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BIOMATERIALS: DESIGN, DEVELOPMENT AND BIOMEDICAL APPLICATIONS

2

Gownolla Malegowd Raghavendra¹, Kokkarachedu Varaprasad^{2,3} and Tippabattini Jayaramudu^{1,3}

¹Synthetic Polymer Laboratory, Department of Polymer Science & Technology, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India ²Department of Materials Engineering, Faculty of Engineering, University of Concepcion, Concepcion, Chile ³Department of Polymer Technology, Tshwane University of Technology, Pretoria, Republic of South Africa

2.1 OVERVIEW

Trauma, degeneration and diseases often bring the necessity of surgical repair. This usually requires replacement of the skeletal parts that include knees, hips, finger joints, elbows, vertebrae, teeth, and other bodily vital organs like kidney, heart, skin, etc. All these materials which perform the respective function of the living materials when replaced are termed as “Biomaterials.” The Clemson University Advisory Board for biomaterials has formally defined biomaterial as “a systemically and pharmacologically inert substance designed for implantation within or incorporation with living systems” [1]. Biomaterial is also defined as “a nonviable material used in a medical device, intended to interact with biological systems” [2]. Other definitions of biomaterial include “materials of synthetic as well as of natural origin in contact with tissue, blood, and biological fluids, intended for use for prosthetic, diagnostic, therapeutic, and storage applications without adversely affecting the living organism and its components” [3] and “any substance (other than drugs) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body” [4]. As the definition for the term “biomaterial” has been difficult to formulate, the more widely accepted working definitions include: “A biomaterial is any material, natural or man-made, that comprises whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function” [5]. The word “Biomaterial” should not be confusing with the word “Biological material.” In general, a biological material is a material such as skin or artery, produced by a biological system.

The study of biomaterials is called ‘Biomaterials Science’ which encompasses the elements of medicine, biology, chemistry, tissue engineering, and materials science. A number of factors, including the aging population, increasing preference by younger to middle aged candidates to undertake surgery, improvements in the technology and life style, better understanding of body functionality, improved esthetics and need for better function resulted in enormous expansion of Biomaterial Science from day to day and it is supposed to be a continuous process. As the field of biomaterials experienced steady and strong growth, many companies are investing larger amounts of money for the development of new products.

Biomaterial is not of a recent origin. The introduction of nonbiological materials into the human body was noted many centuries ago, far back in prehistory. The remains of a human found near Kennewick, WA (often referred to as the “Kennewick Man”) concluded the usage of a spear point embedded in his hip which was dated to be 9000 years old [6]. Some of the earliest biomaterial applications were found as far back in ancient Phoenicia, where loose teeth were bound together with gold wires for tying artificial ones to neighboring teeth. The Mayan people fashioned nacre teeth from sea shells in roughly 600 AD and apparently achieved what we now refer to as bone integration. Similarly, a corpse dated 200 AD with an iron dental implant found in Europe was described as properly bone integrated [7]. Though there was no materials science, biological understanding, or medicine behind the followed procedures, still their success is impressive and highlights two points: the forgiving nature of the human body and the pressing drive, even in prehistoric times, to address the loss of physiologic/anatomic function with an implant [6]. It is understood from the sources that though there were no medical device manufacturers, no formalized regulatory approval processes, no understanding of biocompatibility, and no certain academic courses on biomaterials, yet crude biomaterials have been used, generally with poor to mixed results, throughout history.

In the modern times, early in the 1900s, bone plates were introduced to aid in the fixation of long bone fractures [8]. Many of these early plates broke as a result of unsophisticated mechanical design, as they were too thin and had stress concentrating corners. Also, materials such as vanadium steel though chosen as biomaterial owing to its good mechanical properties, corroded rapidly in the body and caused adverse effects on the healing processes. Hence, better designs and materials were soon followed. With the introduction of stainless steels and cobalt chromium alloys in the 1930s, greater success was achieved in fracture fixation, and the first joint replacement surgeries were performed [9]. As for polymers, poly(methyl methacrylate) was widely used for replacements of sections of damaged skull bones. Following further advances in materials and in surgical technique, in 1950s blood vessel replacements were tried and during 1960s, heart valve replacements and cemented joint replacements came into usage. Recent years have seen many further advances [10–12]. At the dawn of the twenty-first century, biomaterials are widely used throughout medicine, dentistry, and biotechnology. Biomaterials which existed 50 years ago did not exist today as they are replaced by newer ones that give much more comfort indicating the day-to-day advances in the biomaterials field [6]. Hence, keeping all these into consideration, the chapter is aimed to describe the design and development of biomaterials. In addition to these, biomedical applications are also discussed.

2.2 DESIGN OF BIOMATERIALS

Biomaterial is a nonviable (able to function successfully after implantation) substance intended to interact with biological systems. Their usage within a physiological medium is possible with the efficient and reliable characteristics of the biomaterials [13]. These characteristic features are provided with a suitable combination of chemical, mechanical, physical, and biological properties, to design well-established biomaterials [14]. These biomaterials are specifically designed by utilizing the classes of materials: polymers, metals, composite materials, and ceramics. Most of the

biomaterials available today are developed either singly or in combination of the materials of these classes. These classes of materials have different atomic arrangement which present the diversified structural, physical, chemical, and mechanical properties and hence offer various alternative applications in the body. The classes of the materials are illustrated in the following sections.

2.2.1 POLYMERS

Polymers are the convenient materials for biomedical applications and are used as cardiovascular devices for replacement and proliferation of various soft tissues. There are a large number of polymeric materials that have been used as implants. The current applications of them include cardiac valves, artificial hearts, vascular grafts, breast prosthesis, dental materials [15], contact and intraocular lenses [16], fixtures of extracorporeal oxygenators, dialysis and plasmapheresis systems, coating materials for medical products, surgical materials, tissue adhesives, etc. [17]. The composition, structure, and organization of constituent macromolecules specify the properties of polymers [13]. Further, the versatility in diverse application requires the production of polymers that are prepared in different structures and compositions with appropriate physicochemical, interfacial, and biomimetic properties to meet specific purpose.

The advantages of the polymeric biomaterials over other classes of materials are (i) ease to manufacture, (ii) ease of secondary processability, (iii) availability with desired mechanical and physical properties, and (iv) reasonable cost. Polymers for biomedical applications can be classified into two categories namely synthetic and natural. The synthetic polymeric systems include acrylics, polyamides, polyesters, polyethylene, polysiloxanes, polyurethane, etc. Though the processability is easy in case of synthetic polymers, the main disadvantage of these synthetic polymers is the general lack of biocompatibility in the majority of cases and hence their utility is often associated with inflammatory reactions [18]. This problem can be overcome by the usage of natural polymers. For example, the natural polymers such as chitosan, carrageenan, and alginate are used in biomedical applications such as tissue regeneration and drug delivery systems [18].

2.2.2 METALS

Metallic implant materials have gained immense clinical importance in the medical field for a long time. Many of metal and metal alloys which were used for medical requirements include stainless steel (316L), titanium and alloys (Cp-Ti, Ti6Al4V), cobalt chromium alloys (Co Cr), aluminum alloys, zirconium niobium, and tungsten heavy alloys. The rapid growth and development in biomaterial field has created scope to develop many medical products made of metal such as dental implants, craniofacial plates and screws; parts of artificial hearts, pacemakers, clips, valves, balloon catheters, medical devices and equipments; and bone fixation devices, dental materials, medical radiation shielding products, prosthetic and orthodontic devices for biomedical applications [13]. Though there are other classes of materials from which biomaterials can be prepared, engineers prefer metals as a crucial one to design the required biomaterial. The main criteria in selection of metal-based materials for biomedical applications are their excellent biocompatibility, convenient mechanical properties, good corrosion resistance, and low cost [19].

The type of metal used in biomedical applications depends on functions of the implant and the biological environment. 316L type stainless steel (316L SS) is the mostly used alloy in all implants ranging from cardiovascular to otorhinology.

The mechanical properties of metals have a great importance while designing load-bearing dental and orthopedic implants. However, when the implant requires high wear resistance such as artificial joints, Co Cr Mo alloys are used to serve the purpose. The properties of high tensile strength and fatigue limit of the metals allow them the possibility to design the implants that can carry good mechanical loads compared with ceramics and polymeric materials. In comparison to polymers, metals have higher ultimate tensile strength and elastic modulus but lower strains at fail-ure. However, in comparison to ceramics, metals have lower strengths and elastic modulus with higher strains to failure [20].

In the biologic medium, when the metal-based biomaterial is implanted, the surface of material can change and degrade to release some by-products. Owing to this releasing process, interactions between metallic implant surface and cell or tissues occur [13]. This factor has stimulated the present day researchers to give great importance in understanding the surface properties of metallic products in order to develop biocompatible materials.

2.2.3 COMPOSITE MATERIALS

Composites are engineering materials which contain two or more physical and/or chemical distinct, properly arranged or distributed constituent materials that have different physical properties than those of individual constituent materials. Composite materials have a continuous bulk phase called matrix and one or more discontinuous dispersed phases called reinforcement, which usually has superior properties than the matrix. Separately, there is a third phase named as interphase between matrix and reinforced phases [21]. Composites have unique properties and are usually stronger than any of the single materials from which they are made, hence are applied to some difficult problems where tissue in-growth is necessary. In recent years, scientific research has been focused to develop variety of biomedical composite materials because they are new alternative solutions for load-bearing tissue components.

Composite scaffolds with porous structure tailored from combinations of bioglass particles and biodegradable polymers with mechanical properties that are close to cancellous bone are potentially in use. Hard-tissue applications such as skull reconstruction, bone fracture repair, total knee, ankle, dental, hip, and other joint replacement applications are possible with fiber-reinforced composite materials [22].

The main advantage of the composite biomaterials is though the individual metals or ceramic materials suffer from disadvantages like exhibition of low biocompatibility and corrosion by metals, brittleness, and low fracture strength by ceramic materials, the composite materials provide alternative route to improve many undesirable properties of homogenous materials (metals or ceramics).

The properties of the constituent materials have significant influence on composite biomaterials. One of the factor “linear expansion” plays a crucial role in designing composite biomaterial. Often composites are made from constituents that have similar linear expansion constants. If the constituent materials possess distinct linear expansion constants, contact area (interface) between reinforcement and matrix materials can generate large voids through the contact surface, which blots the

purpose of the implant. Therefore, more care is required in selection of individual constituents while processing the composite biomaterial by bone tissue engineers.

2.2.4 CERAMICS

Ceramics are another class of materials used for designing biomaterials. The use of ceramics was motivated by their inertness in the body, their assay formability into a variety of shapes and porosities, high compressive strength, and excellent wear characteristics. Ceramics are used as parts of the musculoskeletal system, hip prostheses, artificial knees, bone grafts, dental and orthopedic implants, orbital and middle ear implants, cardiac valves, and coatings to improve the biocompatibility of metal-lic implants. Though ceramics are utilized for designing biomaterials, yet they have been preferred less commonly than either metals or polymers. Applications of ceramics in some cases are severely restricted due to brittleness and poor tensile strength. However, bioceramics of phosphates are widely used to manufacture ideal biomaterials due to their high biocompatibility and bone integration, as well as being the materials that are most similar to the mineral component of the bones [23].

Among the ceramics, apatites occupied a prominent role. The calcium phosphate-based biomaterials are used in a number of different applications throughout the body, covering all areas of the skeleton. A few of its applications include dental implants, transdermic devices and use in periodontal treatment, treatment of bone defects, fracture treatment, total joint replacement, orthopedics, cranio-maxillofacial reconstruction, otolaryngology, and spinal surgery. Second, hydroxyapatite has been used as filler for bone defects and as an implant in load-free anatomic sites such as nasal septal bone and middle ear. It is also used to develop bio-eye hydroxyapatite orbital implants [24] and hydroxyapatite block ceramic [25]. In addition to these applications, hydroxyapatite has been used as a coating material for stainless steels, titanium and its alloys based implants, and on metallic orthopedic and dental implants to promote their fixation in bone. In this case, the fundamental metal surfaces to the surrounding bone strongly bonds to hydroxyapatite. However, care has to be taken to avoid delamination. Since, delamination of the ceramic layer from the metal surface causes serious problems and results in the implant failure [26].

The classes of the material that are used for designing biomaterials, their advantages and disadvantages are shown in [Table 2.1](#).

2.3 BASIC CONSIDERATIONS TO DESIGN BIOMATERIAL

Though a variety of devices and implants are designed to treat a disease or injury from the above-mentioned classes of materials, the fundamental aspects involved in designing of all the biomaterials are viewed from the following basic considerations:

- A proper specification for which a biomaterial is necessarily opted to design.
- An accurate characterization of the environment in which the biomaterial is desired to function and the effects that environment exhibit on the properties of the biomaterial.
- A delineation of the length of time up to which the material must function.
- A clear understanding of the biomaterial with respect to the safety concerns prior to usage.

Table 2.1 Different Classes of Materials are Used for Designing Biomaterials and Their Advantages and Disadvantages in Application Filed		
Class of the Material	Advantages	Disadvantages
Polymers		
Nylon, silicones, PTFE	Resilient Easy to fabricate	Not strong, Deform with time, May degrade
Metals		
Titanium, stainless steels, Co Cr alloys, gold	Strong, Tough, Ductile	May corrode, High density
Composites		
Various combinations	Strong, Tailor-made	Difficult to make
Ceramics		
Aluminum oxide, carbon, hydroxyapatite	Highly biocompatible, Inert, high modulus, Compressive strength, Good esthetic properties	Brittle, Difficult to make, Poor fatigue resistance

2.4 CHARACTERISTICS OF BIOMATERIALS

The fundamental characteristics that a biomaterial should possess in order to run successfully as an implant in the living system are mentioned below.

2.4.1 NONTOXICITY

A designed biomaterial should serve its purpose in the environment of the living body without affecting other bodily organs. For that, a biomaterial should be nontoxic. Toxicity for biomaterials deals with the substances that migrate out of the biomaterials. In general, nontoxicity refers to noncarcinogenic, nonpyrogenic, nonallergenic, blood compatible, and noninflammatory of biomaterial. It is reasonable to say that a biomaterial should not give off anything from its mass unless it is specifically engineered to do so. In some cases, biomaterial is designed to release necessary amount of masses that is considered toxic. This toxicity of the designed biomaterials gives an advantage. Example a “smart bomb” drug delivery system that targets cancer cells and destroys them.

2.4.2 BIOCOMPATIBLE

Biocompatibility is generally defined as the ability of a biomaterial, prosthesis, or medical device to perform with an appropriate host response during a specific application. All materials intended for application in humans as biomaterials, medical devices, or prostheses undergo tissue responses when implanted into living tissue. “Appropriate host response” includes lack of blood clotting, resistance of bacterial colonization, and normal healing. For a biomaterial implant to function properly in the patient’s body, the implant should be biocompatible.

2.4.3 ABSENCE OF FOREIGN BODY REACTION

The reaction sequence that generates due to the presence of a foreign body in a living biological system is referred as “foreign body reaction.” This reaction will differ in intensity and duration depending upon the anatomical site involved. Practically, a medical device should perform as intended and presents no significant harm to the patient or user; however, there will be chance to develop foreign body reaction, since any material other than autodeveloped bodily material (a biological material) is a foreign material. Hence, the biomaterial should show nil foreign body reaction.

2.4.4 MECHANICAL PROPERTIES AND PERFORMANCE

The most important requirement of the biomaterial is matching of its physical properties with the desired organ/tissue in the living system where it is to be implanted. Biomaterials and devices should necessarily possess suitable mechanical and performance requirements equivalent to that of the replacing organ/tissue. Hence, the materials are designed according to the tissue features where they are going to be used. The basic mechanical and physical requirements for the designed biomaterial are categorized in three ways that are mentioned below and tabulated in [Table 2.2](#).

1. Mechanical performance

Mechanical performance of the biomaterial indicates the mechanical characteristics of the designed biomaterials as medical devices for intended function in the living body environment. The mechanical characteristic of the biomaterial varies depending on the site of application. The biomaterials with strong and rigid properties that find applications to develop hip joints are completely unsuitable to develop heart valves, which should require biomaterials with properties like flexibility and toughness. The mechanical performance of some of the biomaterials is depicted in [Table 2.2](#).

2. Mechanical durability

Durability indicates the minimum period of duration up to which a biomaterial performs its intended function effectively. Obviously, a leaflet in heart valve must flex without tearing for the lifetime. The mechanical duration of some of the biomaterials is mentioned in [Table 2.2](#).

3. Physical properties

Biomaterials should possess specific physical property in order to perform its intended function. [Table 2.2](#) shows the required physical properties of a few biomaterials.

Mechanical Performance	
Biomaterial	Mechanical Characteristics
Hip prosthesis	Strong and rigid
Tendon material	Strong and flexible
Heart valve leaflet	Flexible and tough
Articular cartilage	Soft and elastomeric
Dialysis membrane	Strong, flexible, and nonelastomeric
Mechanical Durability	
Biomaterial	Mechanical Durability
Catheter	3 days
Bone plate	6 months or longer
Leaflet in heart valve	Must flex 60 times per minute without tearing for the lifetime of the patient
Hip joint	Must function under heavy loads for more than 10 years
Physical Properties	
Biomaterial	Physical Characteristic
Dialysis membrane	Permeability
Articular cup of the hip joint	High lubricity
Intraocular lens	Clarity and refraction

2.5 FUNDAMENTAL ASPECTS OF TISSUE RESPONSES TO BIOMATERIALS

All materials intended for application in humans as biomaterials, medical devices, or prostheses when implanted into living tissue undergo tissue responses. The fundamental aspects of tissue responses to materials are commonly described as tissue response continuum, where a series of actions that are usually initiated by the implantation procedure and by the presence of the biomaterial, medical device, or prosthesis are considered. These actions involve fundamental aspects of tissue responses including injury, inflammatory and wound-healing responses, foreign body reactions, and fibrous encapsulation of the biomaterial, medical device, or prosthesis.

2.5.1 INJURY

The process of implantation of a biomaterial, prosthesis, or medical device results in injury to the tissues or organs [27,28]. The response to injury is dependent on multiple factors including the extent of injury, the loss of basement membrane structures, blood material interactions, provisional matrix formation, the extent or degree of cellular necrosis, and the extent of inflammatory response. In situations where injury occurs with exudative inflammation and without cellular

necrosis or loss of basement membrane structures, the process of resolution occurs. Resolution is the restitution of pre-existing architecture of a tissue or organ. On the other hand, if the injury occurs with necrosis, then the granulation tissue grows into an inflammatory exudate and the process of organization with development of fibrous tissue occurs leading to formation of fibrous capsule at the tissue/material interface. The process of resolution or organization formation is determined on basis of the proliferative capacity of cells within the tissue or organ.

2.5.2 BLOOD MATERIAL INTERACTIONS AND INITIATION OF THE INFLAMMATORY RESPONSE

Early responses to the injury mainly involve blood material interactions which occur mainly through vasculature [27 30]. Regardless of tissue or organ into which a biomaterial is implanted, the initial inflammatory response is activated by injury to vascularized connective tissue. Apart from inflammatory response, changes in the vascular system induce changes in blood and its components and cellular events [29 32]. As blood and its components are involved in the initial inflammatory responses, thrombi and/or blood clots are often formed. Thrombus or blood clot formation on the surface of a biomaterial is related to the well-known Vroman effect of protein adsorption [33].

2.5.3 PROVISIONAL MATRIX FORMATION

Injury to vascularized tissue in the implantation procedure leads to immediate development of provisional matrix at the implant site. From a wound-healing perspective, blood protein deposition on a biomaterial surface is described as “provisional matrix formation.” The provisional matrix consists of fibrin, produced by activation of coagulated thrombosis systems and inflammatory products. It is expected to be released by the complement system, activated platelets, inflammatory cells, and endothelial cells [34 36]. Fibrin initiates resolution, reorganization, and repair processes such as inflammatory cell and fibroblast recruitment [33]. Fibrin has also been shown to play a key role in the development of neovascularization, i.e., angiogenesis. New vessel growth is expected to develop within 4 days from the implanted porous surfaces which are filled with fibrin. The provisional matrix may be viewed as a naturally derived, biodegradable, sustained release system where mitogens, chemoattractants, cytokines, and other growth factors are released to control subsequent wound-healing processes, during biomaterial device implantation [37 41].

2.5.4 ACUTE INFLAMMATION

Acute inflammation is of relatively short duration, lasting from minutes to days, depending on the extent of injury. The main characteristics of acute inflammation are the exudation of fluid and plasma proteins (edema) and the emigration of leukocytes (predominantly neutrophils). Neutrophils and other motile white cells emigrate or move from the blood vessels to the perivascular tissues and the injury (implant) site [42 44]. The major role of the neutrophils in acute inflammation is to phagocytose microorganisms and foreign materials. The tissue injury and fibrosis are usually mild and self-limited.

2.5.5 CHRONIC INFLAMMATION

Chronic inflammation is less uniform histologically than acute inflammation. In general, chronic inflammation is characterized by the presence of macrophages, monocytes, and lymphocytes, with the proliferation of blood vessels and connective tissue [29,30,45,46]. It must be noted that many factors modify course and histological appearance of chronic inflammation. Persistent inflammatory stimuli lead to chronic inflammation. Although chemical and physical properties of the biomaterial may lead to chronic inflammation, motion in the implant site by the biomaterial may also produce chronic inflammation. The chronic inflammatory response to biomaterials is confined to the implant site. The tissue injury and fibrosis are usually severe and progressive.

2.5.6 GRANULATION TISSUE

Within 1 day following implantation of a biomaterial (i.e., injury), healing response is initiated by action of monocytes and macrophages, followed by proliferation of fibroblasts and vascular endothelial cells at the implant site, leading to formation of granulation tissue [33]. Granulation tissue is the hallmark of reduced inflammation. The characteristic histological features of granulation tissue include proliferation of new small blood vessels and fibroblasts. Depending on the extent of injury, granulation tissue may be seen as early as 3–5 days following implantation of a biomaterial.

2.5.7 FOREIGN BODY REACTION

If the foreign body, either implant or biomaterial that remains inside the body is biocompatible, it can get along well with the surrounding tissues and may survive inside the organism. On the contrary, if the chemical signals of implanted material are recognized as a threat and cannot be terminated, defense system in the body activates the rejection mechanism to withdraw the foreign body. This rejection mechanism is called foreign body reaction [13]. The foreign body reaction consisting mainly of macrophages and/or foreign body giant cells may persist at the tissue implant interface for the lifetime of the implant [18,27,28,47,48]. The macrophages are activated upon adherence to the material surface at the early stage in the inflammatory and wound-healing response. Although it is considered that chemical and physical properties of the biomaterial are responsible for macrophage activation, the nature of the subsequent events regarding the activity of macrophages at the surface is not clear. Implants with high surface-to-volume ratios such as fabrics or porous materials will have higher ratios of macrophages and foreign body giant cells at the implant site than with smooth surface implants, which will have fibrosis as a significant component of the implant site.

2.5.8 FIBROSIS AND FIBROUS ENCAPSULATION

The end-stage healing response to biomaterials is generally fibrosis or fibrous encapsulation. Repair of implant sites involves two distinct processes: regeneration, which is the replacement of injured tissue by parenchymal cells of the same type, or replacement by connective tissue that constitutes the fibrous capsule [29,49,50]. Based on the regenerative capacity, cells can be classified into three groups: labile, stable (or expanding), and permanent (or static) cells. Labile cells continue

to proliferate throughout life, stable cells retain this capacity but do not normally replicate, and permanent cells cannot reproduce themselves after birth. The perfect repair with restitution of normal structure theoretically occurs only in tissues consisting of stable and labile cells, whereas all injuries to tissues composed of permanent cells may give rise to fibrosis and fibrous capsule formation, with very little restitution of the normal tissue or organ structure. Tissues composed of permanent cells (e.g., nerve cells, skeletal muscle cells, and cardiac muscle cells) most commonly undergo an organization of the inflammatory exudate, leading to fibrosis. Tissues composed of stable cells (e.g., parenchymal cells of the liver, kidney, and pancreas), mesenchymal cells (e.g., fibroblasts, smooth muscle cells, osteoblasts, and chondroblasts), and vascular endothelial and labile cells (e.g., epithelial cells and lymphoid and hematopoietic cells) may also follow fibrosis or may undergo resolution of the inflammatory exudates, leading to restitution of the normal tissue structure.

2.6 EVALUATION OF BIOMATERIAL BEHAVIOR

Prior to clinical use of any biomaterial, which is going to be used as an implant, in contact with living conditions of an organism, it should be strictly tested and proven to be nonhazardous. The obvious implication of that statement is that the body receiving the implant should not reject it physiologically. The evaluation of the biomaterial with regard to its applicability into the biological system is significantly assessed through three ways, before to its use.

2.6.1 ASSESSMENT OF PHYSICAL PROPERTIES

The first requirement is the matching physical properties of the substance, desired to be the same or at least compatible with the organism. These materials, therefore, are produced according to the tissue features, where they are going to be used. According to tissue type for which the biomaterial is utilized; physical strengths (tensile, compressive, shear strength, elasticity modulus), thermal properties, photoreactivity or translucency, color, calcification potency, surface structure, chemical features, or degradation resistance, materials are modified for ideal adaptation to the biological environment. Those features are examined under laboratory conditions before biologic behavioral tests [51].

2.6.2 IN VITRO ASSESSMENT

In vitro term is used to define a test setup that produces cells extracted from a living organism outside the body in controlled laboratory conditions [13]. Initially, cells are harvested from a living organism (animal, human, or even plants), kept in proper environmental conditions containing all the necessary organic and inorganic substances, water, temperature, etc., to maintain their survival. The designed biomaterials which are to be used in the biological systems are necessarily evaluated for their biologic performance through *in vitro* methods. These methods are followed prior to *in vivo* methods. They are helpful to assess the biologic behavior of a material without killing plenty of animals used in *in vivo* experiments. As the *in vitro* study settings result rapidly and can be performed in many different models, the time taken to conduct *in vitro*

experiments is lesser. Additionally, these can be repeated more number of times within a short period if necessary.

The *in vitro* pattern of biomaterial assessment involves the initial selection of the tissue cells that form the host environment for the implant material. The evaluation is conducted to find if the material has a potential to damage them or not. Even materials which are previously proven to be safe to be used inside the body are also tested to check any chemical or major physical modifications. By following the cell culture systems (*in vitro*), toxicity assessment of the material is done for all the related cell types. Besides cell cultures, bacterial or fungi cultures can also be used to evaluate antimicrobial effect of the test material. In this typical process, microorganisms cultured in a medium (on a surface or in a solution) are exposed to the test material and surviving microorganisms are counted to evaluate their lethal efficacy [52,53].

Mutation effect on cell DNA due to exposure of test material may also change cellular behavior or character and cause cancer. Such mutagenic evaluations can also be made through *in vitro* studies by observing the morphological change of the cells that are taken for these studies. The cells are scanned with scanning electron microscopy and ultrastructural transformations are visualized with transmission electron microscopy. In some cases, gene modification is used to transform those cells to cancer cells and work is preceded on such illnesses. Then cultured cells are passaged and exposed to test material or stored at 2196 C for further studies. Time-dependent toxicity rate can be measured as the count of surviving cells, their physical appearance, functions like replication rate or energy production ability (mitochondrial activity) [54].

2.6.3 IN VIVO ASSESSMENT

The third step in assessing biologic behavioral of biomaterial is *in vivo* (animal) experiments. The concluded approved (nontoxic) substances from *in vitro* examinations are subjected to *in vivo* experiments. This minimizes the excessive scarification of animals. The goal of the *in vivo* assessment of biomaterial medical devices is to determine and predict whether such devices present potential harm to the patient or user by evaluations under conditions simulating clinical use. FDA and regulatory bodies, i.e., ASTM, ISO, and USP, have provided procedures, protocols, guidelines, and standards that are followed in the *in vivo* assessment of tissue compatibility of medical devices [55-58].

Depending on the purpose of biomaterial, the substance is tested for allergic reaction (sensitization), tissue inflammation, and rejection reaction (irritation). Depending on the requirement, the material is also implanted under the skin (intracutaneously) or in a prepared bone cavity (intrabony) to evaluate local tissue response. The acute or subacute damages to all organ systems are evaluated through microscopic imaging or biochemical analyses. Genotoxicity potential of the material is investigated by observing changes of genetic structure of the cells that are exposed to the material. Local reaction to the material can be measured with comparison of healthy tissue, physiologic tissue healing, or healing process of a standard material well known and documented previously. Comparison of those tissue samples exposed to the substance is made with healthy tissue as reference in order to detect any morphological changes *via* light microscopy, scanning electron microscopy, transmission electron microscopy, confocal microscopy, etc. Some physical tests (tensile strength, shear strength, or compression tests) are used to evaluate physical strength of hard tissues like bone and tooth, or even (sometimes) soft tissues. Calcified tissues like bone or tooth are

Device Classification	Animal
Cardiovascular	
Heart valves	Sheep
Vascular grafts	Dog, pig
Stents	Pig, dog
Ventricular assist devices	Calf
Artificial hearts	Calf
Ex vivo shunts	Baboon, dog
Orthopedic/bone	
Bone regeneration/substitutes	Rabbit, dog, pig, mouse, rat
Total joints—hips, knees	Dog, goat, nonhuman primate
Vertebral implants	Sheep, goat, baboon
Craniofacial implants	Rabbit, pig, dog, nonhuman primate
Cartilage	Rabbit, dog
Tendon and ligament substitutes	Dog, sheep
Neurological	
Peripheral nerve regeneration	Rat, cat, nonhuman primate
Electrical stimulation	Rat, cat, nonhuman primate
Ophthalmological	
Contact lens	Rabbit
Intraocular lens	Rabbit, monkey

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followed up via X-ray images (radiographically). Study designs are based on time-dependent comparative research methodology and can use assessment of half-life of radioactively singed atoms implanted to the study substance to be placed into the body [59]. It should be noted that the sam-ples which are selected for in vivo examination should be pure, free from contamination, and steril-ized according to their structure.

The various animals that are used for the in vivo experiments to assess the medical devices are presented in Table 2.3 [33].

2.7 PROPERTIES OF BIOMATERIALS ASSESSED THROUGH IN VIVO EXPERIMENTS

In vivo experiments are conducted at the final stage, prior to its usage into the human. The selection of tests for the in vivo assessment is based on the characteristics and end-use application of the device or biomaterial under consideration. The brief perspectives on the assessment of biological properties of designed biomaterial through in vivo experiments are presented.

2.7.1 SENSITIZATION, IRRITATION, AND INTRACUTANEOUS REACTIVITY

Exposure or contact of even minute amounts of potential leachable agents in medical devices or biomaterials can result in allergic or sensitization reactions. Sensitization tests that estimate the potential for contact sensitization of biomaterials are usually carried out in guinea pigs. Irritant effects of potential leachables are determined by utilizing extracts of the biomaterials. Localized reactions of tissue to the extracts of biomaterials are determined through intracutaneous (intradermal) reactivity tests. Preparation of the test material and/or extract solution and the choice of the solvents chosen are used to determine sensitization or irritation or intracutaneous reactivity that must have physiological relevance.

2.7.2 SYSTEMIC, SUBACUTE, AND SUBCHRONIC TOXICITY

Systemic toxicity tests estimate the potential harmful effects of either single or multiple exposures of the medical devices, biomaterials and/or their extracts, during a period of 24 h. These tests also include pyrogenicity (fever-producing) testing. The basic considerations involved in testing protocol are the form and area of the material, the thickness and the surface area to extraction vehicle volume.

Depending on the intended application of the biomaterial, oral, dermal, inhalation, intravenous, intraperitoneal, or subcutaneous application, animals of choice like mice, rats, or rabbits are used along with the test substance. Acute toxicity is considered to be the adverse effect, which occurs after administration of a single dose or multiple doses of a test sample given within 24 h. If the adverse effects occur after administration of a single dose or multiple doses of a test sample per day given during a period from 14 to 28 days, then it is called subacute toxicity (repeat dose toxicity) and the adverse effects occurring after administration of a single dose or multiple doses of a test sample per day given during a part of the life span, usually 90 days but not exceeding 10% of the life span of the animal is called subchronic toxicity.

2.7.3 GENOTOXICITY

Determination of genotoxicity is carried out through *in vitro* and *in vivo* tests. If *in vitro* test results indicate potential genotoxicity, then *in vivo* genotoxicity tests are carried out. Initially, at least three *in vitro* assays are used, and two of these assays should utilize mammalian cells. While the initial *in vitro* assays cover the three levels of genotoxic effects: DNA effects, gene mutations, and chromosomal aberrations, the *in vivo* genotoxicity tests cover the micronucleus test, the mammalian bone marrow cytogenetic tests, chromosomal analysis, the rodent dominant lethal tests, the mammalian germ cell cytogenetic assay, the mouse spot test, and the mouse heritable translocation assay tests. Genotoxicity tests are performed with appropriate extracts or dissolved materials using media as suggested by the known composition of the biomaterial. The most common *in vivo* genotoxicity test is the rodent micronucleus test.

2.7.4 IMPLANTATION

Implantation tests assess the local pathological effects on living tissue of a sample of a material that is surgically implanted into tissue appropriate for the intended application of the device.

Evaluation of the local pathological effects is carried out both at the gross level and the microscopic level. Histological (microscopic) evaluation is utilized to characterize various bio-logical response parameters. Mice, rats, guinea pigs, or rabbits are the usual animals utilized for short-term implantation evaluation within 12 weeks. For longer term testing in subcutaneous tissue, muscle or bone, animals such as rats, guinea pigs, rabbits, dogs, sheep, goats, pigs, and other animals with relatively long-life expectancy are usually chosen. To evaluate a medical device, larger species are utilized. In general, sheep are chosen for testing substitute heart valves, whereas for ventricular assist devices and total artificial hearts, calves are usually the animal of choice.

2.7.5 HEMOCOMPATIBILITY

Hemocompatibility tests of *in vivo* examination evaluate the effects on blood and/or blood components by blood-contacting medical devices or materials. The five test categories indicated from the ISO standards perspective for hemocompatibility evaluation are thrombosis, coagulation, platelets, hematology, and immunology (complement and leukocytes). In case of testing on animals, species differences in blood reactivity are considered, and these differences may limit the predictability of any given test in the human clinical situation. Although species differences may complicate hemocompatibility evaluation, the utilization of animals in short- and long-term testing is considered to be appropriate for evaluating thrombosis and tissue interaction.

2.7.6 CHRONIC TOXICITY

Chronic toxicity tests of *in vivo* experiments are considered as an extension of subchronic (sub-acute) toxicity testing, and both may be evaluated in an appropriate experimental protocol or study. Chronic toxicity tests determine the effects of either single or multiple exposures to medical devices, materials, and/or their extracts during a period of at least 10% of the life span of the test animal, e.g., over 90 days in rats [33].

2.7.7 CARCINOGENICITY

Carcinogenicity tests determine the tumorigenic potential of medical devices, materials, and/or their extracts from either single or multiple exposures or contacts over a period of the major portion of the life span of the test animal [33]. If data from other sources suggest a tendency for tumor induction, then the carcinogenicity tests will be conducted. In a single experimental study, both carcinogenicity (tumorigenicity) and chronic toxicity may be studied. Controls of comparable form and shape are generally used in carcinogenicity testing. The use of appropriate controls is essential, since the animals when spontaneously develop tumors, a statistical comparison between the test biomaterial/device and the controls is always necessary. The commonly used control materials are polyethylene implants. The other implants may be of polypropylene and silicone (or polyurethane-coated silicone).

2.7.8 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

The concerned *in vivo* tests evaluate the potential effects of medical devices, materials, and/or their extracts (released substances) on reproductive function, embryonic development (teratogenicity), and prenatal and early postnatal development. The application site of the device must be considered and tests and/or bioassays should only be conducted when the device has potential impact on the reproductive potential of the subject [33].

2.7.9 BIODEGRADATION

The effects of a biodegradable material and its biodegradation products on the tissue response are determined through biodegradation tests. The amount of degradation; the nature, the origin, and the qualitative and quantitative assessment of the degradation products (e.g., impurities, additives, corrosion products, bulk polymer, etc.); and leachable agents in adjacent tissues and distant organs are specially focused through biodegradation tests. The test materials comparable to that of degradation products may be prepared and studied to determine the anticipated biological responses of the products in long-term implants. This provides advance supporting information on the effects of the implant within short duration rather than anticipation for longer time.

2.7.10 IMMUNE RESPONSES

Determination of immune response is needed with modified natural tissue implants or some other biomaterial device. Immunotoxicity is any adverse effect on the function or structure of the immune system or other systems as a result of an immune system dysfunction. The potential immunological effects include hypersensitivity, chronic inflammation, immunosuppression, immunostimulation, and autoimmunity. The potential immunological responses are histopathological changes, humoral responses, host resistance, clinical symptoms, and cellular responses.

2.8 APPLICATIONS OF BIOMATERIALS

Although the tissues and structures of the body perform for an extended period of time in most people, they do suffer from a variety of destructive processes, including fracture, infection, and cancer that cause pain, disfigurement, or loss of function. Hence, under these circumstances, it has become necessary to remove the diseased tissue and replace it with some suitable synthetic materials that perform the normal activity of the regular living tissue. The purpose of this is fulfilled through biomaterials. The primary reason using biomaterials is to physically replace hard or soft tissues that have become damaged or destroyed through some pathological process [60]. Some of the areas of applications where the biomaterials are successfully utilized are dealt here.

2.8.1 ORTHOPEDIC APPLICATIONS

The use of biomaterials for orthopedic implant devices is one of the major achievements in the field of medicine. The structure of freely movable (synovial) joints, such as the

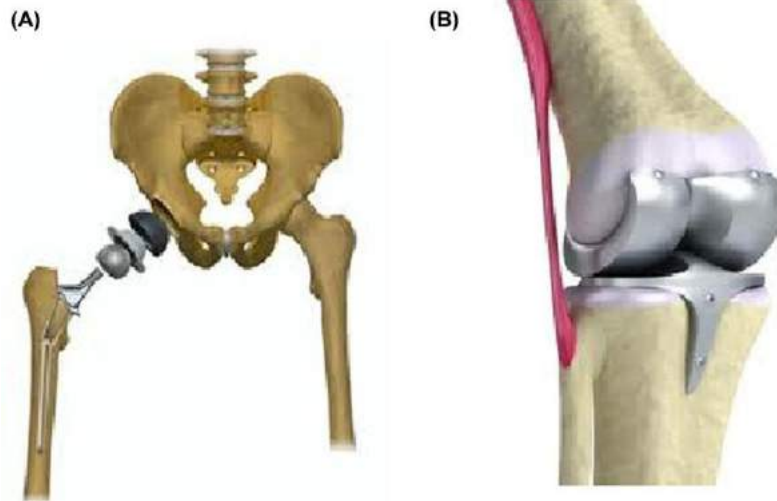


FIGURE 2.1

Orthopedic applications of biomaterials (A) artificial hip and (B) artificial knee.

hip, knee, shoulder, ankle, and elbow, is affected by both osteoarthritis and rheumatoid arthritis. It has been possible to replace these joints with prostheses since the advent of anesthesia, antisepsis, and antibiotics, and the relief of pain and restoration of mobility is well known to hundreds of thousands of patients. Some of the biomaterials used for the orthopedic applications are shown in [Figure 2.1](#).

2.8.2 OPHTHALMOLOGIC APPLICATIONS

The tissues of the eye can suffer from several diseases, leading to reduced vision and eventually, blindness. The devices such as spectacles used to correct the eye vision are the external devices. However, the contact lenses being intimate contact with the tissues of the eye are subject to the same regulations that govern the use of implant materials. Apart from this, artificial cornea, artificial endothelium, intraocular lenses, and implants for vitreous and glaucoma are also available. Artificial cornea and intraocular lenses are shown in [Figure 2.2](#).

2.8.3 CARDIOVASCULAR APPLICATIONS

One of the most prominent application areas for biomaterials is cardiovascular applications. The problems that arise from failure of heart valves and arteries can be successfully treated with implants. The heart valves suffer from structural changes that prevent the valve from either fully opening or fully closing, and the diseased valve can be replaced with a variety of substitutes. The problem of atherosclerosis occurs by blocking fatty deposits in the vessels and the obstruction of arteries can be solved by replacing segments with artificial arteries. Some of the biomaterial devices used for

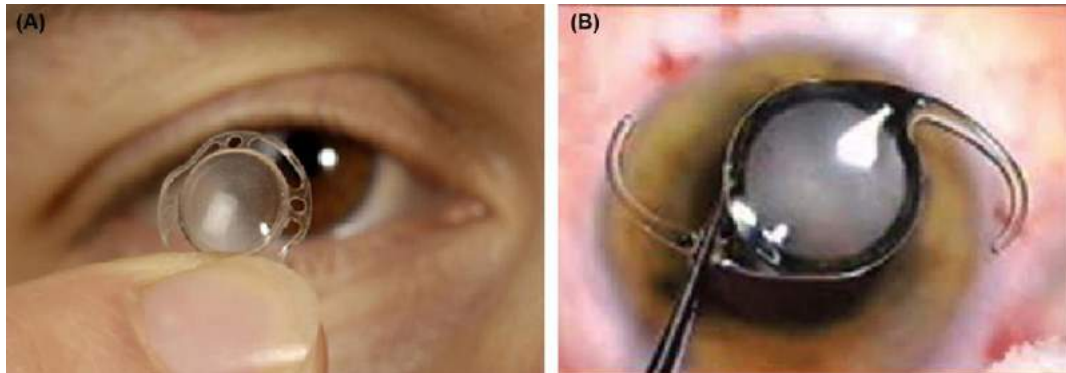


FIGURE 2.2

Ophthalmologic applications of biomaterials (A) artificial cornea hip and (B) intraocular lenses.

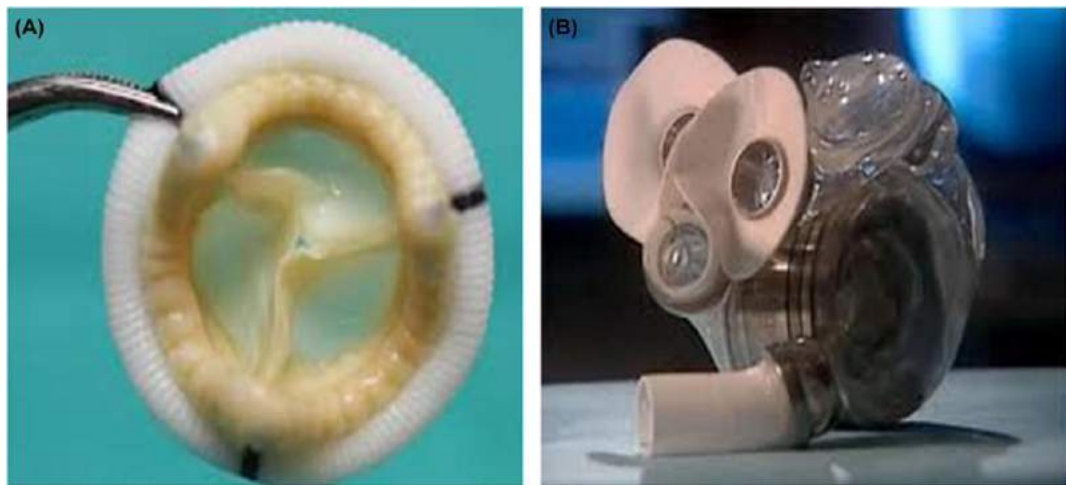


FIGURE 2.3

Cardiovascular applications of biomaterials (A) heart valve and (B) artificial heart.

cardiovascular purpose is cardiopulmonary bypass system, heart valves, vascular grafts, stents, pacemakers, and complete artificial heart. Heart valve and artificial heart are shown in [Figure 2.3](#).

2.8.4 DENTAL APPLICATIONS

The tooth and supporting gum tissues have maximum chances to get destroyed by bacterially controlled diseases, since the tissues are present in the mouth which is the only passage for food and liquids. Dental caries (cavities), the demineralization and dissolution of teeth associated with the

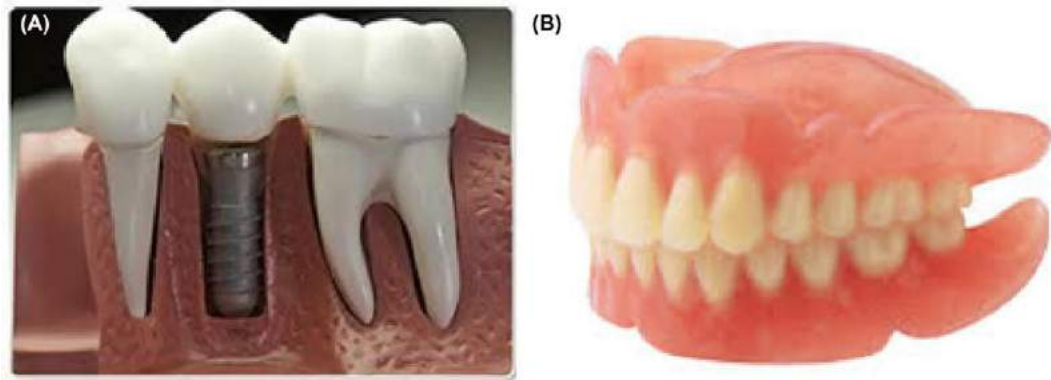


FIGURE 2.4

Dental applications of biomaterials (A) the endosteal root form dental implant and (B) biomaterial tooth gums.

metabolic activity in plaque (a film of mucus that traps bacteria on the surface of the teeth), can cause extensive tooth loss. Teeth in their entirety and segments of teeth both can be replaced and restored by a variety of materials that form the biomaterials. Depending upon requirement, there are many varieties of dental implants. The endosteal root form dental implant and biomaterial tooth gums are presented in [Figure 2.4](#).

2.8.5 WOUND DRESSING APPLICATIONS

Wound dressings are usually the external medical support given to the wound by the surgical team to prevent the flowing of the blood, exudates, and the proteins. The scaffolds that provide the relief at the wound site such as cellulosic materials or the hydrogels or the films developed either from natural or artificial materials are broadly considered as wound dressing materials [61–65]. The antimicrobial wound dressings also came into existence which not only act as normal wound dressing but also provide a hygienic atmosphere around the wound. Apart from this, multiple therapies employing biomaterials for wound management have been developed. One of the examples for commercially available biosynthetic wound dressing is Biobrane^S (Smith & Nephew, London, UK), a biocomposite dressing composed of a silicon/nylon matrix in which porcine dermal collagen has been embedded. [Figure 2.5](#) showing biosynthetic wound dressing Biobrane and its application on wound.

2.8.6 OTHER APPLICATIONS

The other applications of biomaterials include drug delivery, organ implantations like breast and development of artificial organs like artificial skin, artificial kidney (hemodialyzer), artificial pancreas, heart lung machine, etc. For drug delivery applications, the products are designed to increase the duration of orally administered drugs and consist of small spheres with a soluble coating. By using coatings having varying thicknesses, dissolution times could be varied,

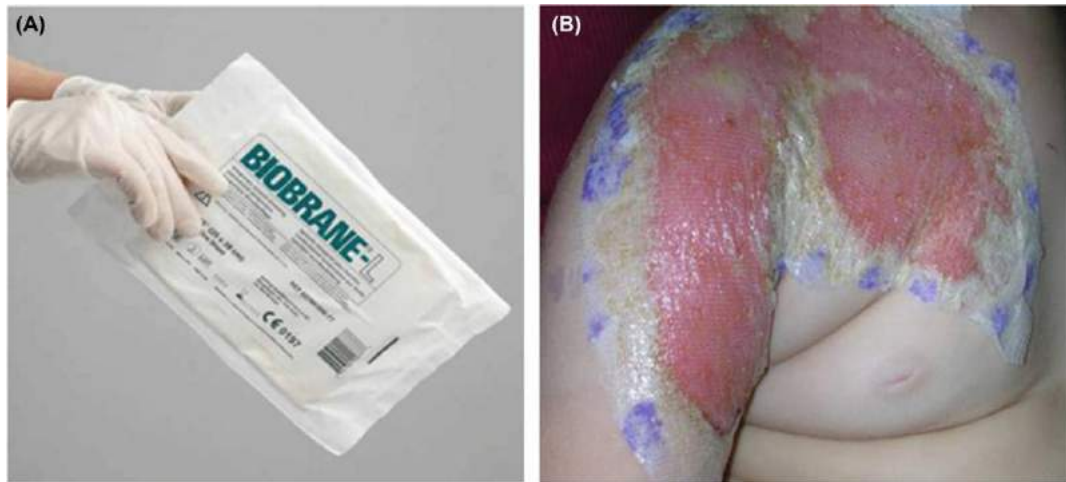


FIGURE 2.5

Wound dressing applications of biomaterials (A) biosynthetic wound dressing Biobrane and (B) application of Biobrane as dressing on the wound.

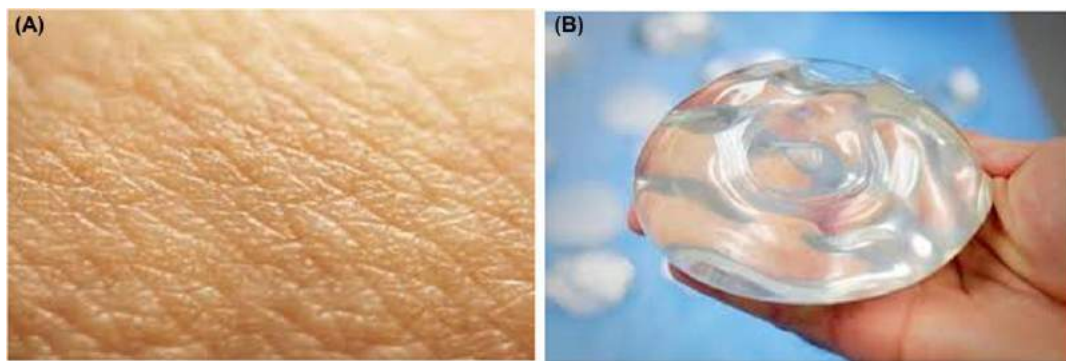


FIGURE 2.6

Other applications of biomaterials (A) artificial skin and (B) breast implant biomaterial.

prolonging the action of the therapeutic agent. The artificial skin and biomaterial breast implant material are shown in [Figure 2.6](#).

2.9 FUTURE DIRECTIONS IN BIOMATERIALS

Biomaterials are the backbone of the medical device industry, a critical element of health care. They have made a great impact on medicine. Currently, there are thousands of hard and soft tissue

products, biomedical devices, pharmaceutical and diagnostic products, and disposable materials that are available at the medical market for human benefits. However, numerous challenges are there to be solved. Nowadays, modern clinical procedures such as preventing and curing main genetic diseases have become significant and new medical demands cause the change of the biomaterial products. Hence, the future trend has to combine the mechanically superior metals and the excellent biocompatibility and biofunctionality of ceramics and polymers to obtain the most desirable clinical performance of the implants. The biomaterial scientists and engineers should increase the vicinity of applications by integration of biomaterials with molecular biology, biochemistry, genetics, physics, and other areas of sciences. This integration supports the researchers, material scientists, and tissue engineers to design the products from molecular level from cell to tissue.

2.10 CONCLUSIONS

The day-to-day enormous increased demand in the medical field for the bioalternatives which could perform the living activities of bodily organs in their absence has raised the interest of the researchers to design novel biomaterials. Based on this, in this chapter, discussion on biomaterials and its design and development has been carried out. Apart from this, the necessary requirements or characteristics of the biomaterials are also discussed. Further, the assessment of designed biomaterial devices or implants and the applications of the biomaterials, which play a significant role, are also discussed. Over all, detailed information has been covered ranging from the basic definition of biomaterial to the design, development, and applications. The crucial study on biomaterials presented here provides the necessary basics required for designing biomaterial devices and implants by the emerging researchers, tissue engineers, and material scientists.

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