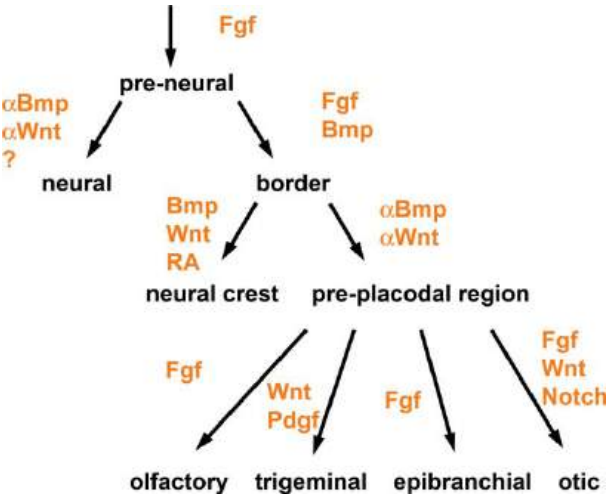

Figure 8.3: Morphogens involved in the induction of various placodes. Image credit: "Signaling pathways in placode formation" by Andrea Streit, Stembook.org is licensed CC BY 3.0

Induction (or initiation)

The first step is poorly understood. **Neuro-mesenchymal stem cells** coordinate with one another through **planar cell**

polarity[←] signals to **induce** the correct number of **tooth buds**, at the correct distance apart from one another. Neuro-mesenchymal stem cells secrete a number of **morphogens**[←] forming complex gradients. Overlying **ectoderm** responds to the morphogen gradients.

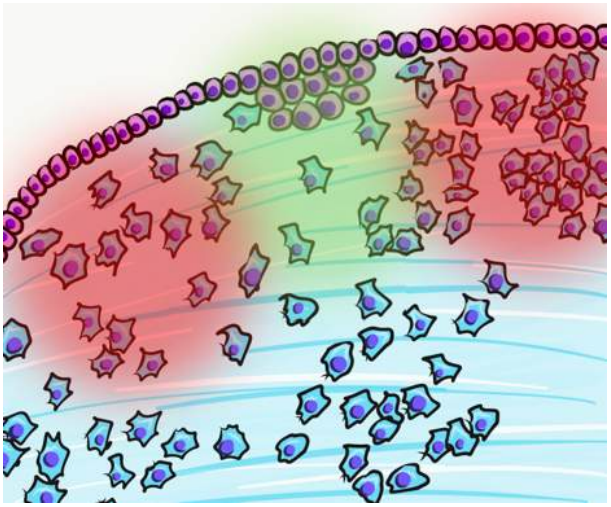

Figure 8.4: Illustration of FGF and BMP gradients.

Two important **morphogens**[←] in tooth development are members of the **FGF** and the **BMP** families of signaling molecules. There are more signals involved, which is illustrated in Fig. 8.3 (further reading). As you might guess from their full names, BMP and FGF aren't unique to tooth formation. These two morphogens **induce** different cells to do different things at different times and locations. At this time and location, they induce **ectoderm** to **differentiate**[←] into **tooth buds** instead of **oral mucosa**. Other morphogens inhibit tooth bud induction, ensuring proper spacing is

established. One of the first visible responses in the ectoderm is **proliferation—** certain regions begin to grow thicker, called tooth **placodes**. Not every book (or exam) includes *placode* as its own stage, be aware. Furthermore, placodes may be called the **dental lamina** or may be listed as a part of the dental lamina—fate mapping experiments can't easily be done on humans to determine which is more correct. The important concept is most of the oral mucosa described in chapter 3 is derived from ectoderm and **mesoderm**, while the teeth and periodontium are derived from ectoderm and the **neuro-mesenchyme** of a tooth bud.

---

animated illustration of tooth development

Figure 8.5: Animated overview of the early stages of tooth development: Placode, bud, cap, bell.

Bud stage

Continued **proliferation** of the **ectoderm** allows us to see the next stage of tooth development under the microscope more easily, the **bud stage**. The name *bud* comes from the fact that tooth buds look like leaf buds on a plant. In the spring, you can see where leaves are **fated** to grow on plants by the location of leaf buds. Leaf buds are not leaves yet, just bumps. Tooth buds begin to appear around week 6, and ultimately 10 tooth buds form on the **maxillary processes** and **mandibular arch**. In addition to proliferation of ectodermal cells, **neuro-mesenchymal stem cells** also proliferate. This makes the **mesenchyme**[←] in a tooth bud appear denser than the mesenchyme in nearby regions that form the **lamina propria** of the **oral mucosa**. Those regions have more of the **mucous ground substance**[←] and fewer cells.

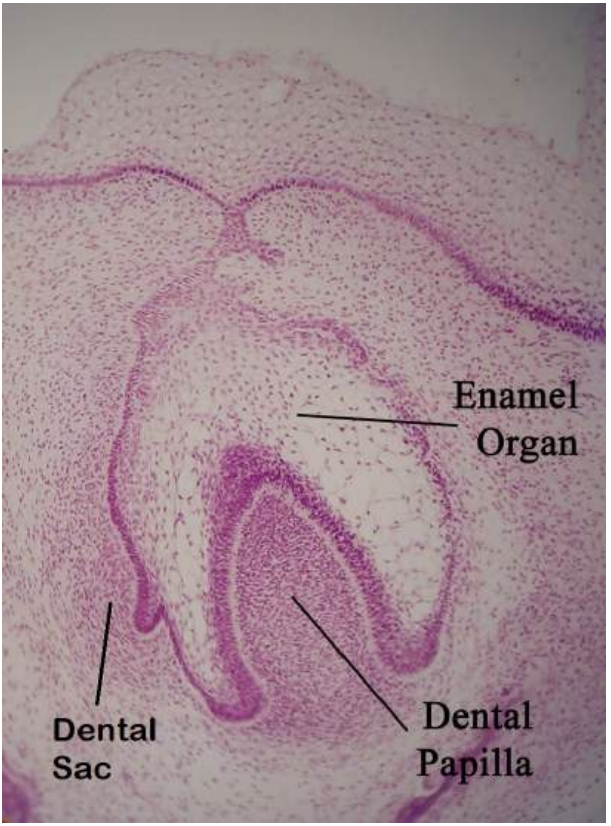

Figure 8.6: Histology of tooth germ, marking the enamel organ, dental papilla and dental sac. Image credit: "Enamel organ" by Dozenist is licensed under CC BY-SA 3.0

Cap stage

Around week 10, the **ectoderm** continues to **proliferate** and it bumps into the cluster of **neuro-mesenchymal stem cells** below. This cluster of neuro-mesenchymal stem cells is called the **dental papilla**. The dental papilla forces the ectoderm

of the **tooth bud** to grow around it. As this happens, the ectoderm resembles a hat, hence the name **cap stage**. At this time, the **dental lamina** has two prominent parts—an inner and an outer layer. The epithelial cells near the dental papilla are referred to as the **enamel organ**, because some of them **differentiate**← into cells that produce enamel. Below the dental lamina, the **neuro-mesenchyme** of the **dental papilla** forms dentin and pulp. The neuro-mesenchyme on the outside of the dental lamina is called the **dental sac** (or *dental follicle*), which forms cementum, **PDL** and alveolar bone. All three together—the enamel organ, dental papilla and dental sac—are collectively called **tooth germ**.

At this time, the process repeats (or **recapitulates**). Cells of the **dental lamina** on the lingual side of the cap are induced to form another **placode**, and the succedaneous teeth bud off the developing primary **tooth germ**. Imagine taking Fig 8.5, rotate it 90^{o}, superimpose that onto the **cap stage**, and you have succedaneous **tooth bud** formation. Exceptions to this include the 2^{nd} and 3^{rd} molars, which do not succeed primary teeth. Their tooth buds develop from **ectoderm** similar to the primary dentition. Furthermore, the tooth germ of succedaneous teeth can move as they develop—**tooth eruption** does not always occur from the lingual side. For instance, the maxillary incisors generally erupt from the facial direction.

Bell stage

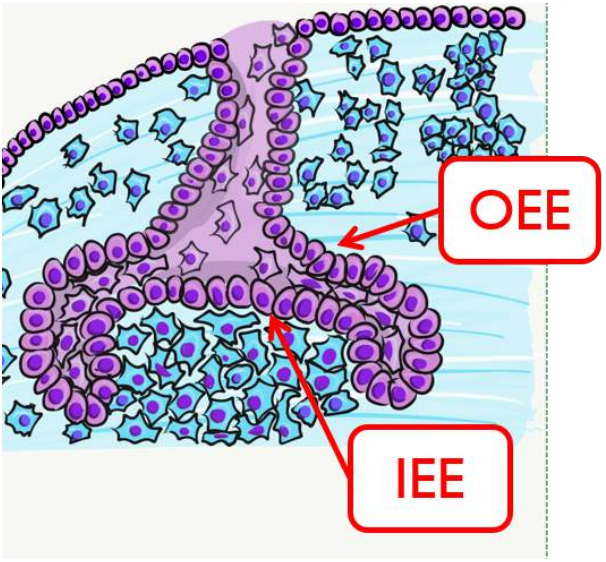

Figure 8.7: Bell stage with inner enamel epitheliu m (IEE) and outer enamel epitheliu m (OEE) labelled.

By the 11th or 12th week, **ectoderm** and **neuro-mesenchyme** continue to **proliferate**. As the epithelial cap becomes larger, it resembles more of a bell shape, hence the name **bell stage**. Hats worn by people are usually smaller than bells on clock towers, which may help you remember which stage comes before the other. At this time, the **enamel organ** contains two layers of cuboidal cells named the inner enamel epithelium (**IEE**) and the outer enamel epithelium (**OEE**). The IEE is next to the **dental papilla**, with a basement membrane physically separating the two. The IEE **differentiates**[←] into **ameloblasts** and produce enamel, while the OEE is involved in

tooth eruption and forms the **junctional epithelium**[←] that bridges the tooth surface and **oral mucosa**.

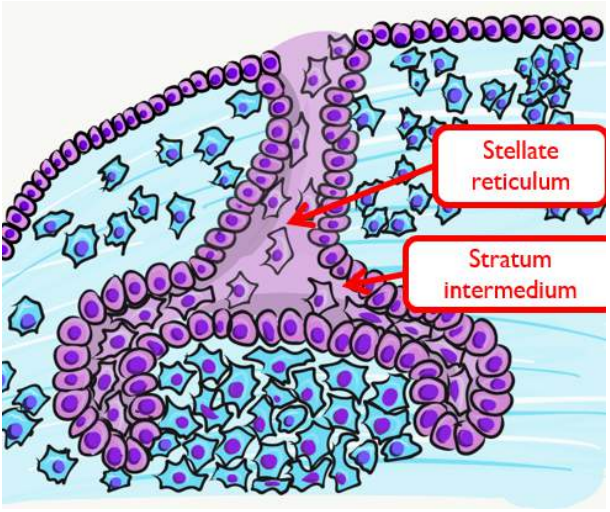

Figure 8.8: Cap stage with stellate reticulum and stratum intermedium labeled

Two additional regions of the **enamel organ** develop at this time. The **stratum intermedium** is on the opposite side of the **IEE** from the **dental papilla**. These cells work with the IEE to form enamel. Past the stratum intermedium are ectodermal cells called **stellate reticulum**, named for their star-shaped rather than cuboidal appearance. These cells help **induce** the IEE to **differentiate**[←] and begin enamel production. These cells are found in the superficial but not the deeper regions of the growing **tooth germ**, which is why enamel is only produced in crowns and not in roots of teeth.

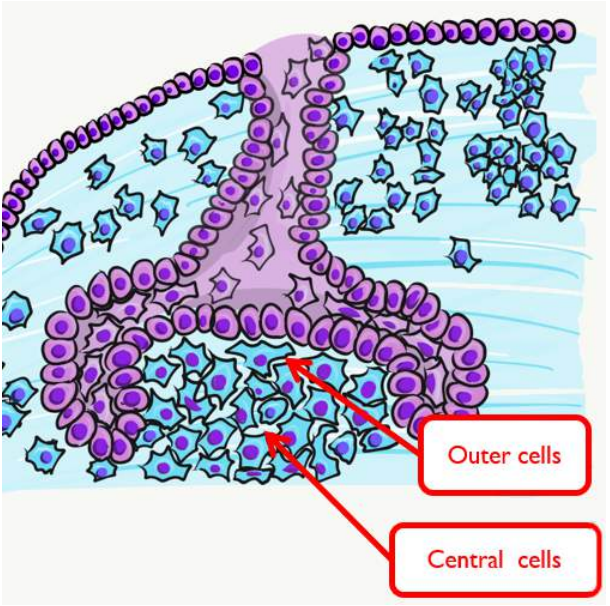

Figure 8.9: Cap stage with two regions of the dental papillae marked, the outer and central regions.

The **dental papilla** may be subdivided at this time into an outer region and a central region. The outer cells **differentiate**[←] into **odontoblasts**, while the central cells form many cell types found in pulp. It is worth noting that cells are not **ameloblasts** or odontoblasts at this time. These cells are epithelial and **neuro-mesenchymal stem cells**. Based on their relative location, we know what they are **fated** to become in the near future, assuming they receive the correct **morphogens**[←].

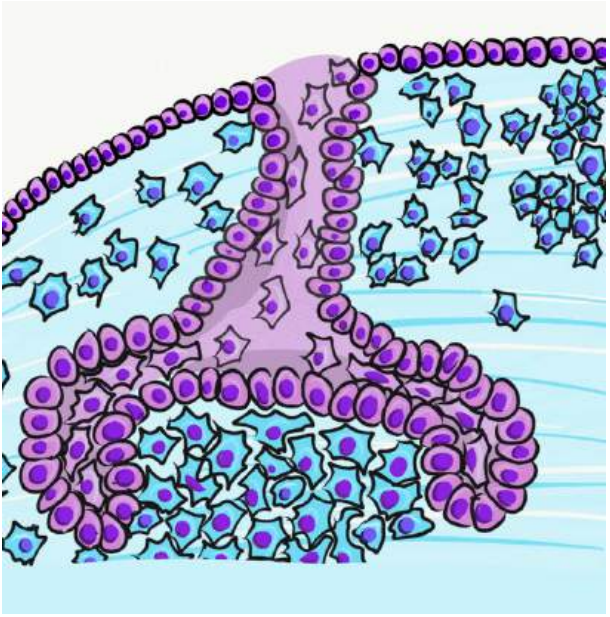

Figure 8.10: Animated representation of odontoblast and ameloblast differentiation, including the induction signals (red arrows) for each process, during the bell and crown stages of tooth development.

A brief overview of the formation of ameloblasts and odontoblasts

During the **bell stage**, interactions between the **IEE**, **stellate reticulum** and outer cells of the **dental papilla induce** the **differentiation**[←] of **ameloblasts** and **odontoblasts**. First,

the **basement membrane** that physically separated the IEE from the **neuro-mesenchymal stem cells** of the dental papillae disintegrates. The IEE contacts **ECM** made by neuro-mesenchymal stems cells, am=nd they differentiate into **pre-ameloblasts**. The prefix *pre-* indicates that differentiation is not considered complete at this time. The IEE looks different: cells elongate, changing from a **simple cuboidal epithelium**← to a **simple columnar epithelium**←. Pre-ameloblasts then produce **morphogens**← that induce neighboring neuro-mesenchymal cells to differentiate into **odontoblasts** (the cells that make dentin). Newly formed odontoblasts begin producing a squishy immature form of dentin (**pre-dentin**). Molecules in pre-dentin signal back to the pre-ameloblasts. Morphogens from the **stellate reticulum** also signal to pre-ameloblasts. The combination of these 2 signals induces the differentiation of pre-ameloblasts into **ameloblasts** (the cells that make enamel).

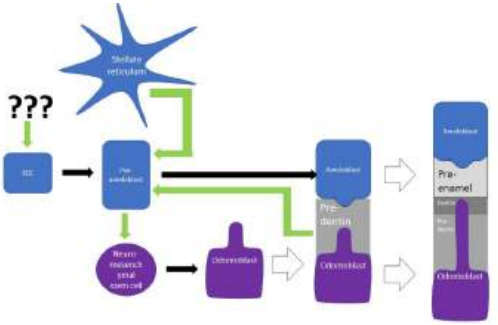

Figure 8.11: Diagram of the reciprocal induction of odontoblasts and ameloblasts. Legend: Green arrows = morphogen signal, Black arrows = differentiation, White arrows = ECM secretion.

This is a common pattern in developmental biology. A dual effort is known as reciprocal induction. This is significant because it makes it hard to re-create one type of cell later in life (such as an **ameloblast**) without also re-creating its partner (in this case, an **odontoblast**). Furthermore, one must also re-create the conditions these cells were in during reciprocal induction (in this case, being close to **stellate reticulum**).

Dentinogenesis, the formation of dentin, therefore begins before **amelogenesis**, the formation of enamel. This results in dentin being thicker than enamel. As **odontoblasts** secrete **pre-dentin**, their cell bodies are pushed deeper, but they leave a log tube-like extension called the **odontoblastic process** within the pre-dentin. By the time a tooth is done growing, the odontoblastic processes extend through nearly the entire layer of mature dentin. **Ameloblasts**, on the other hand, do not grow an extension. There is a little bump on each ameloblast facing the enamel, and this bump is called **Tomes' process**. There is important clinical significance related to odontoblastic processes coming up in subsequent chapters. Tomes' process, on the other hand, is a name of a bump histologists use to identify ameloblasts during embryonic development (and a name prospective dental hygienists get asked about on license exams).

Crown stage

The second half of the **bell stage** is the late bell stage, or the **crown stage**. During this time the crowns of teeth form by the secretion of molecules by **ameloblasts** and **odontoblasts**. No new cells of these types form, teeth continue to grow by the addition of **ECM**. Because the ECM mineralizes and hardens, these tissues must be added **appositionally**. This is somewhat similar to the growth of **bone tissue**[←], only the enamel and dentin-producing cells do not get trapped between layers of

hard tissue. Instead, these cells migrate in a **basal** direction (relative to the cell) as they secrete matrix. Its important to note the process of crown *formation* finishes before mineralization of the mandible and maxilla, but *mineralization* of the crowns takes more time, finishing within the first year or two of life. The timeline for succedaneous teeth is more variable (a timetable may be found on wikipedia).

Root Development

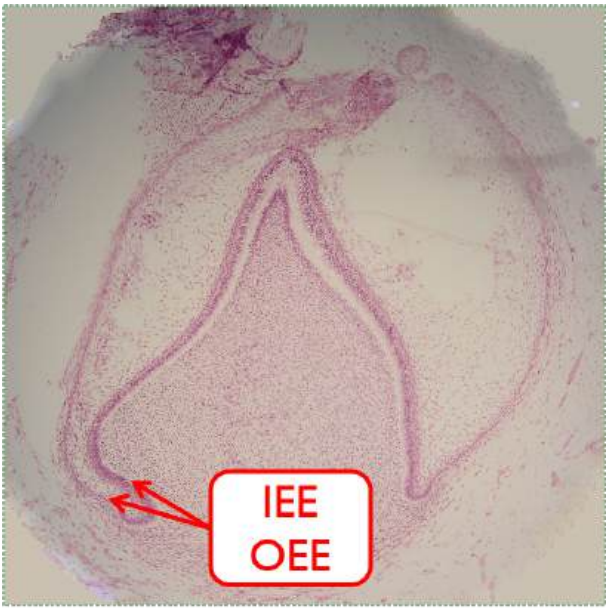

Figure 8.12: Histology of bell-stage tooth germ, with the IEE and OEE labelled. Image credit: "Latebellstage11-18-05" by Dozenist is licensed under CC BY 3.0 / *labels added*

The cervical loop and HERS

We must jump ahead in time to cover root development:

months for primary teeth, and years for succedaneous teeth. After the crown stage, the leading edge of the **IEE** and **OEE** continue to grow around the **dental papilla**. However, they are not separated by **stellate reticulum**. Together they are called the **cervical loop**. As the cervical loop continues to grow deeper, it is called Hertwig's Epithelial Root Sheath (**HERS**). The pattern of HERS growth determines the shape of the root(s). This is similar to a cake mold: HERS does not become the roots, it guide their shape. HERS does not grow evenly around the dental papillae of larger teeth. Instead, HERS grows faster in some regions as it extends over the dental papilla, perhaps the way chocolate fudge drips over a ball of ice-cream unevenly. On larger teeth, this produces multiple roots. The cervical loop forms early in embryonic development, but HERS continues to grow after birth. The roots wont completely mineralize until years after **tooth eruption**.

Cementum formation

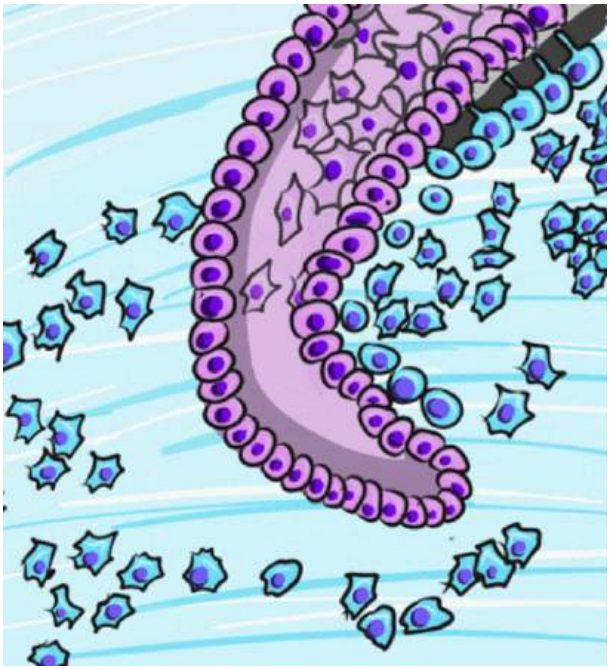

Figure 8.13: HERS and root formation.

HERS contains **IEE** and **OEE**, but no **stellate reticulum**. This leads to key differences between the crown and roots of teeth. The IEE induce the formation of **odontoblasts** in the roots just as it did in the crown, but when odontoblasts form **pre-dentin**, the lack of a signal from stellate reticulum means the **differentiation**[←] of **ameloblasts** does not occur. Hence, roots do not contain enamel. Instead, most of the IEE and OEE cells undergo **apoptosis**[←]. A few are left behind, which get the name the **Epithelial Rests of Malassez** (ERM**)**. These leftover cells may have functions in the **regeneration**

of damaged root tissues, although this is not currently well understood.

After the majority of the epithelial cells are removed, **neuromesenchymal stem cells** of the **dental sac** contact **predentin**. Getting closer to **BMP morphogens** secreted by **odontoblasts induces** them to **differentiate**← into **cementoblasts**. Cementoblasts then cover the root dentin in a relatively thin layer of cementum. At first, the cementum produced is pure ECM. Because it lacks cells it is called **acellular cementum**. Later, in the apical root regions, cementoblasts become trapped within the **ECM** they secrete. This is **cellular cementum**. The cells are called **cementocytes** once trapped within **lacunae**. The presence or absence of cells is easy to identify under the microscope. However, it is the polarity of **collagen**← fibers in the ECM that is more important clinically. This will be covered in Chapter 11.

Tooth eruption

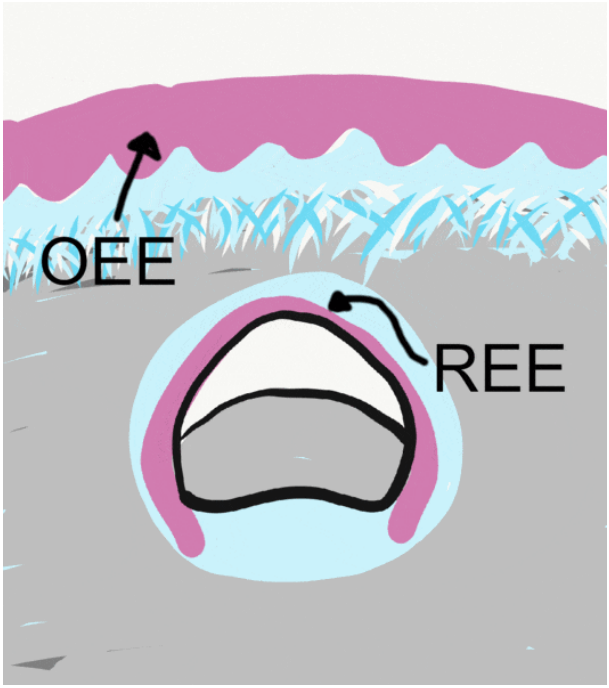

Figure 8.14: Tooth eruption highlighting epithelial fusion. Legend: OE = oral epithelium, REE = reduced enamel epithelium, JE = junctional epithelium.

We now jump forward in time yet again. Even though process of **tooth eruption** happens long after embryological **development**, it should be considered a developmental process. Another way to say that is the teeth continue to undergo **morphogenesis** after birth. Because this process involves cells of the **IEE** and **OEE**, we discuss it here.

After the roots develop, **neuro-mesenchymal stem cells** of the **dental sac** that did not come into contact with **pre-dentin** **differentiate**[←] into **fibroblasts** and **osteoblasts**. These form

the **PDL** and **alveolar bone**. The PDL anchors to cementum first, then to alveolar bone after eruption. Therefore, development of the PDL occurs during tooth eruption, much later than the formation of dentin and enamel.

First, the force or forces that cause teeth to erupt are not agreed upon. It is not even clear if it is a pushing or a pulling force, or a combination of the two. Whatever the cause or causes of tooth eruption are, movement of the crowns into the oral cavity is known as **active eruption**. One might imagine that as a root grows deeper and bumps into the edge of a bony socket, further lengthening of the root would force the crown upwards. This would be similar to jumping: with your legs bent and feet on a hard floor, rapid extension of your legs pushes you upwards. This is known as the root formation theory. To say this causes tooth eruption, it has to be both *necessary and sufficient* (a phrase commonly used in biological research). Because root-less teeth can erupt suggests root growth is not *necessary* for eruption.

We are going to use the terms *necessary* and *sufficient* a few more times. Before we do, here is a simpler scenario: after you stop your car at a red light, to get the car moving again requires taking a foot off the brake, applying a foot to the gas pedal and having gas in the gas tank. All three are *necessary*, but none of them is *sufficient* to move your car through the intersection.

We know bone **remodeling** is *necessary.* Bone remodeling involves coordinated activity between **osteoblasts** and

osteoclasts (the **remodeling unit**). However, tooth eruption is a different than the dynamic equilibrium of bone tissue. During tooth eruption, both osteoclasts and osteoblasts are active. However, they do not work togther as part of the **remodeling unit**, instead they work at different locations. As a result, equilibrium is not maintained. Bone tissue is removed above the tooth, but added below the tooth. We covered evidence for this when we discussed how permanent teeth fail to erupt in people with **cleido-cranial dysostosis**←. There, a faulty **matrix metalloproteinase** enzyme lead to the inability to degrade **ECM** proteins, leaving permanent teeth trapped within their bony crypt. This suggests bone remodeling is *necessary*, but not necessarily *sufficient*.

For the primary teeth, as growing roots encounter mineralized bones tissue below, **hydrostatic** pressure is put on the cells of the **enamel organ**. In response, the **stellate reticulum** secretes **morphogens**← that induce bone resorption. One morphogen is **RANKL**, a morphogen that induces osteoclast differentiation. Another is Parathyroid Hormone (PTH), which is called a **hormone** when it is secreted into the bloodstream. Here, it is secreted into the **ECM**, only stimulating **osteoclast** activity near the enamel organ. Because stellate reticulum is at the surface of enamel, these signals induce the formation of a pathway above the tooth, allowing it to erupt.

Clearing a path is *necessary* for tooth eruption, but it does not cause the tooth to move. As roots grow, they put pressure

on bone tissue in the alveolar sockets below. Pressure on bone tissue triggers release of **BMPs** which leads to the deposition of bone tissue, *pushing* teeth outwards. Bite forces also contribute to BMP secretion. As we mentioned earlier, root growth is not *necessary*, but BMP secretion is *necessary*. That doesn't really make sense, does it? We must be missing some critical information.

We know root growth is *sufficient* to induce BMP secretion, and BMP secretion is *necessary* for tooth eruption. However, there may be something else that can stimulate BMP secretion. That is our mising piece of critical information. Further evidence that pressure is *necessary* comes from observation that teeth fail to erupt in the absence of healthy **PDL**. In fact, pressure *created* by **fibroblasts** of the PDL may trigger the **stellate reticulum** to secrete **morphogens**[←] that trigger **osteoclast differentiation**[←] as well as bone tissue to release **BMPs**. Fibroblasts not only secrete **collagen**[←], they **remodel** it as well. Shortening of collagen fibers anchored to cementum would pressure on both the tooth root and alveolar socket. So does this happen? Polarization of PDL fibroblasts is observed before tooth eruption, suggesting that remodeling of PDL collagen fibers inserted in cementum may actively *pull* the tooth outwards. It is currently unknown whether this involves **planar cell polarity**[←] morphogens, signals which have been well established in their role in polarizing epithelial cells. It seems possible, given the **lineage** of PDL fibroblasts: **neuro-**

mesenchyme (which means **ectoderm**, the same lineage as highly polarized **keratinocytes** and **ameloblasts**).

But we are still left with a major question: how can the **PDL** be *necessary* for tooth eruption if a rootless tooth can erupt? Can a rootless tooth have PDL? Lets refresh what we know about the PDL. The PDL develops from the remaining neuro-mesenchymal stem cells after receiving a signal from the **dental papilla**. We know it is not the BMP signal that induces neuro-mesenchymal stem cells to develop into odontoblasts, cementoblasts or osteoblasts. We know the PDL first connects to cementum during eruption, and connects to alveolar bone after eruption. It seems unlikely that the PDL could develop on a rootless tooth, and even if the PDL did develop, it would have nothing to attach to. So next, let's summarize what we know about tooth eruption:

Action	Reaction	Neccessary or sufficient for tooth eruption?
root growth	pushes down (against alveolar socket)	not necessary*
unknown factor(s)	triggers BMP secretion in alveolar socket triggers PTH and RANKL secretion from stellate reticulum	necessary
PTH and RANKL secretion	clears a pathway above tooth	necessary
BMP secretion	bone deposited in alveolar socket	necessary
bone deposited in alveolar socket	pushes up (against tooth root)	necessary
PDL attaches to cementum	pulls tooth from above	necessary

Table 8.2: Summary of the what is known and potentially unknown about tooth eruption. Note the lack of clarity when it comes to root growth, BMP secretion and whether they are neecessary or sufficient for tooth eruption. The asterisk * indicates data supporting a conclusion is weak.

Now that we have summarized the facts we have, it is a good time to re-visit our troublesome assumption: teeth can erupt without roots. When professionals report the eruption of *rootless teeth,* do they mean these teeth *never* formed roots,

or did the roots undergo resorption *during* eruption? Sadly, waiting until we see the eruption of a rootless tooth is too late to answer this question. We need time-travel, psychic abilities, or lots of radiographs of unerupted teeth. Furthermore, it is difficult to make a strong conclusion that root growth is not *necessary* when the original report of rootless tooth eruption is missing. No wonder the force of tooth eruption are not agreed upon. Luckily, we do not need to solve the controversy here. But it does bring up an important concept: when things don't make sense, question your assumptions! Here is a good article on how and why:

- discussing assumptions using a gender-bias issue

Don't be disappointed if science raises two questions for every question it answers. Let's **recapitulate** our car metaphor as we summarize what we know about tooth eruption. The best evidence at this time is that a foot must be lifted off the brake pedeal (**RANKL** and PTH clear a pathway in bone tissue above), a foot must be placed on the gas pedal (**BMP** causes bone deposition in the alveolar sockets and pushes teeth from below) and there must be gasoline in the gas tank (the **PDL** pulls teeth outwards).

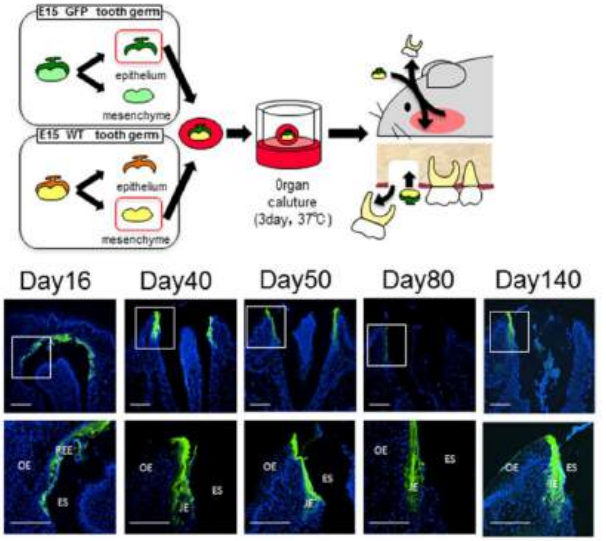

Figure 8.15: A bio-engineered tooth used to show junctional epithelium (JE) develops from the REE (green tracer), not oral epithelium. Legend: OE = oral epithelium, JE = junctional epithelium, ES = Enamel. Image credit: "The JE attached to the bioeng

But wait, there's more. Changes in the gingiva must also occur for tooth eruption to proceed. **Remodeling** of **oral mucosa** after **active eruption** is known as **passive eruption**. Old tissue is removed to clear a path for the tooth to erupt. Teeth do not just tear their way through the gums. But do not think of this like digging a tunnel for cars to drive through. The first difference is oral mucosa is *connected* to the tooth near the **CEJ** as it erupts. Otherwise, oral bacteria could enter the **sub-mucosa** and cause **gingivitis**. The second difference is there is no tunnel. Bone tissue is added behind teeth as they erupt. *So think of this like digging a lacuna for just one car, filling the lacuna behind the car as it travels, and trapping the car just as its front end clears the other side.*

Before eruption begins, the crown of the tooth is topped with 2 layers of epithelial cells, **ameloblasts** and **OEE**. Collectively, these two layers of epithelial cells are called the Reduced Enamel Epithelium (**REE**). Like the **invagination** and separation of **ectodermal** cells during **neurulation**←, the REE becomes separated from the **oral epithelium** during **dentinogenesis**. During tooth eruption, the REE re-joins the oral epithelium. As the REE **fuses** it re-forms **desmosomes**← and pairs up **CAMs**← with oral epithelial cells, but maintains contact with enamel. Many REE cells undergo **apoptosis**←, but those that remain become the

junctional epithelium. This special epithelium maintains **hemi-desmosome** contacts on both sides of the tissue: **apical** (enamel) and **basolateral** (**lamina propria**).

Some of the **REE** may not **fuse** with the **oral epithelium** nor undergo **apoptosis**, and remain partially stuck to the surface of a newly erupted tooth. This is known as the **primary enamel cuticle** (or the more old-fashioned name Nasmyth's membrane). It can easily be removed by gentle brushing or mastication.

Because **ameloblasts** in the **REE** are lost with tooth eruption, no new enamel is produced by human cells afterwards. **Amelogenesis** is faster and more organized than passive re-mineralization because it involves a protein **scaffold** which catalyzes crystal formation. Furthermore, the lack of REE means that gingiva cannot form **junctional epithelium** with **dental implants**. Understanding how junctional epithelium forms in the first place improves our ability to trick **oral mucosa** cells into behaving more like junctional epithelium.

Lastly, it is interesting to consider why only two sets of teeth erupt, making us (and most mammals) `diphyodonts`. The teeth of many other animals are **exfoliated** and replaced repeatedly throughout life (`polyphyodonts`). Why don't tooth buds **recapitulate** the exfoliation pattern of **hair follicles**? The answer is likely our oldest mamallian ancestors were tiny and did not live long. They lived long enough to benefit from replacing hair follicles seasonally (you dog owners

understand). But multiple sets of teeth would not have been beneficial. So why *two* sets of teeth? For that, we must go back even futher in evolutionary time. The ancestors of the very first mammals **developed** one set of teeth for chewing their way out of a hard eggshell, then a second set for eating food. Apparenly, our **ontogeny** recapitulates that **phylogeny**. So why did we say *most* mammals? The teeth of elephants and manatees grow very differently from our own. Their teeth erupt from the distal end of the jaws and pushing older teeth mesially, not twice but repeatedly throughout their lifetime. *Should* we study elephants and manatees to see if we can repeat this in humans? That is up to you, but time is running out.

Summary of dental tissues

	enamel	dentin	pulp	cementu
produced from	enamel organ	dental papilla	dental papilla	dental sac
lineage	ectoderm	neuro-mesenchyme	neuro-mesenchyme	neuro-me
tissue type	epithelial	connective	connective	connectiv
formative cells	ameloblasts	odontoblasts	fibroblasts	cementob
cells in mature tissue		(odontoblastic processes)	odontoblasts fibroblasts NMSCs	cementoc
resorptive cells		odontoclasts		cementoc
resorptive cell activity		exfoliation		exfoliatio
Mineral%	96%	70%		50%
Organic & water%	1% protein 3% water	20% protein 10% water		38% prot 12% wate
major protein(s)	amelogenin enamelin	collagen	collagen fibronectin	collagen

Table 8.3: Summary of dental tissue characteristics covered in chapters 4, 8, cells, PDLSCs = PDL stem c

Forms during	embryonic	embryonic (crown) post-natal (roots)	embryonic (crown) post-natal (roots)	post-nata
Vascularity			vascular	

Clinical considerations

Induction stage complications

Problems with the **induction** of **tooth germ** leads to the formation of too few or too many teeth.

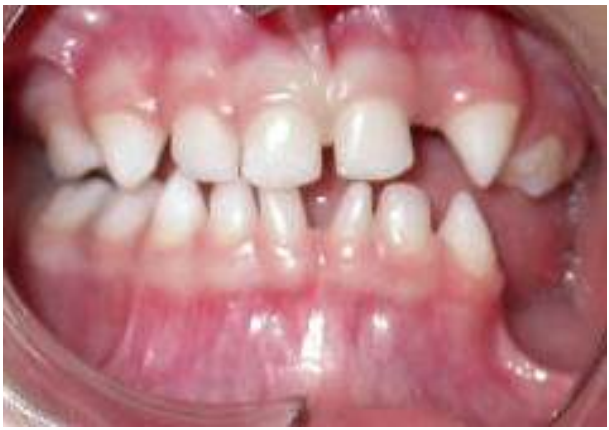

Figure 8.16: An example of partial anodontia (hypodontia). Image credit: "Incontinentia pigmenti presenting as hypodontia in a 3-year-old girl: a case report" By Kitakawa et al, Journal of Medical

Anodontia

Failure during the **induction** stage results in missing teeth, or **anodontia**. This may include inadequate release of **morphogens**← by **neuro-mesenchymal stem cells**, mutations in the morphogen **receptor** proteins, or **teratogens** that interfere with activation of **signal transduction cascades**←. Partial anodontia (also called **hypodontia**), where one or a few teeth are missing, is most common. The most commonly missing teeth are permanent maxillary lateral incisors, 3rd molars, and mandibular 2nd pre-molars.

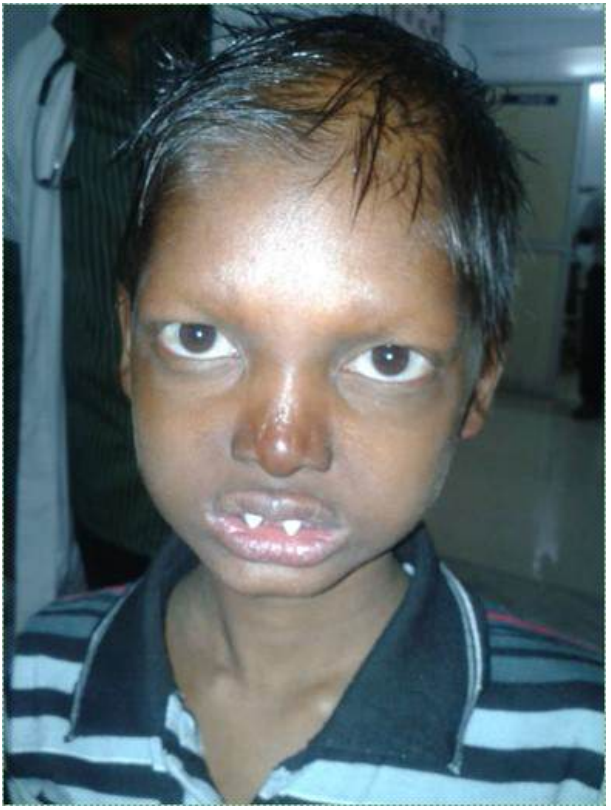

Figure 8.17: A patient with ectodermal dysplasia. Image credit: "Ectodermal dysplasia", by Pratheeba227 – photographed at Govt Vellore Medical College, is licensed CC BY SA 3.0

Ectodermal dysplasia (part 2 of 2)

Ectodermal dysplasia is covered in chapter 6 where we discuss the **induction** of **neural crest cells**. When signals from **neural crest cells** are reduced, regions of **ectoderm** that are supposed to grow faster than others do not get the boost in growth they need. This includes **hair follicles**, finger

and toenails, **tooth buds**, sweat glands and salivary glands. This may cause some or all of these structures to not develop at all (for teeth, **anodontia**), to develop in reduced number (for teeth, **hypodontia**), or reduced in size (for teeth, **microdontia**).

For those who cannot afford or are too young for **dental implants**←, complete or partial anodontia may be visible, as well as microdontia (Fig. 8.16). Children do not receive dental implants because implants do not grow in size to match a growing jawline. Multiple sets of dentures are an option (again, for those who can afford the treatment). Now is a good time to double-check you can distinguish between **hypodontia** and **microdontia**. If you haven't had Latin or a good medical terminology class, remember a *hypo*-dermic needle goes lower than the dermis, while *micro*-biology is the study of small stuff.

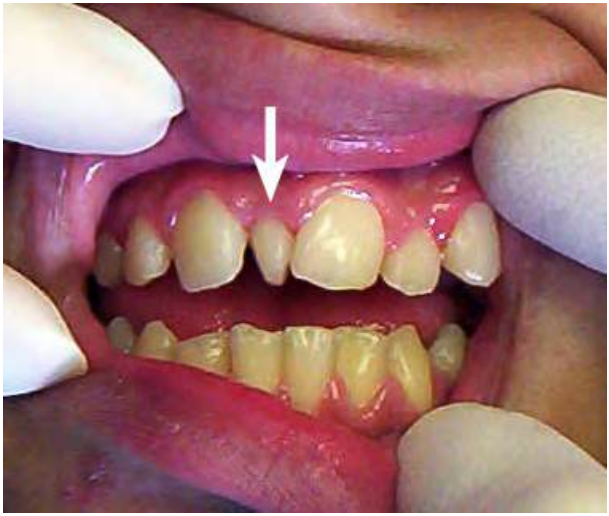

Figure 8.18: An example of hyperdontia. Image credit: "Fig. 5" by Toby Hughes et al., J. Dental Anthropology is licensed under CC BY 4.0

Hyperdontia

Hyperdontia, the formation of extra (supernumerary) teeth, occurs when **induction** of **tooth germ** occurs where it shouldn't. This is often a genetic condition. The most common extra teeth are between the central incisors (**mesiodens**), distal to the maxillary 3rd molar (4th molar, or **distomolar**) and the premolar region of either dental arch (**perimolar**).

Bud and cap stage complications

Problems in the **bud stage** of tooth development may lead to teeth that are too large (**macrodontia**) or too small (**microdontia**). This may affect all the teeth, some teeth, or a single tooth.

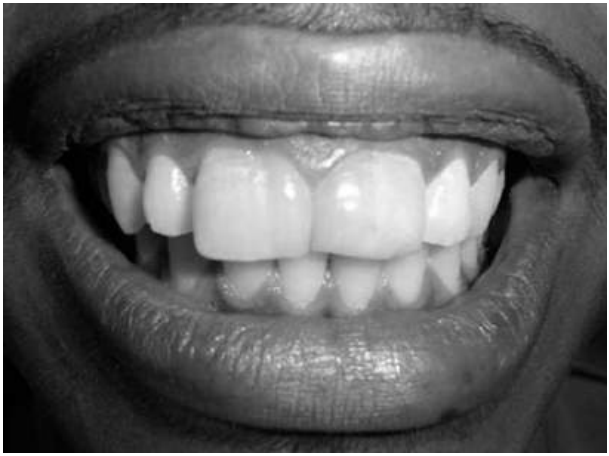

Figure 8.19: An example tooth geminati on. Image credit: "Periapical radiography of superior central incisive permanent geminated" by Katia Simone Alves dos Santos et al., Intl J Morphology is

Gemination

Tooth **gemination** (twinning, as in the constellation Gemini) occurs when a single **tooth germ** is partially divided in two. This may occur if the growing tooth germ bumps into a small dense region in the dental arch. Under healthy conditions, teeth grow within loose **mesenchyme**[←] tissue, which later **differentiates**[←] into osseous tissue and calcifies. A geminated tooth, coming from a single tooth germ, is larger than average, but has a single pulp cavity, hence this patient has an instance of **macrodontia** (but not **hypodontia**).

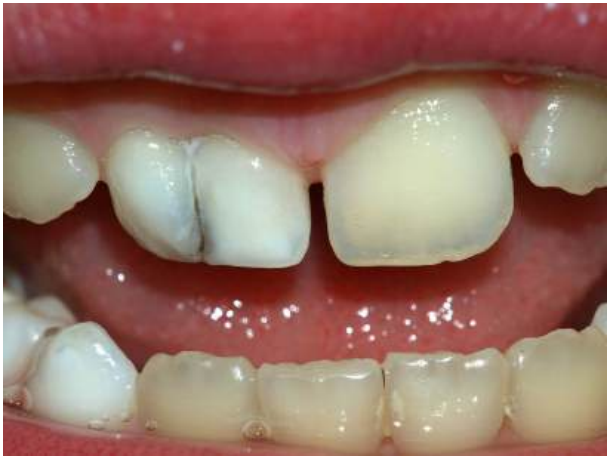

Figure 8.20: An example of two teeth fusing. Image credit: "milk. teeth. fusion" by Sarefo is licensed under CC BY-SA 3.0

Fusion

Tooth fusion (joining) of two **tooth germs** into a single germ can also cause a larger-than-average tooth to develop. In this case, **macrodontia** is accompanied by **hypodontia**. The fused tooth has two separate pulp cavities. This occurs when **induction** of two tooth germs occurs close to one another, or when external pressure forces two tooth germs closer together. Tooth germs can fuse during early stages of tooth development because the tooth germ is soft epithelial and **mesenchymal**[←] tissues. Getting too close together during later stages, when

enamel and dentin are calcified, leads to plain-old tooth crowding (malocclusion).

It is worth a mention that the fusion of teeth involves two separate buds growing into one large structure when their secretions meet. Syndactyly, the fusion of toes or fingers, is not really a fusion but a lack of separation due to diminished or absent **apoptosis**← signals.

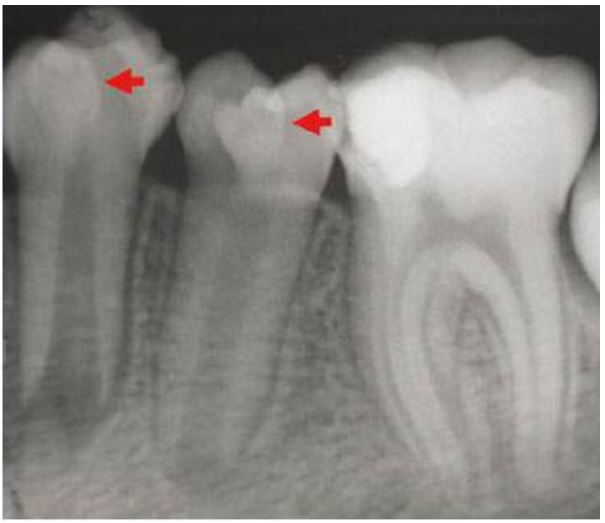

Figure 8.21: A radiograph of dens in dente (arrows). Image credit: "Radiographic view of Teeth" by Eduardo Borie E, et al, Intl J Morphology is licensed under CC BY 4.0

Dens in dente

Dens in dente (tooth within a tooth, or *dens invaginitus*) occurs when a small region of the **enamel organ** grows too fast and **invaginates** a second time, into the **dental papilla**, during the **cap stage**. This sub-region of the enamel organ continues to develop like a smaller version of the original

enamel organ, creating what looks like a miniature tooth within the main tooth. This can complicate a root canal surgery, but otherwise only makes for interesting radiographs.

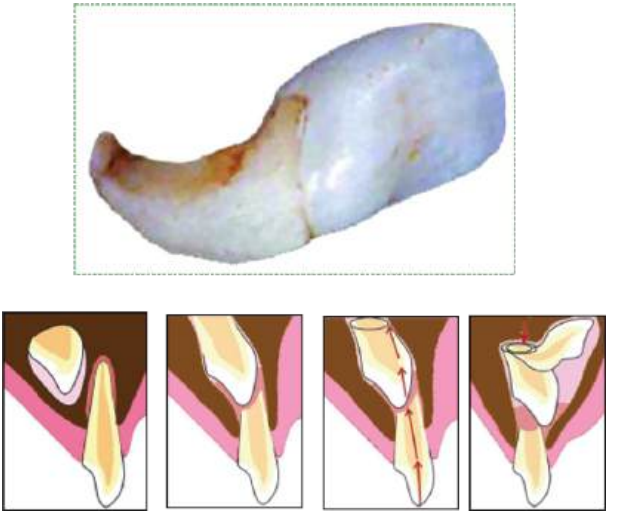

Figure 8.22: Example and illustration of root dilaceration. Image credit: "Figs 1-5" by Pawanjit Singh Walia, Intl J Clinical Ped Dentistry is licensed under CC BY 3.0

Dilaceration

Dilaceration, or a bend in the shape of a tooth, occurs when there is an impediment to the growth of **HERS**. A delay in **tooth eruption** can cause dilaceration. This can occur because the crowns develop and calcify first, and the roots develop later. If a developing tooth bumps into calcifying osseous tissue and is forced to change its direction of growth, the older calcified part of the tooth (the crown) will have grown at a different angle from the newer, softer root(s). Alternatively, trauma to a deciduous tooth can be transferred to the deeper succedaneous tooth, causing dilaceration of the succedaneous tooth (Fig 8.21).

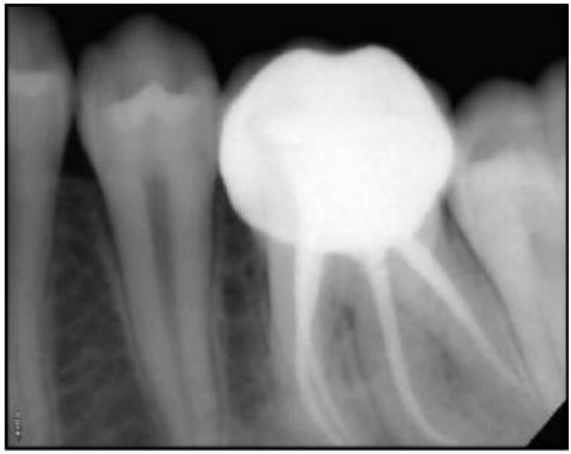

Figure 8.23: Radiograph of a root canal involving more roots than normal. Image credit: "Postoperative IOPA of 36 and 46." by Muktishree Mahendra et al, Case Reports in Dentistry is licensed under CC BY 3.0

Supernumerary roots

Teeth may develop extra, or **supernumerary roots**. As with **dens in dente**, this may complicate root canal surgery, but otherwise has limited clinical significance. When the shape of the tooth is altered but no pathology results, clinicians may note there is an alteration to the **morphology** of the tooth.

Eruption complications

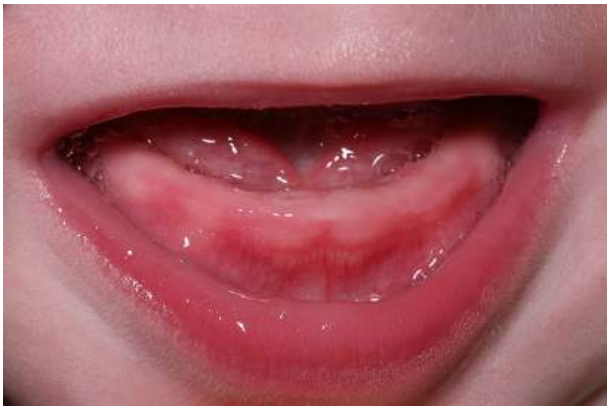

Figure 8.24: Teething. Image credit: "Own work" By Daniel Schwen – is licensed under CC BY-SA 4.0

Teething

During **tooth eruption**, several tissues undergo significant remodeling, including the **REE**, **oral epithelium** and **bone tissue**. This involves removal of **ECM** by the secretion of

digestive enzymes, and removal of cells by **apoptosis**. However, enzymes aren't smart enough to remove only ECM, they tend to kill cells as well. For instance, when the **enamel organ** secretes **matrix metaloproteinases** to clear a path for the erupting tooth, this triggers inflammation as **plasma membrane** proteins are destroyed along with ECM, leading to cell death. Inflammation can cause discomfort, tenderness or **edema** in the affected area.

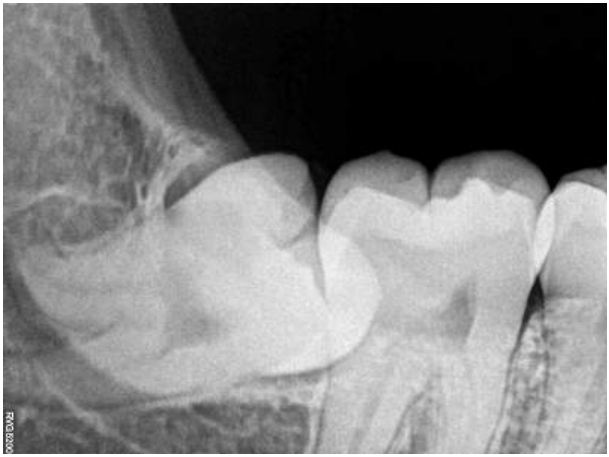

Figure 8.25: Impacted third molar. Image credit: "Impacted Wisdom Tooth aka Lower Right Third Molar 48 RVG IOPA Xray" by Nizil Shah is licensed under CC BY-SA 4.0

Primary failure of eruption

Primary failure of eruption (**PFE**) is the partial or complete

failure of a tooth to erupt (otherwise known as an impacted tooth) despite a healthy eruption pathway. While there are likely many causes of PFE, one major heritable cause is a mutation that disrupts parathyroid hormone signaling from the **tooth germ** to **alveolar bone** tissue. Parathyroid hormone activates **osteoclasts** and inhibits **osteoblasts**, therefore inhibiting parathyroid hormone signals leads to increased bone deposition. This leads to **ankylosis** of the tooth prior to eruption. It also makes tooth extraction difficult or impossible, especially as time progresses. Impacted teeth may cause inflammation and pain, or may not be detected until an x-ray is performed. The 3rd molars are the most commonly impacted teeth. Complications include crowding of neighboring teeth, infection, malocclusion, and– rarely– neoplasia, making extraction an important option.

Mechanical failure of eruption

A tooth may also become impacted *after* erupting partially because of ankylosis of the tooth, which is termed mechanical failure of eruption (**MFE—** it seems odd that it is not secondary). In contrast to **PFE**, there is a physical barrier to the eruption pathway. Absence of the **PDL** indicates **ankylosis** and can indicate MFE. With MFE, orthodontic or other therapies may allow the impacted tooth to erupt eliminating the need for extraction and replacement.

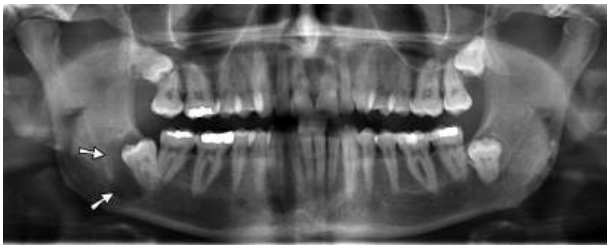

Figure 8.26: Example of a dentigerous cyst. Image credit: "Jaw cyst" By: Coronation Dental Specialty is licensed under CC BY 3.0

Dentigerous cyst

An impacted tooth can produce a cyst around the crown, called a **dentigerous cyst**. As the motionless tooth attempts to digest its way through **oral epithelium**, fluid accumulates between the crown and **REE**. The cyst may continue to grow in size, causing pain and trauma to the jaw bone (usually the mandible). Extraction of the impacted tooth will solve the issue. Orthodontic facilitation of **tooth eruption** may be an option as well. Rarely, the REE in a dentigerous cyst develops

into a benign tumor known as an ameloblastoma. The main risk of this cancer is that similar to that of the cyst—it can cause pressure which may cause bones to break or to grow malformed. The reason this tumor is rare is that the REE cells are **terminally differentiated** (non-mitotic). Furthermore, because these epithelial cells are anchored to one another by **desmosomes**←, this type of tumor rarely metastasizes.

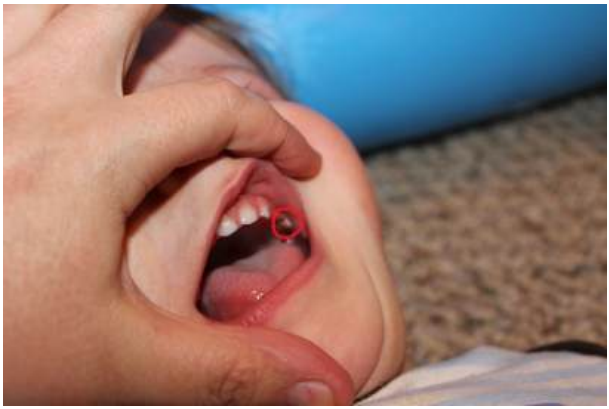

Figure 8.27:A Bohn's nodule is keratin, not a tooth. Image credit: "Own work" by West Exchange is licensed under CC BY 3.0

Bohn's nodules and Epstein pearls

Bohn's nodules are masses of **keratin** trapped in the gingiva. They may be referred to as epithelial rests of Serres in older texts, and are also called **Epstein pearls** when they are located

within **oral mucosa**. Bohn's nodules are produced by the **REE**, while Epstein pearls are produced by **oral epithelium**. Furthermore, remnants of **minor salivary glands** can be called Bohn' nodules as well, despite lacking keratin. Their presence along the alveolar ridge may cause parents to mistake them for erupting teeth. They occur more commonly along the maxillary ridge. Bohn's nodules and Epstein pearls are transient—they go away on their own, usually within 3 months, and produce few if any disturbances.

Lastly, if you are interested in the history of embryology and long-dead European men, we offer the following. Antoine Étienne Serres collaborated with the embryologist Johann Friedrich Meckel, who named the cartilage in the **mandibular arch** that develops into the mandible (**Meckel's cartilage**). Together, they attempted to unify embryology and the **lineage** of different species (later called *evolution by heritable selection* by Charles Darwin and the less-well-known work of Alfred Russel Wallace). Serres' and Meckel's theory was developed further by Ernst Haeckel, who coined the memorable phrase "**ontogeny recapitulates phylogeny**," which we have quoted several times in this text.

< Chapter 7 * navigation * Chapter 9 >

Chapter review questions

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https://openoregon.pressbooks.pub/histologyandembryology/?p=53

9.

ENAMEL DEVELOPMENT

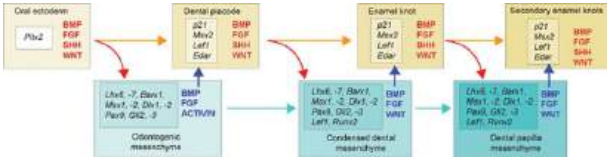


Figure 9.1: An illustration of the large number of and complicated interplay between morphogens involved in amelogenesis. Image credit: "The sequential and reciprocal regulatory signaling between epithe

Overview

lium (red) and mesenchyme (blue)" by Thesleff, I. and Tummers, M, Stembook.org, is licensed under CC BY 3.0

Sola dosis facit venenum (the dose makes the poison) — Paracelcus

Even though dentin formation begins before enamel, we begin with enamel formation. This way, the chapter on dentin formation can remain close to pulp, which is important because they share the same **lineage** from **neuro-mesenchyme**. Enamel is produced by cells derived from the **ectoderm**. This leads to key molecular differences between the **ECM** of enamel versus the ECM of dentin, cementum and bone tissue.

One major concept we focus on is that **ameloblasts** and **odontoblasts** undergo **reciprocal** signaling, meaning you can't get odontoblasts without ameloblast *signals*, and you can't get ameloblasts without odontoblast signals. It takes a team effort. In this chapter, we mention three **morphogens**[←] (ones we've covered before), and two **transcription factors**. Hopefully, Fig. 9.1 illustrates that the process is more complex. Don't memorize the morphogens unless your instructor indicates they are on your exams. The important concept is that when we get to chapter 10 and discuss how dentin undergoes regeneration, we should question how it is similar to embryonic **development**, and how it is different as well.

Figure 9.2: Fluorapatite crystals form in nature, shown here with quartz crystals. "Apatite-(CaF), Quartz" by Rob Lavinsky, irocks.com is licensed under CC BY-SA 3.0

One reason to study embryology is to learn about how tissues are made in the first place. When we do, we often learn how to repair tissues better. For bone and **oral mucosa**, learning how **stem cells**[⇐] **differentiate**[⇐] into adult cell types has led to advances in **bone grafting**, **guided tissue regeneration**[⇐] and **bio-active membranes**. Could we one day re-grow enamel using stem cells? In this chapter, think about why creating new ameloblasts might not be as useful to us as new osteoblasts or fibroblasts (hint: location, location, location).

Then, focus on how enamel matrix is formed. Secrete some proteins and minerals and turns into teeth and bones, right? Fig. 9.2 shows fluorapatite crystals. A mouth full of those would look impressive, but wouldn't be very functional. Calcium and phosphate react to form a powder, you can probably find a bottle of it in your school's chemistry lab. To get these chemicals to react in a way that creates *human teeth* requires more than chemistry. So perhaps the biggest concept in this chapter is how ameloblasts create **calcium hydroxyapatite** crystals in the shape and density of a tooth, rather than in the shape of Fig. 9.2. To understand tooth **morphology**, you must understand how its **ECM** is created.

In this chapter and the next, we discuss **Tomes' process**, **Tomes' granular layer** and Tomes' fibers (we use the name **odontoblastic process** instead). If you wish, take a look at:

- Tomes' collection of teeth, skulls and dental instruments
    - type "tomes, sir john" into the search tool
    - the Royal College of Surgeons of London (*just don't ask where he acquired these, apparently*)

Amelogenesis

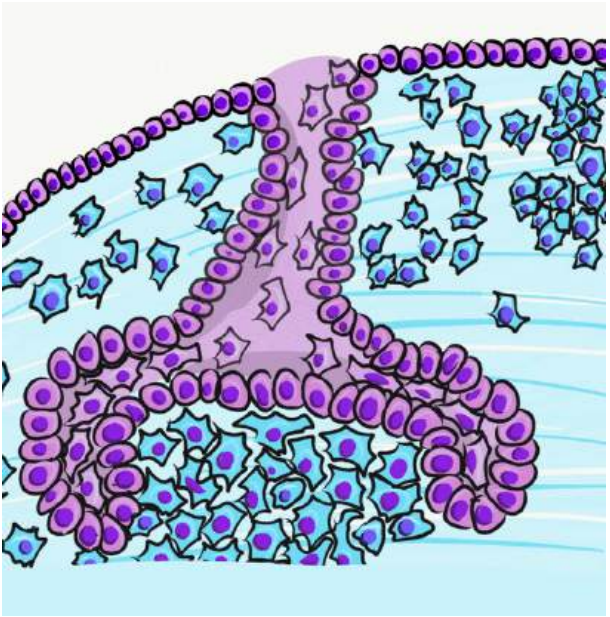


Figure 9.3: Animated representation of odontoblast and ameloblast formation.

Induction (or initiation)

This paragraph should be review. **Amelogenesis** starts at the **bell stage** of tooth development. By this stage, **ectodermal** cells have **invaginated** to produce two layers of **simple cuboidal cells**[←] called the inner enamel epithelium (**IEE**) and outer enamel epithelium (**OEE**). Between the IEE and OEE, more epithelial cells are scattered, namely the **stratum intermedium** and **stellate reticulum** cells. Collectively, these cells are known as the **enamel organ**.

Now let us add to what we learned in the previous chapter.

The **enamel organ** grows around a cluster of **neuro-mesenchymal stem cells** known as the **dental papilla**. Initially, a **basement membrane** separates the two. The first visible sign of **differentiation**← occurs when the cuboidal **IEE** cells next to the dental papilla elongate, becoming more columnar in shape. Their nuclei move to the **apical** side of the cell, while organelles needed for secretion accumulate on the **basal** side. We call these elongated cells **pre-ameloblasts**. It is not certain what triggers this initial change in **morphology**, evidence suggests a member of the **Wnt** family of **morphogens**← activates **planar cell polarity**← signals. The ability to undergo **mitosis**← halts, meaning no new pre-ameloblasts form after the bell stage. The disappearance of the basement membrane allows these cells to move more easily, due to the loss of **hemi-desmosome** attachments.

Pre-ameloblasts secrete a number of short-range **morphogens**←, including a member of the **BMP** family (Fig. 9.3). This **induces** neighboring **neuro-mesenchymal stem cells** of the **dental papilla** to **differentiate** into **odontoblasts** and begin secretion of **pre-dentin**. This puts pre-ameloblasts in direct contact with pre-dentin. **Integrins** on pre-ameloblasts contact a form of **collagen**← found in pre-dentin (collagen type I), as opposed to the type in the basement membrane (collagen type IV). In addition, newly formed odontoblasts secrete several morphogens, including **BMPs**. The important concept is that half of the signal that induces

the differentiation of **ameloblasts** comes from the pre-dentin side.

The second half of that signal comes from the opposite direction, the **stellate reticulum**. One such signal produced by stellate reticulum cells includes a member of the **FGF** family. This leads to the activation of **transcription factors** in the pre-ameloblasts, which up-regulate the **expression** of **genes** involved in the secretion of enamel. Transcription factors involved in amelogenesis include MSX-2, a **homeobox**↩ gene, and RUNX2, a transcription factor also involved in the differentiation of **osteoblasts**. Visible under the microscope, pre-ameloblasts change shape again, becoming shorter, and develop a bump on the basal side known as **Tomes' process**. A summary of some molecules involved is in Table 9.1. The names are less important than the general order of events: from **morphogen**↩ to **receptor** to transcription factor to gene expression.

Morphogens	Receptors	Transcription factors	Genes up-regulated
Collagen (pre-dentin) BMP (odontoblasts)	Integrins BMP-R	MSX-2 DLX1 RUNX2	Enamelin Amelogenin Matrix Metalloproteinase
FGF (stellate reticulum)	FGF-R		

Table 9.1: A partial list of molecules involved in ameloblast differentiation.

Figure 9.4: A thin section highlights enamel's colorless state. Image credit: "18036" is in the Public Domain, CC0 / *cropped*

Apposition

After changing **morphology**, **ameloblasts** secrete enamel matrix from **Tomes' process**. This ECM is similar to the **ECM** of **bone tissue**, but with important differences. Like bone matrix, enamel matrix is first secreted in a softer form (**pre-enamel**), consisting mostly of proteins and water, and it mineralizes later. Enamel matrix contains Calcium-Phosphate crystals and organic components. Enamel, after it matures, has

a higher mineral percentage, making it the hardest substance in the human body. Unlike bone tissue, however, enamel matrix has very little or no **collagen**. Instead, ameloblasts secrete other proteins known as **amelogenins** and **enamelins**. These proteins act as nucleation centers which help induce Calcium and Phosphate to form crystals. We can think of these proteins as **scaffolds** for non-living chemicals rather than cells. The important fact to remember here is that collagen is a protein commonly made by **fibroblasts** (and other cells derived from **mesenchyme**), which most often **differentiate** from the **mesoderm**. Ameloblast **lineage**, on the other hand, is from the **ectoderm**. Presumably the connective tissue **genes** like collagen are **methylated** and packed away around **histones**, and are not available for **transcription**.

The shape of the **enamel organ** in the **bell stage** determines the shape of the crown. No new ameloblasts form because they lose the ability to undergo **mitosis** once they **differentiate** into **pre-ameloblasts**. Furthermore, no **stem cells** can contact both pre-dentin and **stellate reticulum** once thick layers of enamel and (mature) dentin push the two apart. Therefore, no new ameloblasts form by differentiation, either. Because of how the pre-ameloblasts line up in the bell stage, mostly pointing towards the oral cavity, enamel is thicker in the masticatory surfaces and thinner in the cervical areas.

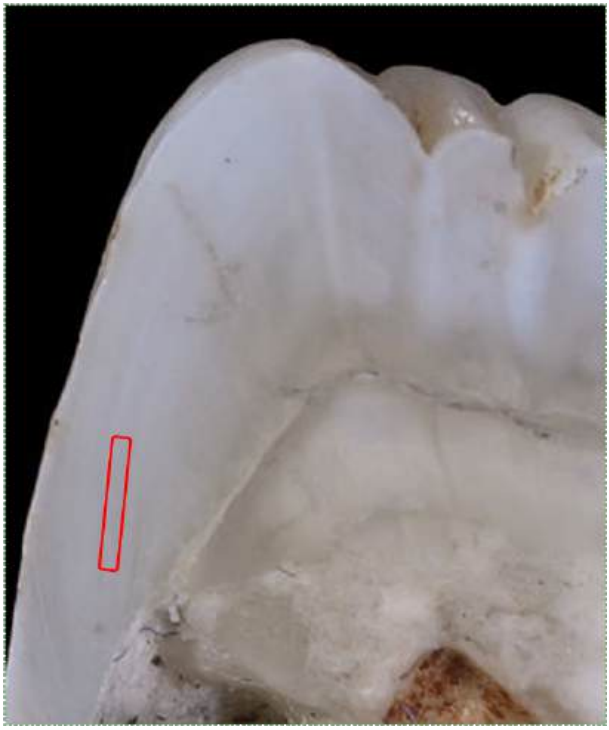


Figure 9.5: Enamel is deposited appositionally, as seen by the Lines of Retzius. Image credit: "Tooth of Paranthropus robustus SKX 21841 from Swartkrans" by Didier Descouens is licensed under CC BY 3.0 / *red box added*

Enamel is secreted by **ameloblasts** from **Tomes' process**, laying down new layers of **pre-enamel** over older layers, pushing the ameloblasts further away from dentin. Ameloblasts, like many human cells, do not work at a uniform speed. About every 4-12 days there is a change in the enamel deposition rate. As a result of deposition of enamel speeding up and slowing down, visible bands of lighter and darker enamel (less dense and more dense) are visible when viewed in cross section. These visible bands are known as **Lines of Retzius** (or striae of Retzius, Fig. 9.5). One such band, the **neonatal line**, is particularly dark because the ameloblasts rested during the day we were born. After birth, the edges of the Lines of Retzius may be visible on the surface of teeth, especially the lingual surface of anterior teeth, in which case they are called **perikymata** (in older texts, the imbrication lines of Pickerill). Over time, chemical and physical abrasion wear perikymata away.

animation of enamel rods

Figure 9.6: Enamel rods are each produced by one small group of ameloblasts.

Together in a line, all of the **ameloblasts** produce *horizontal* layers of enamel, new on top of old. But roughly one ameloblast produces a single **enamel rod** (or prism) in a *perpendicular* direction (it takes 4 ameloblasts to make one enamel rod, but each ameloblast works on 4 different rods). Because the rods are rounded (almost hexagonal), and not square, there are gaps between them. These gaps aren't empty, they are filled with a slightly different form of enamel, called **inter-rod enamel** (while the enamel within rods is called **rod enamel**).

Figure 9.7: Hunter-Schreger bands are the enamel produced by a group of ameloblasts, and are lines that run perpendicular to the DEJ. Image credit: "18036" is in the Public Domain, CC0 / *cropped*

If you look closely to Fig. 9.6, you might notice **Hunter-Schreger bands** (HSB), which are lines running perpendicular to the **Lines of Retzius** and the **DEJ**. Each line represents the contributions of a small group of ameloblasts. If it's a little hard to see, it is because each Hunter-Schreger band is only 1/10th of a millimeter thick. What you are looking for is a pattern similar to the fore edge of a textbook (all the

pages as a group). Ameloblasts do not work in straight lines, the bands curve slightly– like the pages of that $375 textbook you dropped in water and can't sell back to the bookstore. Furthermore, different regions have different curve angles and patterns. These curves aren't accidental, they increase the tensile strength of enamel. If you have ever chopped wood, you know it is easy to do if you land the axe along the grain. Hit at an angle, and it can hurt you instead. The curving of Hunter-Schreger bands ensures there is no single grain along which a tooth can easily shear. Cracks develop in enamel, but cracks usually stop part-way through. The **planar cell polarity**← signals that guide rod curvature have been identified (antagonists of Wnt morphogens). The end result is that enamel, despite having the brittleness of glass, is the most resilient tissue in the human body. Dentin has similar curves (plural) which, unlike enamel, get specific names. As a fun side-note, or of relevance to those planning on pursuing a career as a veterinary dental hygienist at your local zoo, the Hunter-Schreger bands of hyenas occurs in a zig-zag pattern, increasing the tensile strength of their enamel even more, allowing them to chew through bones.

Maturation

Enamel is first secreted as a protein-rich gooey substance called **pre-enamel**. Two major organic components of enamel are

the proteins **amelogenin** and **enamelin**. **Ameloblasts** also secrete a digestive enzyme. This enzyme, a **matrix metalloproteinase**, digests **collagen**, making it easier for ameloblasts to migrate though the **ground substance** of overlying **mesenchyme**. This is necessary because as ameloblasts deposit pre-enamel from **Tomes' process**, it pushes the cells outwards. If you recall, matrix metalloproteinases are also involved in the proper migration of tissues during formation of the palate as well as in **tooth eruption**. Matrix metalloproteinases serve a second role by processing enamel proteins made by the ameloblasts after they are secreted into the **ECM**. The enzymes modify (not digest) amelogenins and enamelins, improving their ability to catalyze the crystallization of calcium and phosphate. This creates a delay between the secretion of pre-enamel and its mineralization. Without a delay, ameloblasts could become trapped by their own ECM, halting **amelogenesis**.

Unlike dentin and cementum, enamel undergoes a significant amount of **remodeling** after mineralization. Once pre-enamel has been laid down and mineralizes, ameloblasts switch tasks and cause the hard enamel to mature into even harder enamel. Most of the proteins are removed, replaced by fluids which crystallize. **Amelogenin** and **enamelin** are removed by endocytosis and by secretion of protein-digesting enzymes. These enzymes include the **matrix metalloproteinase** whose first cuts to amelogenin and enamelin improved their function, but more cutting breaks

them down. Removal of protein increases the relative mineral content of enamel, increasing its hardness compared to bone, dentin and cementum. We can therefore think of the protein secreted by ameloblasts as a **scaffold**[←] that ensures enamel is made quickly, at about 4 μM per day. In contrast, fluoride-based re-mineralization of tooth enamel is not measured using a small ruler, suggesting the rate of re-mineralization is too slow to see under the microscope.

A number of different electrolytes and molecules are incorporated into enamel in small amounts during the maturation stage. One important electrolyte is fluoride (F^-). Fluoride ions are negatively charged, and incorporate into calcium hydroxyapatite crystals by replacing a small number of hydroxide (OH^-) ions. This has important clinical effects. First, a small amount of fluoride increases the strength of **calcium hydroxyapatite** crystals. Secondly fluoride ions found in either saliva or in enamel neutralize acids released by bacteria, reducing enamel demineralization and preventing caries. Excessive fluoride levels, however, affect the activity of ameloblasts during the maturation stage of amelogenesis, reducing their removal of protein **scaffolds**[←]. This may create a visual change in the form of white lesions on the surface of teeth. These lesions are where the enamel has a higher protein and lower crystalline content, known as **dental fluorosis**. As with any chemical, `the dose makes the poison`. Topical application, from toothpaste or gels, allows fluoride to incorporate into the surface layers of enamel, which has

considerable benefits. The greatest potential benefit is when fluoride exposure occurs during the apposition and maturation stages of amelogenesis, early in embryonic development.

Clinical considerations

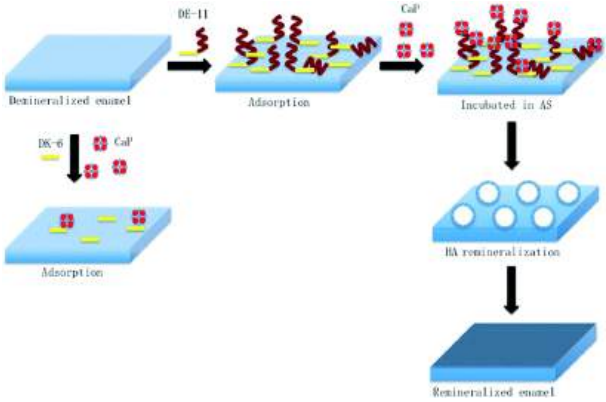
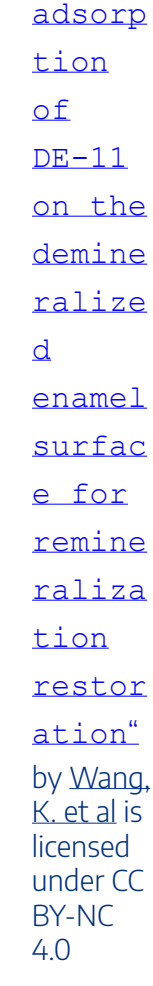


Figure 9.8: Schematic of enamel re-mineralization using a bioactive compound. Legend: DK-6 = control (inactive) peptide. DE-11 = active peptide. CaP = calcium phosphate. HA = carbonated hydroxyapatite. AS = acetic acid solution (to mimic caries). Image credit: "Schematic

Enamel re-mineralization



of the adsorption of DE-11 on the demineralized enamel surface for remineralization restoration" by Wang, K. et al is licensed under CC BY-NC 4.0

Certain chemicals help induce calcium and phosphate to react and add to the crystalline matrix of enamel. The most common is fluoride. In addition to its role in neutralizing acids, Ffuoride nucleates **calcium hydroxyapatite** crystals (see also Fig. 9.2). Topical gels and toothpastes that promote re-mineralization frequently contain Fluroride plus calcium and phosphate in forms that dissolve in saliva. Other agents are added to help fluoride to stick to the surface of enamel. After all, promoting the formation of calcium-phosphate powder in saliva won't help teeth. One such agent is casein-phosphopeptide. Casein is a protein found in milk. Its stickiness makes it useful in cheese-making and glue (including the original formula of Elmer's glue).

Increased understanding of how protein **scaffolds**[⇐] facilitate the growth of mature tissues has allowed researchers and companies to develop better tools for assisting in enamel re-mineralization. The goal is to mimic the ability of **amelogenin** and **enamelin** proteins to nucleate **calcium hydroxyapatite** crystals from a solution containing calcium and phosphate. These products do not need to

contain amelogenins, enamelins, or even proteins. For instance, xylitol and certain glass compounds nucleate calcium hydroxyapatite crystals. Xylitol, a sugar alcohol extracted from birch trees, has the dual benefit of activating sweet taste buds. It can be used to replace simple sugars in foods, removing substrates which harmful oral bacteria use for energy when secreting enamel-dissolving acids (just don't let your dog eat any).

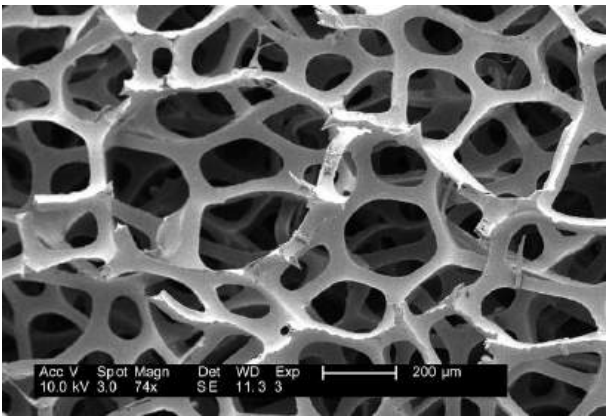


Figure 9.9: Acid etching demineralizes rod and interrod enamel at different rates, producing a rough surface suitable for bonding. Image credit: "This work" by Janice Carr, USCDCP is in the Public Domain, CC0

Surface etching

Because **rod enamel** is slightly different from **inter-rod enamel**, applying an acid to the crown surface wears the two at different rates—a process called **surface etching**. This creates

an uneven surface, providing more surface area to create a stronger bond between the tooth and a surface sealant (think about how **dermal papillae**← and **rete pegs**← interdigit with one another for a stronger connection). Treatment with a weak acid also removes surface debris which interferes with bonding of an adhesive, known as a smear layer.

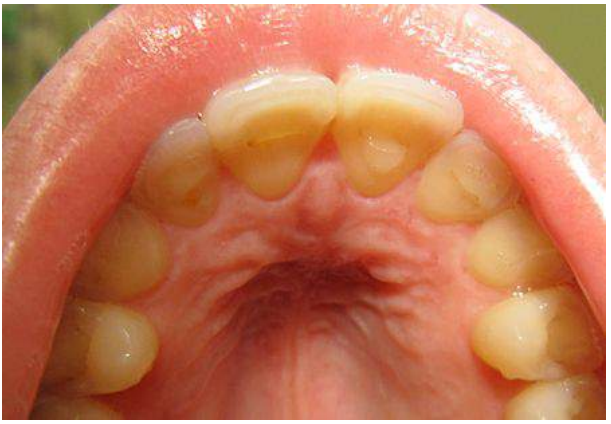


Figure 9.10: Acid erosion of enamel. Image credit: "Loss of enamel from the inside of the upper teeth as a result of bulimia" by James Heilman, MD is licensed under CC BY-SA 4.0

Erosion

Prolonged exposure to a weakly acidic environment, or a shorter exposure to a highly acidic environment, can cause **erosion** of tooth enamel. Positively charged H^+ ions of an acid react with negatively charges OH^- and OPO_3^{3-} ions of **calcium hydroxyapatite** crystals, dissolving them. Bulimia and acid reflux bring highly acidic hydrochloric acid of the stomach in contact with teeth, causing erosion. **Xerostomia**, caused by the abuse of drugs such as methamphetamines, the use of certain medications, chemotherapy, or that associated with old age, reduces the protective buffering ability of saliva to protect tooth enamel. Acidic foods or sugary foods may also contribute to enamel erosion. The simple sugars in many foods are nutrients oral bacteria metabolize and use to produce energy, which they then expend to create and secrete enamel-eroding acids.

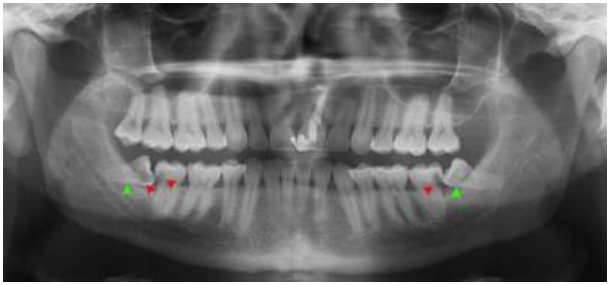


Figure 9.11: Dental caries (red arrows) caused by impacted 3rd molars (green arrows). Image credit: "Impacted wisdom teeth caries" by Coronation Dental Specialty Group is licensed under CC BY-SA 3.0

Decalcification (a **dental caries**, or tooth decay) can be detrected earlyduring a regular examination. Early signs of decalcification include the appearance of a white lesion on the surface of enamel, which may be softer to the touch, and appear more radiolucent on a dental radiograph. Treatment often involves removal of the carious tissue followed by replacement with a **bio-compatible** material.

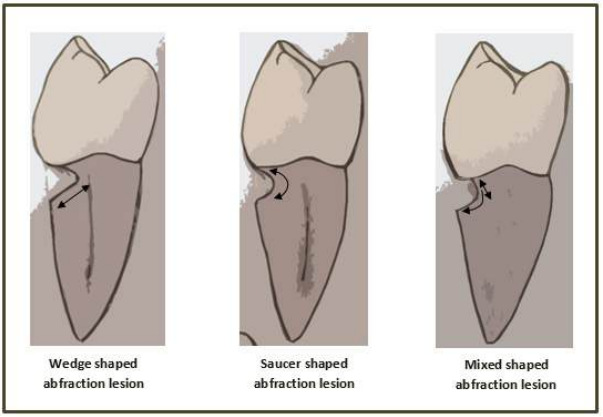


Figure 9.12: Three common patterns of tooth abfraction, all along the dentin-enamel junction. Image credit: "Own work" By Ebd2015 is licensed under CC BY 3.0

Abfraction

When non-carious tooth material is lost by force, it is called **abfraction**. Abfraction frequently occur along the **DEJ**. Chewing causes flexion forces at the cervical region of the tooth. Treatment of this type of lesion should involve reducing the stress that caused it in the first place, such as parafunctional habits or excessivetooth occlusion. Because enamel is stronger than dentin, dentin is frequently lost in response to force transmitted through the enamel to the DEJ.

Developmental disturbances

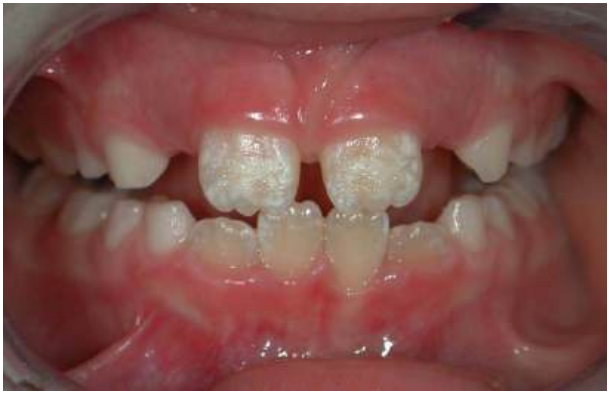
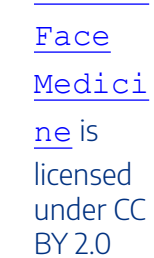


Figure 9.13: An example of the pitting and white lesions caused by enamel hypoplasia. Image credit: "Symmetrical enamel hypoplasia of grade I on permanent incisors in a CD patient" by Maurizio Procaccin

Enamel hypoplasia



Reduced enamel formation during embryonic development is called **enamel hypoplasia**. This occurs to primary and/or succedaneous teeth. Hypoplasia in an embryo is often triggered by health changes in the mother, such as a gastrointestinal disorder or a syphilis infection during pregnancy. If ameloblasts cannot acquire enough minerals during the time they are active, upon tooth eruption the surface enamel may contain white lesions, or exhibit pits and grooves. The enamel is more brittle and susceptible to **erosion** and **abfraction**. Furthermore, increased sensitivity to temperature and increased incidence of dental caries and periodontal disease are expected. Because **amelogenesis** is complete long before birth, there is currently no way to significantly assist in enamel production after tooth eruption occurs. Therefore, preventative treatment becomes much more important, and full-coverage crowns might be required.

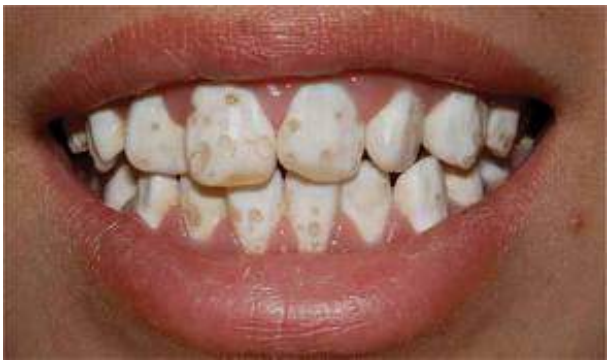


Figure 9.14: An example of a patient with amelogenesis imperfecta. Image credit: "Amelogenesis imperfecta" by 신연아 is licensed under CC BY 3.0

Amelogenesis imperfecta

Another cause of enamel hypoplasia is the genetic condition **amelogenesis imperfecta**. There are a number of different **genes** that, when mutated, cause this disease. For instance, mutations in genes for **amelogenins** and **enamelins** leads to forms of amelogenesis imperfecta by reducing the ability of scaffolds to rapidly nucleate **calcium hydroxyapatite** crystal formation. This results in enamel that is hypo-mineralized.

Additionally, gene mutations to the **matrix metalloproteinases** necessary for proper enamel development can result in amelogenesis imperfecta. Without removal of ameloginins and enamelins by the matrix metalloproteinase enzymes, ameloblasts cannot create the high mineral content of healthy, mature enamel. Because amelogenesis imperfecta is caused by different mutations to a number of different genes, the symptoms occur on a spectrum, from mild to severe.

Figure 9.15: An enamel pearl. Image credit: "Own work", By Wicketcity is licensed under CC BY-SA 4.0 / *cropped*

Enamel pearl

The **IEE** within **HERS** does not normally **differentiate**← into **ameloblasts** because of the lack of **morphogens** from **stellate reticulum**. However, a small region of the IEE might mistakenly differentiate into ameloblasts along the root and produce a small amount of enamel over cementum, known as an **enamel pearl**. There may be no harm in having pearls, other than they may be confused with a calculus during a scaling procedure. If the wrong technique is used, the instrument tip may break off when it encounters the much harder enamel pearl. However, if an emalep pearl forms near or in a fucrcation, there will be a void in the **attachment apparatus** at that site. This results most often in a periodontal pocket which can result in tooth loss, depending on the extent of the void. Enamel pearls can be identified by radiography, because enamel is more radiopaque than cementum. Enamel pearls generally occur on the furcations of the multi-rooted molars.

< Chapter 8 * navigation * Chapter 10 >

Chapter review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:

https://openoregon.pressbooks.pub/histologyandembryology/?p=55

10.

DENTIN-PULP COMPLEX DEVELOPMENT

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animation of dentinal hypersensitivity

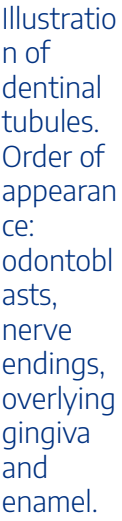

Figure 10.1: Illustration of dentinal tubules. Order of appearance: odontoblasts, nerve endings, overlying gingiva and enamel.

Overview

Dentin and pulp are covered together because of their shared **lineage**: they are derived from the **neuro-mesenchyme** of the **dental papilla**. Dentin resembles enamel chemically. But don't focus too much on chemical composition by weight. Humans are made up of mostly the same atoms as thalidomide— the **morphology** of those atoms matters! By the end of this chapter you should see similarities between dentin and pulp. The two are often referred together as the **dentin-pulp complex**.

One important concept in this chapter involve the cells in (or near) dentin. The **ECM** of dentin contains long tunnels

filled with fluid and cytoplasmic extensions of dentin-producing cells, the **odontoblasts**. These are significant when it comes to dental hypersensitivity, as well as in the repair of dentin following damage.

Another major concept involves focusing on the ECM of dentin. Like the formation of enamel matrix, studying how dentin matrix is formed teaches us about how it can be repaired. Unlike enamel, dentin can be repaired by cellular activity after eruption. As a result, dentin comes in forms made before birth, shortly after birth, and long after birth. Before you get too comfortable thinking that means there are only 3 types of dentin, be aware there are multiple forms made both before and after birth. Enamel only comes in one form: the stuff made during the embryonic period.

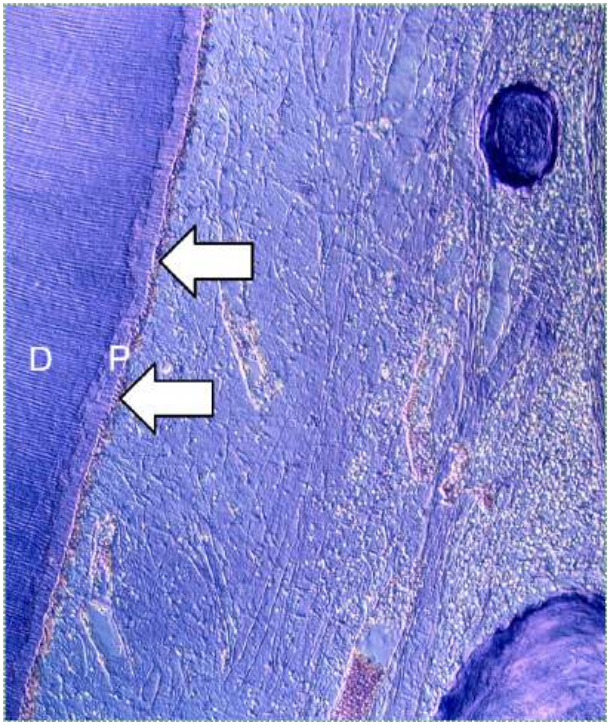

Figure 10.2: Important regions during dentinogenesis (developing tooth). Legend: P = pre-dentin, D = mature dentin, arrows = cell bodies of odontoblasts found at the edge of the pulp. Image credit: "Histological section of tooth" by Doc. RNDr. Josef

Dentin formation

Dentinogenesis is the process of dentin formation (and should not be confused with the word **odontogenesis**). Dentinogenesis begins during the **bell stage** of tooth development. **Odontoblasts** (arrows in Fig. 10.2) are the cells that produce dentin. The first step is the secretion of proteins, including **collagen**↩, which act as a **scaffold**↩. The second step is mineralization around the scaffold. The initial protein-rich material is **pre-dentin** (P in Fig. 10.2), after it mineralizes it is called dentin (D in Fig. 10.2). Unlike **amelogenesis**, there is no third step of protein removal (**remodeling**).

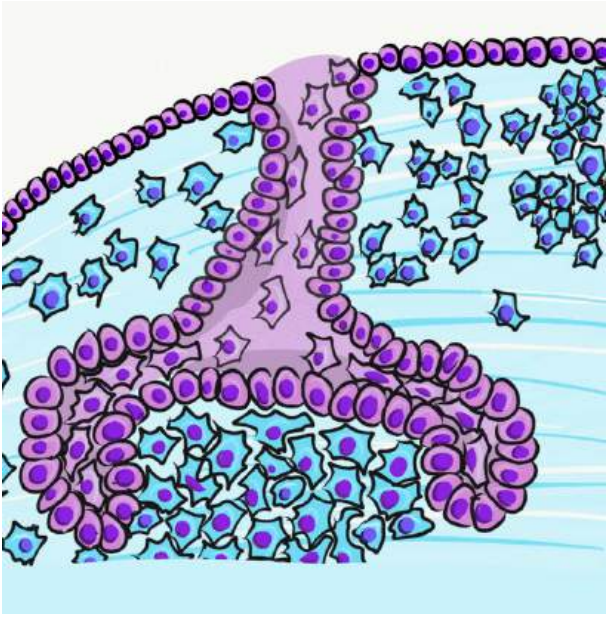

Figure 10.3: Animated view of amelogenesis and dentinogenesis.

Induction (or initiation)

During the **induction** phase, the **odontoblasts** appear. This happens in the **bell stage** of tooth **development**, as the **enamel organ** grows around the **dental papilla**. Cells of the **IEE differentiate**[←] into **pre-ameloblasts** and secrete **morphogens**[←], including **BMP**. The closest **neuro-mesenchymal stem cells** of the dental papilla receive BMP morphogens and differentiate into odontoblasts. Odontoblast differentiation involves a **morphological** change. The **amorphous** neuro-mesenchymal stem cells line up, forming what looks like a **simple cuboidal epithelium**[←], complete with **apical-to-basal polarity**[←]. This sort of polarization is

unusual for a connective tissue. Odontoblasts, however, are not derived from **mesoderm**, they are derived from **neuromesenchyme**. It is common for neurons and glial cells to be polarized, and odontoblasts share this morphology. Inside odontoblasts, large amounts of **rER** and **Golgi apparatus** are forming in preparation for the secretion of large amounts of protein.

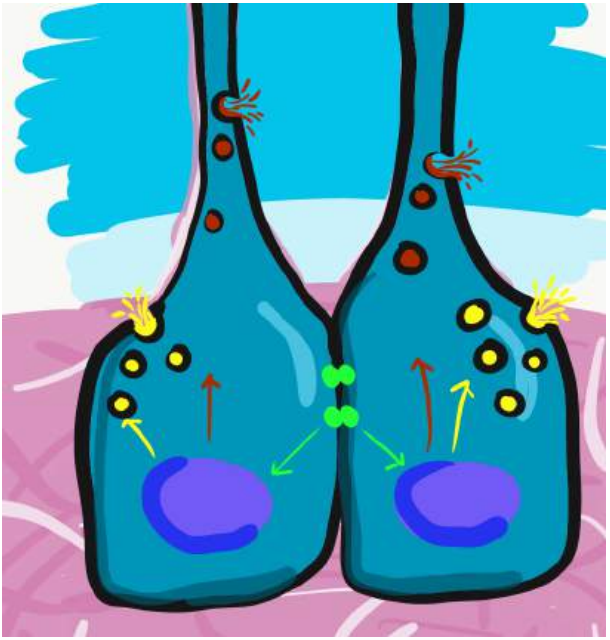

Figure 10.4: Polarization of odontoblasts, including cell-to-cell junctions that trigger activation of genes for proteins secreted into dentin. Legend: Green = cell-cell junctions help establish cell polarity by triggering intra-cellular signals. Yellow= secretion of collagen-filled

Apposition

vesicles to form pre-dentin (light blue layer) occurs at the apical surface of the cell body. Brown = secretion of enzyme-filled vesicles from the odontoblastic process triggers mineralization of pre-dentin into dentin (darker blue layer). Pink = pulp.

Newly formed **odontoblasts** begin secreting proteins that act as a **scaffold**←. This scaffold guides the mineralization of dentin. The initial protein-rich substance is **pre-dentin** (lighter blue layer in Fig. 10.4). It is mostly **collagen**← plus a few other dentin-specific proteins. Pre-dentin mineralizes later. Calcium and phosphate react in pre-dentin to form **calcium hydroxyapatite** crystals, similar to enamel and **bone tissue**← (darker blue layer in Fig 10.4). As layers of dentin mineralize, odontoblasts continue secreting new pre-dentin, pushing the layer of odontoblast cell bodies deeper into the jaw (Fig. 10.3). Unlike **ameloblasts**, odontoblasts leave behind an **odontoblastic process** in the dentin they secrete. This arm-like extension contacts nearly every layer of dentin that odontoblast creates. Because dentin mineralizes around the odontoblastic process, a **dentinal tubule** runs through nearly the entire length of dentin. If you removed the odontoblasts, dentin would be perforated by millions of hollow tubes (Fig. 10.8).

Collagen← and dentin-specific proteins are secreted by the side of the odontoblast cell body facing the enamel (yellow **vesicles**, Fig 10.4). This ensures the cell body does not get

cemented in place by hard dentin, and instead always touches a thin layer of gelatinous **pre-dentin**. **Glycoproteins** and enzymes like **matrix metalloproteinase** are secreted a short distance up the **odontoblastic process** (less than 1 mm, brown vesicles in Fig. 10.4) which trigger mineralization of dentin. This traps the odontoblastic process within mineralized dentin, but leaves the cell body free to move.

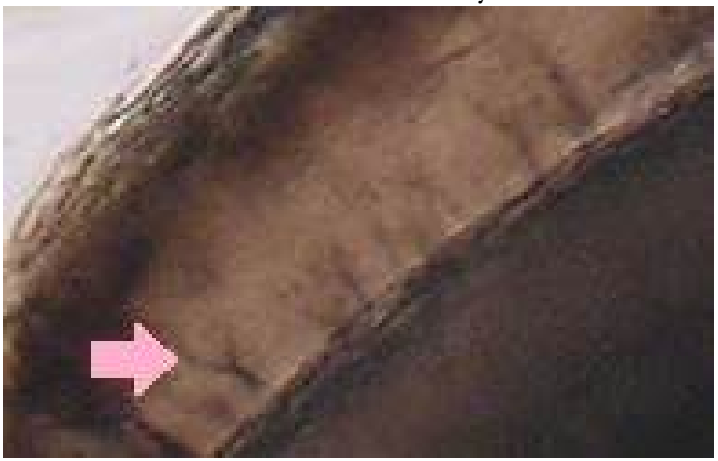

Figure 10.5: Histology of the dentin-enamel junction, with an enamel tuft indicated (pink arrow). Image credit: "Histologic cross-section of tooth showing enamel, labeled A, and dentin, labeled B." by Dozenist is licensed under CC BY-SA 3.0 / *cropped and arrow added*

A few **odontoblastic processes** extend past the **DEJ**. They become trapped in enamel after **amelogenesis** begins (which happens after **dentinogenesis**). These are known as **enamel spindles**. Both enamel spindles, and the bushier-looking

enamel tufts (Fig. 10.5) are referred to as hypo-mineralized regions or defects in enamel. There is something important missing in those descriptions, however. Teeth that exert more force have more enamel tufts (such as human molars, or the teeth of animals who eat nuts), suggesting enamel tufts *strengthen* enamel. It is not impossible for enamel spindles and tufts to both be less mineralized and increase the strength of enamel. Recall how hard but brittle **calcium hydroxyapatite** crystals of **bone tissue**← are strengthened by the flexible protein **collagen**←. It is also attractive to hypothesize enamel spindles and tufts act similar to the interdigitation of **rete pegs**← and **dermal papillae**← in the skin and **oral mucosa**.

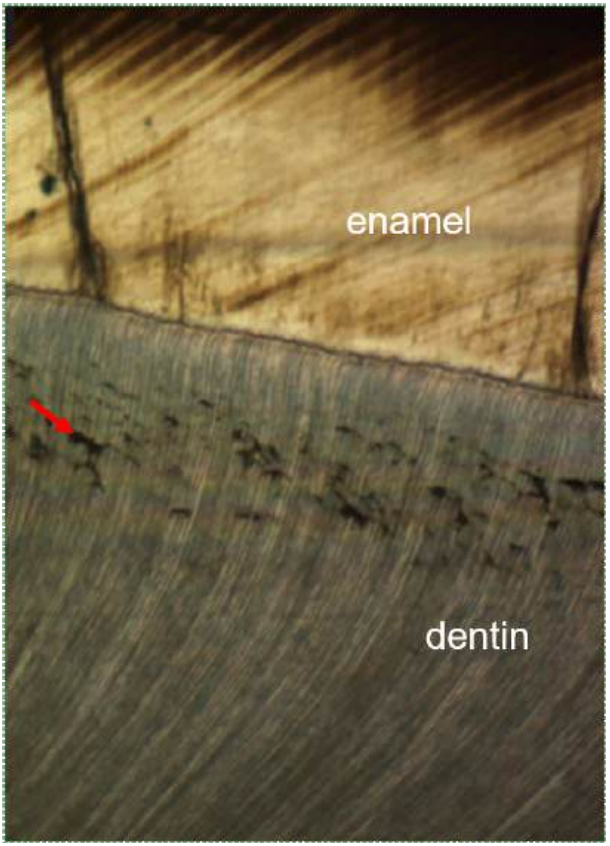

Figure 10.6: Inter-globular dentin (arrowhead) in an adult tooth. Image credit: "Figure 1a" by Monaogna Vangala is licensed under CC BY-SA 3.0 / *labels added*

Dentin mineralizes in one of two ways: in globs or in lines. The first dentin produced mineralizes in globs because extra-large **collagen**[←] fibers are secreted in spheres (there are older theories of what spherical globs are made of). Around the collagen globs, **pre-dentin** mineralize into mature dentin. Some regions do not mineralize fully, and are called **inter-globular dentin**. The fully mineralized dentin found between inter-globular dentin is **globular dentin**. Globular and inter-

globular dentin are visible between **mantle dentin** and **circum-pulpal dentin** (see below). Low levels of phosphate during the first trimester can increase the amount of inter-globular dentin.

After the first collagen fibers are secreted in globs, fibers are lengthened by **odontoblasts**. This dentin mineralizes more uniformly, one layer at a time, in a process called **linear mineralization**. In regions that mineralize linearly, collagen fibers run parallel to each other, but perpendicular to the dentinal tubules. Only after linear mineralization begins do odontoblasts leave behind an **odontoblastic process**.

The lines that should be clearly visible in the layer of dentin in Fig. 10.6 are **dentinal tubules**, the tubes in which the **odontoblastic process** are found. Notice that they do not run in a straight line. **Odontoblasts**, like **ameloblasts**, move in a slightly curved direction as they produce **ECM**. Like the curves you see on most bridges, this curvature is no accident. Curves increase the strength of dentin. The large curves are called the **primary curvature**. If you zoomed out you would see the primary curvature has a sinusoidal (S) shape. The curvature is more pronounced in the crown than the root. If you zoomed in on a single odontoblastic process, you might see areas where it briefly curves opposite of the primary curvature. That is called a **secondary curvature**. In contrast, the bending of enamel rods has no names for bigger or smaller curves.

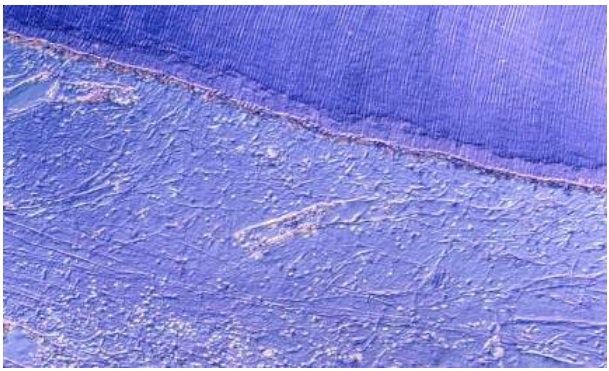

Figure 10.7: Imbrication (incremental) lines of Von Ebner. Image credit "histological section of tooth" by Doc. RNDr. Josef Reischig, CSc. is licensed under CC BY-SA 3.0 /animations added

Similar to the formation of enamel, **odontoblasts** undergo daily patterns of faster and slower **pre-dentin** deposition. As a result, light and dark bands are visible in dentin, called the **Imbrication lines of Von Ebner** (or the incremental lines of Von Ebner). The Imbrication lines of Von Ebner are comparable to the **Lines of Retzius** in enamel. Don't ask how Retzius discovered lines in enamel but missed the same

pattern in dentin a few millimeters away, leaving it to Von Ebner to name a hundred years later. People get credited for *re-discovering* things all the time. These should both be renamed incremental lines in enamel and the incremental lines in dentin, anyway. Furthermore, prominent imbrication lines can be called **contour lines of Owen**. This includes the neonatal line, similar to the one found in enamel. These represent big changes in nutrition that lead to changes in the density of dentin produced that day. You can read part of Owen's treatise on the comparative anatomy of the tooth, someone scanned 30 pages if you are curious about what it was like to learn histology without pictures.

Maturation

Unlike enamel, dentin does not undergo significant changes after it mineralizes. As a result, some textbooks call the mineralization of **pre-dentin** into dentin as the "maturation" step, while others (including this one) have chosen to use the words apposition and maturation consistently. Why do **ameloblasts** take an extra step removing some **scaffolding**[←] after mineralization occurs, and **odontoblasts** do not? One possibility is ameloblasts are epithelial cells, and epithelia generally secrete very little **ECM**. Perhaps they are not very good at it. In contrast, odontoblasts **differentiate**[←] from **neuro-mesenchymal stem cells**. It is likely during their **epithelial-to-mesenchymal transition**[←] they unpack **genes**

that make them efficient at creating ECM. A second possibility is that the high percentage of minerals in mature enamel (96%) can only occur after protein scaffolding is removed. Remember, calcium and phosphate react to form crystals on their own, but to get these crystals to adopt the **morphology** of a tooth requires scaffolds. The reason is not as important as the illustration of another difference between enamel and dentin due to their different cell **lineages**.

Classification of dentin

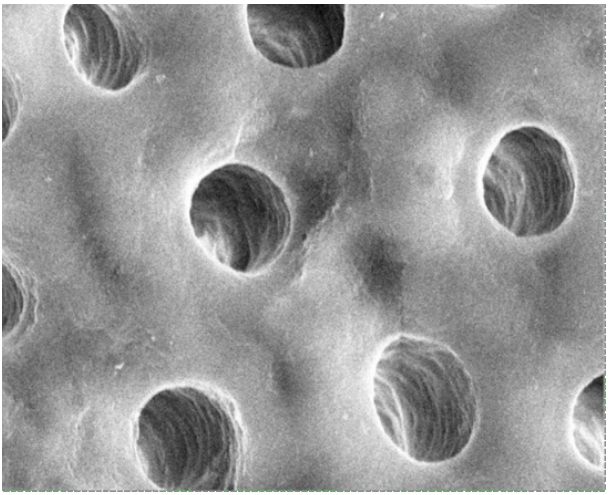

Figure 10.8: Dentinal tubules without odontoblastic processes. Image credit: "Dentinal tubule occlusion of dentine discs after treatment" by Peiyan Yuan is licensed under CC BY-SA 3.0 / *cropped and animated labels added*

In Fig. 10.8, **dentinal tubules** should be easily visible. In a living tooth, each tubule contains an **odontoblastic process**.

Not all regions of dentin are the same, and there are several ways of classifying different types of dentin. One way to classify different types of dentin is based on how close the dentin is to a dentinal tubule. The thin white area of dentin immediately surrounding each dentinal tubule is called **peri-tubular dentin**, while the rest is **inter-tubular dentin**. Despite a similarity in names, this is not **homologous** to **rod enamel** and **inter-rod enamel**.

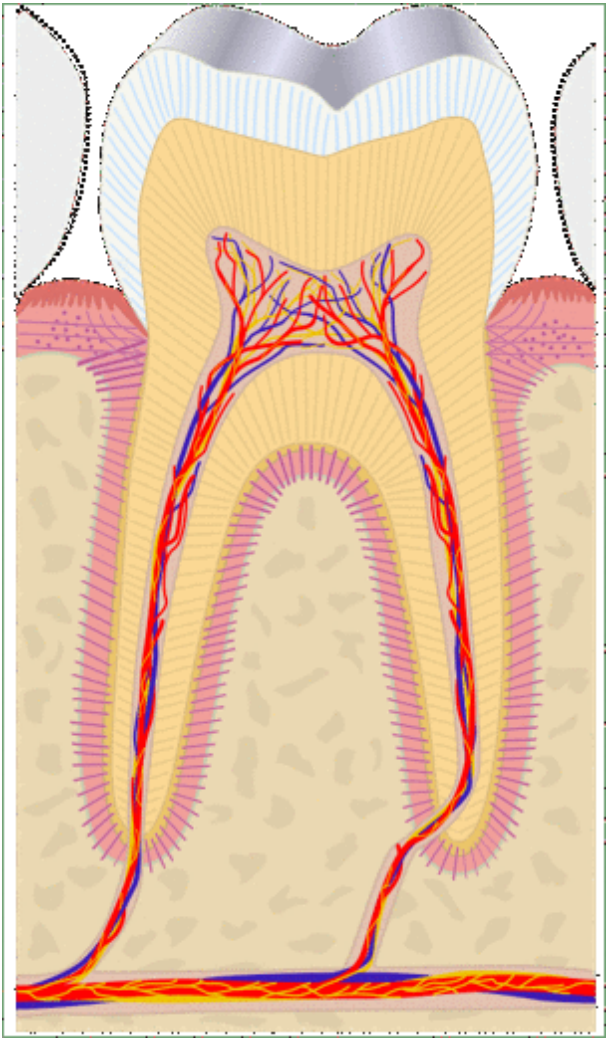

Figure 10.9: Types of dentin by location. Image credit: "cross sections of teeth" by Gorak Tek-en is licensed under CC BY 3.0 / *animation and text added*

Another way to classify types of dentin is on its location relative to the pulp cavity. **Mantle dentin develops** first. It is a thin layer (15 to 30 μm) next to enamel (therefore, only in the crown). Mantle dentin contains few **dentinal tubules**,

they are filled in during the maturation stage. Mantle dentin is where globular mineralization occurs. The **globular dentin** mineralizes and fuses together, creating a uniform appearance. Just below mantle dentin is where you find crescents of **inter-globular dentin** between globular dentin. Got that? If not, see Fig. 10.6. Mantle dentin is thought to be different from other dentin because it is here that **odontoblasts** first begin secreting proteins (near the **DEJ**), at which time the odontoblasts aren't fully mature. The **collagen**← fibers that remain in mantle dentin run perpendicular to the DEJ. The rest of the dentin (in both the crown and roots) is **circum-pulpal dentin,** which mineralizes **linearly**, leaving dentinal tubules intact. Collagen fibers run parallel to the DEJ in circum-pulpal dentin. Mantle and circum-pulpal dentin have slightly different levels of mineralization and protein content. Mantle dentin is more elastic, which provides a cushioning effect for the enamel above (like a yoga mat).

animation of types of dentin

Figure 10.10: Types of dentin by time of production. Original image "cross sections of teeth" by Gorak Tek-en is licensed under CC BY 3.0 / *animation and text added*

Yet another way to classify dentin is based on when it is formed relative to the apical foramen. If this seems redundant to **mantle dentin** versus **circum-pulpal dentin**, you are mostly right. **Primary dentin** is the dentin formed before completion of the apical foramen, therefore it is formed prior to **tooth eruption**←. **Secondary dentin** is formed after the

completion of the apical foramen (after tooth eruption). Unlike mantle versus circum-pulpal dentin, there are no major histological differences between primary and secondary dentin.

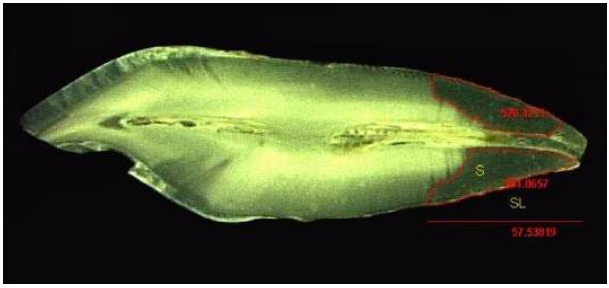

Figure 10.11: Sclerotic dentin (S). Note how loss of dentinal tubules affects how light pases through sclerotic versus healthy dentin. Image credits "Stereomicroscope image (5X) of tooth with measurement of Sclerotic

Much later, a tooth may suffer damage and new forms of dentin are produced to repair the injury. **Odontoblasts** can produce **tertiary dentin** and repair small amounts of damage. Tertiary dentin produced by the original odontoblasts (still in the pulp) is called **reactionary dentin** (or sclerotic dentin). Formation of this form of dentin involves secretion of **matrix metalloproteinases** from **dentinal tubules**. These enzymes are also used during the formation of dentin during embryogenesis. Therefore, this is another example of how wound repair **recapitulates** embryonic **development**. During embryogenesis, however, dentin is formed **appositionally**. Reactionary dentin mineralization occurs within dentinal tubules, causing the tubules to become occluded (to become blocked). Therefore, fewer dentinal tubules are found in reactionary dentin. Furthermore, it is the parallel dentinal tubules that give primary and secondary dentin their yellow-ish hue. With reduced or no tubules, reactionary dentin becomes more translucent (see Fig 10.11).

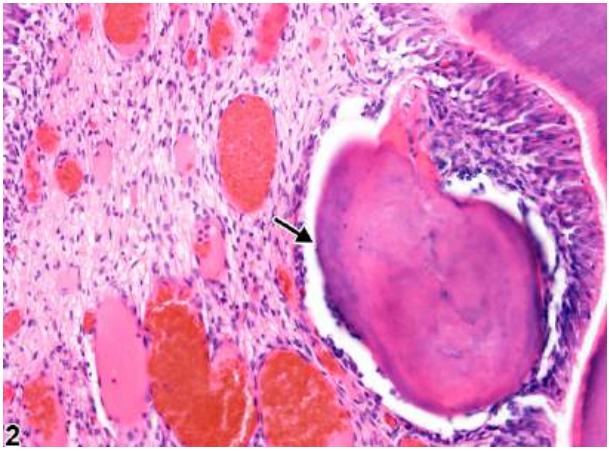

Figure 10.12: Osteodentin (arrow) inside of the pulp cavity. Image credits "Tooth, Pulp - Osteodentin in a male F344/N rat from a chronic study" by Cora MC, Travlos GS, at the National Toxicology Program is in the

If a large enough injury occurs as to expose the pulp chamber, destroying **odontoblasts**, a much more robust response is needed. First, new odontoblasts are needed, but how can we get new odontoblasts without **morphogens** from **pre-ameloblasts**? The answer is pretty cool: there is a backup system. **Mesenchymal stem cells** in the pulp **differentiate** into odontoblasts when they come into contact with a dentin-specific protein (not **collagen**←, but a molecule called dentin sialoprotein 2). This does not happen in a healthy tooth because odontoblast cell bodies are a barrier between dentin sialoprotein 2 and pulp mesenchymal stem cells. When those odontoblasts die (as long as there is some pulp left), new odontoblasts are **induced** to differentiate. The new odontoblasts form a type of tertiary dentin called **reparative dentin**. Reparative dentin does not form the same way dentin does during **development**. **Dentinogenesis** began at the **DEJ**, and layers of dentin were added **appositionally**, *towards* the pulp chamber. To form reparative dentin, odontoblasts and **fibroblasts** start *from* the pulp chamber, migrate throughout the injured area (after a hematoma forms) and secrete proteins and electrolytes. No **dentinal tubules** are created, these new odontoblasts secrete dentin in all directions. Some of the odontoblasts and fibroblasts become trapped within the **ECM**. For this reason, reparative dentin is sometimes referred to as **osteodentin**, because it resembles **bone tissue**← under the microscope more than it does tubular dentin. In fact, osteodentin may

represent the **phylogeny** of dentin. The teeth of some species (such as eels) and the fossils of our ancient ancestors' teeth is made of osteodentin, not mantle or peri-tubular dentin.

Type of dentin	Location	Features
Peri-tubular	Walls of tubules	Found in circum-pulpal dentin
Inter-tubular	Between walls	Found in circum-pulpal dentin
Mantle	Thin border next to DEJ	No tubules Collagen perpendicular to DEJ
Circum-pulpal	Rest of tooth	Tubules Collagen parallel to DEJ
Primary	Formed before apical foramen	Made by original odontoblasts Contains tubules
Secondary	Formed after apical foramen	

Table 10.1: Summary of the types of dentin.

Tertiary	Reactionary, Sclerotic	Formed after injury	Made by original odontoblasts Tubules filled in
	Reparative	Formed after large injury	Made by new odontoblasts and fibroblasts Cell trapped in calcified tissue (osteodentin) No tubules form

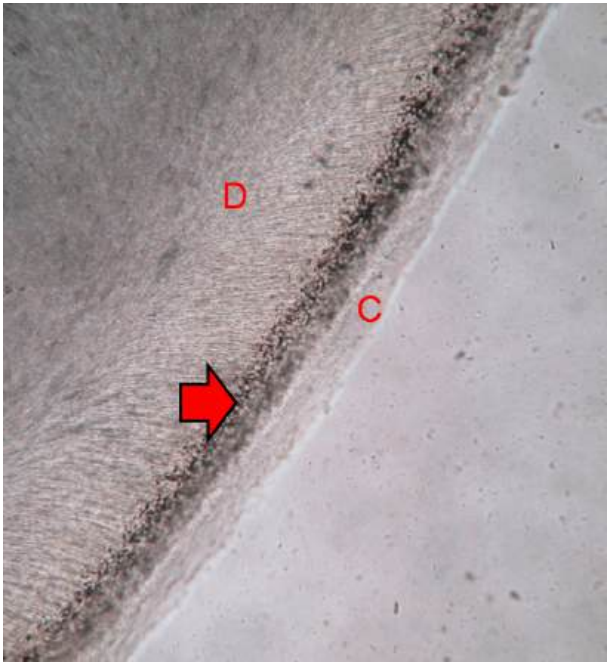

Figure 10.13: Tomes' granular layer (arrow) found in root dentin (D), just deep to cementum (C). image credit "Tomes granular layer" by Shaik Mohamed Shamsudeen is licensed under CC BY-SA 3.0 / *letters and arrow added*

Root dentin

Roots do not contain **mantle dentin**, but they do contain a superficial layer of dentin that is visually distinct from the **circum-pulpal dentin**. Close to the border with cementum, spots are visible in a band of dentin known as **Tomes' granular layer**. This layer has no known clinical significance. It is useful for orienting yourself when looking at histological sections of teeth. Those grain are not the nucleuses of cells. Older data suggested the grains are loops of dentinal tubules, but re-analysis using more advanced microscopes suggests the grains are loops of **collagen**[←] fibers. This suggests they are similar to **globular dentin**. Whatever the cause of the granulations is, the important concept to remember is the order of events in root formation. Odontoblasts are less mature (newer) when they are secreting dentin in Tome's granular layer than when they are closer to the root canal, similar to globular dentin formation in the mantle region of the crown.

Clinical considerations of dentinogenesis

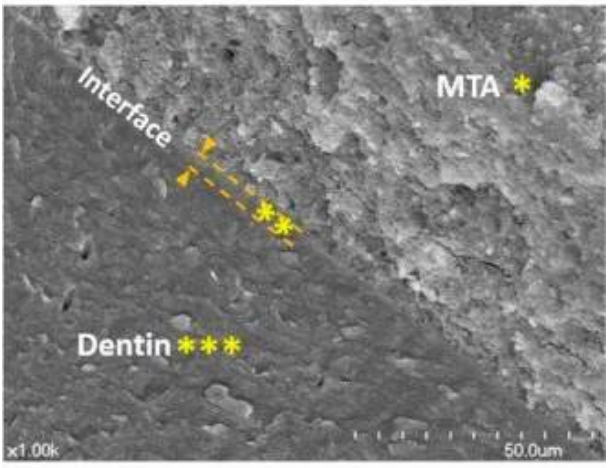

Figure 10.14: Electron micrograph of the interface between dentin and a mineral aggregate (MTA) used in the a root canal therapy to boost replacement of resorbed root dentin. Image credit: "Scanning electron microscopic images that are

Mineral aggregates

representative of the root canal dentin and mineral trioxide aggregate (MTA) interface" by Yoo, Yeon Gee et al is licensed under CC BY 3.0

If there is a large amount of damage to dentin, the production of **tertiary dentin** may be too slow. When treating root resorption, or for root end filling during **endodontic therapy**, artificial **ECM** can be used. Unlike some of the artificial tissues discussed in gingival healing, solid crystals do not make good **scaffolds**[←]. The goal is to mimic something more gelatinous like **pre-dentin**, not crystalline dentin, whose dense matrix would inhibit migration of **stem cells**. Mineral aggregates provide the necessary materials required by **odontoblasts** without inhibiting their movement during **dentinogenesis**. One such compound, Mineral Trioxide Aggregate (MTA), was developed in California by Dr. Mahmoud Torabinejad. MTA contains a purified version of Portland cement plus calcium-containing minerals. The addition of mineral aggregates speeds up the formation of tertiary dentin. MTA slowly releases calcium hydroxide, which provides a raw material for mineralization of tertiary dentin, as well as attracting phosphate from the blood or ECF. *Author's note: Portland cement was invented in Portland, England. Do not apply Portland cement from your hardware*

store to teeth, it is caustic and may contain arsenic or other heavy metals. The Portland cement used in dentistry is highly purified.

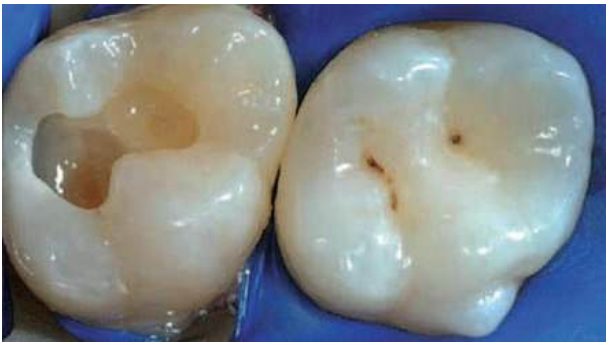

Figure 10.15: The appearance of dentin should be glistening and moist. Image credit: "Dentin appearance" by Pinkmanggis is licensed under CC BY-SA 4.0

In one type of dental restoration (a tooth filling), a wet resin is bonded (glued) to dentin and hardened using a special light. To bond a resin to dentin, one should take into account the chemical structure of dentin. Dentin **ECM** contains **calcium hydroxyapatite** crystals, **collagen**[←] and other proteins, plus water molecules. The water molecules,

along with collagen, allow dentin to bend and compress in response to stress, as opposed to harder enamel which resists stress (summarized in Table 8.3). Water molecules give dentin a moist appearance. Many bonding agents do not adhere well to a wet surface, and require dentin to be dried first. This is usually done with a volatile solvent such as ethanol. Mineral acids may be used to remove an amount of the mineral ECM of dentin, leaving behind the more porous collagen framework. This can improve bonding, somewhat similar to acid-etching of enamel. However, acid etching took advantage of the mineral differences between **rod** and **inter-rod enamel**. There is not enough of a difference between **peri-tubular** and **inter-tubular dentin**, both lose minerals when mineral acids are applied.

There is interest in the research community for developing bonding agents that adhere to wet surfaces (all living human tissues are wet). One promising area is the study of mollusk mucus. **Mucus** is secreted *from* the body, but it is similar to **ground substance**← *in* human connective tissues, especially **mesenchyme**←. Mucus secreted by limpets adheres to wet surfaces with great strength, and it stimulates tertiary dentin formation (link to pdf download). One of the most abundant molecules in mucus is **hyaluronic acid** (a major component of ground substance).

Lastly, bonding agents typically adhere to **primary** and **secondary dentin** more readily, because of the higher degree of surface area from **dentinal tubules**. In contrast, bonding

agents do not adhere as well to **tertiary dentin**, where tubules are filled in or never form.

Morphogens

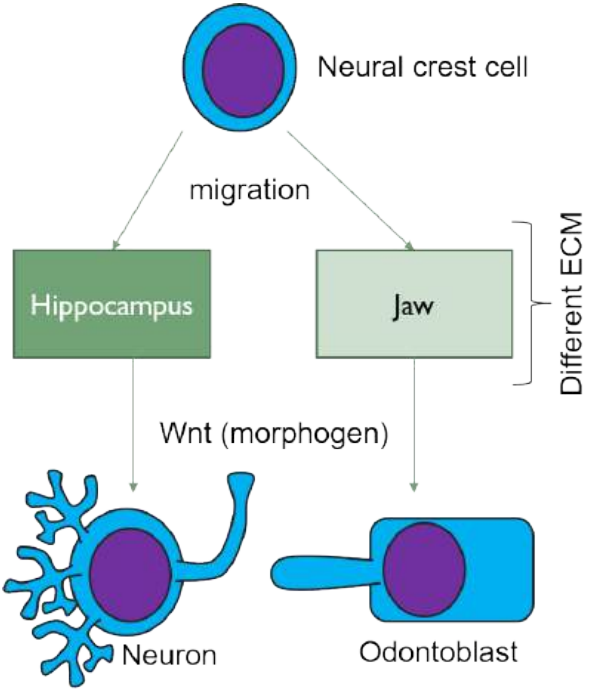

Figure 10.16: Wnt morphogens induce the differentiation of different cell types in different environments.

The formation of **tertiary dentin** requires **morphogens** to **induce** the **differentiation** of **mesenchymal stem cells** into new **odontoblasts**. As more is learned about these morphogens, it opens up the possibility to administer morphogens to speed up natural healing processes, potentially reducing the need for inorganic cements or caps. One such morphogen is a **Wnt**. In the brain, Wnts induce the

differentiation of **neural crest cells**[←] into specific types of neurons and glia. But when neural crest cells migrate to the face and become parts of the **pharyngeal arches**[←], the same Wnt induces them to **differentiate**[←] into odontoblasts. The difference arises because of a second morphogen. This second signal comes from the **ECM**, and ECM is very different in the developing brain than it is in developing **neuro-mesenchyme** of the pharyngeal arches. The significance of this is that a drug used to treat Alzheimer's Disease (AD) [Tideglusib is used off-label in dentistry to boost tooth repair. We suspect it is very rare to randomly test psychoactive drugs for their use in dentistry. Prior knowledge of shared **lineages**, however, makes new avenues of treatment more obvious.]

Attrition and erosion

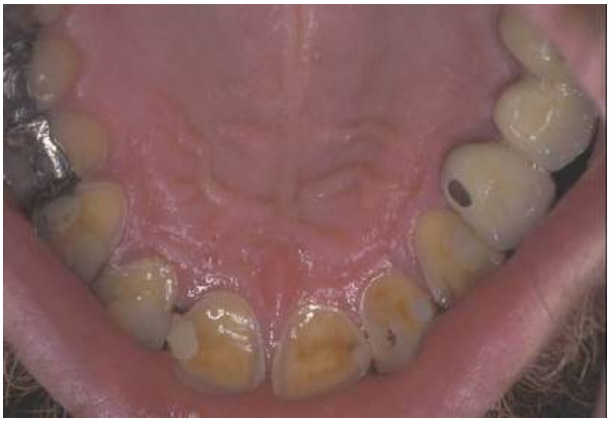

Figure 10.17: Erosion of enamel and dentin. Image credit: "Own work" By Klaus Limpert, Scuba-limp is licensed under CC BY-SA 3.0

Because dentin has a lower mineral content than enamel, dentin **erodes** more quickly than enamel. If **mantle dentin** is lost, this exposes **dentinal tubules** in the underlying **circumpulpal dentin**. Exposed tubules increases the surface area for acids to dissolve **calcium hydroxyapatite** crystals, speeding up dentin **erosion**. Surface exposure of dentinal tubules also leads to increased sensitivity of the teeth (see below).

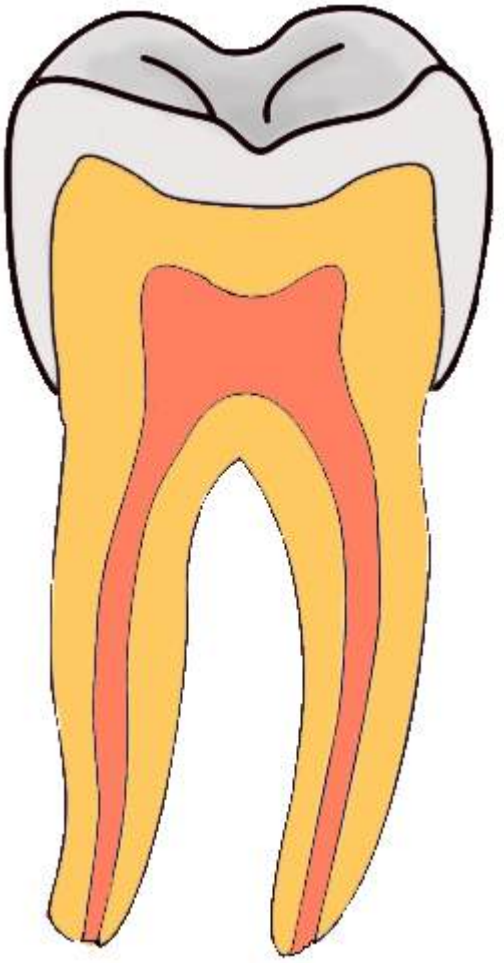

Figure 10.18: Caries in enamel versus dentin. Image credit "Tooth "By ADuran is licensed CC BY-SA 3.0

Dentin caries

Once **dental caries** spread through enamel and reaches the **DEJ**, it spreads at an increased rate. The triangle pattern of enamel loss is quickly repeated in a new triangle of **dentin**

caries. It is possible, but not agreed upon, that a small enough enamel caries might spread to the DEJ without causing changes visible at the surface, and upon reaching the DEJ the rate of tooth loss accelerates. Whatever the exact cause, caries can start *below* enamel and spread through dentin. This **hidden caries** is harder to detect than one at the surface of a tooth.

---

animation of dentinal hypersensitivity

Figure 10.19: Dentinal hypersensitivity is caused by exposed dentinal tubules.

Dentin hypersensitivity

The loss of enamel or cementum covering dentin may expose **dentinal tubules**. In the crown, the few millimeters of **mantle dentin** at the outer surface contain few open dentinal tubules. But if enamel and mantle dentin are lost, tubules in the **circum-pulpal dentin** are exposed. These tubules extend to the root pulp, where nerve endings are located. Changes in

the environment of the oral cavity, including the temperature, pH, alcohol level or osmotic concentration, now affect the temperature, pH, alcohol level or osmotic concentration of the pulp **ECF**. There is a small amount of ECF between the **odontoblastic process** and peritubular dentin (**dentinal fluid**). Changes to the chemical composition of this ECF can be detected by nerve endings, which in turn relay painful stimuli to the brain. This can be called tooth, root, cervical or cemental hypersensitivity, but it is more accurate to call it **dentin hypersensitivity** . Treatments for dentinal hypersensitivity include special toothpastes, mouthwashes or chewing gums that deposit minerals into exposed dentinal tubules and occlude them. Such products likely contain salts and fluoride. Alternately, varnish mineral aggregates such as Portland cement might be applied to the affected area. Conversely, with age, **odontoblasts** add layers of **peri-tubular dentin** inside dentinal tubules, causing the tubules to become narrower. As this happens, teeth become less sensitive. This may not sound so bad until you take into consideration the role that proprioception has in preventing excessive occlusal forces. Cranial nerve V is one of the larger cranial nerve, it transmits a lot of important sensory information to the brain from the teeth. With diminished sensitivity comes an increased risk of occlusal trauma.

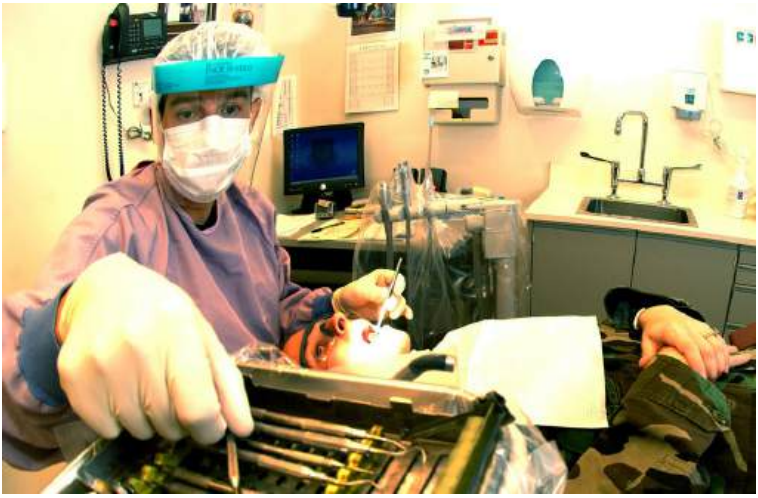

Figure 10.20: The importance of proper dental hygiene procedures. Image credit: "Dental details" by Senior Airman Kristi Emler, US Air Force is in the Public Domain, CC0

Dentin may become exposed due to loss of cementum or enamel, or if the edge of enamel does not meet/overlap cementum. Furthermore, **gingival recession** exposes the thin layer of cementum to environments it is not designed to handle, making dentin exposure along the neck of the tooth the most likely area for dentinal hypersensitivity. Improper technique on the part of dental hygienists or dentists may inadvertently remove protective layers of cementum as well.

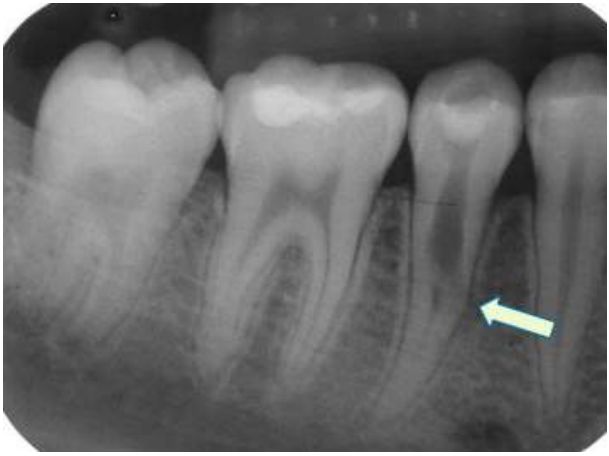

Figure 10.21: Dentin resorption (arrow), also known as internal resorption. Image credit: "An irregular radiolucency in the coronal third to middle third of the root" by Fang-Chi Li is licensed under CC BY-SA 3.0

Dentin resorption

During **exfoliation** of primary teeth, **dentin resorption** (the loss of dentin due to cell-mediated demineralization) assists in the loss of attachment between the tooth root and alveolar bone. This is mediated by **odontoclasts**. Odontoclasts are related to **osteoclasts**. Osteoclasts have baseline activity throughout life maintaining bone tissue as part of a **remodeling unit**. Odontoclasts, on the other hand, are not always present, their differentiation from **mesenchymal stem cells** as well as their activity is regulated by different **morphogens**. Dentin resorption occurring at any time other than the shedding of primary teeth is **idiopathic** (of unknown cause). We do understand that the **dentinal tubules** found throughout dentin create a large amount of surface area for odontoclasts to adhere to and trigger demineralizion. This is similar to what we observe in spongy bone: spongy bone is lost at a faster rate than compact bone because it has a higher amount of surface area (thus symptoms of osteoporosis appear first in bones with higher amounts of spongy bone, like the mandible).

Dentin resorption triggered from the pulp-cavity side is referred to as internal resorption. Conversely, external resorption occurs from the **DEJ** or **CEJ** side.

Fig 10.22: Photographs of a patient with dentinogenesis imperfecta. Image credit: "Oral photographs from the affected individual" by unknown is licensed under CC BY 2.0

Dentinogenesis Imperfecta

A mutation in the gene for one of the dentin-specific proteins (dentin sialophosphoprotein) leads to a genetic condition known as **dentinogenesis imperfecta** (types II and III). In

this condition, the dentinal tubules are wider than normal. This in turn alters the color of dentin, causing teeth to appear more grey or bluish (similar to how **reactionary dentin** has a different color from healthy dentin because it lacks dentinal tubules). Unlike **amelogenesis imperfecta**, the teeth of people with dentinogenesis imperfecta do not have a higher susceptibility to **dental caries**. The teeth are weaker than normal, and are more prone to fracture and loss. The lack of one **scaffold**← protein leads to reduced dentin mineralization. Reduced mineralization leads to higher levels of inter-globular dentin.

Mutation to the gene for type I **collagen**← leads to a condition called osteogenesis imperfecta, or brittle bone disease. About 50% of people with osteogenesis imperfecta have dentinogeneis imperfecta (type I). These individuals have the dentin sialophosphoprotein, but the defect in collagen leads to brittle dentin.

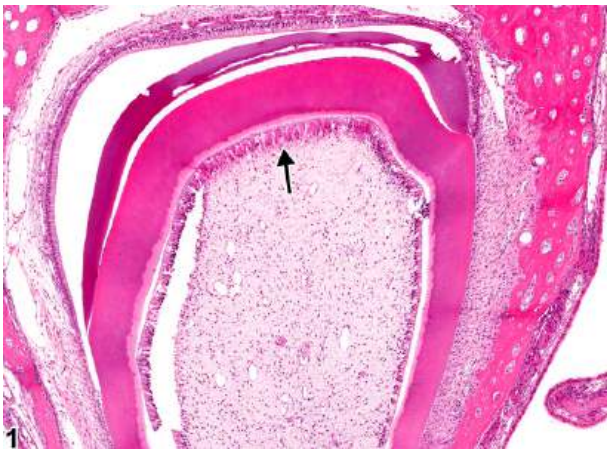

Figure 10.23: Histology of pulp. Arrow indicates odontoblastic layer. Image credit "Tooth, Odontoblast necrosis" by Cesta MF, Herbert RA, Brix A, Malarkey DE, Sills RC (Eds.), National Toxicology Program Nonneoplasti

Pulp overview

The dentin and pulp both develop from **neuro-mesenchymal stem cells** of the **dental papilla**. **Odontoblasts** make a specialized tissue, containing just one **terminally differentiated** cell type. The rest of the neuro-mesenchymal cells make **areolar connective tissue**←, which contains numerous cell types, including **adult stem cells**. It is here that blood vessels, nerve fibers and lymphatic vessels have space and structural support.

Histology of pulp

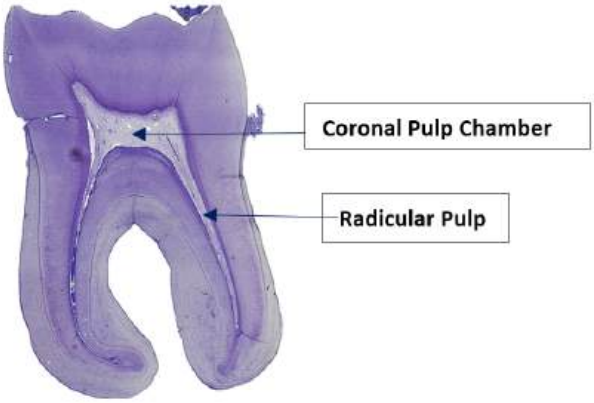

Figure 10.24: Coronal and radicular pulp. Image credit: "Longitudinal tooth 5" by Natdental is licensed under CC BY-SA 4.0

Pulp can be divided into **coronal pulp** and **radicular pulp**. The coronal pulp is in the crown of the tooth and contains smaller **pulp horns** beneath the cusps. Radicular pulp is in the roots, and may extend into **accessory canals**. Accessory canals connect the pulp to connective tissue external to the tooth, traveling laterally rather than out the apical foramen. Accessory canals form when **HERS** runs into a blood vessel and is forced to grow around it. Otherwise, radicular pulp terminates at the apical foramen.

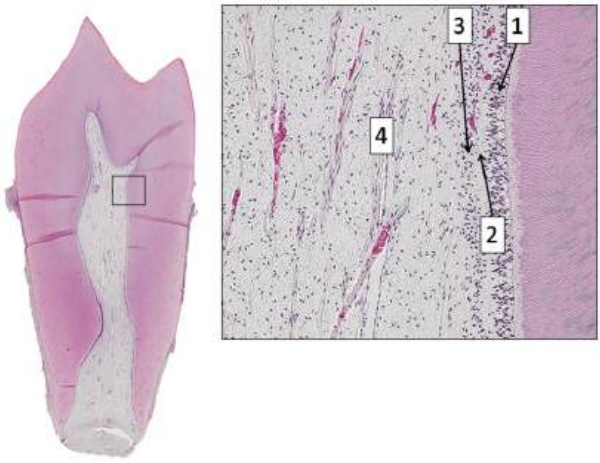

Figure 10.25: Layers of the pulp. Legend: 1) odontoblast layer 2) cell-free layer 3) cell-rich layer 4) pulp core. Image credit: "Pulp Histology" by Dododopamine at the School of Dentistry, University of Dundee is licensed under CC BY-SA 4.0

Pulp layers

Pulp is a single tissue, but there are four layers that look different from one another under a microscope. The first is the **odontoblast layer**, which is closest to the dentin. Odontoblasts initially form a single layer of cells along the broad region of the DEJ. But as they add dentin, the pulp cavity becomes smaller. As the pulp cavity becomes smaller, odontoblast cell bodies crowd together, ultimately forming a thicker layer of cell bodies at the edge of the pulp cavity. Deep to the odontoblasts is what is called the **cell-free zone**. This zone contains cells, but they are not visible under a traditional **H&E** stain. Deep to that is a **cell-rich zone**, composed primarily of **fibroblasts**, **mesenchymal stem cells**, white blood cells and other connective tissue cells. Lastly is the **pulp core**, which contains the same types of cells, but more **ground substance**← spaces them apart. The pulp core is where most of the larger blood and lymphatic vessels are located.

Clinical considerations of pulp

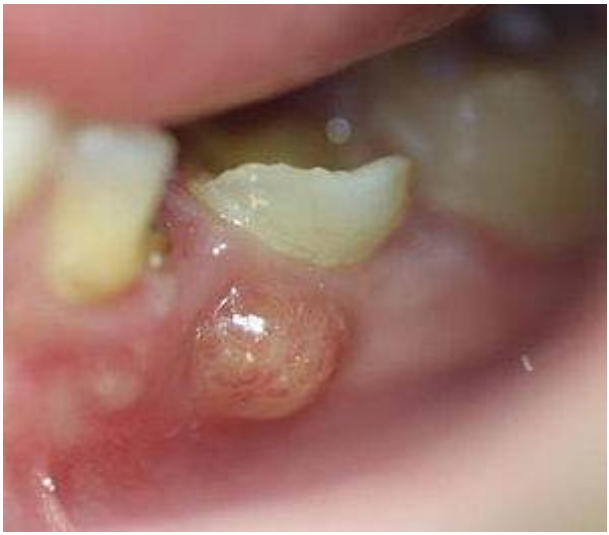

Figure 10.26: Gum boil caused by an underlying periapical abscess. Image credit "abcès paruli que" by Damdent is licensed under CC BY-SA 3.0

Periapical abscess

If damage to dentin exposes pulp to oral bacteria, an infection of the pulp may occur. This usually triggers inflammation of pulp tissue, known as **pulpitis**. Death of pulp tissue can lead to accumulation of pus around the roots, which is called a **periapical abscess**. The release of inflammatory molecules from damaged tissue can lead to swelling of the overlying

gingival tissue, often referred to as a **gum boil**. Less commonly, a gum boil may also form due to an infection of periodontal tissues, in which case it is not a periapical abscess, it is a **periodontal abscess**. It is possible to remove necrotic pulp surgically. If some healthy pulp remains, regeneration of the pulp may occur. This is because of the high degree of vascularization of **areolar connective tissue**[←] and the high mitotic potential of **mesenchymal stem cells**.

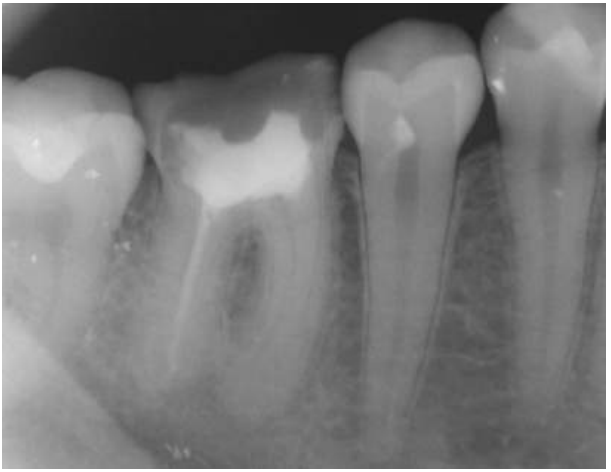

Figure 10.27: Endodontic therapy (root canal). Image credit: "Tooth #30" By DRosenbach at English Wikipedia is in the Public Domain CC0

Endodontic therapy

A **periapical abscess** likely requires the removal of the

infected pulp tissue and replacement by a **bio-compatible** material that is similar in density and elasticity. This procedure is known as **endodontic therapy**. The preferred material used is gutta-percha, a naturally-occurring latex polymer from the sap of trees in Malaysia. It was once widely used as an insulator for electronics, but has been replaced by synthetic polymers, except in endodontic therapy. It is radiopaque because of an additive, barium sulfate. Otherwise, latex does not show on radiographs, which would make it difficult to confirm gutta percha had completely filled the pulp cavity.

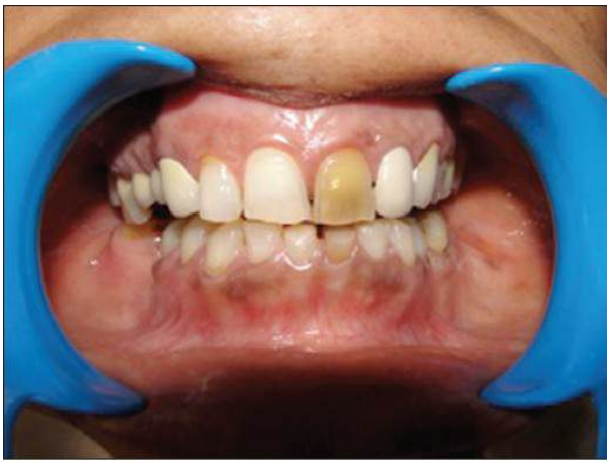

Figure 10.28: Discoloration of a non-vital tooth after endodontic therapy. Image credit "Discolored maxillary left central incisor tooth" by Anandkumar Patil is licensed under CC BY-SA 4.0

Removal of living pulp tissue removes all cells from the tooth,

including **odontoblasts**. This tooth is referred to as **non-vital**. A non-vital tooth cannot make repairs to dentin, which leads to increased brittleness and the accumulation of stains over time. Stain molecules may invade from the enamel side down **dentinal tubules**, or molecules from pulp necrosis may invade dentin by traveling up dentinal tubules.

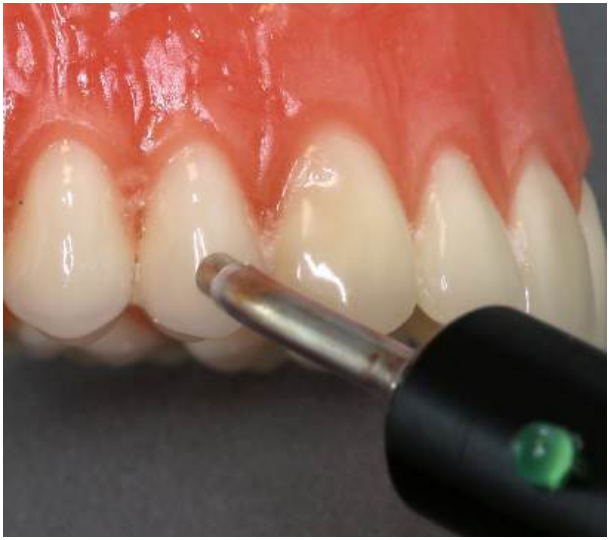

Figure 10.29: Pulp vitality testing. Image credit: "Own work" By Coronation Dental Specialty Group is licensed CC BY-SA 3.0

Pulp vitality testing

Painful stimuli at the surface of the teeth are detected by nerve endings located within the pulp. **Pulp vitality testing** (or pulp sensitivity testing) takes advantage of this to estimate the health of the tooth pulp. An electrical stimulus is applied at the

surface of teeth followed by self-reporting of the discomfort level experienced by the patient. Loss of nerve endings reduces tooth sensitivity, and indicates pulp tissue has undergone fibrosis or necrosis. However, a reduction in the diameter of **dentinal tubules** also reduces the ability of electrical signals at the surface to be detected within the pulp. Conversely, loss of enamel and exposure of dentinal tubules may increase sensitivity without any changes to pulp vitality.

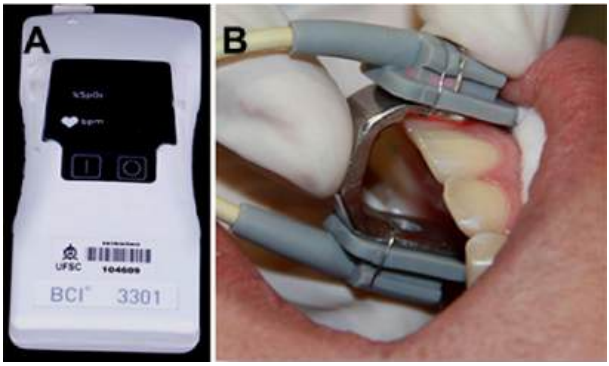

Figure 10.30: A pulse oximeter (A) and dental adapter (B used instead of pulp vitality testing). Image credit: "Pulse oximeter and sensor with specially manufactured adapter" by Lorena Ferreira Lima, et al is licensed under CC BY 4.0

Because different patients report pain differently, and because factors besides the health of the pulp affect the degree of discomfort reported, false-positive and false-negative results are possible with pulp vitality testing. Other less-invasive tests exist, including pulse oximetry and laser doppler flowmetry. These measure the vascular supply to each tooth, which correlates with the vitality of the tooth. Pulse oximetry takes advantage of the color change **hemoglobin** undergoes as it picks up oxygen. Laser doppler flowmetry takes advantage of the doppler shift waves exhibit when they bounces off moving objects (such as flowing red blood cells) but not stationary ones– similar to the way the sound of a motorcycle changes pitch when it is rapidly travelling toward you or away from you.

Age-related changes

Because a vital tooth contains a layer of living **odontoblasts**, dentin becomes gradually thicker with age. This is most noticeable in the fine **pulp horns**, which recede with age. Couteracting this, as with many cells, the **mitotic** ability of **mesenchymal stem cells** within the pulp decreases with age. As a result, older pulp tends to contain more **scar tissue** (cross-linked **collagen**[←] fibers) and has decreased regenerative capability and diminished sensitivity.

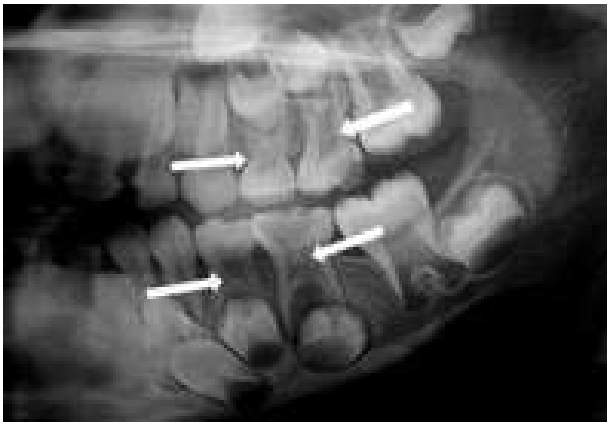

Fig 10.31: Radiograph of multiple pulp stones. Image credit: "OPG revealing pulp stones and taurodontism in all primary molars" by Mohita Marwaha et al, Case Reports in Dentistry is licensed under CC BY 4.0 / *cropped*

Pulp stones

Elsewhere in the body, damage to connective tissue may lead to the formation of **scar tissue**. In the pulp, however, the presence of **odontoblasts** causes scar tissue to mineralize. The formation of calcified scar tissue is a rare condition known as Dystrophic calcification elsewhere in the body, but is common in pulp. Localized regions of calcification in pulp are called **pulp stones** (or denticles). These occur in both **radicular pulp** and **coronal pulp**. The major clincal significance of pulp stones is they may complicate **endodontic therapy**. Pulp stones can not only be found in pulp (free), they also appear on dentin (adherent) and within dentin (embedded).

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Chapter review questions

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11.

PERIODONTIUM DEVELOPMENT

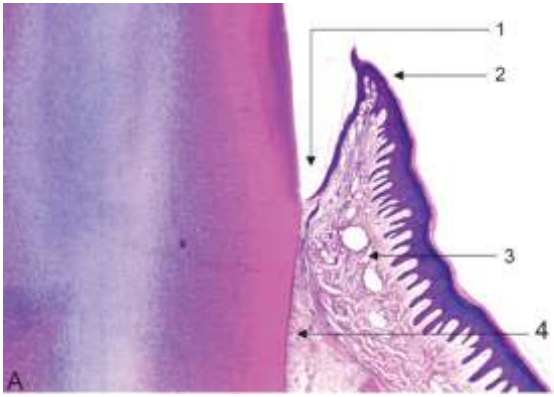


Figure 11.1: H&E stain of the periodontium. Figure legend: 1) gingival sulcus, 2) gingival crest, 3) lamina propria, 4) PDL. Image credit: "Histological section of an incisor tooth of a Rhesus monkey displaying

Overview

The **periodontium** includes cementum, **PDL**, alveolar bone and gingival tissues. The gingiva contain a **stratified squamous epithelium**← which develops from the **ectoderm** of the **pharyngeal arches**←. Underlying connective tissue layers develop from **mesoderm** (as does most of the skull). The **ECM** of these tissues contains **collagen**← and **elastic** fibers. Cementum, PDL and alveolar bone develop from the **neuro-mesenchymal stem cells** of the **dental sac**. These three tissues share extracellular components, thanks to their shared **lineage**, making for a strong connection between them. Like mesoderm-derived connective tissues, these tissues have a lot of collagen, but instead of elastic fibers, these tissues produce a special protein fiber type called **Oxytalan** fibers. Oxytalan fibers are found in a few other places in the human body, such as the aorta, whose lining is also derived from **neural crest cells**←. **HERS**← plays an important role in the induction of these tissues, even if it is mostly fated to undergo **apoptosis**←.

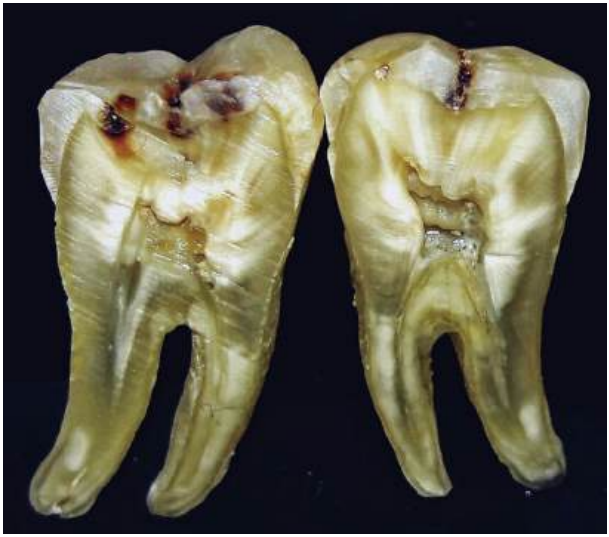


Figure 11.2: Cross section of a tooth to show hard tissues, including cementum. Image credit: "What is inside a human tooth" by Mohamed A.M. Ahmed is licensed under CC BY-SA 4.0 / *cropped*

Cementogenesis

Cementum forms a thin layer on the roots of teeth, attaching the teeth to the alveolar bone by way of the **PDL**. The mineral content of cementum is lower than that of dentin, but not

enough to make it appear different from dentin on a radiograph. The surface of cementum will feel grainier than enamel when explored with instruments.

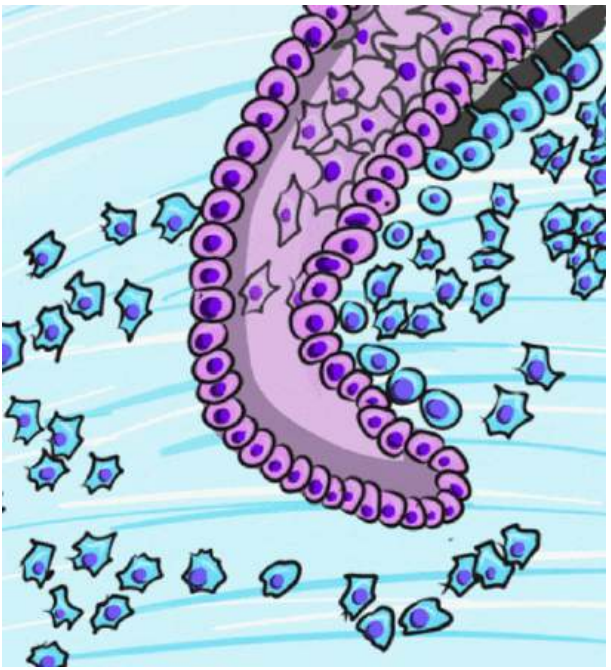


Figure 11.3: Disintegration of HERS and the formation of cementum.

After crown formation is nearly complete, **HERS** grows apically, separating the **dental papilla** from the **dental sac**. The **IEE** of HERS **induces** the **differentiation** of **odontoblasts**, which begin **dentinogenesis**. Without **stellate reticulum**, the IEE cannot be **reciprocally** induced to form **ameloblasts**. Instead, the IEE and **OEE** secrete **morphogens** onto the surface of dentin, including members of the **FGF** and **Wnt** families, but after that, these cells mostly undergo **apoptosis**. A few of the epithelial cells of HERS

may undergo an **epithelial-to-mesenchymal transition**← and differentiate into **cementoblasts**. However, most cementoblasts arise differently. With HERS out of the way, **pre-dentin**← made by root odontoblasts contacts **neuro-mesenchymal stem cells** of the dental sac. Contact with pre-dentin and **BMP** morphogens secreted from the dental papilla induces the neuro-mesenchymal stem cells of the dental sac to become cementoblasts. These cells, like odontoblasts, align next to one another, behaving more like a tissue derived from **ectoderm** (an epithelium) than a connective tissue.

Cementogensis is the process of forming cementum. Cementoblasts secrete the protein components of cementum. This immature matrix is commonly referred to as cementoid, which breaks from the pattern we used for **pre-enamel** and **pre-dentin**, or it may be referred to as **pre-cementum**. Layers of pre-cementum are laid down **appositionally**, and soon mineralize, at which time it is called cementum. Some cementoblasts become trapped in cementum, after which they are referred to as **cementocytes**. Other cementoblasts remain near the surface of the cementum. These cells continue to lay down layers of pre-cementum throughout life, and can become more active during times of injury and repair to cementum. Because of the shared lineage between cementoblasts and **odontoblasts**, and the similarity of their **ECM**, the **CDJ** is less distinct than the **DEJ**. In fact, the CDJ was once considered to be imaginary (reviewed here).

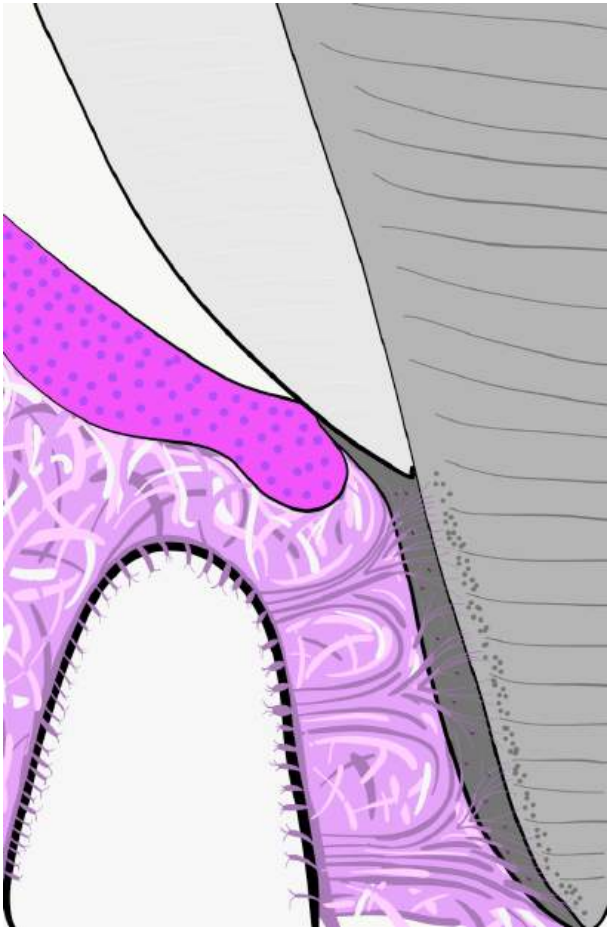


Figure 11.:4 Illustration of the periodontium, highlighting bundles of collagen fibers (dark pink) that extend from Tomes' granular layer through to alveolar bone.

The major protein in cementum is **collagen**. Collagen fibers extend out of cementum into **Tomes' granular layer** of root dentin, and in the other direction become the collagen fibers of the **PDL**. Collagen fibers of the PDL, in turn, become **Sharpey's fibers** embedded in alveolar bone. Rather than thinking about alveolar bone, PDL, cementum and dentin as distinct tissues bonded to each other, think of their borders

as a gradient, thanks to their shared **lineage**. This makes for a stronger connection than bonding 4 separate tissues, especially ones that are too thin for large **rete pegs**[←] and **dermal papillae**-like digitations. Other proteins secreted by cementoblasts include two **glycoproteins** (bone sialoprotein and osteopontin) which help collagen adhere to **calcium hydroxyapatite** crystals. Cementoblasts also secrete enzymes that catalyze the formation of crystals, similar to those active in **dentinogenesis**[←].

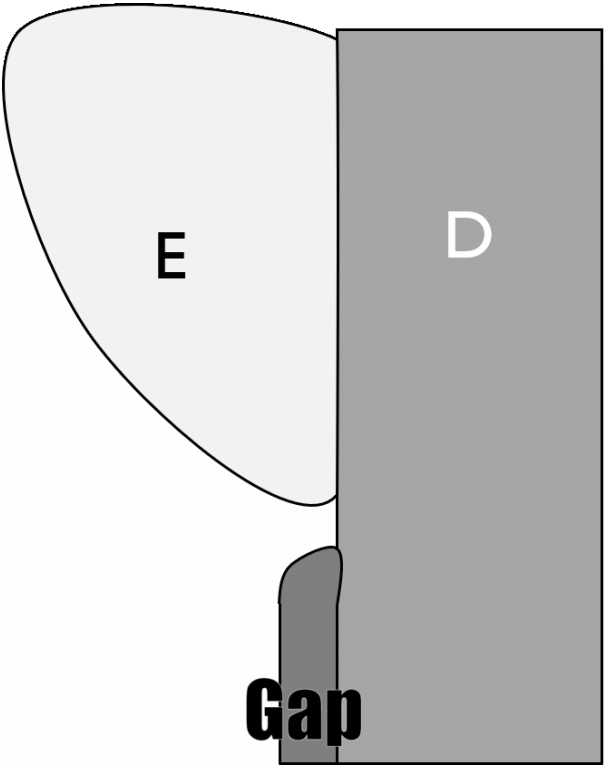


Figure 11.5: Patterns of cementum at the CEJ

Types of cementum by area of the root

For most teeth, the apical one third of the root contains **cellular cementum** over **acellular cementum**. More cervical regions contain only acellular cementum. Most of the time, roughly 60%, cementum extends over the enamel a short distance. About 30% of the time, cementum completely covers root dentin, all the way up to the enamel. Roughly 10% of the time there is a gap between the cementum and enamel,

with underlying dentin exposed. Rarely, about 1% of the time, enamel may overlap cementum (rare because **amelogenesis** begins much earlier than **cementogenesis**). These percentages are not for individual teeth, but for any area of the cervical region on a single tooth. Therefore, a single tooth may have all patterns. The gap pattern is the most significant because of the increased risk of **dentin hypersensitivity**. Cementum found overlapping enamel contains no **collagen** fibers, and does not connect to the **PDL**.

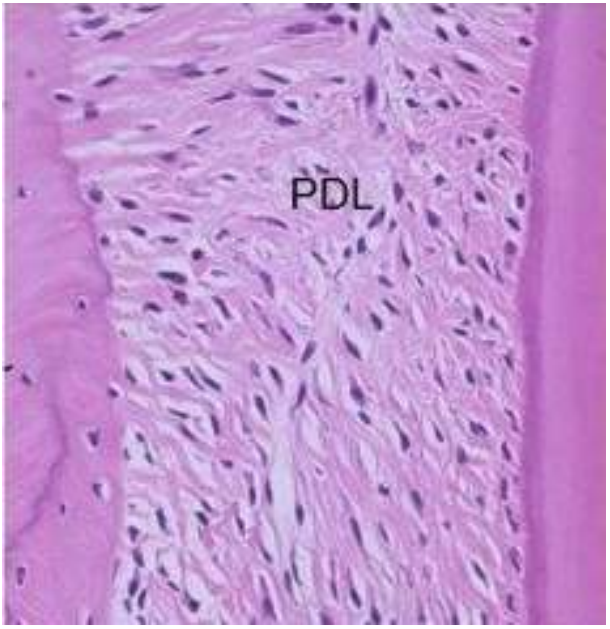


Figure 11.6: Histology of the PDL. Image credit "Immunohistochemistry of CXCL12 in PDL of rat molars" by Yashiro Y et al is licensed under CC BY-SA 4.0 / *cropped*

Periodontal ligament development

The **PDL**, like other ligaments, is a **dense regular connective tissue**, composed primarily of **collagen** fibers in the **ECM**, plus the special **oxytalan** fibers. These fibers are made

by **fibroblasts** that differentiate from **neuro-mesenchymal stem cells** of the **dental sac**. In figure 11.6, fibroblast nuclei stain purple. From this image, you should be able to determine which side of the PDL is anchored to bone tissue versus anchored to cementum. Look at either border, are there cells within **lacunae** or not? The pink color is mostly collagen in all 3 of these tissues.

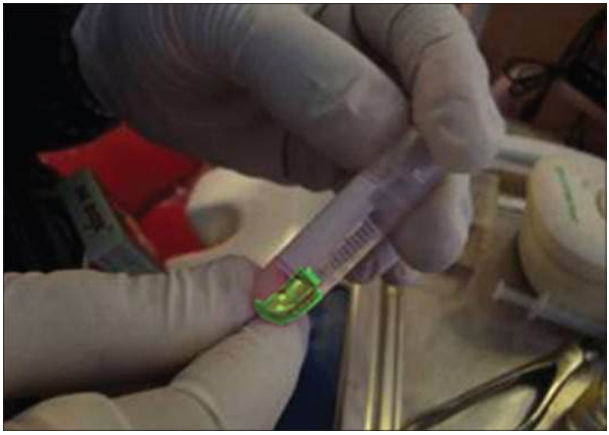


Figure 11.7: Collection of stem cells from an extracted tooth (the fluorescent green color lets you know this is science!). Image credit: "Sample embedded in tooth preservation cocktail and nourishment media" by PM Sunil et al

Like other ligaments, the **PDL** contains **osteoblasts** and **osteoclasts** (mostly at the border with alveolar bone), but unlike other ligaments, the PDL also contains **cementoblasts** and a unique population of **stem cells**←. The stem cells are different from **mesenchymal stem cells**, and may be referred to as Periodontal Ligament Stem Cells (PDLSCs). These stem cells can differentiate into **fibroblasts**, osteoblasts, **odontoblasts**, cementoblasts, **cementoclasts** or **odontoclasts** given the correct **morphogen**←. This allows the PDL to play a role in the **remodeling** and repair of bone, cementum and dentin. Whether these stem cells are different from the **neuro-mesenchymal stem cells** found in the **dental sac** and **dental papilla** is unclear. Their unique properties, however, make them useful: stem cells from extracted teeth can be used to promote the healing of many other (non-tooth) tissues.

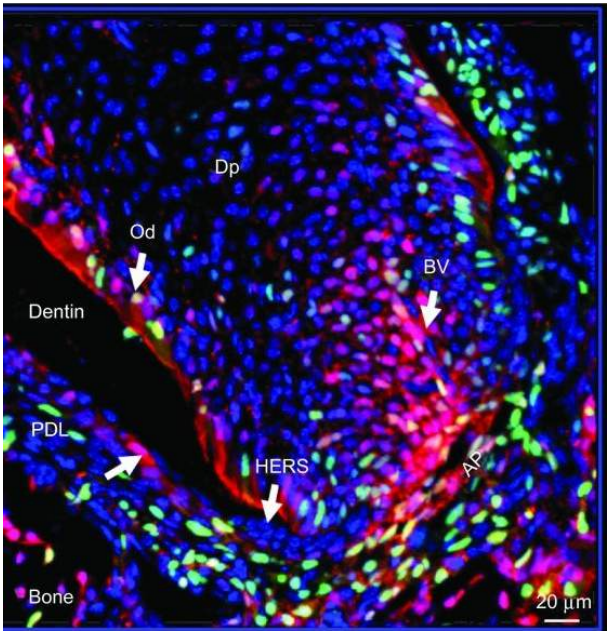


Figure 11.8: Confocal image of developing mouse tooth. Legend: BV = blood vessel, Od = odontoblasts, Dp = dental pulp, PDL = periodontal ligament. Colors: purple = DNA (nuclei), red and green = experimental trans-genes. Image credit: "Lineage studies by

mapping Creactivity in the root region of a second molar in a 2-week-old mouse by confocal microscopy using the Olympus FV 1000." by Rakian A, et al is licensed under CC

 Unlike cementum and dentin, the **PDL** is **vascular**. The PDL is significantly more vascular than other ligaments, thanks to its **lineage**. PDL **fibroblasts** secrete **morphogens**[←] inlcuding vascular endothelial growth factor (VEGF) to promote **angiogenesis**. The **expression** of the VEGF **gene** is controlled by morphogens of the **BMP** and **FGF** families. BMPs **induce** the **differentiation** of **odontoblasts** and **osteoblasts**, whgingival ile FGF induces fibroblast differentiation. Both morphogen signals are necessary to specify PDL fibroblast differentiation. What also makes PDL fibroblasts different from other fibroblasts is that they have a **neuro-mesenchymal** lineage. They have a higher capacity to undergo renewal (new ones forming from PDL **stem cells**[←]) and trigger tissue repair than fibroblasts in other ligaments. In other ligaments, fibroblasts develop from **mesoderm**, and are induced to differentiate from mesenchymal stem cells by FGF alone.

Oddly, the tissues that can best be repaired by cells from the PDL are bone and cementum, not the PDL itself. Keep this in mind as we discuss differentiation of the PDL. The first important concept is PDL fibroblasts are not like other fibroblasts. In addition to VEGF, PDL fibroblasts **express genes** shared by other cells with a **neuro-mesenchymal lineage**. For instance, PDL fibroblasts share features with **cementoblasts**, expressing RUNX2 and **pre-cementum** proteins. PDL fibroblasts also have similarities to neural tissue,

expressing Neuronal **Cell Adhesion Molecules** (NCAMs)[←] and N-cadherin (a neuronal **desmosome**[←] protein) (scientific review article). These genetic details are less important to us, what is important is the PDL **develops** from neuro-mesenchyme.

One more unique feature of PDL fibroblasts is their ability to participate in an immune response. Unlike other ligaments, the PDL is often exposed to bacteria and bacterial toxins. In response to certain inflammatory signals, PDL fibroblasts down-regulate **expression** of **genes** involved in bone **remodeling** and behave more like white blood cells (scientific article for further reading). This is not entirely unexpected given their **lineage**. **Neural crest cells** guide the **development** of the thymus from the 3rd **pharyngeal pouch** (The thymus is where T cell lymphocytes develop).

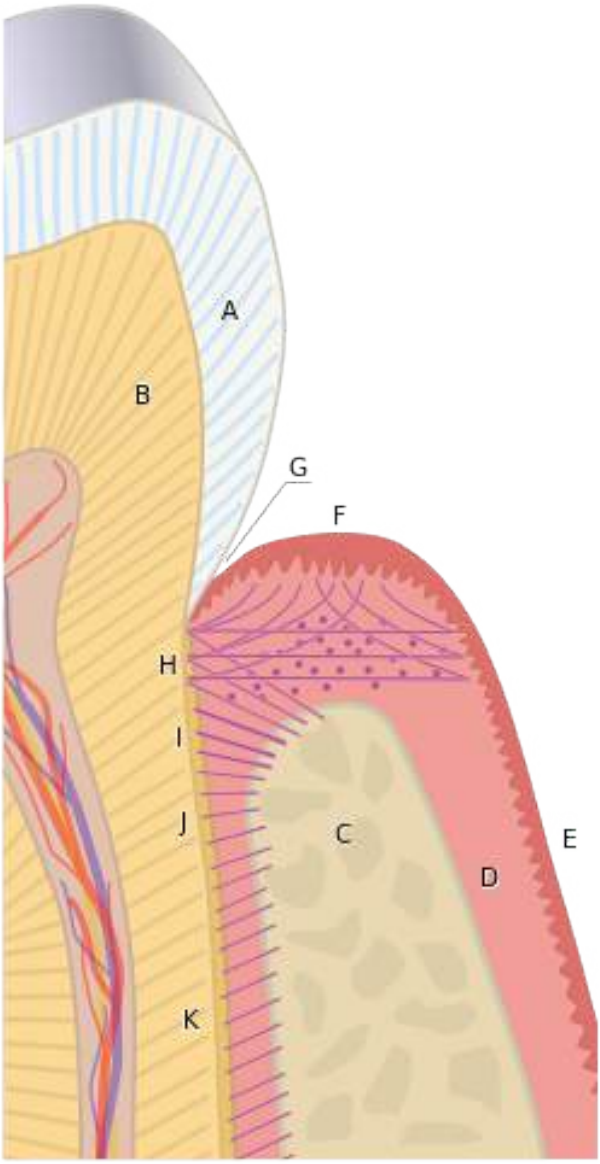
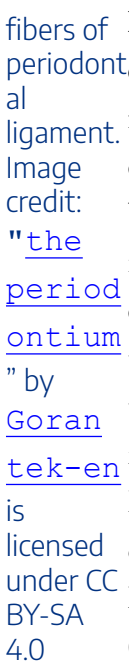


Figure 11.9: The periodontium. Legend: A. Enamel, B. Dentin, C. Alveolar bone, D. Gingiva, E. Oral epitheliu m, F. Free gingival margin, G. Gingival sulcus, H. Principal gingival fibers, I. Alveolar crest fibers of periodontal ligament, J. Horizontal fibers of periodontal ligament, K. Oblique



fibers of periodontal ligament. Image credit: "the periodontium" by Goran tek-en is licensed under CC BY-SA 4.0

Development of the **PDL** begins during **tooth eruption**. PDL **fibroblasts** secrete **collagen** fibers that become embedded in cementum and extend outwards (**principal fibers**). This process begins at the **CEJ** and continues **apically** as the root grows. The other ends of collagen fibers are embedded within alveolar bone later, activated by tooth occlusion. By waiting to anchor one end of the fibers until tooth occlusion results in greater mobility of tooth roots during **tooth eruption**. PDL collagen fiber bundles run in different directions, which can be classified as alveolar crest, horizontal, oblique, (peri) apical, inter-radicular (on multi-rooted teeth) and trans-septal. **Gingival fibers** are also collagen fiber bundles, but anchor cementum to the gingiva. These are also categorized based on their location and fiber orientation. Depositing collagen in the correct orientation requires **morphogens** of the **planar cell polarity** class. One example is a cell-surface membrane protein called CD44 which binds to **hyaluronic acid**, collagen and **fibronectin**. Similar morphogens are involved in the polarization of **odontoblasts** following their induction by the **IEE**. Changes to polarity morphogens are likely necessary for re-orientation of fibroblasts during tooth occlusion and the production of collagen bundles in different directions.

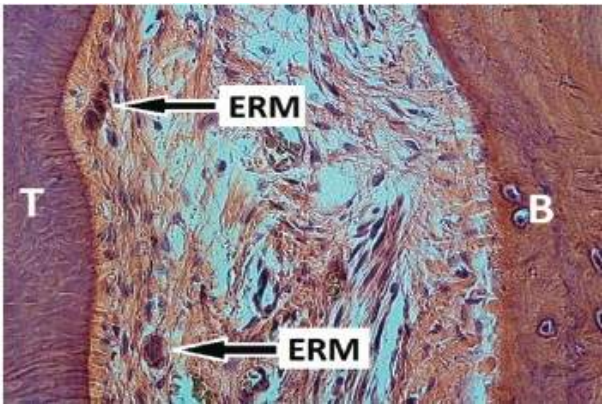


Figure 11.10: Histology of the epithelial Rests of Mallasez (ERM). T = tooth, B = alveolar bone. Image credit "Photomicrograph of the periodontal ligament showing clusters of epithelial cell rests" by HD Miniggio

Remnants of **HERS** may linger after root formation is finished. These cells, the **Epithelial Rests of Mallasez**, *may* be involved in repair of cementum and the **PDL** (the evidence is not definitive at this time). While these cells are epithelial, derived from **ectoderm**, they may undergo an **epithelial-to-mesenchymal transition** and then **differentiate** into **cementoblasts** or **fibroblasts**, if they receive the correct **morphogens**. Evidence for this comes from studying transgenic mice.

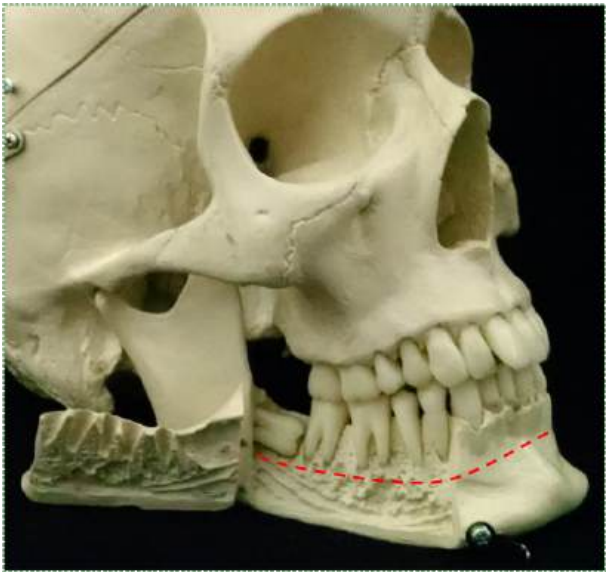


Figure 11.11: Alveolar bone (roughly above dotted red line)

Alveolar bone

The vault of the skull **develops** by **intra-membranous**

ossification (including the maxilla). Thus, skull sutures contain dense connective tissue (unless they fully ossify). The base of the skull, on the other hand, develops by **endochondral ossification**. The development of the mandible is even more complicated. First, **neuro-mesenchyme** forms two bands of cartilage (**Meckel's cartilage**). The posterior portion is fated to become the ramus of the mandible (plus the malleus and incus bones), while the anterior portion disappears. Before the anterior portion disappears, the body of the mandible forms around it by intra-membranous ossification. Later, the condylar process, coronoid process and mandibular symphysis develop by **endochondral ossification**. That is *how* the skull forms. What is forms *from* does not match as nicely. Most bones develop from **mesoderm**, including the entire appendicular skeleton, plus most of the axial skeleton. The exceptions are the facial bones (including the hyoid) and inferior parts of the cranium, which develop from neuro-mesenchyme (the "new head"). In developmental biology classes, this is not a minor issue: ancient fish (either long extinct, or modern-day lampreys) have no lower jaw, they have seven branchial arches (**pharyngeal arches**). Jawed vertebrates (gnathostomes), including humans and bony fish, **remodel** some arches into other structures, including a powerful mandible and ear parts. If you are curious, the lower jaw of a shark (and even the sturgeon) is not a mandible, it is Meckel's cartilage (so sharks

are a closer family member of yours than a lamprey, but more distant relative than a goldfish).

This brings us to alveolar bone. The alveolar ridge of both the mandible and maxilla **develop** from **neuro-mesenchyme** of the **dental sac**. **Neuro-mesenchymal stem cells** in this area are **induced** to **differentiate** into **osteoblasts** by **morphogens**← (including members of the **BMP** family). This in turn activates **transcription factors** such as members of the RUNX family and MSX **homeobox gene**← family. These transcirption factors up-regulate **expression** of bone-specific proteins. From a development standpoint, it is important to understand that the basal bone of the mandible and maxilla are induced by signals from Meckel's cartilage, while alveolar bone develops from the **tooth germ**.

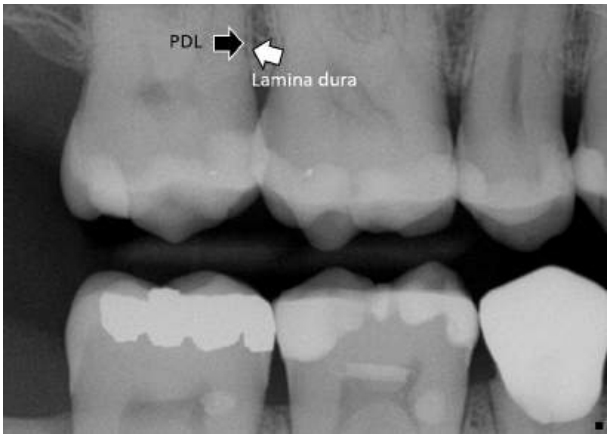


Figure 11.12: Bitewing noting PDL and lamina dura.

Alveolar bone contains numerous small **Volkman's canals**, through which **PDL** fiber bundles insert into bone tissue. This is the same name used for the perforating canals that

run perpendicular to Haversian canals in compact bone, only those Volkman's canals contain blood vessels. Like all bones, the outer portion of alveolar bone is compact bone and below that is spongy bone. The compact bone portion is referred to as the **lamina dura** on a radiograph, as it shows up more radiopaque than the spongy bone deep to it (or the **PDL** superficial to it). The **inter-dental septum** is a triangular-shaped area between two teeth, which should be the height of the alveolar crest. The **inter-radicular septum** is the portion of alveolar bone between roots

Gingival development

The **oral mucosa**, including most of the gingiva, develop from **ectoderm** and **mesoderm** during embryonic development. Ectoderm forms the **oral epithelium**, while mesoderm forms the **lamina propria** and **sub-mucosa**. The exception is **junctional epithelium**[←], which develops from a group of cells more specialized than ectoderm. During **odontogenesis**, ectodermal cells of the **tooth germ** separate from oral ectoderm. During **tooth eruption**[←], it is the **REE** that develops into junctional epithelium.

Clinical applications

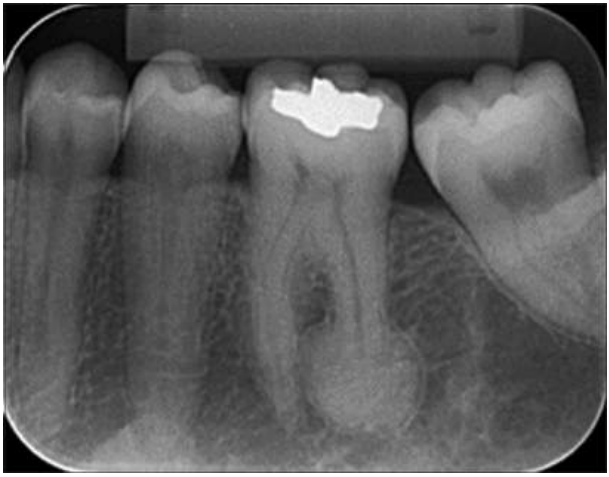


Figure 11.13: Hyper-cementosis. Image credit: "Periapical radiograph showing radiopaque halo around the root of tooth #36" (international numbering system) by Antonione Santos Bezerra Pinto is licensed

Hyper-cementosis

Because **cementoblasts** are located at the surface of adult cementum (within the **PDL**), cementum has the ability to undergo **remodeling** and repair. This makes cementum similar to dentin but different from enamel. Excessive cementoblast function can result in **hyper-cementosis**. This occurs more frequently at the root apex due to excessive occlusal forces on a tooth. It may also be caused by **growth factor**← disturbances, such as gigantism/acromegaly or Paget's disease.

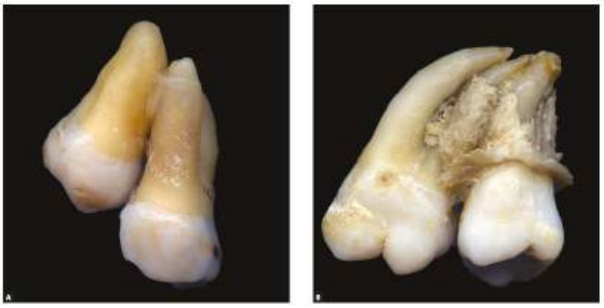


Figure 11.14: Concrescence examples. Image credit : "Second and third molars joined by cement, in teeth with and without hypercementosis" by Consolaro A et al is licensed under CC BY 4.0

Concrescence

Enough **hyper-cementosis** can adhere two teeth together by the roots, which is called **concrescence**. This should illustrate one reason why a radiograph is necessary prior to tooth extraction. This most commonly occurs between the second and third upper molar.

Figure 11.15: Cemental caries. Image credit: "Photograph showing root caries in 33 & 34" (international numbering system) by B Gupta is licensed under CC BY-SA 4.0

Root caries

Gingival recession is worrisome in part because it exposes the roots of teeth to the environment that only enamel is exposed to under healthy conditions. Because of its lower mineral content, **root caries** spread quickly. Caries pass quickly to dentin, and if untreated, pulp. Dental pain may not arise until

infection of the pulp sets in. If that happens, treatment is generally limited to tooth extraction, rather than prevention and maintenance. Root caries can be detected early with radiographs or use of a dental mirror and explorer.

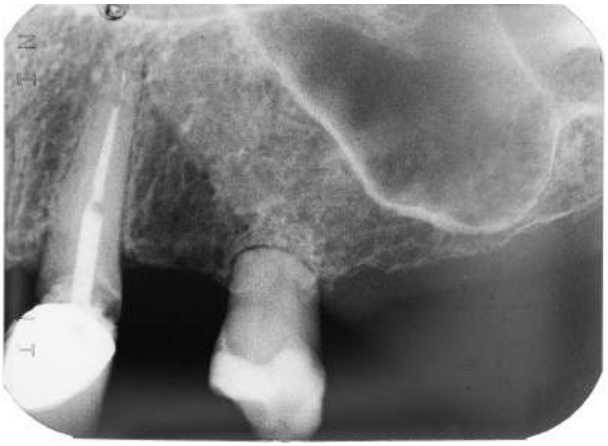


Figure 11.16: Root resorption. Image credit: "Own work" By Bin im Garten is in the Public Domain CC0

Root resorption

Both dentin and cementum have the capability to be repaired in response to mild or moderate trauma. This is because living **odontoblasts** are located in the outer layer of the pulp, and **cementoblasts** in the **PDL**. If necessary, **mesenchymal stem cells** in the pulp or PDL may be induced to **differentiate**[←] and lay down more **ECM**. However, reparative cementum

does not contain **Sharpey's fibers** and does not contribute to tooth attachment to bone tissue.

Severe trauma, on the other hand, can induce the **differentiation**[←] of **mesenchymal stem cells** into **cementoclasts** or **odontoclasts**, leading to the loss of cementum and dentin. It is possible that the precursor to cementoclasts and/or odontoclasts may not be your average mesenchymal stem cell, but a related **stem cell**[←] with a different **cell fate**. Possible candidates include **neuro-mesenchymal stem cells** and **PDL** stem cells.

Figure 11.17: Lateral maxillary incisor has undergone root resorption, evident by its pinkish appearance. Image credit: "Mummery" by DRosenbachis licensed under CC BY 3.0

Loss of dentin and cementum from the roots of teeth is root resorption. Loss of dentin from deep layers is **internal root resorption**. As dentin is lost, the space is filled in by vascular pulp tissue, possibly causing the tooth to have a more pinkish coloration. This tooth may be referred to as a pink tooth of Mummery (named for the anatomist who first described the condition). If internal root resorption is caused by chronic

inflammation, removal of inflamed pulp by **endodontic therapy**← may halt the condition.

External root resorption is the loss of cementum and dentin from the superficial side of the root. External root resorption may be transient and resolve on its own. This occurs because cementum has a high regenerative capacity because of **cementoblasts** in the **PDL**. Chronic inflammation or tooth **ankylosis** may trigger more severe resorption, and replace lost tooth tissues with bone tissue.

For those into crime shows, corpses that have suffered a traumatic death (e.g. asphyxiation) or have spent time in a humid environment before being discovered may have pink teeth. This is due to ruptured pulp blood vessels causing bleeding into dentinal tubules (pdf download— warning: contains photos of a dead body).

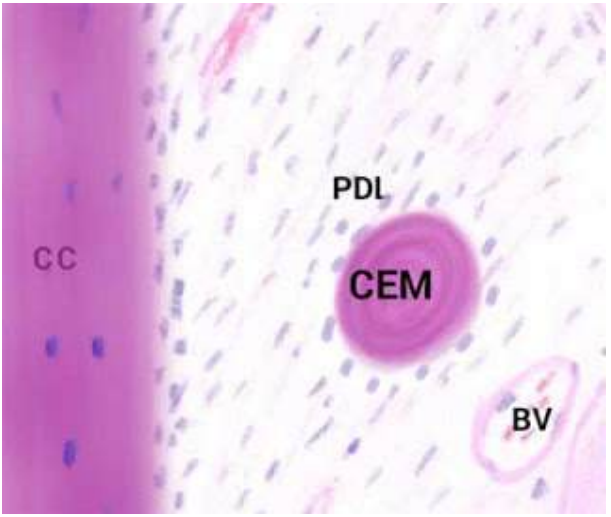


Figure 11.18: Cementicle. Labels: PDL = periodontal ligament, CEM = cementicle, BV = blood vessel, CC = cellular cementum. Image credit: "Cementicles" by Mandana Donoghue is licensed under CC BY-NC-ND 4.0

Cementicles

Masses of **acellular cementum** may develop in abnormal

locations, especially later in life. These are referred to as **cementicles**. Cementicles may be free (within the **PDL** but unattached to the tooth), attached (on the surface of the layer of cementum) or embedded (once attached, but now surrounded by the cemental layer of the root). It is thought cementicles develop when **cementoblasts** encounter debris such as a blood clot (thrombus) lodged in a nearby capillary. This disrupts **appositional growth** of cementum and instead creates a nucleus around which a cementicle forms.

Figure 11.19: Example of the importance of tooth proprioception. Image credit: "Tortoiseshell cat carrying her kitten up a flight of stairs. She grips the kitten's scruff in her teeth" by

Tooth discomfort following endodontic therapy

After **endodontic therapy**←, the pulp—including nerve endings– is replaced with a non-living **bio-compatible** polymer. Nevertheless, tooth discomfort may occur. This is because the **PDL** is present and living. Besides functioning to attach the tooth roots to bone and gingival tissue, the PDL is involved in proprioception (kinesthesia), or the sensation of where the body is located. Nerve endings in the PDL relay information to the somatosensory cortex about where the teeth are located and when they experience occlusal forces. This is very important in reducing or avoiding occlusal forces during mastication and speech. The jaws exert the greatest amount of force the human body can generate, but also exhibit great control. It is routine for people to bite down through substances of varying density and thickness without teeth hitting one another thanks to a large region of somatosensory cortex interpreting information from the teeth.

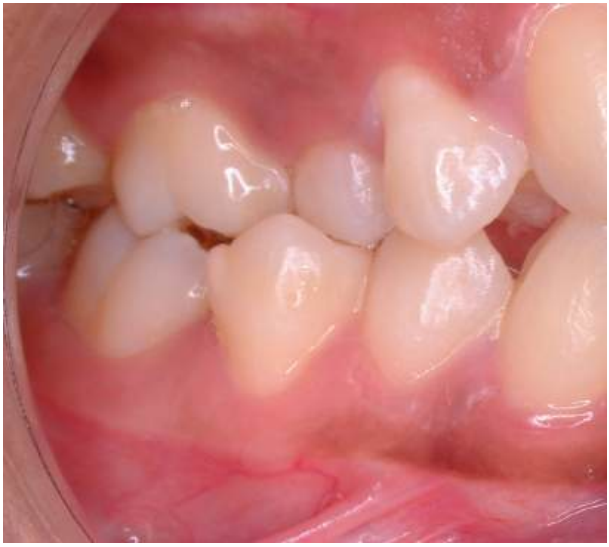


Figure 11.20: Mesial drift. Image credit: "Class II human Molar relationship" by Dr. Vipin C. P. is in the Public Domain, CC0

Mesial drift

Mesial drift (or physiological drift) is the tendency of teeth to migrate in a mesial direction with age. It is a natural phenomenon caused by the assymetrical **remodeling** of alveolar bone tissue. There are two major concepts relevant to mesial drift. One, alveolar bone undergoes more bone remodeling than other bone tissue. Two, this remodeling responds to occlusal forces: removing force causes bone loss to accelerate, adding force causes bone deposition to accelerate.

When occlusal forces are assymetrical on a tooth, it leads to one side of the alveolar socket undergoing bone depostion and the other side bone resorption. Assymetrical remodeling, in turn, causes tooth movement (see Fig. 11.29 for a similar phenomenon). With age, teeth tend to wear down at their contact points, which leads to gaps between teeth, and a reduction in force on the gap side. Mesial drift causes teeth to move closer together, closing gaps as they form from wear. Too much movement can lead to crowding. Missing teeth can accelerate drift, causing neighboring teeth to lean lingually, changing the occlusal plane. This, in turn, can lead to abnormal forces on the temporo-mandibular joint, causing pain, discomfort and reduced range of motion.

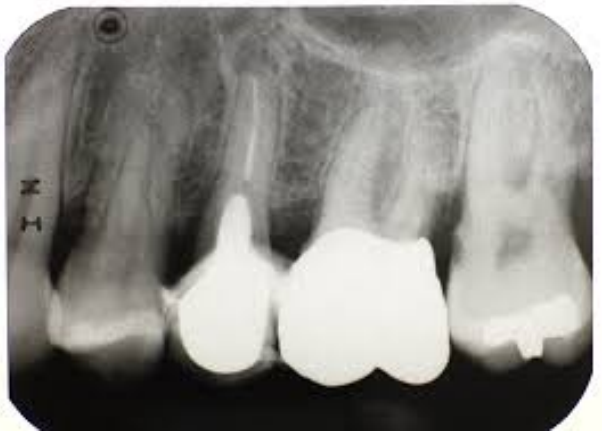


Figure 11.21: Loss of alveolar bone. Image credit: "Own work" by By Bin im Garten is licensed under CC BY-SA 3.0

Loss of alveolar bone

The loss of alveolar bone leads to an uneven level of the **interradicular septa** or **inter-dental septa**. The consequence of this is reduced connection between tooth and bone, which can lead to tooth mobility and loss. This is often first observed in reduced opacity of the alveolar crest region(s) on a radiograph (Fig. 11.21). Under healthy conditions, alveolar bone—especially spongy bone—undergoes contant replacement by the **remodeling unit**. Chronic inflammation, such as from **periodontitis**, inhibits the rate of **osteoblast** function without reducing the rate of **osteoclast** function. This is likely due to the fact osteoclasts are related

to white blood cells, their **lineage** is from the bone marrow. White blood cells are more active during inflammation, unlike most other cells in the body.

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animated illustration of fenestration

Figure 11.22: Illustration of fenestration and dehiscence of alveolar bone tissue, exposing underlying tooth roots.

Fenestration and dehiscence

With significant alveolar bone loss, portions of tooth roots may become exposed. A small window of root may become exposed, known as a **fenestration** (the Latin word for window). If the exposed root area connects all the way to the **CEJ**, the region is known as a **dehiscence** (a general term for a wound that cannot close). This is distinct from **gingival**

recession or **periodontal pockets**←, where **junctional epithelium**← migrates to a region more apical on the root of the tooth, exposing cementum. A fenestration or dehiscence may still be covered by **oral mucosa**, but a lack of **epithelial attachment** means oral bacteria may come into contact with cementum and bone tissue, neither of which is designed to resist infection the way partially **keratinized avascular** epithelia are. This occurs much more frequently on the facial side than the lingual side.

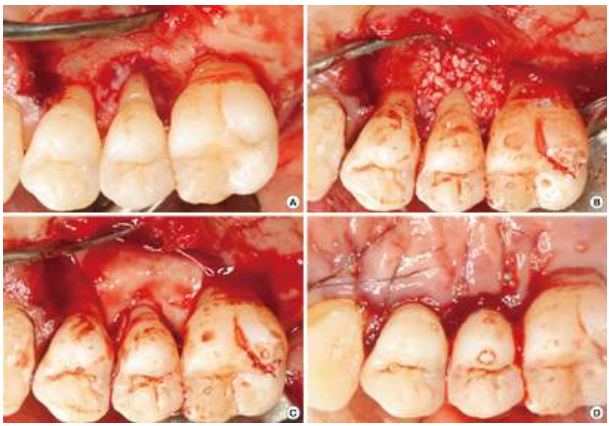
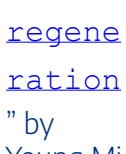


Figure 11.23: Guided tissue regeneration. Legend: A) bony defect after root planing and debridement. B) Bone graft C) Collagen-membrane D) Gingival flap surtured over graft materials. Image credit: "Chronic periodontitis, Collagen, Guided

Guided Tissue Regeneration



There are many techniques that aim to stimulate the body to repair bone loss, including alveolar bone, by filling in the damaged area with a **scaffold**. The proper scaffolding material allows **mesenchymal stem cells** to migrate through the damaged area and **differentiate** into **osteoblasts**. Surgeries involving this method are referred to as Guided Bone Regeneration or **Guided Tissue Regeneration** (GTR). The major difference between these procedures and a **bone graft** is the use of a membrane to prevent neighboring tissue from growing into the damaged area.

First, a membrane must be placed over the injured area. Old-fashioned membranes, such as cotton gauze bandages, form a barrier between pathogens and exposed **vascular** tissue. Membranes can also keep other tissues, such as epithelia or **scar tissue**, from filling up the lost region with the wrong type of tissue. Cotton, however, does not contain molecules that bind to human **integrins** or **CAMs**, and therefore does not act as a scaffold. Without a scaffold, healing of the injury occurs from the edges of the wound. Newer materials may incorporate molecules covered in this book, and be recognized as a scaffold over which stem cells migrate. These molecules may include components of ECM, or **morphogens**. Some morphogens promote the **differentiation** of mesenchymal stem cells into osteoblasts, rather than **fibroblasts** (which

produce scar tissue), others can be used to promote **angiogenesis**, speeding up the healing process.

One consideration when choosing a membrane is whether it is resorbable, or whether it will need to be removed (requiring another surgery). Both organic and synthetic polymers may be capable of being resorbed, or not. For instance, cotton is organic, but is not resorbed readily by the human body. Polylactic acid (PLA) membranes (which are also organic, but synthesized in a laboratory) are readily absorbed during the healing process and get replaced by human tissues. Resorption of large molecules may occur without the activity of human cells. For example, PLA slowly degrades when exposed to heat and UV light. Other polymers might be removed by human enzymes, such as **matrix metalloproteinases**. Guided tissue regeneration is conceptually very different from replacing damaged tissues with a **bio-compatible** but inert material, such as latex in **endodontic therapy**←, or a filling made of metal, porcelain or plastic. The goal is not to fill in a gap, but to promote the healing of a gap.

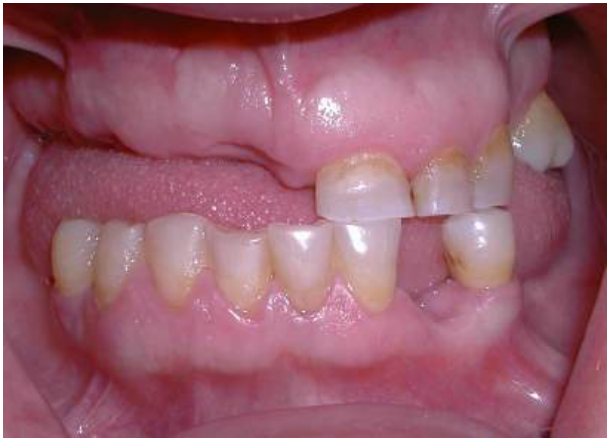


Figure 11.24: Changes to an edentulous alveolar ridge. Image credit: "Own work" by JASFUS assumed in the Public Domain CC0

Resorption of the alveolar ridge

Following tooth extraction or loss, regions of the alveolar ridge no longer anchored to **PDL** and tooth roots undergo resorption (basal bone is unaffected). This occurs because all bone tissue undergoes **remodeling**, and a lack of tension slows down the activity of **osteoblasts**, but not **osteoclasts**. The opposite is why exercise maintains healthy bones. Chewing exercises the alveolar ridge.

animated illustration of changes to alveolar ridge

Figure 11.25: Changes to the vertical dimension of the maxilla and mandible in an edentulous mouth.

Loss of alveolar bone reduces the vertical dimension of the mandible and maxilla. Without teeth to occlude, the mandible and maxilla can move closer together. This causes the mandible to protrude forward, leading to a “popeye chin”.

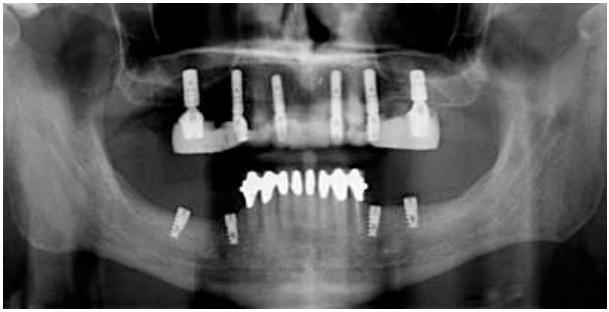


Figure 11.26: Dental implants. Image credit: "Own work" By Danjhon is licensed under CC BY-SA 4.0

Dental implants

Dental implants can prevent loss of alveolar ridge by transmitting force onto bone tissue during mastication. **Osteoblasts** respond to force by increasing the deposition of calcium and phosphate, increasing bone density and maintaining bone health. If bone loss has already occurred, a **bone graft** or **Guided Tissue Regeneration** procedure may need to be done allow for regeneration of lost bone tissue before the implants are installed. Afterwards, **gingival grafting** (including **SECT**) may be involved if gingival tissue has receded significantly.

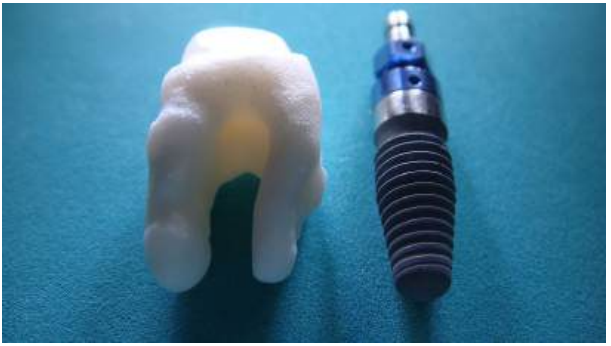


Figure 11.27: Ceramic root analog implant (RAI) compared to a traditional titanium screw-type implant. Image credit: "Own work" by Logicwhatelse is licensed under CC BY-SA 4.0

Dental Implant technology

Each dental implant consists of a core, abutment and crown. The core is traditionally made of titanium and is implanted into bone tissue, although other technologies exist (Fig. 11.27). To get the shape and size of the core to fit properly within the alveolar ridge involves computed

tomography (CT scans) to image bone tissue and computer-aided design (CAD) to specify the core's dimensions. The abutment connects the core to the crown by a screw or dental cement. The crown is usually made of ceramic. Titanium is strong, durable and **bio-compatible**. It is more or less invisible to the cell surface **receptors** that white blood cells use to detect antigens. Unfortunately, this also makes titanium invisible to other cells in the body, including **mesenchymal stem cells**. As a result, titanium does not adhere well to the connective tissue it is embedded in. Knowledge of histology helps: coating the core with **hyaluronic acid** improves the connection between the core and the patient's connective tissue.

Because dental implants fuse to bone tissue, they do not participate in proprioception. There is interest in creating **bio-active** coatings for the titanium core that might allow **PDL** attachment to an implant. For instance, layering a core with with **hyaluronic acid** and PDL **stem cells**[←] promotes the connection of a dental implant to PDL in mice.

Another important connection that is lost with tooth extraction is **junctional epithelium**[←]. Because the junctional epithelium of each tooth is derived from the **REE** during **tooth eruption**[←], new junctional epithelium does not form on an implant. At best, **oral mucosa** may adhere to titanium via **hemi-desmosomes**[←], creating peri-implant mucosa. Coating a ring of the core with **morphogens**[←] (such as **FGF**)

mimics the **induction** of junctional epithlium from REE, improving the connection between oral mucosa and the implant. Coating the titanium core with **BMPs**, on the other hand, promotes integration with bone tissue.

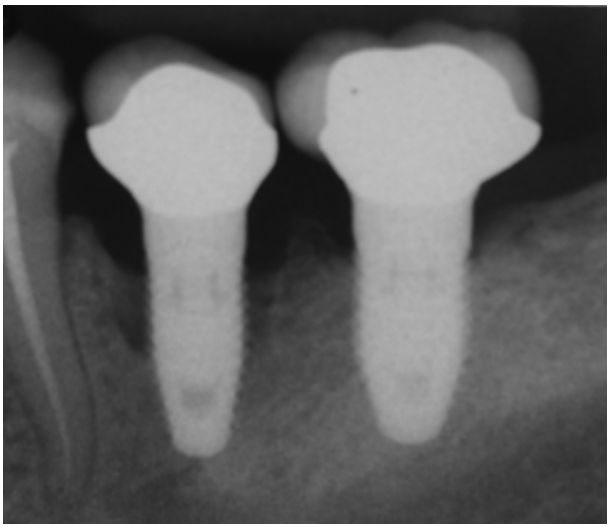


Figure 11.28: Peri-implantitis leading to bone loss. Image credit: "Bone loss following peri-implantitis in a smoker" by Coronation Dental Specialty Group is licensed under CC BY-SA 3.0

Peri-implantitis

Failure to create a complete junction between the **oral mucosa**

and the implant exposes underlying connective tissue to the oral microbiome, frequently leading to **peri-implantitis**. Because the oral microbiome does not go away, chronic inflammation occurs, leading to loss of tissue around the affected implant, including gingival and bone tissue.

animation of orthodontia

Figure 11.29: Changes inthe force applied to bone tissue from the PDL during orthodontic movement leads to changes in the activity of the bone remodeling unit on both sides of the alveolar socket.

Orthodontia

The **remodeling unit** is involved in both maintaining bone health and loss of bone tissue. Its activity is taken advantage of with **orthodontia**. Pressure applied to a tooth causes the

PDL on one side to slacken, and on the other side to tense up. The side with lower amount of tension will undergo bone resorption, as **osteoblasts** are inhibited but **osteoclasts** maintain their speed of bone resportion. Higher tension triggers bone deposition on the opposite side. As a result, the location of the alveolar socket shifts in the jawline, without altering the overall dimensions of the socket itself. It is import that the correct amount of force be applied—to much stress can lead to bone resorption all around, increasing the size of the alveolar socket and increasing tooth mobility.

Common treatments for osteoporosis include drugs than inhibit the activity of **osteoclasts**, such as bisphosphonates. Because the entire **remodeling unit** is required for orthodontia, patients using these medications cannot undergo orthodontic therapy. Another factor to consider with orthodontia is the activity of **odontoclasts** and **cementoclasts**. The **morphogens**[←] that induce odontoclast **differentiation**[←] and activity (RANKL) during orthodontic movement *inhibit* the activity of cementoclasts and odontoclasts. For this reason, there is significantly less resorption of cementum and dentin than there is bone tissue during orthodontic movements, even though force across the PDL is the same on bone and tooth root tissues. It is possible to experience root resorption with orthodontic treatment, especially as the orthodontic force increases, although the causes of root resorption are complex and multi-factorial.

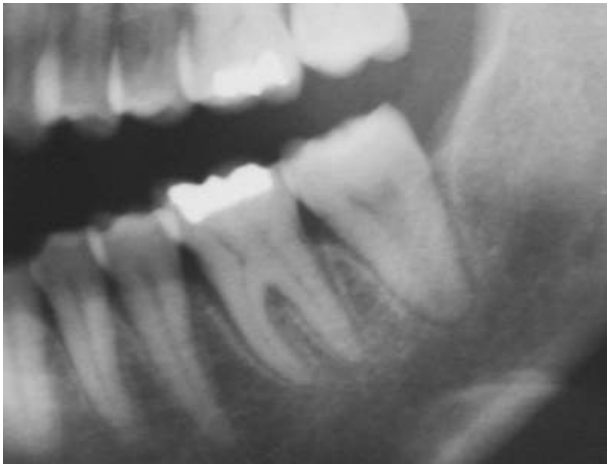


Figure 11.30: Widening of the PDL space. Image credit: "Widening of periodontal ligament space in tooth 36" by CH Chuh is licensed under CC BY-NC-SA 4.0

Widening of the PDL space

With occlusal trauma, **fibroblasts** within the **PDL** responds with increased activity, leading to a widening of the PDL. For example, in **endodontic therapy**, a wider PDL space is

temporarily generated on the side opposite of the direction of force, but this triggers bone deposition and returns the PDL space to its original width. A wider PDL space can be visualized on a radiograph, and may be accompanied by a thickening of the **lamina dura**, bone loss, and/or **hypercementosis**. Other conditions besides occlusal trauma may lead to a widening of the PDL space, such as the use of certain medications. The term wider PDL *space* means that neighboring tissue is lost. It does not necessarily indicate PDL fibroblasts have made more **collagen**← fibers, only that a larger radiolucent gap exists between the root and the lamina dura. This space may contain more **ground substance**← or pus, for instance, and not **dense regular connective tissue**←. This brings us to another example, which may seem contradictory to the first: reduced force can also lead to widening of the PDL space. For example, conditions that negatively affect bone health lead to changes in chewing behavior which in turn reduces occlusal forces. Reduced force is followed by bone loss and a widening of the PDL space. Similarly, chronic inflammation of nearby tissues, such as **pulpitis**, can trigger widening of the PDL space. Whether these conditions stimulate production of **collagen**← by fibroblasts is unknown because **pulpitis** and osteoporosis are associated with increased tooth mobility. Common treatments usually aim to reduce the insult, such as using a nightguard if bruxism is suspected. **Dental implants** help distribute the forces applied

to remaining teeth in a partially **edentulous** mouth, reducing changes to the PDL space and maintaining tooth attachment.

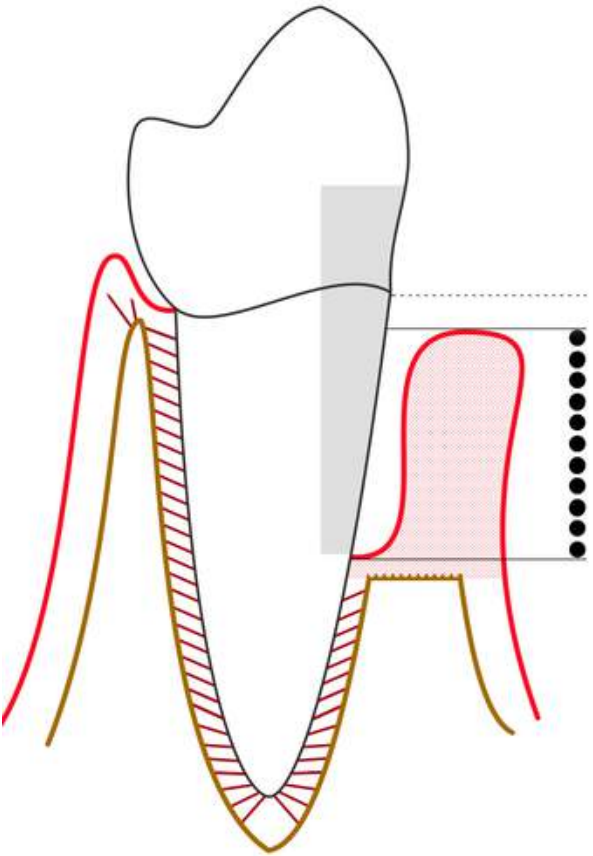


Figure 11.31: Illustration of a healthy pocket versus severse periodontitis, including fibers of the PDL. Image credit: "Line diagram of a tooth showing the gingiva, bone, periodontal ligament with a scale showin

Loss of the PDL apparatus

g the pocket depth of severe periodontitis" by Ian Furst is licensed under CC BY-SA 4.0

Loss of attachment between cementum and alveolar bone, known as **clinical attachment loss**, promotes tooth loss. Two common causes are smoking and bacterial biofilms located within periodontal pockets. With smoking, nicotine plays a role as a toxin. Nicotine activates nicotinic acetylcholine receptors whose activity modulates blood flow, **mitosis**←, chemotaxis and cell attachment to **ECM**. These are all necessary for a **fibroblast** to repair or regenerate the **ECM** of **dense regular connective tissue**←. Bacterial biofilms contain toxins which can cause similar changes in fibroblast activity—but these toxins generally only diffuse 1 to 2 mm through ECM, which means biofilms located more than 2 mm away from the **DEJ** are too far away to harm fibroblasts of the PDL (Fig. 11.31).

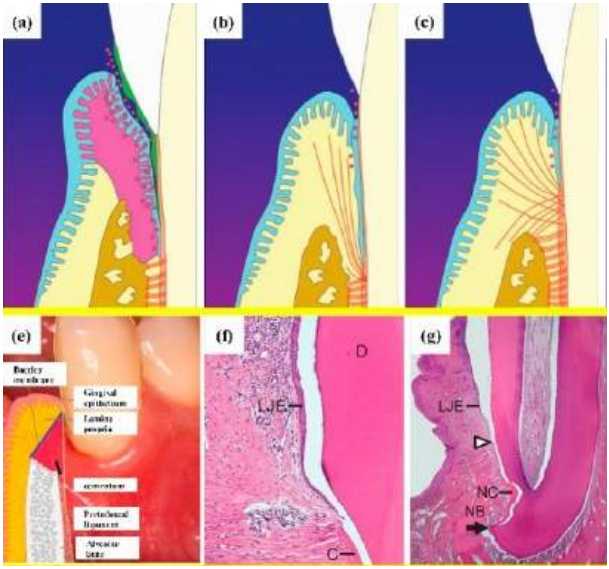
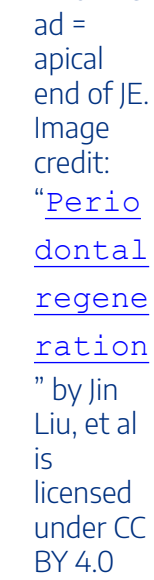


Figure 11.32: PDL regeneration schematic. Figure Legend: a) loss of bone and soft tissue during periodontitis. b and f) Long Junctional Epithelium (LJE) repair. c and g) ideal repair. e) Guided Tissue Regeneration schematic. C = cementum, NC = new cementum, D = dentin, NB = new bone,

Regeneration of the PDL following periodontitis



NPLF = new PDL fibers. Arrowhead = apical end of JE. Image credit: "Periodontal regeneration" by Jin Liu, et al is licensed under CC BY 4.0

We have discussed **regeneration** of bone tissue using **bio-active scaffolds**← (**guided tissue regeneration**), as well as promotion of **epithelial attachment** to **dental implants** using **ECM** protein coatings. The use of similar bioactive membranes to promote regeneration of the **PDL** has only been studied in animals at this time, not humans. PDL regeneration is conceptually more complicated—which is perhaps the reason why PDL regenerates poorly, and why we have made less progress on PDL regeneration than bone or **oral mucosa**. The PDL is good at tissure repair because it contains **stem cells**← and ample vascularity. These two factors that make other connective tissues regenerate well, such as pulp, bone and the sub-mucosa of the oral cavity.

So why is the **PDL** good at repairing bone and cementum, but not itself? First, the PDL is polarized. It is not enough to stimulate **fibroblasts**, the cells must be oriented correctly to create the proper attachments (similarly, a good coach won't tell soccer players to kick the ball into a goal, or even the goal at the north end of the stadium, the target changes at halftime so polarity and time matter). Unfortunately, the **planar cell polarity**← signals involved in this process are poorly

understood. Secondly, the two connections of the PDL form at different times: **collagen** fibers are created within **pre-cementum** during root formation, but fiber connections to alveolar bone are not made until **tooth eruption**. Again, the signals involved are poorly understood, it remains possible that the signals guiding these two connections could be mutually exclusive. We've covered examples where the same **morphogen** does different things at different times throughout this book. For example, **BMP**-2 **induces** the **differentiation** of **odontoblasts**, **ameloblasts**, **cementoblasts** and **osteoblasts**. It may not be possible to get the PDL anchored to cementum and alveolar bone at the same time.

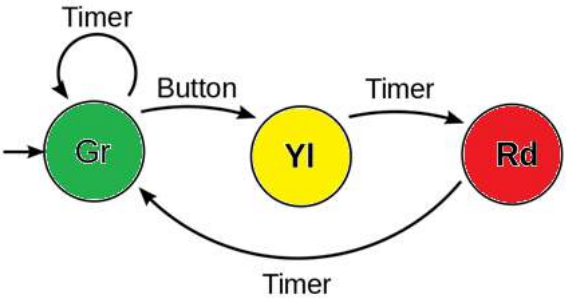


Figure 11.33: Like the programming of traffic lights, development involves timers (such as cyclins) and signal buttons (morphogens), internal electric changes (gene transcription changes) that lead to externally visible changes (morphogenesis). Like a well-planned city traffic light

system, there is significant cross-talk between developing structures in a human embryo not illustrated here. Image credit: "FSA of a traffic light system" by Pluke is in the Public Domain, CC0 / *colors added*

Without knowing more specifics, let us offer a metaphor: the two connections of the **PDL** could be like car traffic at an intersection. For every green light you travel through, cross-traffic has a red light. With proper timing, you might be able to hit green lights all the way down the street, but the traffic light you drove through a minute ago is probably turning red now. It may not be possible to simultaneously get a green light occurring both at the light you are at *and* the light you were at 5 blocks ago. Urban planners realize that would be a horribly inefficient system. The two ends of the PDL might be controlled like traffic lights, a go signal at one end may be the stop signal at the other end.

Our final thought

Don't worry about the red light 5 blocks behind you. You have the green light to go apply knowledge of histology and embryology to your clinical coursework. If the light turns yellow, yellow means go faster.

< Chapter 10 * navigation * Chapter 1 >

Chapter review questions

An interactive or media element has been excluded from this version of the text. You can view it online here:
https://openoregon.pressbooks.pub/histologyandembryology/?p=60

GLOSSARY

abfraction

The loss of tooth structure at the CEJ, often wedge-shaped or V-shaped, and is unrelated to cavities, bacteria, or infection.

accessory canals

Smaller access ways that branch off of the main root canals.

acellular

Without cells

acellular cementum

Cementum covering the cervical portion of the tooth root, important for attachment of the periodontal ligament (PDL) to the root surface.

acini

A small sac-like cavity in a gland, surrounded by secretory cells.

active eruption

Tooth movement in the occlusal direction as the tooth erupts from its osseous crypt.

adipocytes

The cells that primarily compose adipose tissue, specialized in storing energy as fat.

adipose tissue

A type of connective tissue composed primarily of adipocytes, which store high-energy triglyceride and other lipid molecules.

adult stem cell

A stem cell found in adult tissues (as opposed to embryonic stem cells found in embryos)

allele

a variant or different DNA sequence of a gene

alveolar bone

The part of the jaws that holds the teeth.

alveolar mucosa

The lining mucosa covering the alveolar ridges, attached to the buccal, lingual and palatal mucosa in one direction, and gingival mucosa in the other direction.

ameloblasts

Cells present only during the embryonic period that deposit tooth enamel.

amelogenesis

The formation of enamel.

amelogenesis imperfecta

A rare congenital disorder which presents with abnormal formation of enamel, unrelated to any environmental conditions.

amelogenins

Proteins found in enamel matrix which regulate the initiation and growth of hydroxyapatite crystals during the mineralization of enamel.

amorphous

Without shape

angiogenesis

Growth of endothelial cells, creating new blood vessels within a tissue.

ankyloglossia

A congenital malformation that may decrease the mobility of the tongue caused by an unusually short lingual frenulum.

ankylosis

Abnormal stiffening and immobility of a joint (including tooth-to-alveolar bone)

anodontia

absence of primary or permanent teeth.

antagonist

A biological structure or chemical agent that interferes with the physiological action of another.

apical

The top (or apex) side of a cell or tissue, usually facing the lumen.

apical-to-basolateral polarity

Top-to-bottom polarity, where the apical side of a cell (or tissue) is different from the basolateral side

apoptosis

Programmed cell death

appositional growth

The increase in diameter by the addition of tissue at the surface,

areolar connective tissue

A loose connective tissue composed of fibroblasts and other cell type, plus ground substance and all three fiber types.

artifacts

Artificial patterns or damage to a specimen seen under the microscope caused by the sampling or staining procedure.

ATP

Adenosine Tri-Phosphate, the energy molecule of cells.

attached gingiva

Region of oral mucosa firmly bound to the tooth and alveolar process.

attachment apparatus

The tissues that attach the tooth to the alveolar process: cementum, periodontal ligament, and alveolar bone.

avascular

Without blood vessels

basement membrane

A thin sheet of extracellular matrix between epithelial tissues and the underlying connective tissue of the lamina propria or dermis.

basolateral

The side of a cell or tissue oriented away from the lumen or external surface.

bell stage

The stage of early tooth development where the dental organ differentiates into 4 distinct cell types 9IEE, OEE,

stellate reticulum and stratum intermedium), followed by differentiation of odontoblasts and ameloblasts.

benign

(of a disease) not harmful in effect, (of a tumor) not malignant.

bilateral

On both sides

bio-active

A compound that has an effect on a living organism, tissue or cell, such as by mimicking a morphogen or growth factor.

bio-compatible

Materials used in surgical implants, not harmful to living tissue and unlikely to be rejected by the host immune system.

black hairy tongue

A temporary, harmless condition that gives the tongue a dark, furry appearance, resulting from a buildup of dead skin cells on the papillae on the dorsal surface of the tongue.

blastula

A hollow ball of cells that develops after the morula stage of embryogenesis.

Bleeding on Probing

Bleeding that is induced by gentle manipulation of the tissue at the depth of the gingival sulcus.

blood

A type of connective tissue composed of cells (red and white blood cells) and ECM (plasma)

BMP

Bone Morphogenetic Proteins are a group of signaling molecules, initially discovered for their ability to induce bone formation, now known to play crucial roles in all organ systems.

Bohn's nodules

Keratin cysts derived from remnants of odontogenic epithelium over the dental lamina, or remnants of minor salivary glands. They occur on the alveolar ridge, more commonly on the maxillary than mandibular.

bone graft

A medical procedure in which ground up bone tissue or synthetic bone tissue is placed in an injured site to act as a scaffold, promoting the formation of new bone tissue by the patient's own cells.

bone tissue

A hard connective tissue which is mostly ECM, including collagen fibers and a calcium-phosphate crystal.

branchial cleft cyst

A fluid-fille sac causing swelling in the upper part of neck anterior to sternocleidomastoid.

buccal mucosa

The lining mucosa of the cheeks and inner side of the lips.

bud

Outward-folding of an epithelium caused by interstitial growth.

bud stage

The first visible stage of odontogenesis, without a clear arrangement of cells.

calcium hydroxyapatite

Ca5(PO4)3(OH), the inorganic ECM material composed of calcium, phosphate and hydroxide ions that makes up the bulk of bone tissue.

CAMs

Cell Adhesion Molecules: proteins located on the cell surface involved in binding with other cells or with the extracellular matrix.

canaliculi

Microscopic canals between the lacunae of ossified bone or cementum, containing thin cell processes linking distant cells together.

cap stage

An early stage of tooth development where ectodermal cells grow around the dental papilla to resemble a hat, and the first signs of cell arrangement occur.

capsule

Connective tissue casing or outer border of an organ.

carotene

An orange-to-yellow pigment made by plants that accumulates within the dermis of the skin. It is the precursor for Vitmain-A.

cartilage

A firm connective tissue, softer and more flexible than bone composed of chondrocytes and lots of ground substance.

CDJ

Cementum-Dentin Junction

CEJ

Cemento-Enamel Junction, found in the cervical region of the tooth.

cell cycle checkpoints

Control proteins and enzymes which ensure proper progression through the cell cycle, regulating the rate of mitosis.

cell fate

The type or types of cell(s) a stem cell can possibly differentiate into in the future, determined by which genes are methylated and stored around histones, or free to be transcribed.

cell-free zone

A region of pulp with fewer visible cell bodies, and a large amount of capillaries and nerve endings.

cell-rich zone

A region of the pulp containing numerous fibroblasts, mesenchymal stem cells and other connective tissue cells.

cell-to-cell contacts

Direct contact between cells allows the receptors on one cell to bind small molecules on the plasma membrane of different cell. In eukaryotes, many of the cells during early development communicate through direct contact.

cellular cementum

Cementum containing cells and collagen fibers anchoring the tooth to alveolar bone.

cementicles

Spherical calcified bodies lying free in the periodontal membrane.

cementoblasts

Differentiated cell that deposits cementum matrix.

cementoclasts

Resorptive cell capable of demineralizing cementum by the secretion of acids and enzymes.

cementocytes

Terminally differentiated cell found within mature cementum lacunae.

cementogensis

The process of cementum formation which covers the tooth root by cementoblasts of mesenchymal origin.

cervical loop

The location on an enamel organ in a developing tooth where the outer enamel epithelium and the inner enamel epithelium join, fated to become Hertwig's Epithelial Root Sheath (HERS).

cervical lymph nodes

300 (of 800 total) lymph nodes found in the neck.

chondroblast

A cell actively producing the components of cartilage extracellular matrix, and may differentiate into a chondrocyte when trapped in the matrix it produced.

chondrocytes

The mature cells found within cartilage tissue.

chromatin

Unwound DNA and histone molecules, available for gene transcription.

chromosomes

DNA molecule packaged into thread-like structures found during mitosis, visible under a light microscope.

circum-pulpal dentin

The largest region of dentin, containing dentinal tubules, peri-tubular dentin and inter-tubular dentin

circumvallate papillae

Large circular bumps next to the sulcus terminalis on the dorsal surface of the tongue, contain numerous taste buds and minor salivary glands

cleavage divisions

The first few cellular divisions of a zygote are synchronized and divide along longitudinal planes, the second division is at 90 degrees to the plane of the first, and the third is perpendicular to the first two.

cleft lip

an opening or split in the upper lip that occurs when there is incomplete fusion of the maxillary process(es) with the inter-maxillary segment.

cleft palate

An opening or split in the roof of the mouth that occurs when the palatal shelves and/or primary palate fail to fuse completely.

cleido-cranial dysostosis

A rare congenital malformation that affects the collarbones, skull and teeth.

clinical attachment loss

Damage to the structures that support the tooth; results from periodontitis and is characterized by relocation of the junctional epithelium to the tooth root, destruction of the fibers of the gingiva, destruction of the periodontal ligament fibers, and loss of alveolar bone support from around the tooth.

collagen

The main structural protein in the ECM of connective tissues

commissural lip pits

The presence of pits and possibly associated fistulas in the lips.

compact bone

Dense bone tissue composed of osteons, found on the outer edges of bones.

complete cleft

A fully unfused cleft palate, where no bony connection is made.

concrescence

A condition of teeth where the cementum overlying the roots of at least two teeth join together.

congenital disorders

A medical condition that is present at or before birth, which can be acquired during development or from the genetic make up of the parents.

contour lines of Owen

Exceptionally-pronounced imbrication lines of von Ebner

copula

A swelling that forms from the second pharyngeal arch, it quickly gets overgrown by the 3rd and 4th arch during development of the tongue.

coronal pulp

The portion of pulp in the crown of the tooth.

cribriform plate

Sieve-like region of compact bone of the alveolar sockets or ethmoid bone.

crown stage

Sometimes referred to as the maturation stage or the late bell stage, characterized by the calcification of enamel and dentin in the crown region of a developing tooth.

cytokine

a broad and loose category of small proteins (~5–20 kDa) important in immune syhstem responses, including chemokines, interferons, interleukins, lymphokines, and tumour necrosis factors, but generally not hormones or growth factors (despite overlap in the terminology). Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells and fibroblasts.

cytoplasm

the gelatinous material within a living cell, excluding organelles.

cytoskeleton

A dynamic network of interlinking cytoplasmic proteins (mainly actin filaments, intermediate filaments and microtubules), involved in maintaining cell shape, polarity and migration.

de-differentiate

The reversal of differentiation, reverting to a more stem cell-like state.

dehiscence

A lack of the facial or lingual alveolar cortical plate resulting in a denuded root surface

DEJ

Dentin-Enamel Junction

dens in dente

A rare dental malformation found in teeth where there is an infolding of enamel into dentin.

dense irregular connective tissue

A connective tissue that has fibers that are not arranged in parallel bundles as in dense regular connective tissue. Dense irregular connective tissue consists of mostly collagen fibers. It has less ground substance than loose connective tissue.

dense regular connective tissue

A type of connective tissue found in tendons and

ligaments that is composed primarily of parallel collagen fibers, secreted by fibroblasts, with very little ground substance.

dental caries

Damage to a tooth that can happen when decay-causing bacteria make acids that de-mineralize tooth tissues.

dental fluorosis

A condition that causes changes in the appearance of tooth enamel. It may result when children regularly consume fluoride during the teeth-forming years, age 8 and younger.

dental implants

An artificial tooth root that is surgically placed into bone tissue.

dental lamina

A band of epithelial tissue seen in histologic that is is the first evidence of tooth development and begins (in humans) at the sixth week of development.

dental papilla

A condensation of ecto-mesenchymal cells seen in histologic sections of a developing tooth.

dental pellicle

A protein film that forms on the surface enamel by binding of glycoproteins from saliva that prevents continuous deposition of salivary calcium phosphate.

dental sac

Neuro-mesenchymal cells and fibres surrounding the enamel organ and dental papilla of a developing tooth.

dentigerous cyst

A fluid filled pocket or sac around an impacted tooth.

dentin caries

A carious lesion that extends into dentin.

dentin hypersensitivity

Pain derived from exposed dentin in response to chemical, thermal tactile or osmotic stimuli which cannot be explained as arising from any other dental defect or disease.

dentin resorption

The removal of dentin matrix by odontoclasts (as opposed to erosion), which occurs in the roots during tooth exfoliation.

dentin-pulp complex

Dentin and pulp are the same tissue from a function and embryology viewpoint, and are therefore referred to together as a complex.

dentinal fluid

The lymph or ECF found within dentinal tubules, surrounding the odontoblastic process

dentinal tubule

Tiny canals that run through dentin, from the pulp cavity up to near the DEJ or CDJ

dentinogenesis

The formation of dentin.

dentinogenesis imperfecta

A genetic disorder that causes teeth to be discolored

(most often a blue-gray or yellow-brown color) and translucent giving teeth an opalescent sheen.

dermal papillae

Fingerlike projections of the apical side of the dermis, increasing the surface area between the epidermis and dermis, strengthening their connection and increasing exchange of oxygen, nutrients, and waste.

dermis

Th connective tissue layer of the skin, found just deep to the epidermis, composed of areolar CT and dense irregular CT.

desmosome

Trans-membrane structure specialized for cell-to-cell adhesion.

development

The process by which animals and plants grow and change.

deviated septum

A condition where the bone and cartilage that divide the

nasal cavity of the nose in half is significantly off center, or crooked, making breathing difficult.

differentiation

When one cell begins to look different from another. This process involves limiting cell fate by altering gene transcription to become more specialized.

dilaceration

An abnormal bend in the root or crown of a tooth.

distomolar

A supernumerary tooth which is located distal to third molars

DNA

deoxyribonucleic acid is the hereditary material in humans and almost all other organisms.

DNA methyation

Methyl groups added to DNA which change the activity of a gene without changing the sequence, typically repressing gene transcription.

dorsal surface of the tongue

The top surface of the tongue, containing lingual papillae and taste buds.

ECF

Extra-Cellular Fluid is the fluid surrounding cells, but not within blood or lymphatic vessels.

ECM

Extra-Cellular Matrix: Materials in the body, but outside of cells. A network of macromolecules, such as fibers, enzymes, and glycoproteins, that provide structural and biochemical support to surrounding cells.

ectoderm

he most exterior of the three primary germ layers formed in the gastrula, it gives rise to the epithelial tissue covering the body and the CNS.

ectodermal dysplasia

A group of disorders in which two or more of the ectodermally derived structures — the skin, sweat glands, hair, nails, teeth and mucous membranes — develop abnormally.

edema

Swelling caused by excess fluid trapped within tissues.

edentulous

Lacking teeth

elastic fibers

Thin protein fibers in the ECM, capable of stretching and returning to their original length, made of the protein elastin.

embryo

A human offspring during the period from approximately the second to the eighth week after fertilization.

Embryology

The branch of biology and medicine concerned with the study of embryos and their development.

embryonic disc

The part of the inner cell mass of a blastocyst from which the embryo of a placental mammal develops.

embryonic germ layers

The three layers of cells that arise from gastrulation: endoderm, mesoderm and ectoderm.

enamel hypoplasia

A defect of the enamel that occurs during tooth development, it results in thin enamel making teeth vulnerable to decay.

enamel organ

The ectodermal cells that lie above the dental papillae in a cap stage tooth bud.

enamel pearl.

A condition of teeth where enamel is found where enamel is not supposed to be, such as on a root surface.

enamel rod

The basic unit of tooth enamel, 4 μm wide, composed of a tightly packed and organized mass of hydroxyapatite crystals, hexagonal in shape.

enamel spindles

Extensions of odontoblastic processes past the DEJ into enamel, creating thin regions of hypomineralized enamel.

enamel tufts

Similar to enamel spindles, but shorter, bushier-shaped, and do not contain odontoblastic processes.

enamelins

Along with amelogenins, a second protein found in enamel, enamelins are necessary for the adhesion of ameloblasts to the surface of the enamel, and they bind to hydroxyapatite to promotes crystallite elongation.

endochondral ossification

The formation of bone tissue from a cartilage model

endochondralossification

endocrine

Secreting to the inside of the body, either blood or ECF.

endoderm

The innermost of the body's 3 embryonic tissues that

form during gastrulation, it becomes the inner lining of hollow organs.

endodontic therapy

Also known as a root canal, is a treatment for infected pulp of a tooth, result in the removal and replacement of pulp and the protection of the decontaminated tooth from future microbial invasion.

endogenous

having an internal cause or origin; made by human cells.

endosteum

A thin membrane covering the inner surface of compact bone and the trabeculae of spongy bone, containing osteoblasts, osteoclasts and osteo-progenitor cells

endothelium

A single layer of squamous endothelial cells that line the interior surface of blood vessels, and lymphatic vessels, forming the barrier between vessels and tissue and control the flow of substances and fluid into and out of a tissue.

epidermis

The superficial epithelial layer of the skin, composed of a stratified squamous epithelium.

epigenetic

Heritable DNA modifications that do not change the DNA sequence but do affect gene activity.

epithelial attachment

located at the bottom of the sulcus, consisting of approximately 1 mm of junctional epithelium and 1 mm of gingival fiber attachment.

epithelial rests of Malassez

Clusters of epithelial cells found within the PDL, remnants of Hertwig's Epithelial Root Sheath (HERS).

epithelial-to-mesenchymal transition

A process by which epithelial cells lose polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal stem cells, this normally occurs during embryonic development and wound healing.

Epstein Pearls

A white or yellow bump on the oral mucosa which are equivalent to Bohn's Nodules.

erosion

The wearing away of tooth enamel by excess acids.

etiology

the cause, set of causes, or manner of causation of a disease or condition

exfoliation

A general term for peeling or shedding, in humans it refers to the loss of dead epithelial cells or structures (hair, teeth).

Exocrine

Secreting to a surface of the body, either the outer surface (skin) or inner surface (lumen of a hollow organ).

exogenous

Having an external cause or origin; not made by human cells

external root resorption

When the body's own immune system dissolves the tooth root structure. It can occur following tooth infection, orthodontic treatments or in the presence of unerupted teeth in the jaw.

extrinsic collagen fibers

Ends of the principal fibers of the periodontal ligament embedded within cementum.

fenestration

A roughly circular defect of the cortical plate which exposes the underlying root surface, but does not involve the alveolar margin of the bone.

fertilization

The fusion of haploid gametes, egg and sperm, to form the diploid zygote.

fetus

An unborn human baby more than eight weeks after conception.

FGF

Fibroblast Growth Factors are a family of morphogens involved in a wide variety of processes, including important roles in development and tissue regeneration, especially connective tissues.

fibers

The ECM proteins elastin and collagen, visible under the light microscope

fibrin

The insoluble (solid) form of a fibrous protein found in blood plasma that contributes to the formation of a blood clot.

fibroblasts

The basic connective tissue cell type capable of secreting ECM components, including fibers and ground substance proteins.

fibronectin

A large glycoprotein found in the ECM which binds to integrin proteins on the cell surface, involved in cell adhesion, growth, migration, and differentiation.

Filiform papillae

Fingerlike bumps on the dorsal surface of the tongue that contain epithelium for friction, but no taste buds.

floor of the mouth

Portion of the oral cavity underneath the tongue.

foliate papillae

Large bumps on the lateral edges of the tongue, they contain taste buds.

foramen cecum

The small depression at the border between the anterior (oral) and the posterior (pharyngeal) portions of the tongue. It is the point from which the thyroid gland formed by invagination.

Fordyce Spots

Whitish-yellow bumps found on the lips or oral mucosa, caused by the unusual presence of sebaceous glands (or possibly minor salivary glands) trapped below the epidermis.

fungiform papillae

Mushroom-shaped bumps on the dorsal surface of the tongue, they contain taste buds.

fuse

When two or more cells, tissues or organs join to become one.

gain-of-function

A type of mutation in which the altered gene product possesses a new molecular function or a new pattern of gene expression. Gain-of-function mutations are almost always Dominant.

gap junctions

A trans-membrane protein that when paired allows small cytoplasmic molecules to pass through from one cell to another cell.

gastrulation

A phase early in the embryonic development during which the single-layered blastula is reorganized into a multilayered structure known as the gastrula.

gemination

Adental phenomenon that appears to be two teeth developed from one.

gene

A sequence of nucleotides in DNA that encodes the synthesis of a gene product, either RNA or protein.

gene expression

Copying DNA into a functional product, such as a RNA and/or protein. Controlled by the activity of transcription factors binding to gene promoter regions to recruit RNA polymerase.

geographic tongue

A condition where filiform papillae on the dorsal surface of the tongue become non-uniformly hyper-keratinized, creating dynamic regions of more white and more pink coloration.

germinal centers

Sites within lymphoid organs where mature B cells proliferate and differentiate.

gill arches

A series of bony or cartilaginous curved bars along the pharynx, supporting the gills of fish and amphibians.

gingival fibers

Collagen fibers in the gingiva that, in general, attach the tooth to the gingival tissue (rather than the tooth to the alveolar bone like the PDL fibers).

gingival graft

The use of connective and epithelial tissue from the roof of the mouth or neighboring gingiva, attached to the gum area being treated.

gingival hyperplasia

Overgrowth of gum tissue around the teeth, often associated with poor oral hygiene.

gingival recession

The exposure in the roots of the teeth caused by a loss of gum tissue and/or retraction of the gingival margin from the crown of the teeth.

gingival sulcus

Space between the gingiva and tooth.

gingival-crevicular fluid

Fluid exudate from gingival mucosa that collects within the gingival sulcus. Presence of bacterial enzymes, bacterial degradation products, connective tissue degradation products, inflammatory molecules, or extracellular matrix proteins can be detected in higher levels in gingival crevicular fluid during the active phase of periodontitis.

gingivitis

Inflammation of gingival tissue

glia

The numerous and diverse group of cells in neural tissue that provide support for, guide the activity of, and organize the connections between neurons.

globular dentin

Darker-staining regions of dentin, found between mantle dentin and circumpulpal dentin.

glycoproteins

A protein that has had sugar residues attached to it in the Golgi apparatus and is often secreted.

goblet cells

Unicellular glands, or single epithelial cells, that secrete mucus.

Golgi apparatus

A membrane-bound organelle that modifies proteins from the rER and packages them up into vesicles, to be sent to lysosomes, the plasma membrane, or secreted from the cell.

ground substance

Gel-like substances in the ECM, composed of water held in place by large molecules (proteins, glycoproteins and glycosaminoglycans).

growth factors

A signaling molecule capable of stimulating cell proliferation, wound healing, and cellular differentiation.

guidance cues

Signaling molecules which control the navigation of other cells by causing attraction or repulsion.

guided tissue regeneration

Procedures attempting to regenerate (as opposed to replace) lost periodontal structures.

gum boil

A small swelling formed on the gum over an abscess at the root of a tooth.

H&E

Hematoxylin and Eosin stain, typically turns proteins pink and DNA purple.

hair follicles

An infolding of the epidermis that extends deep into the dermis, responsible for producing a hair.

hard palate

The horizontal bony plate that makes the anterior portion of the palate of the mouth, composed of parts of the palatine and maxilla bones.

hemi-desmosomes

Half of a desmosome, specialized for cell-to-ECM adhesion.

hemoglobin

A molecule made by Red Blood Cells that can bind and release Oxygen molecules. It switches from a red to a maroon color as it does so, which in turn contributes to skin color.

Hensen's node

The organizer for gastrulation in the embryo, it is the site where mesoderm migration occurs.

HERS

Hertwig's Epithelial Root Sheath: A proliferation of epithelial cells located at the cervical loop of the enamel organ in a developing tooth which initiates the formation of root dentin.

hidden caries

An occlusal dentine caries that is missed on a visual examination.

histology

The study of tissues under a microscope

histone

Highly basic proteins found in nuclei that compact DNA into a denser form, unavailable for gene transcription.

homeobox genes

A group of 235-300 related genes that code for a transcription factors, which control the activity of other genes involved in development, including directing the formation of limbs and organs along the anterior-posterior axis.

homologue

A similarity due to shared lineage between a pair of structures or genes in different taxa.

hormones

Signaling molecules secreted directly into the blood which travel throughout the body.

Hunter-Schreger bands

When cut enamel is viewed under reflected light, curves in the pattern of enamel rods can be seen; these curves strengthen the enamel and prevent cracks from propagating through the tooth.

hyaluronic acid

A very large glycosaminoglycan distributed widely throughout connective tissue ECM, epithelial, and neural tissues.

hyaluronidase

a family of enzymes that catalyse the degradation of hyaluronic acid.

hydrostatic

The pressure exerted by liquid.

hyoid arch

The second pharyngeal arch.

hyper-cementosis

Idiopathic, non-neoplastic condition characterized by

the excessive buildup of normal cementum on the roots of one or more teeth.

hyper-keratosis

An abnormally high level of keratin production in an epithelium, causing a thickening and whitening of the outer layer of the skin or oral mucosa.

hyperdontia

the condition of having teeth in addition to the regular number of teeth.

hyperplasia

Growth of a tissue by an increased number of cells, an increased amount of ECM produced by cells, or both.

hypo-salivation

Decreased saliva production.

hypodontia

Partial anodontia, or the absence of some teeth.

idiopathic

A disease that arises from an unknown cause.

IEE

Inner Enamel Epithelium: a layer of columnar cells located next to the dental papilla, these pre-ameloblast cells will differentiate into Ameloblasts which are responsible for secretion of enamel during tooth development.

imbrication lines of von Ebner

Incremental lines in the peritubular dentin of the tooth that correspond to the daily rate of dentin formation.

implantation

The process by which a developing embryo makes contact with the uterine wall, attaches and digests its way internally, and remains within it until birth.

incisive foramen

The oral opening of the nasopalatine canal, located in the maxilla at the junction of the medial palatine and incisive sutures.

incomplete cleft

A partially unfused cleft palate, where at least some of the bone portion is intact.

induce

The embryonic process in which one group of cells directs the development of another group of cells.

inner cell mass

The mass of cells inside the blastula-stage embryo that will eventually give rise to the definitive structures of the fetus

integrin

A trans-membrane protein which allows cells to bind to the ECM protein fibronectin, it is involved in cell adhesion, growth, migration, and differentiation.

inter-dental septum

inter-globular dentin

Lighter-staining regions of dentin found between globular dentin spheres.

inter-maxillary segment

Or the globular process, is a mass of tissue formed by the merging of the median nasal processes, fated to become the philtrum and pre-maxilla.

inter-rod enamel

The type of enamel found between enamel rods.

inter-tubular dentin

Thicker layer of dentin found between the thin layers of peri-tubular dentin.

interdental gingiva

Portion of the attached gingiva between teeth, coronal to the free gingival margin on the buccal and lingual surfaces of the teeth

interdental septum

The bony partition across the alveolar process between adjacent teeth that forms part of the tooth sockets.

internal root resorption

A pulp disease characterized by the loss of dentin as a result of the action of osteoclastic cells stimulated by pulpal inflammation.

interradicular septum

The bony septum lying between tooth roots.

interstitial growth

Increase in size by the addition of tissue from the inside.

intra-membranous ossification

Formation of bone tissue from a dense connective tissue model

intrinsic collagen fibers

Fibers found within cementum oriented parallel to the root surface, mainly involved in the repair of cementum.

invagination

Inward folding of an epithelium caused by interstitial growth.

junctional epithelium

Epithelium on the inner wall of the gingiva that attaches to connective tissue of the lamina propria on the basolateral side and to the surface of the tooth on the apical side.

keratin

One of a family of tough fibrous structural proteins, the

key material making up hair, nails, calluses, and the outer layer of skin.

keratinized

Having the protein keratin, which lends resistance to abrasion and water loss.

keratinocytes

A terminally differentiated epithelial cell capable of synthesizing large amounts of the protein keratin within its cytoplasm.

labial mucosa

The oral mucosa of the lips (both inner and outer surface)

lacunae

Lakes, or spaces within cartilage of bone connective tissue where cells reside.

lamina dura

Compact bone that lies adjacent to the periodontal ligament, in the tooth socket.

lamina propria

The areolar connective tissue layer of the oral mucosa (or hollow organ), homolgous to the papillary layer of the dermis.

lateral lingual swellings

Two swellings on the floor of the primitive pharynx which, along with the tuberculum impar, form the anterior 2/3rd of the tongue.

lateral nasal processes

Tissue on the lateral sides of the nasal pits that form the alae of the nose.

leukoplakia

Whitish patches or lesion of the oral mucosa caused by hyper-keratinization.

Linea Alba

A white line. In the oral cavity, refers to a line in buccal mucosa along the occlusal plane.

lineage

The developmental history of a differentiated cell traced back to the embryonic cell from which it arises.

linear mineralization

The calcification of pre-dentin in the circumpulpal region occurs linearly, not in globs.

lines of Retzius

Incremental growth lines seen in enamel.

lingual frenulum

A small fold of mucous membrane extending from the floor of the mouth to the midline of the underside of the tongue.

lingual papillae

Bumps on the tongue, giving it its rough texture

Lining mucosa

A non-keratinized stratified squamous epithelium found in the buccal mucosa, labial mucosa, alveolar mucosa, ventral surface of the tongue, floor of the mouth, and soft palate.

loss-of-function

A mutation that result in the gene product having less or no function (being partially or wholly inactivated).

lower labial pit

Depressions of the lower lip.

lumen

Hollow center or cavity inside a structure.

lymph nodes

A small bean-shaped structure that is part of the body's immune system.

lymphadenopathy

Palpable enlargement of lymph nodes.

lymphatic tissue

A type of connective tissue composed of cells (white blood cells) and ECM (lymph).

lysosomes

Membrane-bound vesicles that contains acids and

hydrolytic enzymes that break down many kinds of molecules.

macrodontia

A condition in which one or more teeth grow to be a larger size than normal.

mandibular arch

The first branchial arch of the vertebrate embryo which in humans develops into the lower lip, mandible, masticatory muscles, and anterior tongue.

mandibular torus

Abony growth in the mandible along the surface nearest to the tongue.

mantle dentin

The outer layer of dentin closest to enamel, contains few odontoblastic processes.

marginal gingiva

A 1.5 mm strip of gingival tissue which surrounds the neck of the tooth.

masticatory mucosa

Areas of oral mucosa that have become partially keratinized due to the friction and abrasion of the masticatory process, including the gingivae and hard palate.

matrix metalloproteinase

Enzymes that degrade all kinds of extracellular matrix proteins, or process a number of bioactive molecules. They play a major role in cell proliferation, migration, differentiation, angiogenesis, and apoptosis.

maturation

The continued development of a cell, tissue, organ or organism as it takes on a more adult form.

maxillary process

A process that grows off the mandibular arch on each side and forms the lateral part of the upper lip, cheek, and upper jaw except the pre-maxilla.

McCall's festoon

Semilunar-shaped enlargements of the marginal gingiva primarily on the labial surfaces of the anterior and premolar teeth.

mechanical failure of eruption

A type of failure of eruption takes place when the affected tooth is ankylosed to the bone around it.

Meckel's cartilage

Apiece of cartilage from which the mandibles (lower jaws) of vertebrates evolved, originally the lower of two cartilages which supported the first branchial arch in early fish.

medial nasal processes

Tissue on the inner side of each nasal pit merge into the inter-maxillary segment and form the philtrum, crest and tip of the nose, and merge with the maxillary processes.

median lingual sulcus

A line that divides the dorsum of the tongue into symmetrical halves, from the anterior tip to the foramen caecum,

median palatine suture

The suture between the two palatine bones.

melanin

A group of red, dark brown or black pigments found in the hair, skin, and other places, capable of absorbing UV-B radiation.

melanocytes

Terminally differentiated cells derived from neural crest cells that produce one of the three forms of melanin.

membranes

A two-dimensional sheet that forms a selective barrier.

mesenchymal stem cells

Multipotent cells of a connective tissue that can differentiate into a variety of cell types, including osteoblasts, fibroblasts, chondrocytes, myoblasts, blood cells and adipocytes.

mesenchymal-to-epithelial transition

A reversible process that involves the transition from motile mesenchymal cells to planar arrays of polarized epithelial cells.

mesenchyme

An embryonic tissue composed of undifferentiated mesenchymal stem cells and mucous ground substance.

mesial drift

The tendency of teeth to move in a mesial direction within the arch, maintaining interproximal contact between teeth.

mesiodens

A supernumerary tooth present between the two central incisors.

mesoderm

The middle of the three embryonic layers that develop during gastrulaton, mesoderm becomes most of the body's muscle and connective tissues.

microdontia

A condition in which one or more teeth appear smaller than normal

mini-valves

Similar to venous valves, mini-valves are on the walls of

lymphatic vessels, allowing them to absorb larger materials than blood vessels.

minor salivary glands

Small salivary glands located throughout the oral cavity that generally get no name.

mitochondria

Membrane-bound organelles that generate most of the chemical energy needed to power a cell's biochemical reactions.

mitosis

The process of cell division, where one cell divides into two identical clones (daughter cells).

morph

Slowly change shape

morphogen

A substance whose non-uniform distribution governs the pattern of tissue development and pattern formation.

morphogenesis

The biological process that causes a cell, tissue or organism to develop its shape.

morphology

The shape (or form) of.

morula

A solid ball of cells that develops by mitosis from the single-cell zygote.

mRNA

Single-stranded RNA molecule complementary to one strand of DNA of a gene. It leaves the nucleus and moves to the cytoplasm where it instructs the synthesis of a protein.

muco-serous acini

Glandular acini that make a mixture of watery and gelatinous secretions.

mucocoele

A mucous cyst

mucogingival junction

Border between the mucosa of the cheeks and floor of the mouth-- which are freely moveable and fragile-- and the the mucosa around the teeth and on the palate-- which are firm and keratinized.

mucous

An adjective used to describe a gelatinous, slimy substance such as loose ground substance or mucus.

mucous acini

Glandular acini that secrete more gelatinous (or mucous) secretions.

mucus

A thin gelatinous secretion composed of primarily of glycoproteins and water.

mumps

A highly contagious viral disease caused by the mumps virus, causing swelling of the parotid salivary gland(s).

muscle tissue

A soft tissue whose cells contains a large amount of actin

and myosin proteins, allowing it to change in size (contraction and elongation).

myo-epithelial cells

Epithelial cells found in glands, containing smooth muscle actin allowing them to contract and expel secretions of exocrine glands.

myo-satellite cell

A multi-potent stem cell that can generate more muscle tissue.

myoblasts

A more differentiated form of a myo-satellite cell that can differentiate into a muscle cell (myofibril)

nature

In the phrase nature vs nurture, nature refers to genetics (heritable traits).

necrosis

The death of most or all of the cells in an organ or tissue due to disease, injury, or failure of the blood supply.

neonatal line

A particularly pronounced incremental line in enamel or dentin, created on the day of birth.

neural crest cells

A temporary group of cells that arise from the embryonic ectoderm, and in turn give rise to a diverse cell lineage—including melanocytes, cranio-facial cartilage and bone, teeth and periodontal tissue, smooth muscle, peripheral and enteric neurons and glia.

neural tissue

The main component of the nervous system – both central and peripheral, composed of two types of cells: neurons and glia.

neural tube

The embryonic precursor to the central nervous system.

neuro-ectoderm

Cells derived from ectoderm fated to develop into the CNS or neural crest.

neuro-mesenchymal stem cells

A subset of mesenchymal stem cells derived from neural crest cells rather than mesoderm, or mesoderm-derived mesenchymal stem cells induced by neural crest morphogens to adopt a more neural fate.

neuro-mesenchyme

Mesenchyme tissue that contains neural crest cell derivatives.

neurons

Terminally differentiated and highly specialized cells of neural tissue, capable of firing electrical signals over long distances and releasing chemical signals across discrete locations called synapses.

neurulation

The folding process in vertebrate embryos, which includes the transformation of ectoderm into the neural tube.

nicotinic stomatitis

Hyper-keratinization of the hard palate mucosa-- but not minor samilvary gnds-- caused by cigarette smoke or other stresses,.

non-keratinized

A stratified squamous epithelium that does not contain the protein keratin, making it softer and moister than the skin.

non-vital

No longer containing living cells.

notochord

A flexible rod formed of a material similar to cartilage, which ultimately disappears in human (and most vertebrate) embryos.

nurture

In the phrase nature vs nurture, nurture means environmental factors that occur after fertilization, not DNA inherited from our parents.

odontoblast layer

The other layer of pulp containing the cell bodies of odontoblasts.

odontoblastic process

Arm-like extension of an odontoblast found within dentinal tubules.

odontoblasts

A cell of neural crest origin that is part of the outer surface of the dental pulp, and whose biological function is the formation of dentin.

odontoclasts

Resorptive cell capable of demineralizing dentin by the secretion of acids and enzymes.

odontogenesis

Formation and eruption of the teeth.

OEE

Outer Enamel Epithelium: a layer of cuboidal cells located on the periphery of the enamel organ in a developing tooth.

ontogeny

The development of an organism or anatomical or behavioral feature from the earliest stage to maturity.

oral epithelium

The stratified squamous epithelium of the oral mucosa.

oral mucosa

The mucous membrane lining the inside of the mouth. It is a stratified squamous epithelium, named the oral epithelium, and an underlying areolar connective tissue named the lamina propria.

oro-nasal cavity

The embryonic form of the oral cavity and nasal cavities before they are separated.

oro-pharyngeal membrane

The region where the ectoderm and endoderm come into direct contact with each other constitutes a thin membrane, which forms a septum between the primitive mouth and pharynx.

ortho-keratinized

An epithelium that is partially keratinized and contains visible nucleuses.

orthodontia

The treatment of irregularities in the teeth (especially of alignment and occlusion) and jaws, including the use of braces.

osteo-progenitor cell

A cell that differentiates from a mesenchymal stem cell, and can further differentiate into an osteoblast.

osteoblast

A differentiated connective tissue cell that secretes bone ECM, including collagen, calcium and phosphate.

osteoclast

A cell derived from bone marrow stem cells capable of demineralizing bone tissue by the secretion of acids and enzymes, releasing calcium into the blood.

osteocytes

The terminally differentiated cell type found in mature bone tissue (both compact and spongy), capable of repairing small amounts of damage.

osteodentin

Another name for reparative dentin, based on its resemblance to bone tissue, with cells trapped within dense ECM.

oxytalan

Elastic-like fibers made of the protein Fibrillin that run parallel to the tooth surface and bend to attach to cementum.

palatal shelves

The portion of the hard palate formed by the growth of two shelves off the maxillary process medially and their mutual fusion in the midline.

palatal torus

A harmless, painless bony growth located on the roof of the mouth.

palate

The roof of the mouth, separating the oral cavity and nasal cavities.

palatine raphe

A ridge running across the palate, from the palatine uvula to the incisive papilla.

para-keratinized

An epithelium that is partially keratinized that does not contain visible nucleuses.

para-nasal sinuses

Spaces within the frontal, sphenoid, ethmoid and maxillary bones surrounding the nasal cavity.

parotid glands

Two salivary glands that sit in front of the ears on each side of the face

passive eruption

Movement of the gingiva apically or away from the crown of the tooth to the level of the CEJ after the tooth has erupted completely.

pattern formation

The mechanism by which initially equivalent cells in a

developing tissue in an embryo assume complex forms and functions.

PDL

Periodontal Ligament, a group of specialized connective tissue fibers that attach a tooth to alveolar bone.

peri-implantitis

A destructive inflammatory process affecting the soft and hard tissues surrounding dental implants.

peri-tubular dentin

The thin layer of dentin found bordering the dentinal tubule.

periapical abscess

A collection of pus at the root of a tooth, usually caused by an infection that has spread from pulp to the surrounding tissues.

pericardial patch

A medical procedure hat grafts connective tissue from cow or pig pericardium to human tissues to boost healing and regeneration.

perikymata

Incremental growth lines that appear on the surface of tooth enamel as a series of linear grooves.

perimolar

A supernumerary tooth in front of the molar teeth.

periodontal abscess

A localized collection of pus within the tissues of the periodontium.

periodontal pocket

A pathologically deepened gingival sulcus around a tooth at the gingival margin.

periodontitis

Inflammation that damages soft tissue and bone that anchors the teeh, including the gingiva plus PDL, cementum or alveolar bone tissue.

periodontium

Specialized tissues that surround and support the teeth, including gingival tissue, PDL, cementum and alveolar bone.

periosteum

A layer f dense regular connective tissue surrounding bones, also containing osteoblasts, osteo-progenitor cells and some osteoclasts.

pharyngeal arches

A series of externally visible anterior tissue bands lying under the early brain that give rise to the structures of the head and neck.

pharyngeal grooves

An ectodermal groove between two pharyngeal arches.

pharyngeal pouches

An endodermal pouch between two pharyngeal arches on the internal surface of the embryo.

philtrum

vertical indentation in the middle area of the upper lip, extending from the nasal septum to the tubercle of the upper lip.

phylogeny

The history of the evolution of a species or group,

especially in reference to lines of descent and relationships among broad groups of organisms.

placode

Small bumps that give rise to bigger structures such as hair follicles and teeth.

planar cell polarity

A polarity axis that organizes cells in the plane (side-to-side) of the tissue.

plasma membrane

The border of every cell, made of a phospholipid bilayer and proteins.

pocket epithelium

The thin, overly-permeable epithelium that replaces junctional epithelium in a periodontal pocket.

positional information

In pattern formation, the development of spatial organization in the embryo that results from cells differentiating at specific positions, which requires that the cells have positional values as in a coordinate system

pre-ameloblasts

An intermediate cell type, differentiate from IEE cells, capable of inducing the differentiation of odontoblasts, which in turn induce pre-ameloblasts to differentiate into ameloblasts,.

pre-cementum

The thin unmineralized layer present on the surface of developing cementum.

pre-dentin

Newly formed dentin before calcification and maturation.

pre-enamel

The initial un-mineralized form of enamel secreted by ameloblasts.

pre-implantation period

The first two weeks of human development, starting with fertilization and ending with implantation of a morula.

primary curvature

The overall curve that odontoblastic processes take as seen in dentin.

primary dentin

The majority of dentin, formed before completion of the apical foramen, contains both mantle dentin and circumpulpal dentin.

primary enamel cuticle

A thin membrane of remaining REE tissue that covers the tooth once it has erupted.

primary failure of eruption

Partial or complete failure of a tooth to erupt despite a healthy eruption pathway.

primary palate

The pre-maxilla, includes that portion of the alveolar ridge containing the four incisors.

primitive foregut

The anterior end of the primitive gut tube, derived from yolk sac endoderm, not yet connected to the oral cavity.

primitive streak

The visible line that forms on the dorsal side of the embryo as Hensen's node travels in a rostral-to-caudal direction, creating the embryos left/right symmetry.

principal fibers

The main principal fiber group is the alveolodental ligament, which consists of five fiber subgroups: alveolar crest, horizontal, oblique, apical, and interradicular on multirooted teeth. Principal fibers other than the alveolodental ligament are the transseptal fibers.

proliferation

The process that results in an increase of the number of cells, and is defined by the balance between cell divisions and cell loss through cell death or differentiation.

prominences

Five swellings that appear on the face in the fourth week

pseudopockets

A pocket depth over 3mm caused by enlargement of the gingival margin, but does not have harmful loss of the epithelial attachment.

pseudostratified epithelium

An epithelium that has more than one layer of cells, but the layers are not organized into distinct rows.

pulp core

The center of the pulp chamber with many cells and an extensive vascular supply; except for its location, it is very similar to the cell-rich zone.

pulp horns

A prolongation of coronal pulp extending toward the cusp of a tooth.

pulp stones

Discrete calcifications found in the pulp chamber of the tooth.

Pulp vitality testing

A test which helps establish the health of the pulp of a tooth.

pulpitis

Inflammation of the pulp.

radicular pulp

The portion of pulp in the roots.

RANKL

Receptor activator of nuclear factor kappa-B ligand, a pro-apoptosis gene expressed in many tissues.

ranula

A cyst that forms in the mouth under the tongue.

Rathke's pouch

An evagination at the roof of the developing mouth in front of the oropharyngeal membrane, which gives rise to the anterior pituitary (adenohypophysis).

reactionary dentin

Tertiary dentin formed from a pre-existing odontoblast, contains narrow or filled-in dentinal tubules.

recapitulate

To state again, or to repeat.

receptors

A cell-surface, trans-membrane or cytoplasmic protein

that binds to (receives) a signaling molecule and transmit the signal further.

reciprocal

Shared equally by two parties.

REE

Reduced Enamel Epithelium: the OEE and ameloblasts found on the surface of the crown prior to tooth eruption.

regeneration

After partial loss of as tissue, based on the remaining part, the tissue grows the same structure and function as the lost part.

remodeling

Reorganization or renovation of existing tissues, either physiological or pathological. The process can either change the characteristics of a tissue such as in blood vessel remodeling, or result in the dynamic equilibrium of a tissue such as in bone remodeling.

remodeling unit

Osteoblasts and osteoclasts working together to remove

old bone tissue and replace it with new bone tissue, necessary for the maintenance of healthy bones.

reparative dentin

Dentin produced by the differentiation of pulp stem cells becoming later odontoblast and/or osteoblast-like cells, which form a bone-like structure

rER

rough Endoplasmic Reticulum, an organelle where membrane and secreted proteins are translated by bound ribosomes on the surface of the rER.

resorptive cells

Cells capable of removing a tissue, such as osteoclasts, cementoclasts and odontoclasts.

rete pegs

Fingerlike projections of the epidermis, the downward-pointing counterparts to the dermal papillae.

reticular connective tissue

A soft connective tissue composed of reticular fibers, ground substance, plus fibroblasts and blood cells.

Reticular fibers

A form of collagen, forms a web-like meshwork in ECM.

rhinoplasty

Cosmetic surgery that alters the appearance of the nose.

ribosomes

A small particle consisting of rRNA and proteins found in large numbers in the cytoplasm. They bind mRNA and tRNA to synthesize proteins.

RNA

Ribonucleic acid is a nucleic acid present in all living cells. Its principal role is to act as a messenger carrying instructions from DNA for controlling the synthesis of proteins

rod enamel

The type of enamel found within an enamel rod

Root caries

A lesion located on the root surface of a tooth, usually close to or below the gingival margin.

root resorption

The progressive loss of dentin and cementum by the action of odontoclasts (and cementoclasts).

saliva

Watery liquid secreted into the mouth, providing lubrication for chewing and swallowing, and protection for the teeth.

scaffold

Extracellular matrix material involved in the repair of injured and missing tissues, allows stem cells to migrate into the injured area. It is usually replaced during regeneration.

scar tissue

A strong but immovable connective tissue quickly made by fibroblasts, consisting primarily of highly cross-linked collagen fibers.

sebaceous glands

Microscopic exocrine glands in the skin that open into hair follicles to secrete an oily or waxy matter, called sebum, which lubricates the hair and skin.

secondary curvature

A small curve to dentinal tubules observed in dentin, in the opposite direction of the primary curvature.

secondary dentin

Dentin formed after completion of the apical foramen, contains only circumpulpal dentin.

secondary palate

The portion of the hard palate formed by the growth of two palatal shelves medially and their mutual fusion in the midline.

septoplasty

A surgical procedure to straighten the bone and cartilage of the nasal septum.

sER

Smooth Endoplasmic Reticulum, an organelle where calcium and lipids are stored.

serous acini

Glandular acini that produce watery secretions.

Sharpey's fibers

Bundles of strong type I collagen that anchor the periosteum to bones by penetrating the outer layers of compact bone tissue

sialograph

Radiographic examination of the salivary glands, involving the injection of a contrast medium into the salivary duct of a single gland.

sialoliths

Calculus produced from saliva.

signal transduction cascade

When a chemical or physical signal is transmitted through a cell as a series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response.

simple columnar epithelia

Epithelia that have one layer of tall cells

simple cuboidal epithelia

An epithelium composed of one layer of square cells

simple squamous epithelium

An epithelium composed of one layer of flat cells

sinusitis

Inflammation of the sinuses.

soft palate

The posterior muscular portion of the roof of the mouth.

somites

Bilateral pairs of blocks of mesoderm that form along the rostral-caudal axis.

specialized mucosa

The epithelium found on the dorsal surface of the tongue, containing lingual papillae and taste buds.

spina bifida

A congenital malformation involving incomplete closure of the neural tube.

spongy bone

Softer bone material composed of thin trabeculae, found deep to compact bone.

stellate reticulum

A group of star-shaped cells located in the center of the enamel organ of a developing tooth, they synthesize glycosaminoglycans.

stem cells

Undifferentiated or partially differentiated cells that can differentiate into various cell types, and proliferate to produce more of the same stem cell.

Stillman cleft

A V-shaped region of gingival recession

stomodeum

A depression between the brain and the pericardium in an embryo, and is the precursor of the mouth and the anterior lobe of the pituitary gland.

stratified squamous epithelia

Epithelia that have more than one layer of cells, with the cells at the apical surface having a flat shape.

stratum intermedium

A stratified layer of cells situated between the IEE and the stellate reticulum.

sub-epithelial connective tissue graft

The most common method used to treat root exposure, involving the transplantation of healthy connective tissue from a donor site to the damaged area, which acts a scaffold aiding in tissue regeneration.

sub-lingual glands

Paired salivary glands on the floor of the oral cavity, underneath the tongue, bordered laterally by the mandible and medially by genioglossus muscle of the tongue.

sub-mandibular glands

Major salivary glands located beneath the floor of the mouth, superior to the digastric muscles.

sub-mandibular lymph nodes

Three to six lymph nodes beneath the body of the mandible in the sub-mandibular triangle, or the superficial surface of the sub-mandibular gland, whose afferent vessels drain the medial canthus, cheek, side of the nose, upper lip, lateral part of the lower lip, gums, and the anterior part of the margin of the tongue, and whose efferent vessels drain ino the superior deep cervical lymph nodes.

sub-mental lymph nodes

Lymph nodes in the mental region whose afferent vessels drain central portions of the lower lip, floor of the mouth and apex of the tongue, and whose efferent vessels drain partly into sub-mandibular lymph nodes and partly into deep cervical lymph nodes.

sub-mucosa

The dense irregular connective tissue layer found below the oral mucosa (or mucosa of a hollow organ), homologous to the reticular layer of the dermis.

sulcular epithelium

Epithelium lining the gingival sulcus.

sulcus terminalis

V-shaped groove separating the anterior two thirds of the tongue from the posterior third and containing the circumvallate papillae.

superior deep cervical nodes

Lymph nodes under the sternocleidomastoid muscle.

supernumerary roots

Extra roots on a tooth.

surface etching

The use of an acids to prepare enamel for the application of an adhesive, which roughens the surface and removes the smear layer, increasing retention of resin sealant.

temporo-mandibular joint

The synovial joint between the temporal bone and the mandible.

teratogens

Agents or factors which cause malformation of an embryo.

terminally differentiated

When a cell has finished its last possible differentiation step and lost the ability to undergo mitosis.

tertiary dentin

dentin formed as a reaction to external stimulation, such as cavities and wear, including both reactionary and reparative dentin.

tight junction

A type of cell-to-cell contact that prevents diffusion between cells.

tissue

A group of cells, all the same type, that work together to perform one or more functions.

Tomes' granular layer

A layer of dark granules that lie parallel to the outer surface of root dentin.

Tomes' process

A histologic landmark identified on an ameloblast.

tonsillitis

Inflammation of the tonsils

tooth bud

tooth buds

A mass of tissue having the potentiality of differentiating into a tooth.

tooth eruption

A process in tooth development in which the teeth enter the mouth and become visible.

tooth fusion

The union of two adjacent teeth at the crown level (enamel and dentin).

tooth germ

An aggregation of cells that eventually forms a tooth, composed of the enamel organ, dental papilla and dental sac.

trabeculae

Rod-like mineralized connective tissue structures found in spongy-bone.

trans-membrane proteins

Proteins embedded within the plasma membrane, with parts both the inside and outside of the cell.

transcription

The process of converting DNA into an mRNA copy

transcription factor

A protein that controls the rate of transcription of DNA to mRNA, by binding to a specific DNA sequence.

translate

The process of turning the sequence of a mRNA into a sequence of amino acids during protein synthesis

trimesters

A period of three months, especially as a division of the duration of pregnancy.

trisomy 21

The most common chromosomal anomaly in humans, also known as Down syndrome, caused by an extra chromosome 21.

trophoblast

Cells that form the outer layer of a blastula which provide nutrients to the embryo and develop into a large part of the placenta.

tuberculum impar

A swelling situated in the midline of the floor of the pharynx between the mandibular arch and of the second branchial arch that contributes to the formation of the anterior part of the tongue.

unilateral

On one side

vascular

Containing blood vessels

ventral surface of the tongue

The bottom surface of the tongue, which contains no lingual papillae.

vermilion zone

The lip, as opposed to adjacent skin or oral mucosa,

named for its more reddish appearance than keratinized skin.

vesicle

A structure inside (or outside) a cell, consisting of liquid enclosed by a lipid bilayer.

Volkman's canals

Small channels in the bone that transmit blood vessels from the periosteum into the bone and that communicate with the haversian canals, or small holes in alveolar bone through which PDL fiber bundles are embedded.

von Ebner salivary glands,

minor salivary glands associated with circumvallate papillae.

Waldeyer's ring

Four tonsils (pharyngeal, tubal, palatine and lingual tonsils) as well as small collections of lymphatic tissue disbursed throughout the mucosal lining of the pharynx.

Wnt

Signaling molecules first identified for their role in

carcinogenesis, then for their function in embryonic development, including body axis patterning, cell fate specification, cell proliferation and cell migration.

xerostomia

The symptom of having a dry mouth due to reduced saliva production.

zygote

A diploid cell resulting from the fusion of two haploid gametes; a fertilized ovum.