Section I

1

FUNDAMENTALS OF BIOMATERIALS AND BIOCOMPATIBILITY

Bikramjit Basu and Shekhar Nath

Department of Materials and Metallurgical Engineering, Indian Institute of Technology Kanpur, Kanpur, India

Contents

Advanced Biomaterials: Fundamentals, Processing, and Applications, Edited by Bikramjit Basu, Dhirendra S. Katti, and Ashok Kumar

1.1 OVERVIEW

In last two decades, impressive progress has been recorded in terms of developing new materials or refining existing material composition and microstructure in order to obtain better performance of designed materials in biomedical applications. The success of such large efforts clearly demands better understanding of various concepts such as biocompatibility, host response, and cell-biomaterial interaction. This chapter reviews the fundamentals for understanding biomaterials development.

1.2 INTRODUCTION

One of the most exciting and rewarding research areas of materials science involves the applications of materials to health care, especially to reconstructive surgery. The importance of biomaterials can be well realized from an economical aspect, that is, in terms of an estimate of total health care expenditure around the world. In the most developed country of the world, the United States, total health care expenditure in the year 2000 was approximately 14 billion US dollars. It was also reported that the US market for biomaterials in 2000 was 9 billion US dollars. It can be further noted that the respective annual expenses in other countries of the world are typically around two-to-three times that of the US expenses[1]. With continuous changes in lifestyle as well as in global scenarios in the health sector, such expenses are definitely on a much higher side today in both developed and, more importantly, developing nations, than at the beginning of this century. To this end, the development of biomaterials and related devices is important.

The field of biomaterials is multidisciplinary, and the design of biomaterials requires the synergistic interaction of materials science, biological science, chemical science, medical science and mechanical science. Such interaction has been schematically illustrated in Figure 1.1. Also shown in Figure 1.1 is the necessity to develop cross-disciplinary approaches in designing new biomaterials. Among different kinds of biomaterials[2], metals and metallic alloys are used in orthopedics, dentistry and other load-bearing applications; ceramics are used[3] with emphasis on either their chemically inert[4] nature or their high bioactivity[5]; polymers are used for soft tissue replacement and research is also being pursued for application in hard tissue replacement. To achieve better biological properties and mechanical strength, composite materials of metals, ceramics and polymers are being developed and clinically assessed to a limited extent. Broadly, all biomaterials are being developed to maintain a balance between the mechanical properties of the replaced tissues and the biochemical effects of the material on the tissue. Both areas are of great importance as far as the clinical success of materials is concerned. However, in most (if not all) biological systems, a range of properties is required, such as biological activity, mechanical strength, chemical durability, and so forth. Therefore, a clinical need often can only be fulfilled by a designed material that exhibits a complex combination of some of the above mentioned properties. Figure 1.2 shows the different organs of a living human body that can

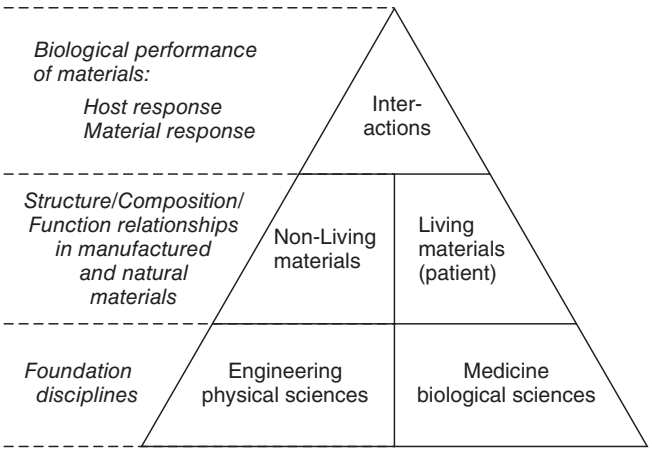


Figure 1.1. Concept triangle illustrating the synergistic interaction of Engineering and Biological science disciplines, involved in designing biomaterials. The schematic also demonstrates the multidisciplinary approach of the science and technology of biomaterials. [Reproduced from, J Black, in chapter: Biocompatibility: Definition and Issues, Biological Performance of Materials: Fundamentals of Biocompatibility, CRC Press, US, 2006.]

be replaced by various biomaterials. In living humans, most orthopedic/prosthetic joints and dental restorations demand the use of hard tissue/cortical bone or analogue materials, such as high-strength metals or high-hardness ceramics. To this end, the use of softer polymeric materials is restricted to the cranial area, blood vessels, heart valves, intraocular lenses, and so on.

In this section, the structure of this introductory chapter has been presented. Section 1.3 discusses necessary biological terms and their importance as well as the materials classification with respect to host response. Specially, in subsections 1.3.1 and 1.3.2, the two important terms *biomaterials* and *biocompatibility* have been defined and their implications are provided. In the subsequent subsection (1.3.3), the host tissue response with biomaterials has been assessed critically. In section 1.4, the cell-material interaction has been discussed with an aim to provide a fundamental idea about the interactions of a specific cell line with implanted materials. The next section (1.5) demonstrates the various *in vitro* and *in vivo* experiments to determine the biocompatibility of the materials. In the subsequent section (1.6), the steps involved in characterizing biomaterials are discussed. At the close, the brief highlights of the various book chapters under 'Fundamentals' section is presented in section 1.7.

1.3 SOME USEFUL DEFINITIONS AND THEIR IMPLICATIONS

1.3.1 Biomaterial

Broadly, biomaterials can be defined as synthetic materials, which have been designed to induce a specific biological activity[6]. The major difference of

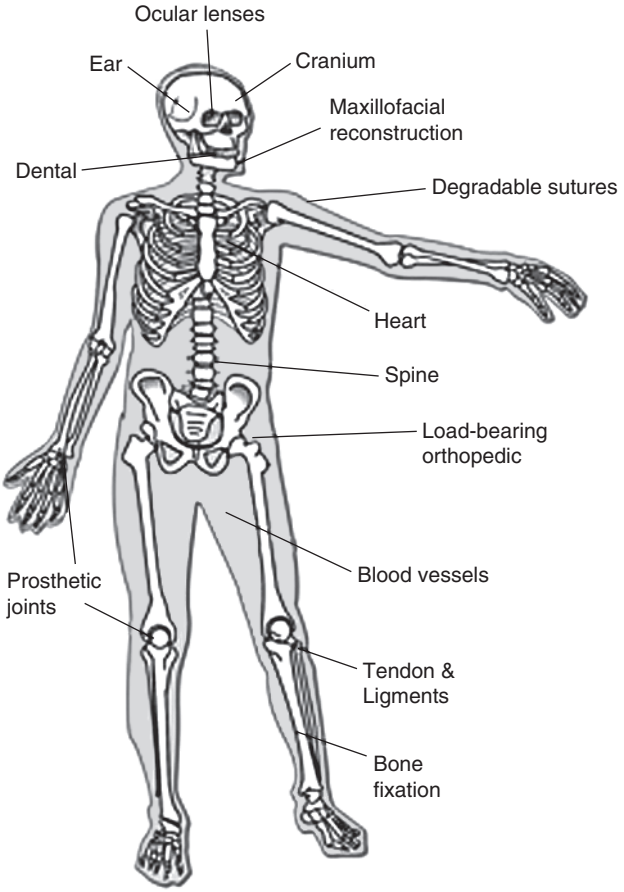


Figure 1.2. A schematic of the various human body parts, which can be potentially replaced by synthetic biomaterials. [Reproduced from, J Black, in chapter: Biocompatibility: Definition and Issues, Biological Performance of Materials: Fundamentals of Biocompatibility, CRC Press, US, 2006.]

biomaterials from other classes of materials is their ability to remain in a biological environment without damaging the surroundings and without getting damaged in that process[7]. Therefore, biomaterials require both biological and materials properties to suit a specific application. It must be emphasized here that biological properties/responses of a material in physiological environments are, by far, the most important consideration, as opposed to superior mechanical properties, for selecting/defining biomaterials. From the health care perspective, it is desirable that a biocompatible material interrupts normal body functions as little as possible. The most important aspect of a biomaterial is, therefore, how a biomaterial interacts when implanted in a human or animal body.

1.3.2 Biocompatibility

The fundamental requirement of any biomaterial concerns the ability of the material to perform effectively with an appropriate host response for the desired application, that is, the material and the tissue environment of the body should coexist without having any undesirable or inappropriate effect on each other. This has also been mentioned in Figure 1.1. Such a requirement is broadly described by the concept known as 'biocompatibility'[8]. Broadly, biocompatibility is defined as 'the ability of a material to perform with an appropriate host response in a specific application.' From a biological point of view, biocompatibility arises from the acceptability of non-living materials (synthetic biomaterial) in a living body (mammal/human). There are three important aspects of biocompatibility that a candidate biomaterial seeks to achieve in diverse environments, such as bone, blood vessel and the eye. In the first place, biomaterials must be biochemically compatible, non-toxic, non-irritable, non-allergenic and non-carcinogenic; second, biomechanically compatible with surrounding tissues; and third, a bioadhesive contact must be established between the materials and living tissues. It needs to be emphasized here that the biocompatibility depends on place of applications. For example, a specific material could be biocompatible in bone replacement, but the same material may not be biocompatible in direct blood contact application. However, as will be discussed later, a range of *in vitro*/*in vivo* tests are suggested to completely describe the biocompatibility property. It must be emphasized hence that for a given biomedical application, only a selected set of relevant tests, among various tests mentioned in section 1.6, should be carried out on potential implant materials.

1.3.3 Host Response

In order to develop new materials, it is desirable to understand the *in vivo* host response of various biomaterials. Ideally, biomaterials should not induce any change or provoke undesired reaction in the neighboring or distant tissues. An important aspect of host response involves the formation of a structural and biological bond between the material and host tissues. When the biocompatibility is lacking, materials cause tissue reactions, which may be systemic or local. Systemic responses can be toxic or allergic and triggered by the products of metallic corrosion and polymer degradation, release of micro particles from materials, and the presence of contaminants.

Different human systems (such as respiratory, circulation, or digestive) respond in different ways to contact with foreign bodies or materials. Depending on the biocompatibility and host reaction, biomaterials can be broadly classified into three main categories on the basis of various types of host responses of biomaterials after implantation into the living body[2]:

a) **Bioinert / biotolerant:** Bioinert materials are biocompatible materials, but cannot induce any interfacial biological bond between implants and bone.

b) **Bioactive:** Bioactive materials are a group of biocompatible materials that can attach directly with body tissues and form chemical and biological bonds during early stages of the post implantation period.
c) **Bioresorbable:** Bioresorable materials are the type of biocompatible materials that are gradually resorbed before they finally disappear and are totally replaced by new tissues *in vivo*.

When a bioinert material is implanted, a capsule-like layer forms on the surface of the implant to keep it isolated from the living part of the body. For example, bioinert ceramics, such as alumina or zirconia, develop fibrous capsules at their interface when implanted. However, it is important to note that the thickness of an interfacial fibrous layer depends upon motion and the extent of required fit at the interface. Therefore, a bioinert material is not useful for long-term application. The most significant class of biomaterial is bioactive material, which can potentially behave as the part of a living body. A few examples of bioactive materials are 45S5 bioglass and calcium phosphates (HA). For bioactive materials, the interfacial bond prevents motion between the implant–tissue interfaces and imitates the type of interface found when natural tissues repair themselves[9]. The third kind of material is bioresorable or degradable, which degrades with time inside the body's environment. The degradation rate should be such that the regeneration rate of new tissue will be same as the material resorption rate. Tricalcium Phosphate (TCP) and bone cement are the two examples of bioresorable materials.

1.4 CELL–MATERIAL INTERACTIONS

The interaction between biomaterials and natural tissues is an important scientific issue and understanding this issue is essential to designing new biocompatible materials. In understanding the interaction and integration of biomaterials in a human body, it is worthwhile to mention the physicochemical conditions of the human body's environment. For example, nominal pH values vary over a wide range of 1.0 (gastric content) to 7.4 (blood)[10]. Additionally, pH values can change depending on health conditions (disease, etc.). The temperature of the normal core of human body is around 37.4 °C; however, deviations over a range of temperature 20–42.5 °C have been reported for diseased patients[10]. As far as the inorganic composition of the human body is concerned, total body burden of Ca, Na, Cl ions is much higher and also traces of Mg, Fe, Zn, Cu, Al, and so on, are present in cytoplasm.

Biologically, a cell is defined as a self-duplicating unit, given the proper nutrients and environment. A cell can alternatively be described as a collection of self-replicating enzymes and structural proteins. In Figure 1.3a, the anatomy of a typical mammalian eukaryotic cell has been provided. Various important organelles can be identified in Figure 1.3a and important organelles include mitochondrion (energy warehouse), Golgi apparatus, Endoplasmic Reticulum (ER), etc. It can be mentioned here that rough ER is one of the preferred locations for protein

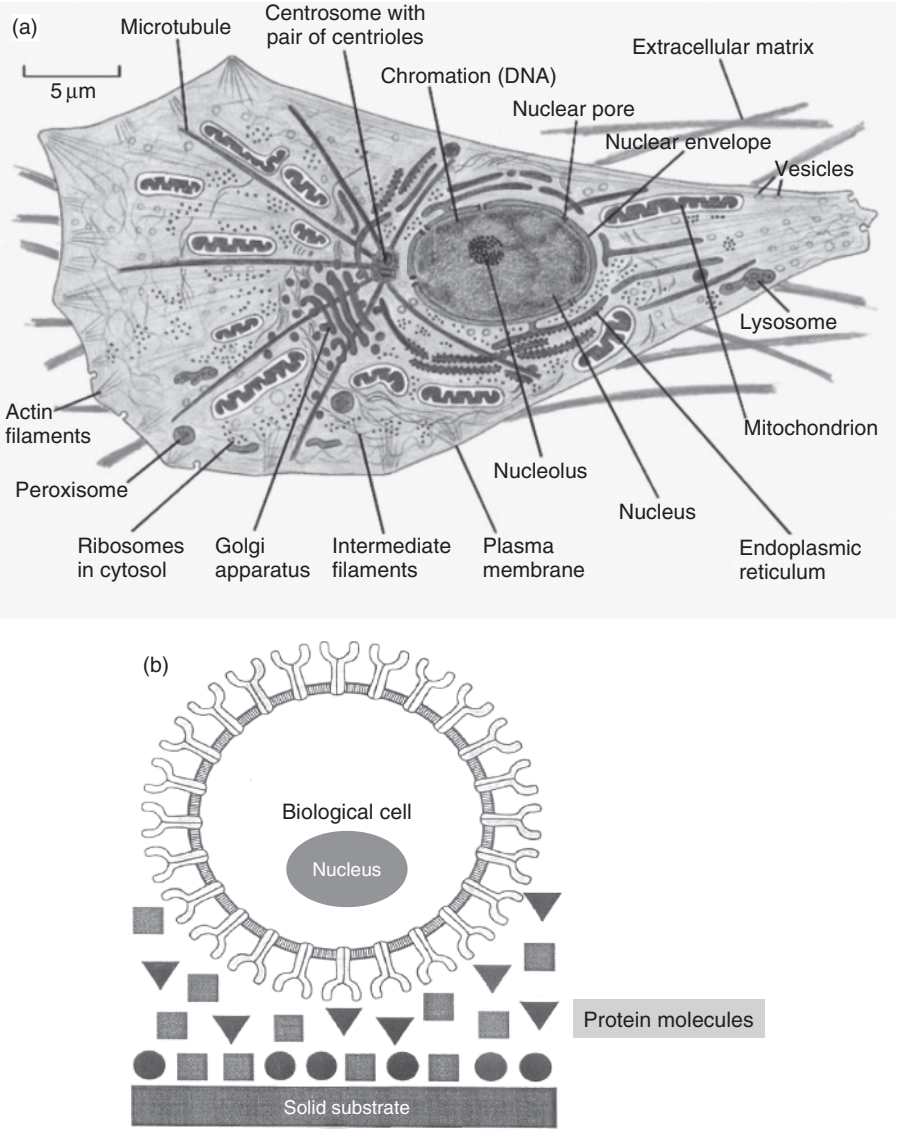


Figure 1.3. Schematic illustration showing the anatomy of an eukaryotic animal cell (a) [Reproduced from Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter, Introduction to the Cells in "Molecular Biology of The Cell".] and the fundamental mechanisms involved in biomaterial-cell interaction, established by the adsorbed proteins (circles, boxes and triangles) with the integrin proteins of a biological cell (b) [Reproduced from BD Ratner, AS Hoffman, FJ Schoen, JE Lemons, Biomaterials Science—An Introduction to Materials in Medicine, 2nd edition, Academic Press, New York, 2004.].

synthesis. The structure of cytoskeleton is also visible. Cytoskeleton is typically made up of three proteinaceous structures: actin filament, microtubule and intermediate filaments. Specific chatrcaterisitcs of cytoskeleton allows the constituent proteins to rearrange or reorganize themselves when desired, e.g., in case of change of cell shape in response to external stimulation.

It is critical that any implant material must, to a minimum extent, elicit a toxic response, that kills cells in the surrounding tissues or releases chemicals that can migrate within tissue fluids and cause systemic damage to the patient. Therefore, it is important, in the first place, to understand biomaterial–cell interaction. A schematic illustration of the fundamental mechanisms involved in biomaterial–cell interaction is shown in Figure 1.3b. It can be recalled that once a material is implanted in an animal, a large number of protein molecules are adsorbed on biomaterial surface. This is because of the abundance of protein as an order of 10^9 number of protein molecules per eukaryotic cell is estimated in the human body. Also, a simple calculation shows an order of 10^{14} number of eukaryotic cells in a healthy human.

From the phenomenological point of view, protein adsorption on an implant takes place first because of faster adsorption kinetics, and this acts as precursor to the cell–material interaction. A schematic of protein adsorption phenomenon as well as experimental results to illustrate the protein adsorption isotherm have been provided in Figure 1.4. Therefore, a material does not "see" a cell directly

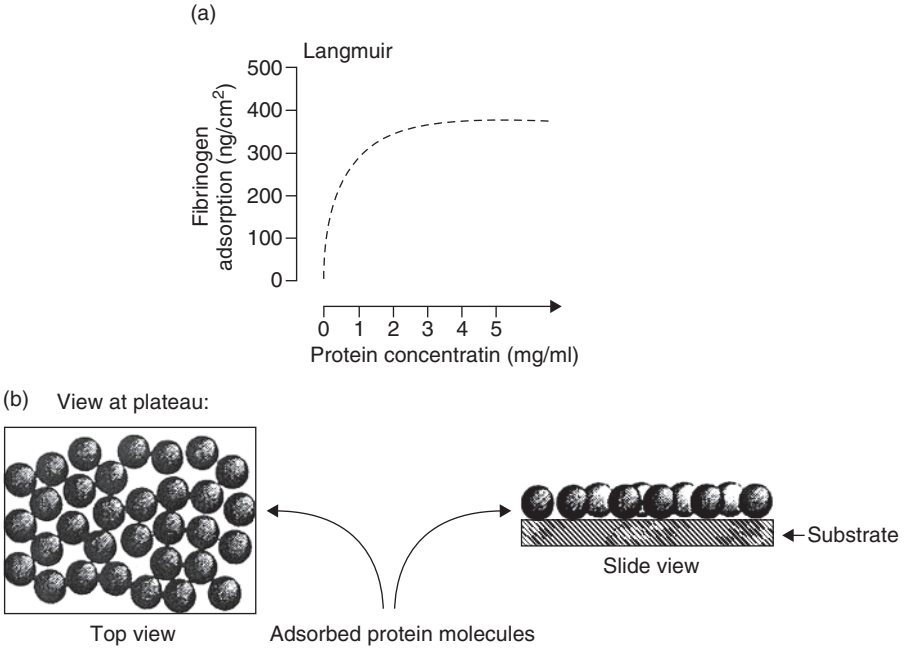
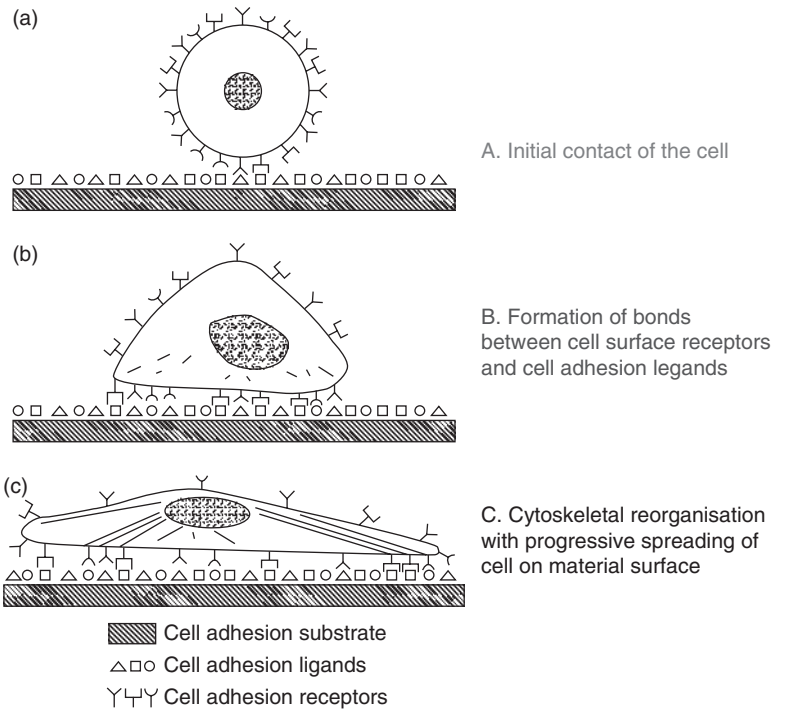


Figure 1.4. Schematic illustration showing the anatomy of a eukaryotic animal cell (a) and the fundamental mechanisms involved in biomaterial-cell interaction, established by the adsorbed proteins (circles, boxes and triangles) with the integrin proteins of a biological cell (b). [Reproduced from BD Ratner, AS Hoffman, FJ Schoen, JE Lemons, Biomaterials Science—An Introduction to Materials in Medicine, 2nd edition, Academic Press, New York, 2004.]



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and the initial interaction is established through the interaction of cell surface receptors with adsorbed protein ligands. Such protein-to-protein binding importantly helps a cell to spread on the material surface.

Subsequent spreading to cover entire implant surface is also facilitated by cytoskeletal reorganization, as shown schematically in Figure 1.5.

After implantation of a biomaterial, a monolayer of protein molecules gets absorbed on the material surface within a minute. Subsequently, the transport of various cell types towards the biomaterial surface occurs and the interaction of the integrin proteins of cell surface with the absorbed protein of biomaterials surface is established. The secretion of cell enzymes of various cell type form an extra cellular matrix (ECM). Depending on cell biomaterial interaction, various cell types can adhere in a self-organized manner to form a tissue. To this end, an important event is the formation of the small blood vessels (angiogenesis) as well as formation of large blood vessels (vasculogenesis) within the newly-formed tissue layer. Such formation is necessary for the supply of nutrients locally to various cell types as well as for the removal of waste from ECM.

1.5 EXPERIMENTAL EVALUATION OF BIOCOMPATIBILITY

Any research program of assessment on biomaterials must include a range of *in vitro* and *in vivo* tests, as stated by various standard agencies (for example, International Organization for Standardization [ISO]). ISO guidelines are also available and such guidelines are followed to select the tests for the biological evaluation of materials and medical as well as dental devices. It may be worthwhile to remember the difference between *in vitro* and *in vivo* tests. *In vitro* tests are lab scale simulated experiments, which are rapid and are a must as initial screening tests. From the results of the *in vitro* tests, one cannot obtain any information of inflammation and immune response of the materials. Also, most of the *in vitro* experiments use single cell lines, which do not reflect the actual tissue interaction (involving multiple cell types) *in vivo*. Although the *in vitro* experiments are inexpensive, such tests do not provide appropriate representation of physiological conditions. These tests, nevertheless, are effective as the first step of biocompatibility evaluations. On the other hand, *in vivo* experiments produce a better approximation to the human environment. Here, the material comes in contact with different cell types and the effect of hormonal factors can be analyzed. Also, *in vivo* tests provide interactions with extracellular matrix, bloodborne cells, protein and molecules. These experiments can be regarded as the second step prior to clinical use.

1.5.1 *In Vitro* Tests

In order to harmonize the existing guidelines, ISO has prepared the guideline document 'Biological testing of Medical Devices—Part 1: Guidance on Selection of Test' (ISO 10933-1), which incorporates all the national and international documents. ISO 10993 requirements for long-lasting tissue/bone implants require various biological tests and the following are the major *in vitro* tests:

- **Cytotoxicity:** This is an *in vitro* test of cell toxicity. The cytotoxicity experiments determine whether the material is toxic in contact with some particular cell lines. The test is generally done in a laboratory using some standard/relevant cell lines and the cells are seeded on the materials. As far as the experimental evaluation of biocompatibility is concerned, the cytotoxicity tests are widely cited as the primary assessment of biocompatibility and therefore are discussed in more detail below.

  As a first step, the sterilization of the samples is carried out in order to remove other micro-organisms, if present on the surface. Depending on type of implant materials (i.e., chemistry and chemical composition), the sterilization is either carried out in steam autoclave (15 psi, 121 °C, 20 minutes) or using γ-ray irradiation. The culture medium used for cell culture testing is DMEM (Dulbecco's modified Eagles' medium), containing 10% serum, 1% antibiotic cocktail. The samples are incubated in the culture

solution containing mammalian cells (direct contact) for 24 hours at 37.4 °C (human body temperature).

The choice of cell types depends on desired application of a given biomaterial. For example, if the material under investigation is to be used as bone analogue material, then human osteoblast (HOB) cell lines are to be used. Nevertheless, as per ISO guidelines, fibroblast cells, which are normally contained in living connective tissue, are to be used for primary assessment of cell adhesion. This is because of the fact that these cells can easily proliferate on material surfaces and also they come in contact at wound/injured area (e.g., implant zone) at the initial stage. Another parameter for cell culture testing is the time of culture. Usually, the tests are widely reported to be carried out for 24 hours; however, slowly growing cells, like HOB, need to be cultured for three to seven days. In order to quantify the number of attached cells or to study cell–material or cell–cell interactions using light/electron microscopy, the cells were fixed in glutaraldehyde/formaldehyde. Before fixing, these are washed twice in PBS (Phosphate buffer saline) to completely remove the culture medium. The formaldehyde solution is (4%) diluted in PBS and it is kept for 20 minutes. Finally, the samples are stored in PBS at 4 °C. Afterward, the samples will be dried in a critical point dryer using liquid CO_2.

MTT assay is a standard colorimetric assay (an assay which measures changes in color) to quantify cellular proliferation (cell growth). It is used to determine cytotoxicity of potential medicinal agents and other toxic materials. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. A solubilization solution (usually either dimethyl sulfoxide or a solution of the detergent sodium dodecyl sulfate in dilute hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore, conversion is directly related to the number of viable (living) cells.

- **Genotoxicity:** In this *in vitro* experiment, it is primarily observed whether any genetic mutation occurs in the cells in direct contact with the biomaterial surface.
- **Hemocompatibility:** Hemocompatibility evaluates the material's compatibility with red blood cells. In particular, thrombogenic property or changes in RBC content in a blood stream flowing over the biomaterials are assessed and a better thrombus material should ideally show limited thrombus formation. Such evaluation is a must for cardiovascular implant materials. The examples of hemocompatibile materials are PolyTetra Flouro Ethylene (PTFE), Diamond Like Carbon (DLC), and so on.

1.5.2 *In Vivo* Tests

The different *in vivo* tests include:

- **Sensitization:** This is an *in vivo* test, where materials are kept in the subcutaneous region of an animal and the observations are the change in skin color, allergic effect, or some other irritations.
- **Implantation:** Implantation is an *in vivo* experiment, where a sample of a predefined shape is placed in the long bone of a mammal (rabbit, rat and mouse). After a specified time frame, the samples and surrounding tissues are examined histopathologically to determine the *in vivo* response of the materials. In general, short term implantation tests are conducted for up to 12 weeks and long term tests for up to 78 weeks. Since the animals are sacrificed, the number of animals is limited to a minimum number from an animal welfare point of view.
- **Carcinogenicity:** Carcinogenicity is a long term *in vivo* experiment, which determines any cancerous effect on the cells in contact with materials. The examples of carcinogenic materials are Pb, Sn, and so on.

ISO-10993 draft categorizes the devices by the nature of their contact with the body:

a) Surface-containing devices, such as electrodes, compression bandages, contact lenses, urinary catheters, and so on.
b) External communicating devices, such as dental cements, arthroscopes, intravascular catheters, dialysis tubing, and so on.
c) Implant devices, such as hip and knee prosthesis, pacemakers, artificial tendons, heart valves, and so on.

Furthermore, ISO-10993 document groups the implants according to the duration of their interaction with the body [limited exposure (<24h), prolonged exposure (>24h and <30 days) or permanent contact (>30 days)]. The interaction duration and contact type between the device and tissues affect the selection of the test to asses the device compatibility.

Sufficient knowledge about the biodegradation[11] of biomaterials is essential in the evaluation of local or systemic effects, which can be caused in patients. For example, the corrosion of orthopedic alloys causes the release of various metal elements in human tissue and has to be assessed with respect to levels, kinetics, and chemical state of the ions. Currently, there are no standard practices, methods or guideline for the evaluation of corrosion of orthopedic alloys, and of the products formed from corrosion. Other important tests for load-bearing implants include friction and wear tests. Besides evaluation of frictional and wear resistance properties, the wear debris particles need to be analyzed in terms of size/size distribution and chemistry. This analysis is critical as far as asceptic loosening

or osteolysis is concerned. In addition, *in vitro* dissolution tests assess the weight change or deposition of any mineralized phase (such as CaP-rich) on the surface of biomaterial. In the absence of prescribed ISO guidelines, all the above mentioned tests are carried out in simulated body fluid solution, such as Ringer's solution or Hank's balanced salt solution (HBSS). Depending on the intended use of biomaterials, different proteins such as bovine serum albumin (BSA) or other serum proteins are added to SBF. During corrosion/wear/*in vitro* dissolution tests, the pH of 7.4 and temperature of 37 °C are closely maintained during the entire test duration. In case of tests with dental restorative material, such as glass-ceramics, *in vitro* dissolution tests are carried out in artificial saliva.

1.6 STEPS FOR CHARACTERIZATIONS OF BIOMATERIALS

In order to evaluate the cell/tissue interactions for biomaterials, certain specific experiments have to be followed. These include:

a) The first step is the material development and its characterization. Previous experience and literatures help in choosing the appropriate system to evaluate a material for a specific application.
b) After evaluating the physical properties and some other necessary tests, the material goes to microbiology section, where the material is sterilized by ethanol or gamma ray or some other techniques. Depending on the desired biomedical application of the materials under investigation, it goes to thrombosis lab and tissue culture lab, to evaluate the *in vitro* properties of materials. In thrombosis lab, materials are tested in contact with blood. The tests are platelet count, platelet adhesion, hematology, coagulation test, and immunology. In tissue culture lab, the interaction of cells as well as different tissues with materials is observed. The tests are cell proliferation and cell adhesion and *in vitro* toxicity. Depending on end use, a specific property of a material is evaluated. For orthopedic implant applications, cell adhesion is mostly desirable, but cell adhesion assessment is not desirable for heart valve materials. For the latter, the desirable property is thromboresistance.
c) Subsequently, material goes to the *in vivo* toxicity lab. Here, materials extract is injected into animal bodies or material is placed in the animal body. After a long-term observation, the animal is sacrificed and the contacting body parts of the animal are taken to the histopathology lab for further experiments
d) In the histopathology lab, animal tissues are prepared for microscopic analysis. Special techniques are adopted to make the sample for optical, scanning and transmission electron microscopy.
e) There are some important aspects to choose the animal for *in vivo* experiments. The animal welfare committee and ethical committee decide

the number of animals and the type of animals for particular *in vivo* experiments. Some guidelines need to be followed.

f) When the material is successfully selected for a particular application, it goes to implant biology section to shape the material into final use. Rapid prototyping using CAD-CAM technology is the new technique for developing these materials.

g) The other important aspects of biocompatibility testing are clean room practice and microbiological evaluation of materials. The laboratory environment should be free from dust and microbes and totally sterilized. Distilled water maintenance plays an important role, because in every step the quality the distilled water, dictates the perfection in experiments.

1.7 BROAD OVERVIEW OF FUNDAMENTALS SECTION

In the **Fundamentals section of this book (section I)**, the topics will cover the structure and properties of calcium phosphates, mechanical properties of bones, interaction of cells with nanobiomaterials, interface tissue engineering, blood compatibility, and polymer-ceramic biocomposites. In particular, the fundamental aspect of structure, processing and properties of the natural bone as well as those related to various approaches to develop or design new biomaterials is presented in the chapters under this section. For example, the chapter by **Bikramjit Basu** and co-workers broadly discusses the various approaches to optimize the processing conditions or material composition to design biomaterials in metals, ceramics and polymeric materials for hard tissue replacement. Limited discussion is also made on biocompatible coatings. It is well known that the inorganic component of the natural cortical bone is calcium phosphate (CaP) compounds, rich with hydroxyapatite (HA) phase. In view of this, concerted research efforts were invested in understanding the structure and properties of HA and subsequently to modify or refining the structure and properties of HA to improve the physical/biological properties. In this context, the chapter contributed by **Racquel Z. Legeros** describes, among many aspects, the fundamental crystal structure of HA and calcium-deficient apatite (CDA). The results obtained with various characterization tools in precisely describing the structure of such complex inorganic compounds are provided. Following the substitution of anion (OH^-) by F^- or Cl^-, $(CO3)^{-2}$ and similarly, incorporation of Sr, Ba, Pb to substitute (Ca^{+2}) cation are discussed along with their implication on structure and properties in reference of stoichiometic HA.

The aspect of synthesis of various types of biologic apatites, including ACP, DCPDTCP, OCP, TTCP is mentioned briefly along with the existing/potential biomedical applications of CaP-compounds. The chapter by **Guy Daculsi** describes the processing strategies to develop micro- and macro-porous biphasic calcium phosphate (BCP) bioceramics (an optimum balance between the more stable HA phase and more soluble TCP) for better osteogenicity and osteoinductive properties. The clinical applications requiring better control of biomaterial resorption

and bone substitution are highlighted along with the existing commercial use of BCP blocks/particulates/designed matrices with bone marrow or mesenchymal stem cells for tissue engineering (hybrid bone). Overall musculoskeletal joint motion depends largely on the synchronized interactions and integration between bone and soft tissues such as ligaments, tendons or cartilage. Therefore an important consideration in the current functional tissue engineering effort is how to achieve tissue–to–tissue integration and as a consequence, the focus in the field of tissue engineering has shifted from tissue formation to tissue function, in particular on regenerating the anatomic interface between various soft tissues and bone.

In the context of interfacial tissue engineering, the chapter by **Helen H. Lu** discusses the following aspects: design considerations in interface tissue engineering and recent research results using the anterior cruciate ligament–to–bone interface model system. It is posited that functional integration of soft tissue grafts can be achieved through the regeneration of the characteristic fibrocartilage interface found between soft tissue and bone. Some model scaffold systems based on biodegradable polymers and calcium phosphate composites for tendon–to–bone integration have been discussed, along with a discussion of the potential mechanism for *ex vivo* development and *in vivo* translation of integrated musculoskeletal tissues with biomimetic complexity and functionality.

The chapter by **Yoshiki Oshida** presents how better osseointegration property in Titanium-based new dental implants can be achieved by introducing functionality. The aspects of biological, mechanical and morphological compatibilities at the ti-implant/hard tissue interface have been utilized and described along with the related processing strategies. Since the last decade, nanotechnology offers exciting alternatives to traditional implants since human tissues are composed of constituent nanostructured entities. The cross-fertilisation of ideas drawn from nanotechnology and bone-tissue engineering offers the opportunity to closely biomimi the cortical bone properties, in terms of the combination of the structure–property–biological performance correlationship.

In this context, the chapter contributed by **T. J. Webster** focuses on the contemporary development of nanomaterials for orthopedic applications. After briefly reviewing the existing problems with the existing orthopedic implants (osteolysis, fractures etc.), the results obtained with synthesized novel nanophase composites (that is, materials with dimensions less than 100 nm in at least one direction) of metals, ceramics, biodegradable polymers, injectable hydrogels are presented. It is demonstrated that the increased regeneration of bone, cartilage, vascular, and bladder tissue *in vivo* is achievable on nanophase compared to conventional materials.

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