Tissue Engineering: Principles, Recent Trends and the Future: State of the Art and Recent Trends

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CHAPTER 2

TISSUE ENGINEERING: PRINCIPLES, RECENT TRENDS AND THE FUTURE

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ABSTRACT

Since the time immemorial or for millennia, humans have been interested in manipulating things surrounding them to suit their needs, and with the advent of technology their inspiration to modify or alter life has increased. Tissue engineering is one such idea that has been practiced by humans for thousands of years and thus most of the achievements in this field that enthralls us today have its origin thousands of years back. Tissue engineering entitled as a multidisciplinary approach which involves the development and replacement of living tissues and organs for diseases and trauma. It applies the principles of both engineering and biology toward the development of viable substitutes which replace, restores, or improves the damaged, missing, or poorly functioning components of human tissues or organs. The success of skin tissue engineering and its commercialization made scientists to put on interest in expanding the research on tissue engineering and this will be one of the significant fields of science/health in the coming centuries. In this chapter, we discuss about the mechanisms involved in tissue engineering, the role of biomaterials, its fabrication methods, different approaches, current status, and various applications.

2.1 INTRODUCTION

Damages and degeneration of tissue happens in every organism now and then but rebuilding or treatment of these body parts becomes a major issue when we consider about the availability of the grafts needed for the

particular replacement and various anatomical limitations. Rebuilding of body parts has a long tradition and was nurtured by the discovery and availability of new synthetics during World War II where, the introduction of various man-made materials for the reconstruction of the tissue damage happened. This reconstructive surgery which focused on the fabrication of living replacements in the laboratory is termed as tissue engineering (Lanza et al., 2011). The practice of tissue engineering seems relatively new but the idea of replacing of a tissue with another dates back to the16th century. It was only in 1988 at the National Science Foundation workshop that the term "tissue engineering" was officially stated at first (O'Brien, 2011).

As discussed, tissue engineering is considered as a highly multidisciplinary approach as it draws experts from various fields like clinical medicine, mechanical engineering, material science, genetics, and life sciences.

2.2 PRINCIPLE OF TISSUE ENGINEERING

According to Langer and Vacanti, tissue engineering utilizes the basic principles of life sciences and engineering for the development of biological substitutes which then restore, maintain, or improve the tissue functions (Langer and Vacanti, 1993). The basic principle of tissue engineering depicted in Figure 2.1 can be demonstrated in this way: at first the cells have to be isolated from a source (allogenic, xenogenic, or autologous source), it is then expanded in a cell culture system or a bioreactor (expansion in vitro) and these expanded cells are then seeded onto a matrix/carrier which then provide structural support along with addition of proper medium (rich with nutrients and growth factors). Here the cells differentiate, proliferate and migrate to the carrier and replace the old tissues by forming new tissues. The tissue engineering construct formed as a result of this is then grafted back into the patient to function as the introduced replacement tissue (Vasita and Katti, 2006).

Tissue engineering can be performed by various approaches as listed hereunder.

1. In vitro tissue genesis for in vivo application
2. In vivo tissue genesis for in vivo application
3. In vitro tissue genesis for ex vivo application
4. In vitro tissue genesis for in vitro application (Irvin, 2003)

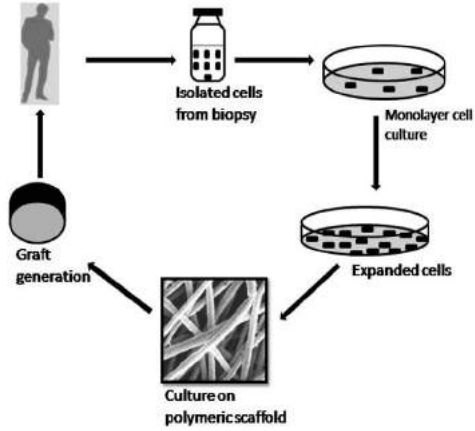

FIGURE 2.1 Basic principle of Tissue Engineering

As compared to the original biologic tissue which involves the co-ordination between the cells, extracellular matrix, and the signaling system, the tissue engineering system also needs a better understanding among the cells, scaffold, and the signal molecules and these three basic components together forms the "triad of the tissue engineering" and shown in Figure 2.2. The scaffold here serves as the mechanical platform onto which the cells get adhered, proliferate, and differentiate and thus it is better to develop a biocompatible and biodegradable scaffold (Rim et al., 2013).

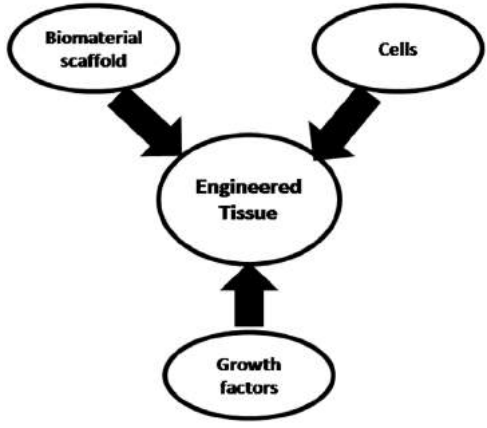

FIGURE 2.2 The Tissue engineering triad

2.3 INTERFACE BETWEEN NANOMEDICINE AND TISSUE ENGINEERING

Tissue engineering is another application of nanotechnology to medicine (nanomedicine). It helps to reproduce or replace damaged tissues by using suitable scaffolds made of nanomaterials and growth factors. Thus, tissue engineering is an area of nanotechnology where we focus on the construction of new tissues or organs for replacement. This effort may replace the existing conventional methods of treatment like organ transplantation or artificial implants.

The immune responses regarding these methods can be avoided by using autologous or isogenic cell sources in the scaffold constructs, by suppression of immune system of host, induction of tolerance in the host, or by immunomodulation of the tissue engineered construct (Fisher et al., 2007). The immune reactions also help in removing cellular debris caused by infections and injury that can lead to additional tissue damage. So to enhance regeneration, strategies have to be developed that exploit the beneficial aspects of the immune reactions while limiting the deleterious aspects. Appropriate application of technologies should be employed that have the potential to turn immune reactions to an asset for regeneration, differentiation, and more regenerative and less inflammatory.

2.4 SCAFFOLD AND TRANSPLANT

A transplant can be classified or defined in many ways; for example, it can be based on the relationship between the recipient and the donor, its location in a recipient, and so on. Different types of transplants include autograft (from one part of the body to other within one individual), isograft (within genetically identical species like identical twins), allograft (from different individuals of same species), and xenograft (from members of different species). The transplantation of organs or parts of organs has been considered as a conventional method for the curative treatment of end stage diseases of liver, kidney, heart, lung etc. Current methods of transplantation and reconstruction are time-consuming and involve very costly therapies like immunosupression therapy. The donor shortage is another problem apart from the serious side effects caused by the lifelong immunesupression therapies. Thus tissue engineering using scaffold bio-

materials has taken an approach toward the replacement of lost tissue or organ function (Ratner, 2004).

An essential component in the tissue engineering approach is the carrier which is a highly porous artificial extracellular matrix (ECM), or scaffold which act as a template for tissue formation. This three dimensional (3D) scaffolds accommodate mammalian cells, regulate, and stimulate the cellular functions of adhesion, migration, growth, differentiation, and tissue organization and also guides their growth and regeneration in 3D. A scaffold in terms of both physical structure and chemical composition should mimic the structural and biological function of the native ECM as much as possible. It is due to the fact that the native ECM does not only just act as a physical support for cells but also provide a substrate with specific ligands and growth factors so that the cell can adhere and grow. Thus we can say that a tissue- engineered scaffold mimicking an ECM will surely play a similar role in promoting tissue regeneration in vitro as native ECM behaves in vivo (Ma et al., 2005).

The common source material for the natural scaffold is the ECM and there exist a practice of using these natural scaffolds as ECM for tissue engineering. These decellularized natural scaffolds derived from native tissues or complex organs by treating them with detergents to remove cellular material (decellularization) which can be then reseeded with healthy autologous or allogenic cells (Badylak et al., 2012). There are numerous examples of successful therapies based on natural ECM scaffolds but have some drawbacks also. These drawbacks can be challenged by using synthetic platforms with desired physical and biochemical properties of the natural ECM. The development and proper function of differentiated cells are contributed by a supportive 3D architecture of the scaffold.

Various biomaterials can be used as scaffolds to direct specific cell types to organize into 3D structures and to perform various differentiated functions of the targeted tissues. The most attractive scaffold materials are the synthetic bioresorbable polymers fully degradable into the body's natural metabolites under physiological conditions and which offer a possibility to create a completely natural organ or tissue equivalent. They overcome the issues like infection and fibrous tissue formation that are associated with permanent implants (Ratner, 2004). Thus a scaffold should be biocompatible with the cells and should guide the cell growth and hence the selection of a scaffold material is very important for a sovereign tissue engineered product. Most of the scaffolds are made from

natural or synthetic polymers but in cases of certain hard tissues like bone and teeth, various ceramics like calcium phosphate compounds have been utilized (Park and Roderic, 2007). Natural polymeric materials used for the scaffold preparation includes collagen, chitosan, hyaluronic acid, silk fibroin, gelatin etc. and synthetic polymers include poly-lactic acid (PLA), polyethylene terephthalate (PET), poly-capro lactone (PCL), poly (lactic-co-glycolic acid (PLGA), poly (ethylene-co-vinylacetate (PEVA) etc.

2.5 BIOPOLYMER SCAFFOLDS FOR 3D CULTURE OF CELLS

Tissue engineering is a flourishing and promising biomedical engineering field which aims to develop viable substitutes to restore and maintain the function of damaged tissues. Culturing of cells in 3D micro-environments simulates normal cellular compartment and enhances adhesion, proliferation, and differentiation of cells than in 2D. Scaffolds can function as a delivery vehicle, a matrix for cell adhesion, and also serve as a mechanical barrier against infiltrating surrounding tissues which hampers tissue repair and regeneration. Scaffolds can be synthetic or natural in origin. Different types of scaffolds like porous, fibrous, customs, ECM, hydrogel, microspheres, and native tissue scaffolds are available and they influence the characteristics of cellular unit processes. A variety of techniques have been developed for fabrication of scaffolds. There are no scaffolds universally suitable for all cells and all applications. The following section of this chapter highlights the application of natural and synthetic biopolymers in 3D culture of cells, their fabrication techniques, and describes the biophysical, biochemical, and biomechanical properties of scaffold which influence cellular processes inside the scaffold.

2.6 BIOMATERIALS FOR TISSUE ENGINEERING

Biomaterials can be defined as materials that are natural or synthetic, and can be used therapeutically to repair, restore, or replace lost function. Such materials have been in use for decades, but the understanding of how the body interacts with implanted materials led to the progression of this field from the use of anything which was surgically available toward the use of materials which are deemed biocompatible. Biomaterials serve as 3D

synthetic framework which is commonly referred to as scaffolds, matrices, or constructs and plays a very crucial role in tissue engineering.

Ceramics, synthetic polymers, and natural polymers are the three important groups of biomaterials that are used in fabrication of scaffolds with their own specific advantages and disadvantages. Thus the use of a composite scaffold comprising different phases is comparatively more (O'Brien, 2011). The biomaterials can be categorized into different sections as shown in Figure 2.3.

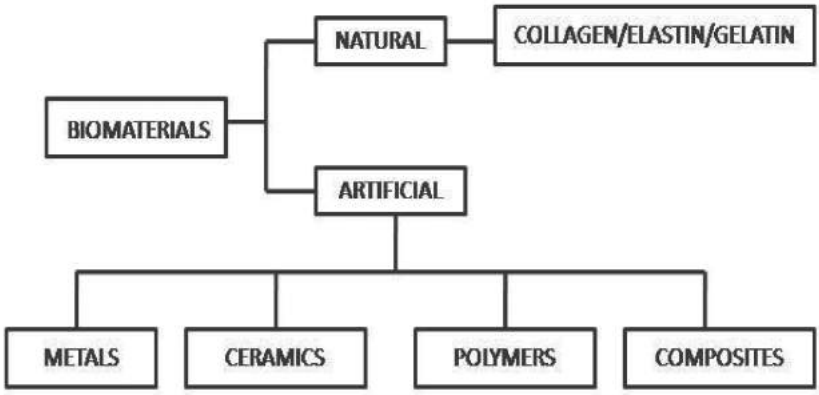

FIGURE 2.3 Classification of biomaterials

2.6.1 PROPERTIES OF A BIOMATERIAL

In order to function appropriately, the biomaterials have to satisfy certain properties like bulk properties and surface properties. The strength of the biomaterial implant can be determined by the bulk properties and the interaction with the biological system by the surface properties. To avoid failure of the implants, the properties of the biomaterial should be adjusted to its presumed function.

Bulk properties: The atomic organization as well as inter-atomic forces (ionic bonding, metallic bonding, and covalent bonding) that keep the atoms together determines the bulk properties of a material. Elastic modulus, yield stress, and ultimate stress are some properties that have to be considered from a mechanical point of view which determines the stiffness,

strength, and deformability of the biomaterial. Fatigue that occurs through the cyclic stress is another important bulk property of a biomaterial.

Surface properties: Like intrinsic/bulk properties, surface properties are also important for the success of an implant. Energy, charge, release of ions, roughness, and composition are some of the factors which determine the surface characteristics of a biomaterial. The interaction between the material and biological constituents should not cause any deleterious effects to the surrounding cells, tissues, or organs and thus the material should not be antigenic, cytotoxic, carcinogenic, pyrogenic, or toxic to the living cells.

2.6.2 CHARACTERISTICS OF AN IDEAL SCAFFOLD

The selection of a scaffold is very important while considering the behavior of cells that has to be grown to form tissues or organs of specific dimensions. Some of the characteristics needed for an ideal scaffold are explained in the following sections.

2.6.2.1 POROSITY

The scaffold architecture should have highly interconnected pore structure and high porosity to ensure the cellular penetration and diffusion of nutrients to the cells within the scaffold and to the ECM formed by these cells. Porous nature also helps in the diffusion of waste products and the products of scaffold degradation to exit the body without interfering the surrounding tissues or organs (O'Brien, 2011). Porosity can be created intentionally by production process such as leaching of salt, sugar or starch crystals, sintering of beads, knitting, and weaving of fibers or it can also occur as a manufacturing artifact. Porous scaffolds are mainly used for artificial skin, blood vessels, drug delivery, bone and cartilage reconstruction, periodontal repair etc. each with fulfilling some specific requirements.

2.6.2.2 MECHANICAL STRENGTH

Mechanical strength is a key factor to consider in designing or determining the suitability of a scaffold in tissue engineering. The mechanical proper-

ties of the biomaterial used for making scaffold should match with that of the host tissue (Chan and Leong, 2008). For the creation of the scaffold, particularly in the construction of load-bearing hard tissues such as bone and cartilage that retains its structure even after implantation the mechanical strength is a very essential factor. The mechanical properties of the biomaterial should be adjusted to its proposed function to avoid failure. For example, for the fixation of a bone fracture it is necessary to have a required strength to avoid breakage. The intrinsic properties needed for a material from a mechanical point of view are elastic modulus, yield stress, and ultimate stress. These three properties determine the stiffness, deformability, and strength of a material. Another property is fatigue which is a process by which structures fail as a result of cyclic stress which is less than the ultimate tensile stress. Cyclic stresses are very common in human body in locations like pumping heart (artificial heart valves), connections of limbs (artificial hips) etc.

2.6.2.3 BIOCOMPATIBILITY

After the preparation of the required tissue-engineered construct, it should be successfully integrated into the living system and only after addressing the issues in the technologies for integration into the living system can achieve success in tissue engineering. This involves the issues of biocompatibility and immune acceptance (Lanza et al., 2011). Biocompatibility is the property of a material by which when it is introduced into a living body to perform a specific function, does not interfere or enhance the functioning of an organ and exerts neither local nor general toxicological actions in the body. After performing the functions it should get biodegraded and the products of the biodegradation should be fully eliminated and natural tissues should be regenerated in the place of the implant (Lipatova and Lipatova, 2000). In simple terms, biocompatibility can be defined as the ability for the performance of a medical device or material within an appropriate host response in a specific situation. The magnitude and duration of any adverse alterations in the homeotic mechanisms which determine the host response can be measured and is termed as biocompatibility assessment (Ratner, 2004). A schematic representation of the possible interactions between a biomaterial and biological components is shown in Figure 2.4.

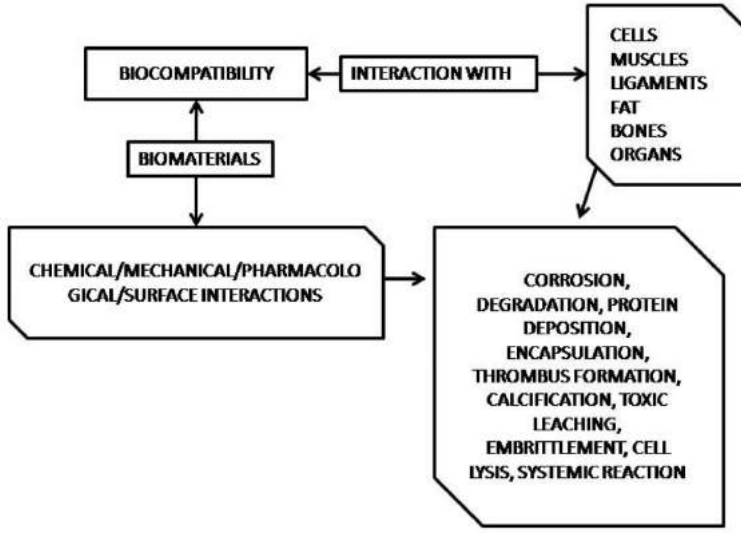

FIGURE 2.4 Interactions between a biomaterial and biological components

Potential biological hazards may include short-term effects, specific toxic effects, and long-term effects. Two perspectives for the in vivo assessment of the tissue compatibility are: first the tests for general biocompatibility of a biomaterial which is necessary for development of a biomaterial in further research and second is the biocompatibility tests for the final products, that is, a condition in which it is implanted. Various in vivo tests for determining the biocompatibility includes sensitization, irritation and intracutaneous reactivity, acute and sub-acute toxicity (systemic and sub chronic toxicity), genotoxicity, implantation, chronic toxicity, hemocompatibility, carcinogenicity, reproductive and developmental toxicity, biodegradation, and immune responses.

2.6.2.4 THE ROLE OF IMMUNE ACCEPTANCE

Tissue engineering of scaffold has become an important area in regenerative medicine and immune response is recognized as an important factor which influences regeneration. The immune reaction will start with an acute response to the injury followed by innate recognition of the foreign materials and a chronic immune response which involves specific recogni-

tion of the antigens like transplanted cells by adaptive immune response. All these together eventually leads to the rejection of the implants. The processes starting from transplantation of cells, implanting biomaterial scaffolds, or delivery of inductive factors may stimulate the immune reactions.

The biomaterial aims at creating a local environment for the growth of tissues but the injuries caused during the time of implantation and certain host inflammatory responses will have negative impacts in creating this environment. The repairing of these injuries can lead to fibrosis. Activation of complement proteins and cellular pattern recognition receptors (PRRs) initiate inflammatory cytokine and chemokine production which then leads to the recruitment of polymorphonuclear neutrophils (PMNs), monocytes, fibroblasts etc. to the injury sites. PMNs remove pathogen and cellular debris. They remove pathogen by phagocytosis, reactive oxygen species (ROS), and causes secondary damages to the surrounding tissues by various cytokines. Macrophages peaks for around one week and can persist at the injured site up to several months and can also secretes ROS and cytokines for the secondary damages. On the other hand, the presence of macrophages is necessary for the growth as they secrete growth factors and phagocyte cell debris (Boehler et al., 2011).

2.7 ENGINEERING BIOMATERIALS FOR TISSUE ENGINEERING

Different methods are available for engineering of biomaterials into a desirable form that is intended by the scaffold for tissue engineering and all of these techniques have advantages as well as drawbacks. Different types of techniques involved in the scaffold preparation are detailed as follows.

1. Solvent casting
   In this method a mold is prepared and this mold is then dipped into a polymer solution. Allowing sufficient time for the solvent evaporation and this will result in the formation of a layer of polymeric membrane. The main drawback of this technique is the toxicity caused by the solvent that is left after evaporation. Vacuum drying of the prepared scaffolds can overcome this to some extent but is very time-consuming.
2. Particulate leaching techniques

Similar to solvent casting method, in this technique also a mold is prepared but porogens like salt, wax, or sugars of desired size are added into the mold. The polymer solution is then added into this porogen-filled mold which is then evaporated and the salts are then leached out using water. The pore size can be controlled to some extent by controlling the size, shape, and amount of porogen taken for the experiment (Subia et al., 2010)

3. Gas foaming
   This technique uses gas as a porogen. A disc is prepared using desired polymer by compression molding and it is then placed in a chamber exposed to high pressure carbon dioxide for a few days during which pores are formed on the discs. Further, 3D porous structures are formed after the foaming process and the porosity can be further controlled by using salts or other porogens (Sachlos and Czernuszka, 2003).
4. Freeze drying
   It is based on the principle of sublimation and is used for preparation of porous scaffolds. Here a polymer is dissolved in an appropriate solvent and water is added into the polymer solution and mixed thoroughly to obtain an emulsion. Before separation of the phases, the emulsion is casted into a mold and frozen by immersing into liquid nitrogen and subsequently freeze dried to remove the solvent and water (Mandal and Kundu, 2009). This method does not use high temperature and no separate leaching step is needed but produces only low pore size and has long processing time.
5. Porogen leaching
   This method is performed by dispersing porogens like wax, salt, or sugar into either powdered materials or liquid particulates by the process of evaporation cross- linking etc. Scaffolds with up to 93% porosity can be produced using this method. It is very difficult to make scaffolds of accurate pore inter-connectivity and only wafers or membranes up to 3 mm thick can be produced by this method (Moore et al., 2004).
6. Fiber mesh (textile technologies)
   Individual fibers are either woven or interweaved into various 3D forms with variable pore sizes for scaffold fabrication (Martins et al., 2008). A polymer solution is poured over a nonwoven mesh of another polymer and is subsequently evaporated. Large surface

area and rapid diffusion of nutrient are some of the advantages while lack of structural stability is a drawback for this technique.

7. Phase separation
   It requires a temperature change that separates the polymer solution into two phases and also a solvent with low melting point and is easy to sublime. The temperature change will separate the polymer solution in the solvent into two separate phases. The solvent is later removed by extraction, evaporation, and sublimation resulting in a porous scaffold. An appropriate phase separation can also produce 3D fibrous structures with nanoscaled architecture.
8. Self-assembly
   When a disordered system forms an organized structure without any external direction, but by the local interaction between its own components, this can be termed as self-assembly. This is used for the production of 3D nanofiber structures. Hydrophobic and hydrophilic domains within the amphiphilic peptide sequence interact together due to the weak non-covalent bond and as the molecules come together they produces fast recovering hydrogel (Joshi et al., 2009; Zhang et al., 2005). Apart from peptides, synthetic polymer nanofibers with very thin diameter can also be formed by self-assembly methods. It is performed in aqueous salt or physiological solution and no solvents are used but the self-assembly process is a very elaborative process.
9. Electrospinning
   Electrospinning is a versatile technique producing continuous fibers of sub-micrometer-to-nanometer scale using electrostatic force. The polymer solution in a solvent is made to eject from a spinneret with high voltage to a collector with opposite or grounded charge forming a highly porous network after drying or solidification (Doshi and Reneker, 1993; Reneker and Chun, 1996). By varying the flow rate, distance between the needle and collector or the applied voltage, the scaffold architecture can be modified.
10. Rapid prototyping (RP)
    Controlling of porosity and pore size of a scaffold is a very difficult task and can be solved to a limit by using computer-assisted design and manufacturing techniques (CAD/CAM technologies). Using CAD software a 3D scaffold can be designed and using various algorithms in this software the porosity can be tailored (Melchels

et al., 2011). Fused deposition modeling (FDM), selective laser sintering (SLS), 3-D printing, or stereo lithography are some of the RP techniques used for the construction of 3D objects in a layer-by-layer layer method. RP technique can control the matrix architecture, mechanical property, biological effects etc. of scaffolds. Low resolution is a drawback of this technique.

11. Melt molding
    A teflon mold is filled with PLGA powder and gelatin microspheres (with specific diameter) and the mold is heated above the glass transition temperature of PLGA applying high pressure to the mixture making the PLGA particle to attach together (Thompson et al., 1995). The mold is then removed and the gelatin is then washed off and the scaffold is dried. This process can be modified to incorporate hydroxyapatite fibers.
12. Membrane lamination
    This method can be used for constructing 3D polymeric foam scaffolds that are biodegradable and have precise anatomical shapes. This uses a layer-by-layer fabrication process and generates porous 3D polymer foams with defined anatomical shapes using computer-assisted mechanisms. Disadvantage of this technique is lesser pore interconnectivity due to layering of porous sheets (Hutmacher, 2001). Only thin membranes are used and thus it is a time-consuming process.
13. Laser-assisted bioprinting (LaBP)
    Multicellular 3D patterns are made in natural matrix using laser-assisted bioprinting (LaBP). A fully cellularized substitute can be prepared using LaBP technique in an exact 3D spatial conformation by setting living suspensions of small cell volumes in patterns of high resolution. The generated tissue constructs may be studied in vivo (Lai et al., 2011). Matriderm® is an example of skin substitute successfully tried in mice (Michael et al., 2013).

2.8 COMMONLY USED BIOMATERIALS FOR SCAFFOLD FABRICATION

Different types of materials like natural, synthetic, biodegradable, or permanent materials have been investigated for the purpose of scaffolding in tissue engineering. Most of these materials have been already in use even

before the advent of tissue engineering in various applications. Scaffolds should be absorbed by the surrounding tissues rather than surgical removal and thus degradability of scaffold is an essential factor, but the rate of degradation of scaffold should coincide with the rate of tissue formation. The problems related to long-term safety of permanently implanted structures can be circumvented by the use of degradable materials. Unlike non-degradable materials, degradable materials should fulfill requirements that are more stringent. Toxicity of the contaminants that leach out from the implants, subsequent metabolites, and degradation products must be taken into consideration and so only a few numbers of starting materials have been implied for the preparation of degradable biomaterials. As of 1999, the Food and Drug Administration (FDA) has approved only five synthetic degradable polymers for clinical application: PLA, PGA, polydioxanone, poly (caprolactone) (PCL), and poly (PCPP-SA anhydride). Some of the commonly used materials are explained in the following sections.

2.8.1 SILK

Silk is a fibrous protein derived from the silkworm, Bombyx mori. It is a natural biomaterial tough and strong, spun by insects and spiders. Different types of silk fibers are available and are composed of peptide molecules conferring distinct mechanical properties (Hinman et al., 2000). Silk fibroin is a potential natural biomaterial for preparing nanofibrous scaffolds. The in vitro biocompatibility of the silk nanofiber is proved by Min et al., 2004, and the fiber diameter, high porosity together with the cytocompatibility makes the silk nanofibers a suitable candidate material for scaffolding technology. Cell culture studies have shown a slow degradation of silk scaffolds in around four weeks as a result of proteolysis (Taddei et al., 2006). Silks can be either modified by using various chemical treatments or can be used with other materials for varying its mechanical properties and surface chemistry. The degradation behavior also can be modified according to the applications. Silk fibroin films and silk fibroin alginates are used in wound healing applications (Kearns et al., 2008).

More recently, spider silk has been explored in the field of both bone and cartilage tissue engineering. Silk, after the extraction of cericin is wound into strands and yarns and have been investigated for the ligament tissue engineering. Silk fibroin films that are heparinized and sulfonated show suitable mechanical properties for the tissue engineering of artificial

blood vessels. Silkworm silk has also shown potential in liver tissue engineering and also supports Schwann cells and dorsal root ganglia and thus can be used as a potential scaffold for nerve tissue engineering (Cirillo et al., 2004; Yang et al., 2007). Thus, silk nanofibers can be a promising biomaterial for the tissue engineering applications.

2.8.2 COLLAGEN

Collagen is the most abundant protein present in the mammalian ECM and this natural polymer has been in use for a variety of tissue engineering applications. Collagen along with other proteins can be electrospun into nanofibers which resembles native state and can be used in tissue engineering applications (Jha et al., 2011). Collagen molecules have a triple helical structure and exist in a fibrillar form with elaborate 3D arrays in the ECM.

Collagen fibrils and their networks function as ECM (highly organized 3D architecture surrounding the cells) for most of the soft and hard connective tissues like bone, tendon, blood vessels, skin, cornea etc. Collagen encourages cellular growth and modifies the morphology, migration, adhesion, and differentiation of cells. Compared to other proteins, collagen is weakly antigenic and the immune reactions always depend on the species to which the tissue is implanted and the site of implantation. It is a biodegradable protein and the biodegradability can be either extended or completely suppressed by modifications. Peptide–amphiphile nanofibers produced by the process of self-assembly have been used for the preparation of hybrid bone implants. Nanofibrillar gels prepared were used to support neuronal cell attachment and differentiation of liver progenitor cells and also for brain repair. Skin substitutes based on cell seeding on 3D scaffolds of bovine collagen type I is a commercialized product. The interactions between cells and collagen have opened a great potential of using collagen with biocompatibility and controlled biodegradability in the field of tissue engineering (Chevallay and Herbage, 2000).

2.8.3 POLY LACTIC ACID (PLA)

PLA is a commonly used synthetic biomaterial. It is polyester and degrades within the human body to form lactic acid which can be easily

removed from the body as it is a commonly occurring chemical in the body. According to Vert et al., (1991), the polymeric backbone of PLA is chemically degraded by simple hydrolysis and independent to any biological method and hence this cannot be described as biodegradation. PLA is a hydrophobic polymer and this hydrophobicity reduces the water uptake and thus reduces backbone hydrolysis also. There exist four distinct morphological polymers for PLA: D-PLA, L-PLA, racemic PLA, and meso-PLA due to the chiral nature of the lactic acid.

PLA is considered safe and non-toxic and its biocompatibility is proven by various regulatory agencies and has been used in a large number of clinically successful medical implants. Thus, devices prepared from PLA are more easily brought into market. A major drawback of PLA is that they are poor substrates for cell growth in vitro. Other factor is that the degraded product, lactic acid which is a relatively strong acid may get accumulated in the implant sites and delayed inflammatory response happens after six months to one year. Apart from all these, many porous scaffolds have been in use for tissue engineering application based on PLA and its copolymers (Netti, 2014).

Polyglycolide (PGA), which is a class of poly (α-esters), has been used to engineer tendons and cornea stroma in animal models. PGA fibers were also used to repair defects of cartilage tissues but a major drawback with PGA is its acidic degradation that results in unfavorable host response. Coral, calcium alginate, and demineralized bone matrix (DBM) are also successfully used as biomaterials for repairing bone tissues.

2.8.4 BIONANOMATERIALS IN TISSUE ENGINEERING

Bionanomaterials are any nanomaterial of biological origin. A myriad of biomedical applications have been associated with these bionanomaterials due to the advancement in their fields. Some of the bionanomaterials used in tissue engineering are described in the following sections.

2.8.4.1 NANOCHITOSAN/NANOCHITIN

Chitin (poly-β-(1-4)-N-acetyl-D-glucosamine), is a natural, renewable, and biodegradable polysaccharide and the rate of degradation is highly dependent on the molecular mass of the polysaccharide. Chitosan is an

important derivative of chitin prepared by the partial deacetylation under alkaline condition. Both chitin and chitosan are non-toxic polymers and have found several biomedical applications in wound healing and tissue engineering.

Scaffolds prepared from chitin have been used in the regeneration of cartilage, bone, and tendon tissues (Wan and Taj, 2013). In order to use chitin in tissue engineering, it is needed to modulate its physical or biochemical properties for a better interaction in the biological environment. Nano-sized chitin/chitosan possesses a high performance because of its high surface area, quantum size effect, and small size compared to the traditional micro-sized chitin/chitosan materials (Qi and Xu, 2004). It can be prepared by many methods like coagulation, covalent cross-linking, precipitation, ionic cross-linking etc. (Berthold et al., 1996). Preparation of porous chitosan nanofibers ranging from nanometer to microns in diameter was performed by electrospinning technique and found many applications in tissue engineering. Blending of chitosan with other polymer makes an easier method for the formation of chitosan (nano) fibers. A wide range of experiments has been performed in case of chitin/chitosan as a biomaterial for tissue engineering but the use of nanosized chitin/chitosan needs further support for it to get established in the field of tissue engineering.

2.8.4.2 NANOCELLULOSE

Cellulose is a ubiquitous structural polymer which confers its mechanical properties to plant cells and is the most abundant organic polymer available on earth. It is an important structural component of cell wall of plants, some algae, bacteria etc. Nanocellulose or microfibrillated cellulose (MFC) is composed of nanosized cellulose fibrils having a high aspect ratio. Nanocellulose can be obtained by an acid hydrolysis of native fibers resulting in highly crystalline rigid nanoparticles (referred to as nanowhiskers) or nanocrystalline cellulose (NCC) (Peng et al., 2011). The purity, web-like nature, long fiber length, high degree of crystallinity, and the nanoscale fibril dimensions has made bacterial cellulose superior to plant-derived cellulose in tissue engineering. Based on the bacterial cellulose (BC) synthesized by Gluconacetobactor xylinus, a skin tissue repair material has been prepared. Nano-composites of BC and chitosan with a

cohesive gel structure have showed excellent results promoting the healing of epithelial tissues and reduced inflammation (Fu et al., 2013).

Bacterial nanocellulose (BNC), being a novel non-degradable, biocompatible, and functionally competent material has been used recently in skin, bone, vascular, and cartilage tissue engineering. Nanocrystalline cellulose (NCC) formed from the acid-catalyzed degradation of cellulosic materials are able to create highly porous silica films and carbon films with chiral nematic organization and thus will have promising applications in the near future. Nanocellulose have been used to prepare 3D macroporous nanocellulose scaffolds by biofabrication technique using porogens and have shown ability to attract endothelial cells, chondrocytes, smooth muscle cells of various origin, urethral cells, as well as osteoprogenitor cells. By using bioprinting techniques, 3D porous nanocellulose scaffolds with large size, unique architecture, and with various surface modifications have been prepared to enhance the cell attachment and differentiation. A myriad of exciting new-cellulosic materials have been developed with nanoscale fibrillar structures having promising applications in the growing field of tissue engineering. On the other hand, the development of advanced biomedical applications for cellulose is still in its infancy (Dugan et al., 2013).

2.8.5 CARBON-BASED NANOMATERIALS

Carbon-based nanomaterials like graphene (G), carbon nanotube (CNT) etc. possess unique mechanical, electrical, and optical properties that present much interest in research fields like tissue engineering. They provide a similar microenvironment like biological ECM, both in terms of physical structure and chemical composition and thus a potential candidate for the development of artificial scaffolds. Typical graphene and CNT structures are shown in Figure 2.5.

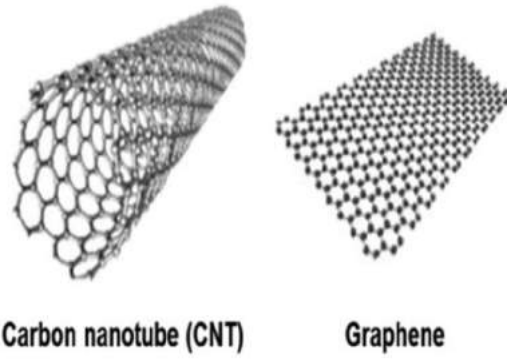

FIGURE 2.5 Structure of graphene and CNT

2.8.5.1 GRAPHENE

Graphene (G) is a 2D carbon crystal sheet of molecular thickness and composed of sp^2 hybrid bonded carbon. It poses a number of diverse and exceptional properties like optical, mechanical, electrical, and thermal qualities. With the rapid development of synthesis and functionalization approaches, graphene as well as its related derivatives have shown exceptional potential not only as physicochemical material but also are extended to biological uses. There is an intensive area of research focusing on the bio-applications of graphene and its related derivatives such as graphene oxide (GO) due to many intriguing properties (Guo and Dong, 2011).

Different types of osteoblast cells have been tested with graphene in the view of bone regeneration and bone treatment scaffolds. Graphene layers modified with artificial peroxidases and laminins have promoted cell adhesion and pseudopodial cell configuration, whereas coating graphene sheets with laminins alone showed eight times more proliferation than those with the pristine graphene (Guo et al., 2010). The high electrical conductivity of graphene could be used for modulating the behavior of neural cells or neural differentiation. The electrochemically active transduction by graphene helps in the bioelectrical signal transmissions in neurons which is essential for the neural activity. Graphene can also be used as electrode materials in neural prosthetic devices (Park et al., 2011).

Graphene polymer composites, Graphene hydrogel etc. were also used for the cell modulation along with structural scaffolding functions. The studies so far demonstrated the ability of graphene-based materials in supporting tissue engineering and other biomedical fields and this trend is believed to continue in the coming years.

2.8.5.2 CARBON NANOTUBE (CNT)

CNTs are graphite sheets that have been rolled into cylindrical tubes with length in nanometer or micrometer range and a diameter in nanoscale. CNT-based materials have high electrical conductivity, chemical stability, and physical strength with structural flexibility and thus they have been studied for neural cell adhesion and proliferation. The activities of the CNT surface can be altered by its chemical functionalization. The similarity of CNTs to that of ECM components makes it a potential candidate as a scaffold material for tissue engineering.

The CNT-polymer composites from the homogenous suspension of polymers and CNTs have been used to fabricate polymeric scaffolds for bone tissue engineering. Some examples include multi-walled carbon nanotubes (MWNTs)/polycaprolactone, ultra-short single-walled nanotubes (SWNTs)/poly (propylene fumarate) (PPF) etc. CNTs incorporated into polymeric nanofibers (both synthetic and natural) improved mechanical strength, thermal stability, and electroactivity. The CNT integration will increase the tensile strength and Young's modulus and decrease elongation at break. Several types of cells such as osteoblast, fibroblast, skeletal myoblast, and mesenchymal stem cells (MSCs) grew well on polymer/CNT nanofibrous scaffolds (Harrison and Atala, 2007)

CNT-inorganic composites and CNT-polymer-inorganic hybrid materials have been formulated and have shown positive results in tissue engineering. Even though the literature suggests that the surface functionalization of CNT can attenuate its toxicity in vivo, long term safety periods longer than six months have not been studied and thus it is very important to investigate its potential health risks. In spite of all these issues and challenges carbon-based materials are promising substrates for tissue engineering scaffolds because of their unique properties (Ku et al., 2013).

2.8.6 VARIOUS OTHER NANOPARTICLES USED IN TISSUE ENGINEERING

Other nanomaterials like ceramic, alumina or titania, and their composites have been used in the design of tissue engineering scaffolds, especially for bone and dental applications.

2.8.6.1 CALCIUM PHOSPHATE NANOPARTICLES

Calcium phosphate (CaP) is a major component of bone and is studied extensively as scaffolds for bone tissue engineering. Different types of CaPs exists but majority of the research is focused on hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), or mixture of HA and β-TCP also known as biphasic calcium phosphate (BCP). CaPs are biocompatible, osteoconductive, and have the ability to bond directly to bone. Addition of dopants like SiO_2 and ZnO into CaP can control the dissolution rates, densification behavior, biocompatibility, mechanical strength, increased compressive strength, and cell viability (Fielding et al., 2012). Various types of calcium phosphate nanoparticles are involved in tissue engineering. However, HA is one of the most important and is explained in the following section.

2.8.6.1.1 HYDROXY APATITE NANOPARTICLES

Hydroxylapatite or hydroxyapatite (HA/HAp) is a mineral form of calcium apatite naturally occurring with the formula $Ca5(PO_4)_3$, usually written as $Ca_{10}(PO_4)_6(OH)_2$. They have the ability to integrate with the bone structures and support bone ingrowth and osseointegration without any local or systemic toxicity and inflammation thus proving its bioactive nature. Dense HA does not have the mechanical strength for long term load bearing applications. They also significantly increase the bioactivity and biocompatibility of the man-made materials. The bone tissue is a natural composite of HA nanocrystals embedded in collagen fibrils. Thus the incorporation of these HA nanofillers in a polymer matrix will mimic the structure of human bone. HAp can be used as a model component to study biomineralization in human body and is a choice for various biomedical applications like replacements for bone and periodontal defects, dental materials, bioactive coatings for osseous implants etc.

As compared with the conventional HAp, nanophase minerals showed improved cytophilicity and greater cell viability and proliferation and have stimulated great interest in tissue engineering application. A 3D scaffold made out of porous hydroxyapatite with interconnected pores have been developed by foam-gel technique and successfully showed osteoconduction with majority of pores filled with newly formed bone (Yoshikawa and Myoui, 2005). Hydroxyapatite macrochanneled porous scaffolds produced using polymer sponge templating method using reactive submicrometer powder, with optimized mechanical strength have shown good results for tissue engineering. Composites made with other materials like chitosan, collagen, and other polymers will reinforce the matrix along with osteoinduction.

2.8.6.2 ZINC OXIDE NANOPARTICLES

ZnO is a conventional semiconductor with a wide band gap and has been explored widely in multiple areas of science and is considered as a safe material by the FDA. The high stability, photo-luminescent properties, wide band gap semiconductor properties, absorption of ultraviolet UV radiation, and optical transparency of ZnO nanoparticles have gained applications in wide areas like photo-catalytic applications, biomedical applications etc. The applicability of ZnO nanoparticles in regenerative medicine and tissue engineering significantly increased the research in this area.

ZnO nanoflowers have shown induced proliferation and migration of endothelial cells leading to the formation of new blood vessels (Kumara Barui et., al., 2012). In another report ZnO nanoparticles with β-chitin hydrogel bandages showed antimicrobial and wound healing application with a good biocompatibility to human dermal fibroblast cells (Kumar et al., 2013). Scaffolds made up of electrospun polycaprolactone (PCL) with ZnO nanoparticles when used as a skin substitute have shown an enhanced rate of wound healing without any scar formation (Augustine et al., 2014b). They also demonstrated that ZnO nanoparticles can act as the key regulators of angiogenesis in the scaffolds in redox signaling mechanism (Augustine et al., 2014c). These reports have explained the significance of ZnO nanoparticles in tissue engineering and wound healing.

Nanofiber meshes prepared by sodium alginate/poly (vinyl alcohol) with different concentration of ZnO nanoparticles when cultured with mouse fibroblast showed good adhesion and spreading of fibroblast and

also confirmed the antibacterial activity of the nanofibers improved by the increased concentration of ZnO nanoparticles. Electrospun membranes of polycaprolactone with varying concentration of ZnO nanoparticles have shown excellent fibroblast cell attachment and proliferation. This proved the efficiency of polycaprolactone/ZnO nanocomposites in tissue engineering applications like the regeneration of damaged skin where rigorous cell proliferation and antimicrobial properties are essential (Augustine et al., 2014a).

2.8.6.3 TITANIUM DIOXIDE NANOPARTICLES

Metals having a high metallic strength and exceptional fatigue resistance like titanium (Ti) have been widely used to produce porous metallic scaffolds. Titanium dioxide (TiO_2) has been studied extensively for bone replacement material due to its light weight and resistance toward corrosion. Bioactivity of the scaffolds can be increased by proper surface modification of these scaffolds (Das et al., 2008). The bio-inert surface of the TiO_2 makes the chemical bonding between the skeletal bones and the implant surface difficult.

The loose and powdery nature of TiO_2 nanoparticle makes it difficult to be used in scaffold; hence, modifications such as blending of TiO_2 nanoparticles with synthetic polymer are performed. TiO_2 NPs have been used as filler materials for biodegradable polymer matrices. Nanocrystals of TiO_2 with grain size<100 nm have showed the ability to stimulate nanometer surface topography and roughness in osseous tissues. Gerhardt et al., (2007) prepared poly (D,L lactic acid) composite films with different compositions of TiO_2 NPs. This showed an increased surface roughness in the films and an improved adhesion of osteoblast cells. Jayakumar et al., (2011) prepared a chitin-chitosan/TiO_2 NPs composite scaffolds for bone tissue engineering where the addition of TiO_2NPs decreases the pore size of the scaffold. Kim et al., (2014) prepared scaffolds from silk fibroin incorporated with TiO_2NPs resulting in a porous scaffold. The TiO_2 incorporation resulted in a decrease in pore size and swelling behavior and improvement in the mechanical property of the scaffolds. Thus TiO_2 can be used as an efficient filler material for the design of scaffolds for tissue engineering but a better result can be obtained with a 3D structure with inter-connective pores.

2.9 NANOFABRICATION TECHNOLOGIES IN TISSUE ENGINEERING

For the successful formation of a tissue-engineered construct, it is very important to consider about the scaffolds which serves as the mechanical platform for the adhesion, proliferation, differentiation etc. of the cells. There are various conventional methods for the fabrication of scaffolds, though there are some important methods too for the fabrication of scaffolds and are explained in the following sections.

2.9.1 ELECTROSPINNING

Electrospinning is an approach to prepare nanofibrous networks and is a cost- effective method for the fabrication of micro-to-nanometer scale diameter fibers, with very high specific surface area (Liang et al., 2007). Applications of electrospinning include tissue engineering scaffolds, catalytic nanofibers, filtration membranes, and fiber-based sensors.

A simple and inexpensive nature of the experimental set up is an attractive feature of the electrospinning. It consists of a syringe pump, a voltage source and a collector. During the process, a polymeric solution in the syringe is held at the tip of a needle due to surface tension. An electric field is applied using high voltage sources which provide a charge to the solution. With the increase in the electrical potential the solution overcomes the surface tension and forms a jet that is ejected out from the tip of the capillary tube or a syringe and gradually thins due to solvent evaporation and elongation and forms randomly oriented nanofibers which are collected on a stationary or rotating collector (Vasita and Katti, 2006). This process can be used successfully to spin synthetic or natural polymers into fibers of many kilometers in length. A schematic representation of electrospinning set up is shown in Figure 2.6.

The typical electrospinning set up can be modified to produce fibers with unique morphologies. Co-axial two capillary spinneret can be used to electrospin hollow nanofibers, and for aligned nanofibers a rotating drum collector can be used.

The process of electrospinning can be controlled or manipulated by many variables such as those as listed hereunder.

1. Solution properties: This includes viscosity, surface tension, polymer molecular weight, dipole moment, conductivity, dielectric

constant etc. Varying of any one of these parameters will affect the other and so the effect of these properties cannot be isolated.

2. Controlled variables: This includes flow rate, field strength of the electric field, design of the needle tip, distance between the tip and the collector, composition and geometry of the collector etc.
3. Ambient parameters: This involves velocity of the air, temperature, and humidity.

The electrospinning technique controls the thickness, composition, and also porosity of nanofibers with a simple experimental set up. The electrospun nanofiber with a high porosity and surface area allows favorable cell interactions and hence becomes potential candidates for tissue engineering application. Electrospinning provides a simple and cost-effective method to produce scaffolds with interconnecting pores and submicron range compared to other techniques like phase separation and self-assembly. It has been used for the preparation of 3D scaffolds using natural polymers, synthetic polymers, composite of both natural and synthetic polymers etc. Functionalized scaffolds for increasing the biocompatibility can also be prepared. However, in spite of the comprehensive experimental and theoretical studies explaining the ability to control fiber formation, fiber diameter uniformity is still a problem that needs to be addressed. Control of fiber morphology is necessary for the improved scaffold design that recreates functions of native ECM. For tissue engineering applications, designer scaffolds with dimensions that are clinically relevant and that support a homogeneous distribution of cells need to be addressed (Pham et al. 2006).

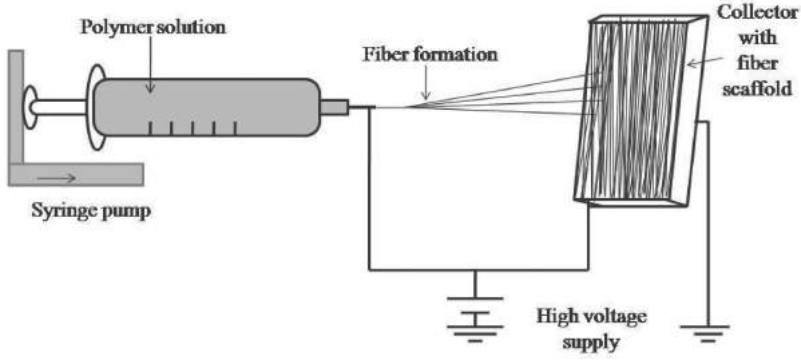

FIGURE 2.6 Schematic representation of electrospinning set up

2.9.2 NANOLITHOGRAPHY

The fabrication of nanometer-scale structures or patterns with at least one lateral dimension in nanometer scale (between the size of an atom and approximately 100 nm) is termed as nanolithography. Many techniques like X-ray, e-beam, imprint etching, and scanning probe lithographies have been used in patterning surfaces for tissue engineering (Laurencin and Nair, 2008). Various instruments used in nanolithography include scanning probe microscope (SPM), atomic force microscope (AFM) etc. for printing and etching in a single atom dimension on the surface. Nanolithography can be utilized in the semiconductor fabrication like integrated circuits, nanoelectromechanical systems, and other scientific fields in nanoresearch.

In electron beam lithography, highly focused beam of electrons are scanned over the surface of the substrate and with the help of a design editor and pattern generator these electron beams are guided. The surface contains an electron beam sensitive resist which generates a resist mask which is then used for the transfer of nanopattern. Dip-pen lithography (DPN) is another kind of lithography in which an AFM is used for the patterning even below 100 nm level. Here, the tip or cantilever of the AFM coated with a chemical compound or mixture (ink) acts as the pen and kept in a substrate contact. DPN emerges as a potential tool for manipulating cells at subcellular level resolutions. Activities like cell adhesion, patterning of subcellular ECM proteins, cell sorting etc. can be performed using DPN (Pulsipher and Yousaf, 2010).

Nanoimprinting lithography (NIL) is another kind of nanolithography used for fabrication of nanoscale patterns. The imprint resist used here is a formulation of monomer or polymer that can be cured by using UV light or heat. Many reports exist that explains the application of NIL in tissue engineering. Guillemette et al. have studied the effect of the surface topography and the interaction between cell-cell and cell-ECM interactions in cultured tissue by patterning polystyrene and a thermoplastic elastomer (TPE) using NIL and replication molding (replication molding is a variation of NIL) (Guillemette et al., 2009). Another work by Matsuzaka et al. (2003) fabricated polyester gratings using replication molding and studied the growth of osteoblast cells. Further, poly (methyl methacrylate) (PMMA) was patterned by NIL by another group for studying the reaction of neuronal process toward the grooves and ridges of the patterns (Johansson et al., 2006). Apart from the 1D gratings and groove structures,

2D pillar or hole structures were also studied and found that even similar features like circular holes or pillars arranged in different patterns like circular or hexagonal array have shown different cell responses toward it. Because of its capability for patterning substrates that are interesting for tissue engineering and also low cost and high throughput realization, NIL has a promising future in tissue engineering. The schematic representation of nanoimprinting lithography is shown in Figure 2.7.

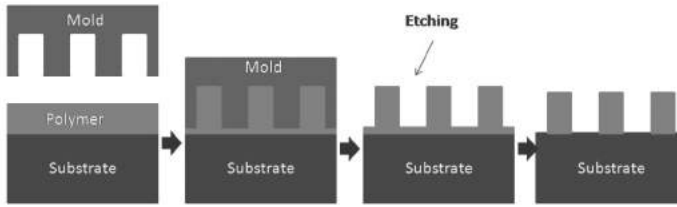

FIGURE 2.7 Schematic representation of nanoimprinting lithography

2.9.3 NANOPRINTING

Nanoprinting is another kind of nanofabrication technology for fabrication of 3D objects which is depicted in Figure 2.8. Further, 3D nanoprinting is a unique benefit of NIL that supports patterning of 3D structures. With this technique of 3D nanoprinting, the products and structures of our need can be constructed independent of the complexity of its shape. The concept of 3D printing at the nanoscale level will have various advantages like less wastage, economic viability, speed etc. (Li et. al., 2001).

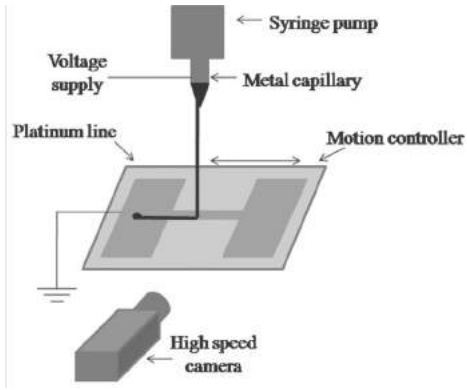

FIGURE 2.8 Schematic representation of nanoprinting

2.10 FUNCTIONALIZATION OF SCAFFOLDS WITH BIOMOLECULES FOR VARIOUS TYPES OF TISSUE ENGINEERING APPLICATIONS

Tissue engineering involves use of porous scaffold that can provide ambient conditions for the growth of target cells that are intended to grow inside or surface of the scaffold. Such growth inside the scaffold is mostly possible only when we follow a tissue engineering triad involving appropriate cells, relevant signaling molecules or biomolecules, and a proper porous scaffold. An effective cell adhesion as well as the growth and retention of differentiated cell's function in a scaffold depend on many factors such as bio-mimetic surface, oxygen tension, growth factor, immobilization or incorporation method of growth factor, controlled combinatorial activity of key signaling molecules or growth factors from scaffold or biomaterial, hydrophilicity of scaffold etc.

Scaffolds are having different surface properties like hydrophilicity or presence of functional groups on the surface which play a key role in cell adhesion, proliferation etc. For achieving an enhanced growth and regeneration in terms of cell attachment, cell proliferation, and matrix secretion, it is important to optimize the cell-biomaterial interactions and this can be achieved by the physical and chemical modification of the scaffolds. One such attempt is the modification with various cell surface receptors like integrin. Integrin receptors have arginine-glycine-aspartic acid (RGD) peptide sequence and mediate cell-matrix interactions and have been used in various studies for enhanced cell attachment (Orlando and Cheresh, 1991). PEG scaffolds with RGD domains also can direct cell regulation and proliferation. Another method is functionalizing the scaffolds with various functional groups like phosphates, amides, sulfonates etc. (Kuo et al., 2010). Addition of collagen-platelet composite (CPC) to a suture in a porcine model has shown an enhancement in suture repair via increased cellularity within the region of healing. Another example is the modification of silk fibroin performed by blending it with hyaluronan. Poly (sodium styrene sulfonate) (PNaSS) has been used as a functionalization agent for PET scaffolds because of the increased adherence shown by fibroblasts onto the surface compared with nonfunctionalized fibers (Ghasemi-Mobarakeh et al., 2010; Zhou et al., 2007). Figure 2.9 depicts the different types of scaffold modification.

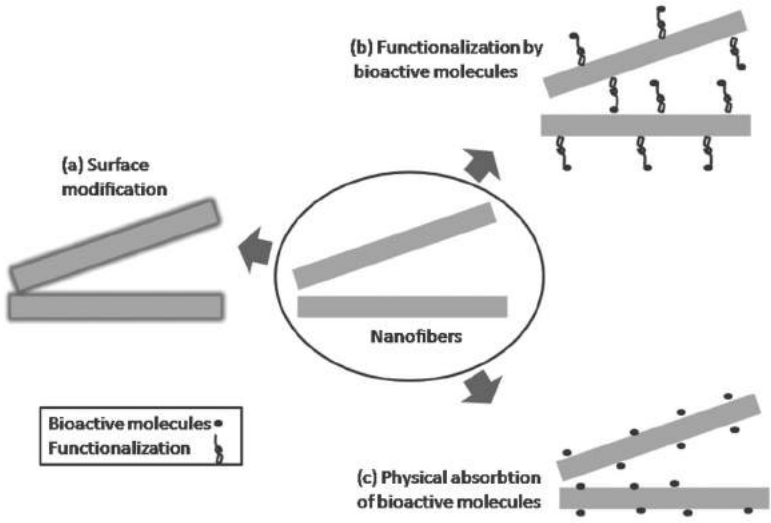

FIGURE 2.9 Different types of scaffold modification

2.11 3-D ARCHITECTURE AND CELL INCORPORATION

Tissue-engineered products traditionally involve seeding of cells on the scaffold, a structure that is capable of supporting 3D tissue organization and development. The scaffold which is either synthetic or biological or both in origin can be conjugated to bioactive materials like ECM or growth factors. But all type of materials available cannot be used for the preparation of 3D scaffolds because of their difference in chemical and physical properties and processability. The cell morphology is highly correlated with the cellular activities and the function and proliferation of cells are favored by strong cell adhesion and spreading. Maintenance of cell polarity is another important factor especially in case of epithelial cells and can be provided by a heterogeneous extracellular environment. In addition to cell morphology, functions of many organs are dependent on the 3D spatial relationship of cells with its ECM, for example, the relation between the shape of the skeletal tissues which is important for its function.

The regulation of gene expression is regulated differentially in 2D and 3D culture substrates. A suitable scaffold is critical that act as a template to direct the cell growth and ECM formation and development of 3D structure (Ratner, 2004). Obtaining uniform cell seeding at high densities and

maintaining nutrient transport to cells in the scaffold are the major obstacles in the in vitro development of 3D cell-polymeric constructs. The hydrodynamic and biochemical factors in the cell environment should be controlled and is necessary to achieve a desired spatial and temporal distribution of cells. The scaffolds produced can be then placed in suitable culture systems like static culture, spinner flask culture, rotary vessel culture, perfusion culture etc. for seeding and growth.

As compared to the conventional approach of diffusion-based chemical modification of the cellular environment (i.e., by adding growth factors directly into the tissue culture media), the 3D physical microenvironment is a better option as a tool to control cell differentiation and significantly improved spatial control and reproducibility (Willerth and Sakiyama-Elbert, 2008). The growth factors needed for the growth and differentiation of the cells can also be incorporated into the 3D scaffolds so that it can be released in a controlled manner and there is no need for the addition of these factors to the culture media itself. This is an advantage when we consider the implantation of the scaffolds into the native environment where they have to support cell differentiation over a repair period in vivo. This acts in a way endogenous factors act in the ECM during development. The goal of creating 3D scaffolds for tissue engineering is to increase the ability to direct stem cell differentiation along specific paths and simultaneously improve the scalability of the cell production to large capacity needed for the clinical applications.

2.11.1 ROLE OF STEM CELLS IN TISSUE ENGINEERING

Tissue engineering involves the combination of living cells with a natural/synthetic support or scaffold to build a 3D living construct and the development of such a construct requires cautious selection of four key factors: scaffold, growth factors, ECM, and cells. The cells selected for tissue engineering should provide a long lasting repair of the damaged tissues. A sufficient number of cells must be produced to fill the defect and (1) they should differentiate into desired phenotypes, (2) must take up a 3D structural support/scaffold and produce ECM, (3) should be structurally and mechanically compliant with the native cells, (4) should be able to integrate with the native cells and must overcome immunological rejection, and (5) should be allied with minimal biological risks (Vats et al., 2002).

2.11.2 STEM CELLS

Stem cells are undifferentiated cells that can differentiate into specialized cells and divide (via mitosis) to produce more stem cells. They have a remarkable potential for developing into different cell types in the body during early life and growth and also serve as an internal repair system by dividing limitlessly and replenishing other cells till the person/animal is alive. When a stem cell divides, each new cell has the potential to remain as stem cell or some other type of cell with a more specialized function like a brain cell, red blood cell etc. Two important characteristics of stem cells are: (1) they are unspecialized cells that are capable of renewing themselves by frequent cell divisions even after a long period of inactivity and (2) they can be induced to form tissue-specific or organ-specific cells with specialized functions under special conditions.

Stem cells can be broadly divided into three types: (1) embryonic stem cells (grown in laboratory from cells of early embryo), (2) adult/tissue stem cells (found in our body for whole life), and (3) induced pluripotent stem cells/reprogrammed stem cells (similar to embryonic stem cells but formed from adult cells/specialized cells). Primary cells taken from the patient have been used in conjugation with scaffolds to produce tissues for re-implantation but the invasive nature of the cell collection and the chances of disease limit this strategy. Thus we focus on use of stem cells like embryonic stem cells (ESCs), bone marrow mesenchymal stem cells (BM-MSCs), umbilical cord-derived mesenchymal stem cells (UC-MSCs) etc. (Howard et al., 2008). The cells for tissue engineering can be from autologous, allogenic, or xenogenic sources. The cell sources can be further portrayed into adult stem cells or somatic stem cells, mature (non-stem) cells, ESCs, and totipotent stem cells or zygotes.

2.11.3 STEM CELLS IN TISSUE ENGINEERING

Because of the high proliferation capacity and the ability to acquire diverse cell fates depending upon the tissue in which they relay, stem cells have been used for tissue engineering and tissue regeneration studies. The stem cells can be (1) directly injected into the injured site or (2) grown in tissue culture flask and then conjugated with the scaffolds for the regeneration of the wound, or (3) can be used as a part for scaffold in therapeutic purposes.

The pluripotent cells utilized in tissue engineering are from hematopoietic cells derived from adult peripheral blood (bone marrow). These cells can be induced to differentiate into osteoblasts, adipocytes, myocytes etc. and are good candidates in tissue rebuilding (Stachowiak and Tzanakakis, 2011). Another type of pluripotent cell is mesenchymal stem cell which multiplies and gives rise to various other cell types and regenerates the damaged tissues. They also produce various compounds for the maintenance of the newly formed tissues and angiogenesis. All these will be mediated by various growth factors and proteins. Studies on a variety of systems highlights great prospects for the future of stem cell-based tissue engineering but only a few areas have shown its translation into clinical reality. A large number of tissue types ranging from epithelial surfaces like skin, mucosal membranes, and cornea to skeletal tissues can be engineered by using stem cells. The two main applications for the stem cells toward tissue engineering are: first the formation of 2D sheets like regeneration of skin and second is the reconstruction of a 3D structure like bone (Bianco and Robey, 2001).

Identification of novel stem cell technologies with materials that are able to deliver a combination of growth factors leading to reconstructive surgery or organ replacement are the important requirements for engineered tissue (Howard et al., 2008). In vitro bioreactors and the development and use of microfabrication technologies for creating vascularized tissues and organs are another important areas being investigated. Since the stem cell researches and its clinical applications are still debated, it is very necessary to consider the social, legal, and ethical issues regarding these experiments.

2.12 TISSUE ENGINEERING FOR REPLACING BODY PARTS

The applications of tissue engineering cover a broad range, but this term will be mostly associated with the repair or replacement of tissues or organs like bone, cartilage, skin etc. some of these applications are explained in the following sections.

2.12.1 ENGINEERING TISSUES FOR REPLACING SKIN

Skin is the largest organ of our body and is vital for the survival of the organism by acting as a barrier to the environment against adverse conditions.

It constantly undergoes regeneration and also possesses the capacity to repair wounds depending on the different types of stem cells in the skin. Engineered skin substitutes serve as an important medical application for the extensive burn wounds. The tissue constructs presently available lacks normal appendages of skin like sebaceous gland, sweat gland, hair follicles, and normal mechanical properties of the skin and thus cannot restore the normal skin anatomy (Wong and hang, 2009).

Skin damages mainly occur by chronic wounds (venous pressure and leg ulcers), burn injuries, skin excision, tumors, and other dermatological conditions. Tissue-engineered substitutes promote the regeneration of epidermis and dermis, prevents fluid loss, and provides protection from contaminations and can deliver ECM components, cytokines, growth factors, drugs etc. to the wound site that enhances the healing process and can be used with autografts. Bruke et al., in early 1980s successfully created artificial skin using fibroblast cells seeded on the collagen scaffolds for treatment of extensive burn injury and this is still being used. Skin substituents that are made from cell-seeded collagen have been commercialized extensively (Cen et al., 2008). Examples of commercially available skin substitutes are provided hereunder.

Epidermal substitutes- Epicel® and CellSpray® (cell based), Myskin™ and Laserskin® (scaffold-containing cells), ReCell® (autologous epidermal cell suspension)

Dermal substitutes- Integra®, Hyalomatrix PA® and AlloDerm® (cell free), Dermagraft®, TransCyte®, and Hyalograft 3D™ (scaffold-containing cells)

Dermoepidermal substitutes- OrCel®, Apligraf® (natural-based scaffold containing cells) and PolyActive® (synthetic scaffold-containing cells)

Bottom-up and top-down approaches are two important strategies for skin regeneration and repair where the top-down or scaffold approach uses a temporary scaffold as a substrate and bottom-up using cell aggregates to produce tissue-engineered construct without using scaffolds. Natural fibers like collagen, chitosan etc. and synthetic polymers like poly-L-lactide (PLLA), polycaprolactone (PCL), polyglycolic acid (PGA) etc. have been used either alone or in combination in skin tissue engineering. PCL membranes containing ZnO nanoparticles haveshown ability to promote wound healing without scar formation. Skin substitutes that are derived from skin stem cells hold promise for feasible gene therapy for skin. Skin

tissue engineering is a maturing field that has benefited patients since the 1990s and it is hoped that new biomaterials will be produced to overcome many problems that exists in current approaches (MacNeil, 2008). Electrospun polycaprolactone membranes incorporated with ZnO nanoparticles perform as skin substitutes with enhanced fibroblast proliferation and wound healing (Augustine et al., 2014b).

2.12.2 ENGINEERING TISSUES FOR REPLACING LIVER

The liver has a complex structure and performs a myriad of functions in the body. It is a highly regenerative organ but the use of drugs, toxins, and viral infections leading to various diseases cause extensive damages to the hepatocytes that reduce its function and regeneration. Liver transplantation is the definitive treatment for the end stage liver failure but the shortage of organs limits the transplantation procedure (Palakkan et al., 2013). Organs like liver are found to be difficult to engineer, partly due to the lack of a well defined circulatory system. Liver cell (hepatocyte) transplantation offers a possible solution to overcome the organ shortage, one of the major limitations in organ transplantation. But the isolated liver cells suffer during the isolation and cryopreservation procedures which is one reason for the limited success of this transplantation procedure.

Tissue engineering approach created new liver tissue providing a potential solution to the obstacles that challenge liver cell transplantation. The primary cell sources for liver tissue engineering have been adult primary hepatocytes but the limited availability of quality human liver hepatocytes limited its use. Hepatocytes from different animals like rat, pig, mouse etc. were used for grafting liver construct. ECM has a very important role in maintaining the structure and function of the liver cells.

In vitro cultures in dishes which are coated with ECM like matrigel, fibronectin, collagen, as well as mixtures of collagen and fibronectin were able to preserve functions of hepatocyte for a short time (Castell and Gomez-lechon, 2009). Naturally derived polymers like collagen, alginate, chitosan etc. and synthetic biopolymers like the polymer family- poly(hydroxyl acid) which include PGA, PLA, PLGA copolymer, and their modified derivatives have been developed for liver tissue engineering. Scaffolds of porous sponge have been the most extensively used type of biodegradable scaffolds for the ex vivo culturing and transplantation

of hepatocytes (Mikos et al., 1993, 1994). These sponges and bonded fiber structure of highly porous surface (83% porosity) are used to support growth of hepatocytes in vitro and in vivo. Generation of advanced bioreactors in the mid-1990's, where the liver cells can be cultured in 3D scaffolds, has revolutionized the liver tissue engineering (also known as bioreactors for tissue engineering) (Catapano et al. 2010). The advent in the field of tissue engineering is limited by the challenge of finding the right scaffold material and architecture to facilitate the function of hepatocyte and survival in vivo. The success in this field seems to provide the promise of creating engineered liver for the clinical transplantation in future (Uygun and Yarmush, 2013).

2.12.3 ENGINEERING TISSUES FOR REPLACING BONE

In populations where aging is coupled with poor physical activity and obesity, there is an expected increase in the incidence of bone disorders and other related conditions. Thus potential alternatives like engineered bone tissue has been viewed due to their infinite supply and lack of disease transmission. By the synergistic combination of biomaterials, cells and factor therapy, the bone tissue engineering aims at inducing new functional bone regeneration (Amini et al., 2012). In a wide range of clinical settings bone grafts are being used to augment bone repair and utilization. Though osseous tissue has a unique internal repair capacity of healing and remodeling without any scar formation, several conditions both acquired and congenital are necessary in bone replacement (Buckwalter et al., 1993).

The process of bone regeneration was uncovered by Prof. Marshall R. Urist, an orthopedic surgeon at the University of California, Los Angeles. Bone, being very alive, constantly rebuilds itself and the porous framework of bone is composed of collagen protein fibers which run through hydroxyapatite (hydroxyapatite is a mineral that makes up 70% of living bone). The problems like lack of bone availability for autografts, immune rejections from recipients, and transmission of diseases made it necessary to find substances more closely related to the real bone, like hydroxyapatite.

In 1992, the FDA approved a synthetic bone implant called "Pro Osteon" which is a calcium phosphate material that mimics hydroxyapatite.

However, this material lacks strength needed for weight-bearing bones but possess zero rejection. Another product "Megagraft 1000", a bioceramic processed from calcium metal, calcium hydroxide, and phosphoric acid encouraged faster bone growth. In 1993, another product "Collagraft", made out of hydroxyapatite/tricalcium phosphate and bovine collagen then mixed with the bone marrow of the patient have been approved by the FDA.

Functional bone tissue engineering is another technique which uses bone morphogenetic proteins (BMPs) which have great importance in increasing the bone regenerative potency and is done with the help of gene therapy. Synthetic polymers like PLA, PGA, PLGA, polyanhydrides such as poly (methacrylated 1, 6-bis (carboxyphenoxy) hexane), poly (methacrylated sebacic anhydride), a non-degradable polymer, poly (ethylene glycol) (PEG) etc. have shown good results in bone tissue engineering (Fisher and Reddi, 2003). A wide range of bioactive inorganic materials like tricalcium phosphate, bioactive glass, and HA and their combinations having a similar composition as that of bone are widely in use.

2.12.4 ENGINEERING TISSUES FOR REPLACING CARTILAGE

Articular cartilage exhibits very less capacity for intrinsic repair (poor regenerative properties), also it is an avascular tissue and so even very minor injuries may lead to progressive damage and osteoarthritic joint regeneration which results in pain and disability. Articular cartilage tissue engineering aims at repairing, regenerating, and/or improve diseased or injured articular cartilage functionality and holds great potential for improvement of the articular cartilage therapy (Zhang et al., 2009). Numerous attempts have been done for the development of grafts for repairing chondral and osteochondral defects but still remains with significant challenges in the clinical application of cartilage repair (Johnston et al., 2013).

The three types of cartilages in human body are hyaline cartilage (e.g., in diarthrodial joints), fibro cartilage (e.g., knee meniscus), and elastic cartilage (e.g., ear). Articular cartilage that covers bone surfaces is a soft and specialized hyaline cartilage which possesses superior lubrication, wear, and low friction properties and also reduces stresses in the joint. Various cell sources for the repair and regeneration of articular cartilage involves chondrocytes which is the sole source of cells, stem cells like mesenchy-

mal stem cells, ESCs, dermis of the skin etc. Due to the biocompatibility of the natural biomaterials, scaffolds used in cartilage tissue engineering includes carbohydrate-based hyaluronic acid, alginate, chitosan, agarose and protein-based fibrin glue, collagen etc. are used and synthetic scaffolds like PLA, PGA, PLGA etc. are also used. Methods like electrospinning, particulate leaching, phase separation, and 3D printing techniques can be used for the preparation of nanofibrous 3D scaffolds (Zhang et al., 2009). Hydrogels based on hyaluron, PEGs etc. are also used for cartilage tissue engineering. Gene therapy is in an infant stage in cartilage tissue engineering, growth factors like insulin like growth factor-19 (IGF-1and transforming growth factor beta 1 (TGF-β1) have been successfully transfected within chondrocytes for the increase in the expression of collagen aggrecan. By the method of press-coating, an in vivo engineered cartilage construct was developed on PLA scaffold in a one-step method (Tuli et al., 2003). Self-assembling peptide hydrogel scaffolds are suitable candidates for cartilage tissue engineering. A better understanding for the development of clinically feasible designs in disease compromised animal models should be the future approach in the cartilage tissue engineering. Various evidences existing today represent a potentially sound approach to treat cartilage injury or trauma by the idea of tissue engineering.

2.12.5 ENGINEERING TISSUES FOR REPLACING TENDONS

Tendon is a connective tissue, physically binds muscles to skeletal structures, permits locomotion, and enhances joint stability. The structure has multiunit hierarchial collagen molecules, fiber bundles, fibrils, fascicles and tendon units, and resists tensile loads. Collagen I is the most abundant molecular component in tendon formed by self-assembly of collagen molecules.

Tendon injuries are difficult to treat and the classical surgical reconstructive methods have significant limitations especially when there is large tendon deficit. So transplantation is the other option which also has certain limitations and tissue engineering has become a newer option for an answer even though the role of tissue engineering in tendon healing is still unclear (Moshiri and Oryan, 2012). Tendon tissue engineering induces self-regeneration of tendon tissue in vivo, or produces functional tissue replacement in vitro which is then implanted in the body. The impact of tissue engineering in tendon healing can be increased by involving in-

formation regarding the structure, injury, healing, host immune response, biomaterial characteristics etc. in the tissue engineering approaches.

Natural scaffolds tested for tendon tissue engineering involves collagen and chitosan. Collagen as it is a major component of tendon has shown good results when used as collagen gels and also as composites with synthetic polymers which showed an improved mechanical property and cell migration. Chitosan-based hyaluronan composite fiber scaffolds also have shown improvements in in vivo models. Synthetic polymers like PLA, PGA, and PLGA were used for tendon repairs. Cell sources include bone marrow derived mesenchymal stem cells, tissue derived mesenchymal stem cells tenocytes, and tendon sheath fibroblasts. Growth factors directly injected into the wound sites showed enhancement in the tendon repair. Tendon injuries can be managed by gene therapy by the delivery of genes that help in healing to the site of injury. In vivo transfer of genes for the production of growth factors like BMP-12, BMP-IGF-1) and in vitro transfer of smad proteins (a group of intracellular proteins) co-expressed with BMP-2 has been practiced recently. Tendon tissue engineering is in an infant stage and translation of various investigations to clinics involves several important concerns like in vivo efficiency of the graft, host immune reactions etc. (Hampson et al., 2008).

2.12.6 ENGINEERING TISSUES FOR REPLACING LIGAMENTS

Like tendons, ligaments are also composed of collagen fibers but less densely packed and woven unlike the parallel arrangement of tendon. They connect two or more bones and are responsible for joint movement and stability. Rupture in ligament leads to abnormal joint kinematics and irreversible damage of the surrounding tissues which lead to degenerative diseases that do not heal naturally and cannot be repaired completely by conventional clinical methods. Various advantages of ligament tissue engineering are minimal patient morbidity, reliable fixation methods, infection or disease transmission, biodegradation along with adequate mechanical stability, rapid return to the preinjury functions etc.

Currently, anterior cruciate ligament (ACL) and medial collateral ligament (MCL) and glenohumeral ligaments are the most frequently practiced ligament tissues for tissue engineering, whereas all ligaments are in the pursuit of tissue engineering and studies are carried out to create functional replacements of tissues (Yilgor et al., 2011). Growth factors

like IGF-1, TGF-β, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), platelet-rich plasma (PRP), and collagen-PRP-complex (CPC) are effective in ligament cell proliferation and matrix formation alone or in combination. The age and origin of the fibroblast also affects the proliferative response to PDGF and bFGF. The major cell source in tendon tissue engineering is MSC which can differentiate into various connective tissues. Both natural and synthetic materials are widely used in the form of gels, membranes, or 3D scaffolds. Collagen, hyaluronic acid, silk, poly (β-hydroxybutyrate) (PHB), poly-3-hydroxy-10-undecenoate (PHUE-O3), poly-3-hydroxybutyrate-co-hydrovalerate (PHBV) etc. are the examples for natural replacements and dacron polyester, PGA, PLLA, poly (lactic acid-co-glycolide) etc. are examples for synthetic materials used in ligament tissue engineering.

Ex vivo bioreactors can be used for the controlled biochemical and physical regulatory signals to guide the tissue development. In order to assemble ligament tissue structures, combinations like braiding, stratifying, knitting, or 3D braiding scaffolds and also merging of scaffolds with different material types and aligning its cellular content, functionalizing the surface, and adding mechanical stimulation have to be done (Yilgor et al., 2011).

2.13 GENE THERAPY AND TISSUE ENGINEERING

Gene therapy is a technique for correcting the defective genes that are responsible for the disease development by supplementing the defective gene with a functional one. Gene therapy can be either germ-line or somatic cell gene therapy. Somatic gene therapy is the most exclusive gene therapy and involves introduction of genes to somatic cells of an affected individual, whereas germ line gene therapy involves the permanent transmissible modification of the genome of a gamete, a zygote, or an early embryo. The prospect of human germ line gene therapy is not sanctioned currently.

It is been around 20 years since the clinical development of gene therapy started and at present over 320 ongoing clinical trials for gene therapy are going on regulated by the FDA. The products of gene therapy initially appear promising as they reflect strong scientific foundation and offers hope for treating rare and life-threatening disorders but despite all this the

field of gene therapy has not yet produced any successful products that have gone through clinical trials and proved safe and effective for marketing approval. The results obtained from a trial for treating X-linked severe combined immunodeficiency (X-SCID) and several reports of encouraging results have created subsequent enthusiasm in the field of gene therapy (Takefman and Bryan, 2012).

By using intraperitoneal or intravenous injection, somatic cells are transfected, but after the implantation in the organism the survival of transfected cells is limited. By implanting vascular implants called organoids or neo-organs into the organism will serve as a support to these modified cells. This helps in the localization and the accessibility of the implant, to record the cell survival and progress of the implants and also improves the survival rate of the cells. If necessary, by removing the implants this treatment could be stopped. Angiogenisis was shown by introducing a sponge of type I collagen impregnated with acidic fibroblast growth factor (aFGF) in the abdominal cavity. In another experiment hepatocytes showed longer survival rates with the intraperitoneal implant of cultured microbeads of dextran (Thompson et al., 1989).

2.13.1 IN HEREDITARY DISEASES

Hereditary diseases like phenylketonuria, hypercholesterolaemia etc. where the organ concerned is not affected by the deficit circulatory protein and a partial correction will be enough to obtain an improvement in the disease conditions, gene therapy is the best suited solution. Neo-organs with polyfluorethylene (PTFE), type I collagen gel and recombinant human bFGF containing autologous cells were used for the long-term correction of genetic defect of β-glucuronidase gene (Moullier et al., 1993). Continuous secretion of erythropoietin and hemophilia factor VIII from a neo-organ in mice has also succeded. Matrices based on nylon and collagen along with cells transfected with genes have been implanted in athymic mice resulted in the production of human transferin (hTf). Another example is the formation of a collagen matrix with cells producing growth hormone implanted in hypophysectomized rats which showed a positive result.

2.13.2 IN CANCER

Neo-organs can be used for the treatment of cancer by increasing the immune response by stimulating immunogenic neo-antigens or immunity stimulating lymphokines like tumor necrosis factor (TNF), interlukin-4, interlukin-12, interferon (INF) α, β, γ etc. Cells transfected with G-CSF (granulocyte colony stimulating factor) gene and cultivated in collagen matrices successfully secreted G-CSF when implanted in mice (Chevallay and Herbage, 2000).

2.13.3 IN TISSUE REPLACEMENT

Tissue engineering constructs can be supplemented with various types of growth factors like BMPs, IGFs etc. by seeding of transfected cells (cells that are transfected with specific genes that codes for the production of various growth factors) into the scaffolds. This approach can in turn increase the rate of cell growth and adhesion and fast healing. But this method of gene transfection in tissue engineering is in a beginning stage and requires more investigations and clinical trials.

2.14 TISSUE ENGINEERING AS ALTERNATIVE TO DRUG SCREENING AND THERAPY

Production of a large number of healthy cells for repopulating a damaged site is the most important aspect of tissue engineering. It can be also considered as an alternative to drug therapy, gene therapy or whole organ transplantation. Tissue engineering can be utilized for the treatment of metabolic disorders. A metabolism can be said as a coordinated ensemble of chemical transformation controlled or regulated by various enzymes and a defective production of even a single enzyme can lead to various metabolic disorders. These missing links can be corrected or the effects of the disorder can be reduced by gene therapy or drug therapy.

The introduction of 3D cultures based on the combination of cells, scaffolds, and biomolecules have integrated microchip and microfluidic approaches to tissue engineering. The techniques such as replica molding, photolithography, and microcontact printing enable us to control cell position morphology and function by creating microscale-level structures.

The microfluidic approaches help in the manipulation of small amounts of fluids in hollow chambers, generates and tune the spatiotemporal gradients of nutrients and oxygen. This combination will lead to the organ-on-chip microdevices and represent a potential substitute for the animals in drug screening process. This in turn reduces the gap between 2D cultures and animal models (Huh et al., 2011).

As the results obtained with the animal models cannot be directly translated to humans because of the species specificity of drug action. 3D cultures are also being introduced in drug screening procedures to analyze the drug action, effectiveness, and to reduce the investment. Thus by transplanting human cells, significant efforts have been made for "humanizing" mice although it is expensive to adopt this in an assay format. Such experiments have been performed by culturing hepatocytes which then regains their morphology and protein expressions (Griffith and Swartz et al., 2006; Meli et al., 2012).

2.15 FUTURE

"Tissue engineering" is the term that represents a new concept focusing on regeneration of new tissues from cells with the support of biomaterials and growth factors. The term was coined around 30 years back for this interdisciplinary engineering method and has attracted much attention as a therapeutic means. This offers hope for a large number of patients with injuries, organ failures, and other clinical issues. These patients are treated currently also with transplantation of organs but as the number of patients increases day by day, there is a great need of donor organs. The new cases of organ failures increase each year and thus scientists in the field of regenerative medicine applies various principles of material sciences, bioengineering, and cell transplantation for constructing substitutes that restores and maintain normal function in diseased and injured tissues.

Various achievements acquired with the bladder, blood vessel, and tracheal replacements using tissue engineering have encouraged scientists to engineer other organs also in the laboratory that can cast light on various unsolved problems in tissue engineering. Some of the problems faced are the lack of innervations of tissues and organs which is a very important part for the full functionality of the neotissues or organs. The clinical application of these engineered constructs is still very limited, including

skin, bone, cartilage, and capillary and periodontal tissues. Moreover tissue engineering is having a deep impact in the development of new therapies. For example, the developments of 3D cultures have reduced the gap between 2D cultures and animal models which facilitates a constant turnover of oxygen and nutrients under extended studies. But an unresolved issue is the translation of these 3D cultures to pharmaceutical level.

Tissue engineering has captured many advantages of normal cell culture over whole animal experiments. The relative transparency of the engineered tissue may allow the visualization of the structures and processes happening in the cells. It has a major role in physiological genomics, which link various pathways and products to phenotypes and physiological systems. The studies at the level of transcriptome, metabolome, proteome, and population levels have succeeded in identifying various genes responsible for the development as well as progression of various diseases. Later, various studies in recombinant, knock-out animals etc. assigned various phenotypes to the genome. Thus for various molecular level studies particularly those related to gene expression where the changes at the genomic level depicts changes in the phenotype, the 3D culture systems can be utilized. Several reports on the gene expression of the 3D cultured cells suggest that the expression levels of engineered constructs more closely parallels the in vivo situations. Many engineered skin substitutes utilized for toxicological testing and other clinical applications also showed similar level of expressions in case of both native and engineered tissues. All these reports points toward the use of engineered tissue as a model system for testing the gene expression and the effects of altered gene expression (Birgersdotter et al., 2005; Ghosh et al., 2005; Smiley et al., 2005).

Tissue engineering and human genomics together have a great potential in personalized medical care. There is a need of very intense analysis regarding the gene expression of healthy as well as diseased cells for creating molecular fingerprints of the disease stages. As the knowledge base regarding stem cell technology alarms, it may be also possible to produce engineered tissues by the differentiation of stem cells yielding an unlimited source of grafts for tissue replacement and repair. Since these grafts will be produced from the autologous cell source, the possibility of immune response and graft rejection can be minimized.

2.16 CONCLUSION

Taking into consideration the contributions given by tissue engineering toward science and human race and those that are going to happen in future, the 21st century can be considered as a revolutionary era that has marked its importance by this area of nanomedicine. In a wide sense, any manipulation that involves an alteration in structure and function of a tissue which may also include gene manipulation, surgical interference, hormonal therapy etc. can be considered as tissue engineering. A large number of obstacles and questions has to be resolved regarding the biocompatibility and establishment and functioning of the constructed tissues at present. The proper delivery of regulatory molecules remains another challenge to this, whereas the computer modeling for predicting the outcomes of the tissue product add up an advantage to the establishment of tissue engineering systems. The possible inevitable advantages of tissue engineering are the reduction in post-operative patient costs, improved patient care at less expense, and an enhancement in the quality of life with a reduction of cost. In conclusion, tissue engineering is an emerging field of science offering tremendous promise with the proper implementation of quality assurance.

KEYWORDS

- **Biomaterials**
- **Electrospinning**
- **Nanolithography**
- **Nanomedicine**
- **Stem cells**
- **Tissue engineering**

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