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6 BIOMATERIALS

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Chapter Contents

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At the conclusion of this chapter, the reader will be able to:

- Understand the complexity of natural tissue construction.
- Describe several different types of biological responses to implanted materials.
- Understand the benefits and differences between the various classes of materials used in medicine.
- Design bio-inspired medical device features.
- Describe how biomaterials can be modified to enhance or modify cellular interactions.
- Understand the various methods to prepare scaffolds for tissue engineering.
- Know where to find the appropriate established testing protocols to demonstrate medical product safety.

6.1 MATERIALS IN MEDICINE: FROM PROSTHETICS TO REGENERATION

Throughout the ages, materials used in medicine (biomaterials) have made an enormous impact on the treatment of injury and disease of the human body. Biomaterials use increased rapidly in the late 1800s, particularly after the advent of aseptic surgical technique by Dr. Joseph Lister in the 1860s. The first metal devices to fix bone fractures were used as early as the late eighteenth to nineteenth century; the first total hip replacement prosthesis was implanted in 1938; and in the 1950s and 1960s, polymers were introduced for cornea replacements and as blood vessel replacements. Today, biomaterials are used throughout the body (Fig. 6.1). Estimates of the numbers of biomedical devices incorporating biomaterials used in the United States in 2002 include

- Total hip joint replacements: 448,000
- Knee joint replacements: 452,000
- Shoulder joint replacements: 24,000
- Dental implants: 854,000

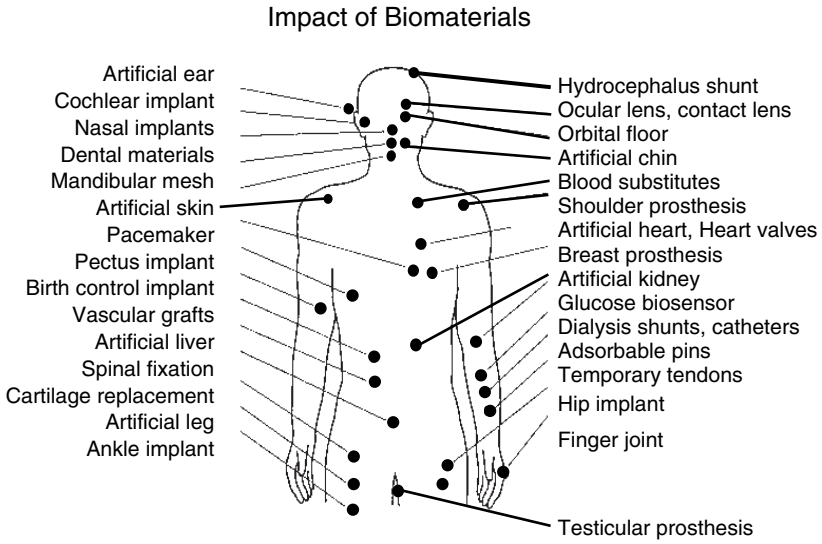

Figure 6.1 Biomaterials have made an enormous impact on the treatment of injury and disease and are used throughout the body.

- Coronary stents: 1,204,000
- Coronary catheters: 1,328,000

Millions of lives have been saved due to biomaterials and the quality of life for millions more is improved every year due to biomaterials. The field remains a rich area for research and invention because no one material is suitable for all biomaterial applications and new applications are continually being developed as medicine advances. In addition, there are still many unanswered questions regarding the biological response to biomaterials and the optimal role of biomaterials in tissue regeneration that continue to motivate biomaterials research and new product development.

Over most of history, minimal understanding of the biological mechanisms of tissues meant that the biomedical engineering approach was to completely replace the tissue with lost function with a simple biomaterial. As our understanding of tissues, disease, and trauma improved, the concept of attempting to repair damaged tissues emerged. More recently, with the advent of stem cell research, medicine believes it will be possible to regenerate damaged or diseased tissues by cell-based tissue engineering approaches (see Chapter 7). The notion of a biomaterial has evolved over time in step with changing medical concepts. Williams in 1987 defined a biomaterial as "a nonviable material used in a medical device, intended to interact with biological systems." This definition still holds true today and encompasses the earliest use of biomaterials replacing form (e.g., wooden leg, glass eye) as well as the current use of biomaterials in regenerative medical devices such as a biodegradable scaffold used to deliver cells for tissue engineering. While the definition has remained

the same, there have been dramatic changes in understanding of the level of interaction of biomaterials with the biological system (in this case, the human body). The expectations for biomaterial function have advanced from remaining relatively inert in the body to being "bioactive" and assisting with regeneration. Bioactive materials have the capability to initiate a biological response after implantation such as cell adhesion, proliferation, or more excitingly, the differentiation of a stem cell leading to regeneration of a damaged tissue or whole organ.

Due to the complexity of cell and tissue reactions to biomaterials, it has proven advantageous to look to nature for guidance on biomaterials design, selection, synthesis, and fabrication. This approach is known as biomimetics. Within the discipline of biomaterials, biomimetics involves imitating aspects of natural materials or living tissues such as their chemistry, microstructure, or fabrication method. This does not always lead to the desired outcome since many of the functionalities of natural tissues are as yet unknown. Furthermore, the desirable or optimal properties of a biomaterial vary enormously depending on the biomedical application. Therefore, in addition to presenting general strategies for guiding tissue repair by varying the chemistry, structure, and properties of biomaterials, this chapter includes application-specific biomaterials solutions for several of the major organ systems in the body and for drug delivery applications. This chapter also includes a section on the standards and regulatory agencies that play an essential role in establishing and ensuring the safety and efficacy of medical products.

6.2 BIOMATERIALS: PROPERTIES, TYPES, AND APPLICATIONS

6.2.1 Mechanical Properties and Mechanical Testing

Some basic terminology regarding the mechanical properties of materials is necessary for a discussion of materials and their interactions with biological tissues. The most common way to determine mechanical properties is to pull a specimen apart and measure the force and deformation. Materials are also tested by crushing them in compression or by bending them. The terminology is essentially the same in either case—only the mathematics are different. Standardized test protocols have been developed to facilitate comparison of data generated from different laboratories. The vast majority of those used in the biomaterials field are from the American Society for Testing and Materials (ASTM). For example, tensile testing of metals can be done according to ASTM E8, ASTM D412 is for rubber materials, and ASTM D638 is for tensile testing of rigid plastics. These methods describe specimen shapes and dimensions, conditions for testing, and methods for calculating and reporting the results.

Tensile testing according to ASTM E8 is done with a "dog bone" shaped specimen that has its large ends held in some sort of a grip while its narrow midsection is the "test" section. The midportion is marked as the "gage length" where deformation is measured. A mechanical test machine uses rotating screws or hydraulics to stretch the specimen. Force is measured in Newtons (N), and how much the specimen stretches—deformation—is measured in millimeters. Since specimens of different dimensions

can be tested, measurements must be normalized to be independent of size. Stress, σ (N/m^2 or Pascals), is calculated as force divided by the original cross-sectional area, and strain, ε (%), is calculated as change in length divided by the original length.

$$\sigma(N/m^2) = \text{force/cross-sectional area} \tag{6.1}$$

$$\varepsilon(\%) = [(\text{deformed length} - \text{original length})/\text{original length}] * 100\% \tag{6.2}$$

A stress–strain curve can be generated from these data (Fig. 6.2), and there are a number of material properties that can be calculated. Region A is known as the elastic portion of the curve. If a small stress is applied to a metal, such as up to point (1), it will deform elastically.

This means that, like a rubber elastic band, it will return to its original length when the stress is removed. The slope of the elastic portion of the stress–strain curve is a measure of the stiffness of the material and is called the elastic modulus (*E*) or Young's modulus.

$$E = \sigma/\varepsilon \text{ initial slope} = \text{stress/strain} \tag{6.3}$$

As the applied stress is increased, a point is reached at which the metal begins to deform permanently, the yield point (YS). If at point (2) the stress is now released, the

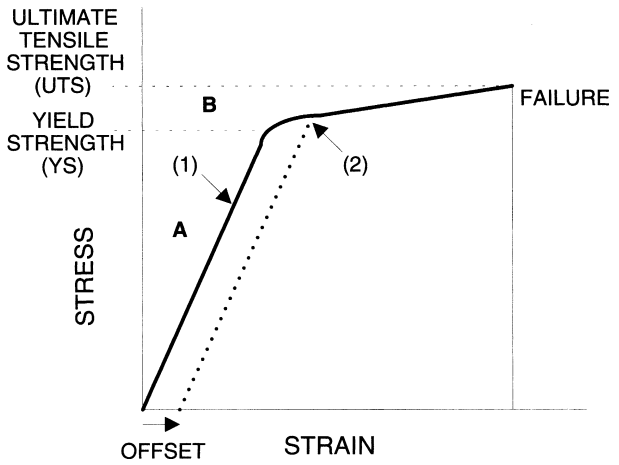

Figure 6.2 Typical stress–strain curve for a metal that stretches and deforms (yields) before breaking. Stress is measured in N/m^2 (Pa) while strain is measured as a percentage of the original length. The minimum stress that results in permanent deformation of the material is called the yield strength (YS). The ultimate strength (UTS) is the maximum stress that is tolerated by the material before rupturing. The stress at which failure occurs is called the failure strength (FS). Region A represents the elastic region since the strain increases in direct proportion to the applied stress. If a small stress is applied (e.g., to point 1), the material will return to its original length when the stress is removed. Region B represents the plastic region in which changes in strain are no longer proportional to changes in stress. Stresses in this region result in permanent deformation of the material. If a stress is applied that results in the strain at point (2), the material will follow the dotted line back to the baseline when the stress is removed and will be permanently deformed by the amount indicated by the offset.

stress–strain recording will come down the dotted line parallel to the elastic region. The permanent amount of deformation is now shown as the offset yield. Since it may be difficult to determine the yield point for a material, an offset yield point often is used in place of the original yield point. For metals, yield is typically defined as 0.2% while a 2% offset is often used for plastics. If the metal is loaded again, the recording will follow the dotted line starting at the offset yield, reaching the upper curve, and continuing to show a gradual increase in stress with increasing strain. This is known as the plastic region of the curve. The peak stress that is attained is called the tensile or ultimate tensile strength (UTS). Eventually the metal will break at the failure or fracture strength (FS), and the percentage of elongation or compression to failure can be determined.

Example Problem 6.1

A 7-mm cube of bone was subjected to a compression loading test in which it was compressed in increments of approximately 0.05 mm. The force required to produce each amount of deformation was measured, and a table of values was generated. Plot a stress–strain curve for this test. Determine the elastic modulus and the ultimate tensile strength of the bone.

Deformation (mm)	Force (N)
0.00	0
0.10	67.9
0.15	267.6
0.20	640.2
0.26	990.2
0.31	1265.1
0.36	1259.9
0.41	1190.9
0.46	1080.8
0.51	968.6
0.56	814.2

Solution

First, determine the cross-sectional area of the cube in meters ($0.007\,\text{m} \times 0.007\,\text{m} = 49 \times 10^{-6}\,\text{m}$). Use this value to determine the stress at each measuring point where stress (σ) equals force divided by cross-sectional area. For example, the stress when the cube was compressed by 0.10 mm was $67.9\ \text{N}/0.000049\,\text{m}^2 = 1.39\,\text{MPa}$ ($1\ \text{N/m}^2 = 1\,\text{Pa}$). Next, determine the strain at each measuring point. The deformed length when the cube was compressed by 0.10 mm was 7 mm − 0.10 mm equals 6.9 mm, so the strain was [(6.9 mm − 7.0 mm)/7.0 mm]* 100% equals 1.43%. This is the same value that would be obtained by dividing the amount of deformation by the original length. The minus sign is ignored because it merely indicates that the sample was subjected to compression rather than to tension.

Strain (%)	Stress (MPa)
0.00	0
1.43	1.39
2.14	5.46
2.86	13.07
3.71	20.21
4.43	25.82
5.14	25.71
5.86	24.30
6.57	22.06
7.29	19.77
8.00	16.61

The resulting stress–strain curve is shown in Figure 6.3. Linear regression was used to determine the line shown in Figure 6.3. The elastic modulus (i.e., the slope of the line) was 8.4, and the ultimate tensile strength was 25.82 MPa. ■

Several other terms are applied to the test results. The slope of the elastic portion, the elastic modulus, is often called stiffness. If the metal stretches a great deal before failure it is said to be ductile (Fig. 6.4). If the material does not deform or yield much before failure, it is said to be brittle. The area under the curve has units of energy and

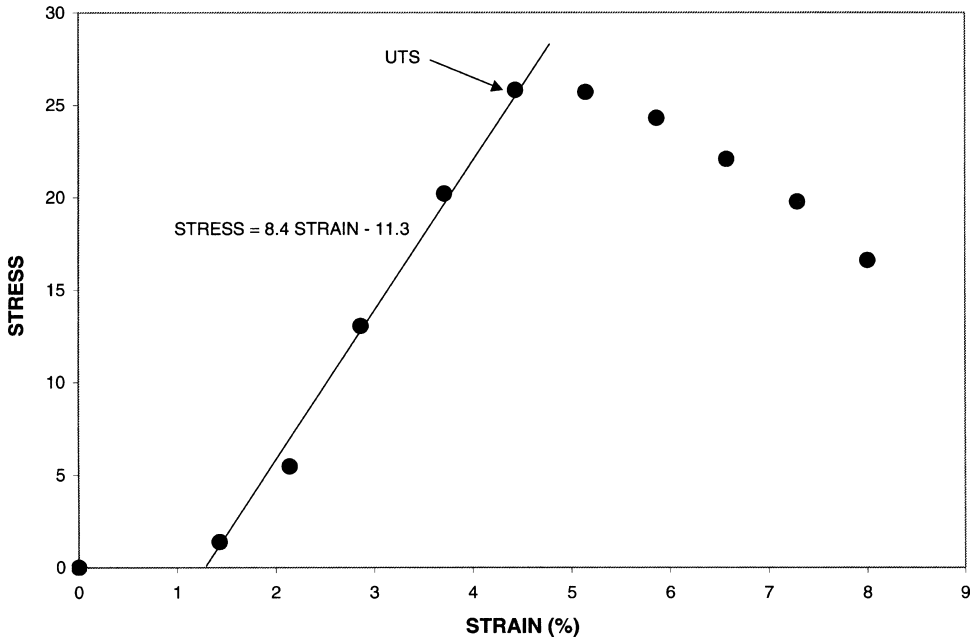

Figure 6.3 The stress–strain curve for the bone data from Example Problem 6.1. Linear regression analysis was used to find the line that best fit the data for strains of 1.43% to 4.43% (i.e., the linear portion of the curve). The slope of the line, 8.4, represents the elastic modulus of the bone. The ultimate tensile strength of the material (UTS) was 25.82 MPa.

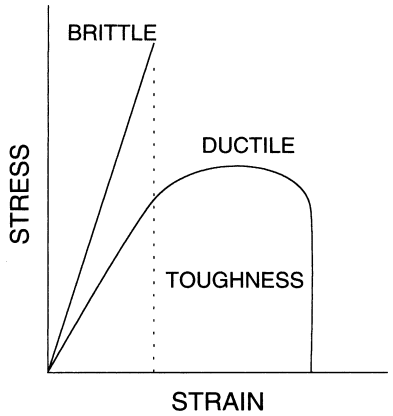

Figure 6.4 Brittle materials reach failure with only a small amount of deformation (strain) while ductile materials stretch or compress a great deal before failure. The area under the stress–strain curve is called toughness and is equal to the integral from ε_0 to $\varepsilon_f \sigma d\varepsilon$.

is called toughness. Although not directly available from a stress–strain curve, the strength of a material can be related to its hardness. Stronger materials are typically harder. Hardness is tested by measuring the indentation caused by a sharp object that is dropped onto the surface with a known force. Hardness is perhaps the most important property when considering a material's wear resistance.

An additional property that is not depicted in the figure is the fatigue strength or endurance limit of a material. If the material were tested as in Figure 6.2, but was loaded to point (2) and unloaded, it would become permanently deformed. If this were repeated several times, like bending a paper clip back and forth, the material would eventually break. If, however, the metal were loaded to point (1) and then unloaded, it would not be deformed or broken. If it were loaded again to point (1) and unloaded, it still would not break. For some metals, there is a stress level below which the part can theoretically be loaded and unloaded an infinite number of times without failure. In reality, a fatigue limit is defined at a specified number of cycles, such as 10^6 or 10^7. Clearly, fatigue strength is a critical property in the design of load-bearing devices such as total hips which are loaded on average a million times a year or heart valves which are loaded 40 million times a year.

6.2.2 Metals

Metals used as biomaterials have high strength and resistance to fracture and are designed to resist corrosion. Examples of metals used in medical devices and their mechanical properties are shown in Tables 6.1 and 6.2. Many orthopedic devices are made of metal, such as hip and knee joint replacements (Figs. 6.5 and 6.6). The implants provide relief from pain and restore function to joints in which the natural cartilage has been worn down or damaged. Plates and screws that hold fractured bone

TABLE 6.1 Materials and Their Medical Uses

Class of Material	Current Uses
Metal	
Stainless steel	Joint replacements, bone fracture fixation, heart valves, electrodes
Titanium and titanium alloys	Joint replacements, dental bridges and dental implants, coronary stents
Cobalt-chrome alloys	Joint replacements, bone fracture fixation
Gold	Dental fillings and crowns, electrodes
Silver	Pacemaker wires, suture materials, dental amalgams
Platinum	Electrodes, neural stimulation devices
Ceramics	
Aluminum oxides	Hip implants, dental implants, cochlear replacement
Zirconia	Hip implants
Calcium phosphate	Bone graft substitutes, surface coatings on total joint replacements, cell scaffolds
Calcium sulfate	Bone graft substitutes
Carbon	Heart valve coatings, orthopedic implants
Glass	Bone graft substitutes, fillers for dental materials
Polymers	
Nylon	Surgical sutures, gastrointestinal segments, tracheal tubes
Silicone rubber	Finger joints, artificial skin, breast implants, intraocular lenses, catheters
Polyester	Resorbable sutures, fracture fixation, cell scaffolds, skin wound coverings, drug delivery devices
Polyethylene (PE)	Hip and knee implants, artificial tendons and ligaments, synthetic vascular grafts, dentures, and facial implants
Polymethylmethacrylate (PMMA)	Bone cement, intraocular lenses
Polyvinylchloride (PVC)	Tubing, facial prostheses
Natural Materials	
Collagen and gelatin	Cosmetic surgery, wound dressings, tissue engineering, cell scaffold
Cellulose	Drug delivery
Chitin	Wound dressings, cell scaffold, drug delivery
Ceramics or demineralized ceramics	Bone graft substitute
Alginate	Drug delivery, cell encapsulation
Hyaluronic acid	Postoperative adhesion prevention, ophthalmic and orthopedic lubricant, drug delivery, cell scaffold

together during healing also are made of metal and are shown in Figure 6.7. Sometimes the metallic plates and screws are retrieved after successful healing, but in other cases they are left in place. Metallic devices are also used to fuse segments of the spine together when the disk has degenerated (Fig. 6.8) and as dental root prosthetic implants (Fig. 6.9).

Materials selection for a medical device is complicated. The selection depends on a number of factors, including the mechanical loading requirements, chemical and

TABLE 6.2 Mechanical Properties of Materials with Literature Values or Minimum Values from Standards

	Yield	UTS	Deform	Modulus
	MPa	MPa	%	GPa
METALS				
High-strength carbon steel	1600	2000	7	206
F138[1], annealed	170	480	40	200
F138, cold worked	690	860	12	200
F138, wire	-	1035	15	200
F75[2], cast	450	655	8	200
F799[3], forged	827	1172	12	200
F136[4] Ti64	795	860	10	105
Gold		2-300	30	97
Aluminum, 2024-T4	303	414	35	73
POLYMERS				
PEEK		93	50	3.6
PMMA Cast		45-75	1.3	2-3
Acetal (POM)		65	40	3.1
UHMWPE		30	200	0.5
Silicone rubber		7	800	0.03
CERAMICS				
Alumina		400	0.1	380
Zirconia, Mg partially stabilized		634		200
Zirconia, Yttria stabilized		900		200
CARBONS AND COMPOSITES				
LTI pyrolytic carbon + 5–12% Si		600	2.0	30
PAN AS4 fiber		3980	1.65	240
PEEK, 61% C fiber, long		2130	1.4	125
PEEK, 61% C fiber, +-45		300	17.2	47
PEEK-30% C fiber, chopped		208	1.3	17
BIOLOGIC TISSUES				
Hydroxyapatite (HA) mineral		100	0.001	114–130
Bone (cortical)		80–150	1.5	18–20
Collagen		50		1.2

[1]F138, wrought stainless steel: 17–19 Cr, 13–15.5 Ni, 2–3 Mo, <2 Mn, <0.08 or <0.03 C
[2]F75, cast cobalt-chromium-molybdenum alloy: 27–30 Cr, <1.0 Ni, 5–7 Mo, <1 Mn
[3]F799, wrought Co-Cr-Mo alloy: 26–30 Cr, <1.0 Ni, 5–7 Mo, <1.0 Mn, <1.5 Fe, <1.5 C
[4]F136 Titanium 6Al-4V alloy: 5.5–6.5 Al, 3.5–4.5 V, <0.015 N, < 0.13 O, <0.08 C

Figure 6.5 A typical total hip joint replacement is made primarily of metal. The ball of the femoral hip stem fits into a pelvic acetabular cup that is lined with ultra high molecular weight polyethylene (UHMWPE) for friction-free motion. (Photograph of the PROFEMUR ® Z minimally invasive hip stem with modular necks courtesy of Wright Medical Technology, Inc.)

Figure 6.6 A metallic artificial knee joint with an ultra high molecular weight polyethylene bearing surface. (Photograph of the ADVANCE ® medial-pivot knee system courtesy of Wright Medical Technology, Inc.)

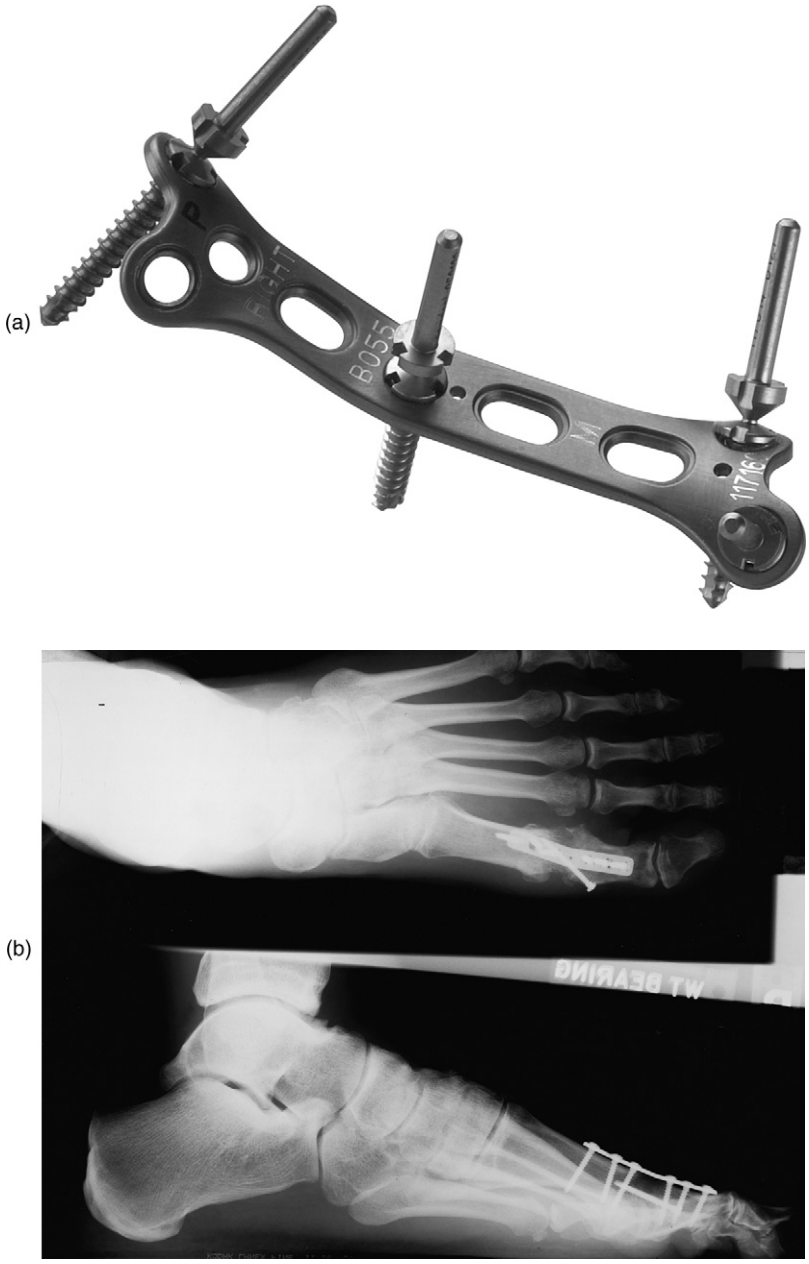

Figure 6.7 (a) Metal plates and screws are used to hold fractured bone segments together during healing. Depending on the extent of injury, the plates and screws or rods may be removed when the bone is fully repaired. (Photograph of the HALLU®-FIX MTP Fusion System (registered mark of NEW-DEAL) is courtesy of Wright Medical Technology, Inc.) (b) Through the use of x-rays an implanted metal plate with screws can be visualized in this patient's foot and hand. (X-ray courtesy of Wright Medical Technology, Inc.)

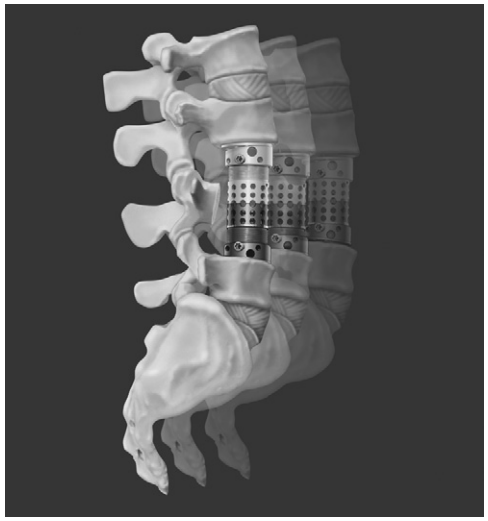

Figure 6.8 Metallic devices are used to fuse segments of the spine together when vertebral bones are fractured due to osteoporosis or back injury. The metal cage can accommodate the patient's own bone particles to assist with new bone formation which will eventually span and fuse the adjacent vertebral bones. (Photograph of the VERTESPAN® spinal fusion cage courtesy of Medtronic Sofamor Danek.)

Figure 6.9 As an alternative to dentures, patients can have metallic dental root prosthetics implanted to replace each missing tooth. The implant is then topped with a porcelain crown. One advantage of dental implants over dentures is that the implant transmits mechanical forces into the jaw bone and stimulates it, resulting in less bone recession over time. (Photograph courtesy of Dr. Martin Freilich of the University of Connecticut Health Center.)

structural properties of the material itself, and the biological requirements. The longstanding use of metals for knee and hip joints, bone plates, and spinal fusion devices is due to the high mechanical strength requirements of these applications and proven biocompatibility in these settings. The advantages of metals over other materials such as ceramics and polymers are that they are strong, tough, and ductile (or deformable, particularly as compared to ceramics). Disadvantages include susceptibility to corrosion due to the nature of the metallic bond (free electrons). In fact, the steels that were used in the early 1900s for hip implants corroded rapidly in the body and caused adverse effects on the healing process. This has led to the preferred selection of alloys of titanium or cobalt-chrome for hip, knee, and dental implants. Other typical properties of metallic materials include a high density and much greater stiffness than most natural materials they replace, which lead to undesirable stress shielding. Stress shielding has been observed after implantation of metal joint replacements and leads to loss of adjacent bone because the bone is not exposed to normal levels of mechanical loading. Certain metals known as shape memory alloys (e.g., nitinol) can be bent or deformed and still return to their original shape when the stress is released. These metals have found application in eye glasses and coronary artery stents that can be inserted through a catheter while collapsed and then spring into a cylindrical shape once they are pushed beyond the confines of the catheter.

Metallic devices are typically made by investment casting, computer-aided design and machining (CAD/CAM), grinding, or powder metallurgy techniques. The specific steps involved in the fabrication of a medical device will depend on factors such as final geometry of the implant, the forming and machining properties of the metal, and the costs of alternative fabrication methods.

Example Problem 6.2

A stent is a helical, woven device that is implanted into an occluded artery to permit increased blood flow. A permanent yet flexible device is needed for use as a vascular stent. What material meets that need? In addition to information contained in this chapter, search the web for information on current materials selections using keywords such as ''stents'' and ''metals.'' The National Institutes of Health PUBMED website catalogs scientific publications within the biological, biomedical, and medical sciences (http://www.ncbi.nlm.nih.gov/entrez). Corporate web pages can provide additional information. Guidant and Boston Scientific are two companies that currently produce coronary stents. The United States patent office provides another very useful web page for researching uses of materials in surgical and medical devices (www.uspto.gov).

Solution

The preferred material for a stent is a metal such as platinum or titanium. Shape memory alloys such as nitinol also have been used. Stents made of nitinol are self-expanding and ''remember'' their manufactured shape when they are deployed in the

body. They are particularly good for curved or tapered vessels. Metal materials have the strength required for this application and have been shown to be biocompatible after implantation. ■

6.2.3 Ceramics and Glasses

The advantages of the class of materials known as ceramics are that they are very biocompatible (particularly with bone), are inert, have low wear rates, are resistant to microbial attack, and are strong in compression. Some disadvantages include brittleness, the potential to fail catastrophically, and being difficult to machine. These properties arise from the atomic structure of ceramics: unlike metal, in which atoms are loosely bound and able to move, ceramics are composed of atoms that are ionocovalently bound into compound forms. This atomic immobility means that ceramics do not conduct heat or electricity. Two very obvious properties that are different from metals are melting point and brittleness. Ceramics have very high melting points, generally above 1000°C, and are brittle. Examples of ceramics used in medical devices are shown in Table 6.1. A photograph of a ceramic femoral head of a hip implant is shown in Figure 6.10 and an example of a pelletized calcium sulfate bone graft substitute is shown in Figure 6.11.

Certain compositions of ceramics, glasses, glass-ceramics, and composites have been shown to stimulate direct bone bonding, which is important in securing orthopedic medical devices such as replacement hips and knees and spinal fusion devices. These types of materials are known as bioactive ceramics. Studies on retrieved implants have shown that a biologically active calcium phosphate forms on the biomaterial surface upon implantation in the body. Since the calcium phosphate that forms is much like that found in our bones, bone cells are able to form an intimate attachment to the biomaterial surface after this bone mineral-like layer has formed. The same results are attained by implanting a material that already has a bonelike calcium phosphate surface.

There are several different atomic structures (or phases) of calcium phosphate that have been used in medical applications, including hydroxyapatite, carbonated apatite, di-calcium phosphate or brushite, beta-tricalcium phosphate or tetracalcium phosphate, and amorphous calcium phosphate. The stability of a given calcium phosphate medical device depends on the crystal phase, the crystal size and perfection, the temperature used during processing, the density, and the in-use environment. At physiological temperature and pH, hydroxyapatite is the stable phase, and it generally takes a long time to resorb via physiochemical dissolution. However, bone cells and other cells called macrophages can initiate cell-mediated resorption by changing the local pH to acidic. Resorbable ceramics are typically nonhydroxyapatite phases of calcium phosphate or other calcium-based biomaterials such as calcium carbonate or calcium sulfate.

Due to the high melting point of most ceramics, which prevents them from being cast or extruded, ceramic components are typically made from powdered stock. The powders are formed by wet synthesis methods or by pulverizing raw materials. The

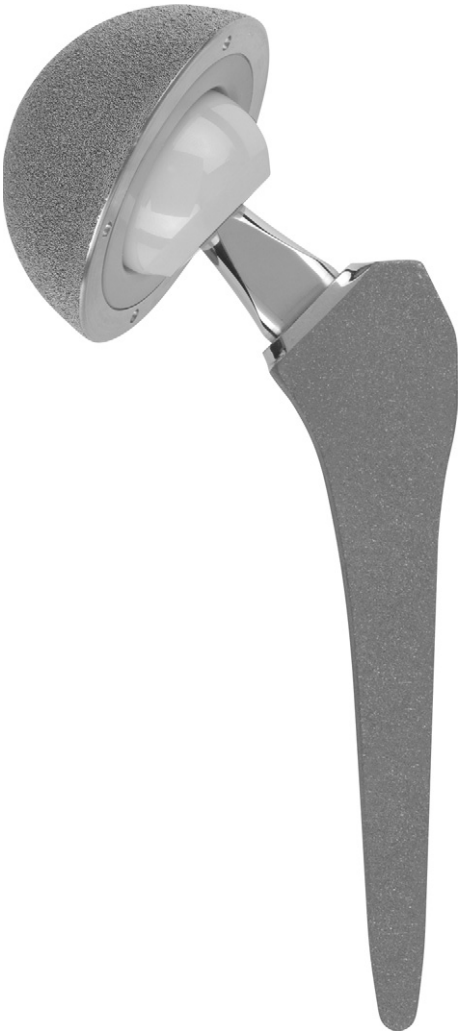

Figure 6.10 In this artificial hip joint, the polymer bearing surface and some of the metallic components have been replaced by ceramics to improve the durability of the joint replacement. This design features a ceramic femoral head and acetabular cup. (Photograph of the LINEAGE ® ceramic–ceramic acetabular cup system is courtesy of Wright Medical Technology, Inc.)

ceramic powder is either added to a liquid with binders to form a slurry that is cast in a mold or dry pressed to form ''green ware.'' The green ware must be finally sintered or fired to densify the powders and remove the porosity between the powder particles. In weight-bearing applications, the porosity must be nearly totally removed or the residual porosity acts as microcracks within the material and weakens it. In other applications such as bone graft substitutes it is desirable to have large pores like those in trabecular or cancellous bone so that cells can infiltrate the material and grow new

Figure 6.11 If there is an insufficient amount of the patient's own bone or donor bone available to fill a bone defect, synthetic bone graft substitutes made of calcium phosphate or calcium sulfate may be used. (Photograph of OSTEOSET® surgical grade calcium sulfate resorbable beads is courtesy of Wright Medical Technology, Inc.)

vital tissue. In this case, pores are typically created by using second phases, such as polymer beads, that maintain pore space during the early processing steps and are then burned out during the final sintering stage. More detailed descriptions of how porous scaffolds are formed are included later in this chapter. Glasses are silica based. Silica is a network-forming oxide that can be heated to its melting point and, unlike most ceramics, is more easily manufactured.

Example Problem 6.3

What material is preferred for the acetabular cup of a hip implant? What design parameters are utilized during the selection process? Use scientific, corporate, and patent websites to locate information on this topic using keywords such as "ceramic" and "hip replacement."

Solution

Acetabular cups are currently made with a metal support structure and a polyethylene cup; however, problems with wear debris from the soft polyethylene have led to new products with ceramic acetabular cups and femoral balls (alumina or zirconia). The cup must resist wear and deformation and be a low-friction surface because it is in contact with the ball component of the artificial joint. The ceramic materials generate less wear debris during use than does the traditional metal on plastic design. Thus, in

theory, the risk that ceramic total hips will fail is low compared to the traditional metal on plastic design. Since the ceramic components are fragile relative to the metallic components and not well tolerated by osteoporotic bone, both types of hip replacements are utilized today. ■

6.2.4 Polymers

Polymers are well suited for biomedical applications because of their diverse properties. For example, polymers can be flexible or rigid, can be low strength or high strength, are resistant to protein attachment or can be modified to encourage protein attachment, can be biodegradable or permanent, and can be fabricated into complex shapes by many methods. Some disadvantages of polymers are that they tend to have lower strengths than metals or ceramics, deform with time, may deteriorate during sterilization, and may degrade in the body catastrophically or by release of toxic by-products. Examples of polymers used in medical devices and their mechanical properties are listed in Tables 6.1 and 6.2.

The large macromolecules of commercially useful polymers are synthesized by combining smaller molecules (mers) in a process termed *polymerization*. Polymerization may proceed by addition (or chain reaction) polymerization, in which monomer units are attached one at a time and then terminated, or by condensation (or step reaction) polymerization, in which several monomer chains are combined and a by-product of the reaction, such as water, is generated. Additives such as fillers, plasticizers, stabilizers, and colorants typically are used in polymer synthesis to enhance the mechanical, chemical, and physical properties.

Polymers can be classified as thermoplastic or thermosetting. A thermoplastic polymer has a linear or branched structure. As a solid it is like a bowl of spaghetti in that the chains can slide over one another. With heating, the chains can slide more easily, and the polymer melts or flows. Thus, thermoplastic polymers can be heated, melted, molded, and recycled. Differences in properties can be achieved with the addition of different ligands. PVC is more rigid than PE because the chlorine atoms are larger and tend to prevent the sliding of one molecule over another. PMMA, as shown in Table 6.2, is stronger, stiffer, and much more brittle than UHMWPE. In this case, 2 of the 4 hydrogen atoms are replaced, one with a methyl group (CH_3) and the other with an acrylic group ($COOCH_3$). These large side-groups make sliding much more difficult, hence the increase in strength and modulus. They also make it difficult for the molecules to orient in an orderly, crystalline pattern. As a result of this amorphous structure, PMMA (Plexiglas® or Lucite®) is optically transparent.

In contrast, a thermosetting polymer is composed of chains that are cross linked. They do not melt with heating but degrade. The term *thermoset* implies that there is a chemical reaction, often involving heat, which results in setting a three-dimensional cross-linked structure. A common example is "5-minute epoxy." When the two parts are mixed, the catalyst causes setting and cross linking of the epoxy. Once set, it cannot be heated and reused. The amount of cross linking affects the mechanical properties. Few cross links are used to make rubber gloves. Adding more sulfur and cross linking

produces a car tire. Even more cross links are added to make the hard casing of a car battery.

Hydrogels are water-swollen, cross-linked polymeric structures that have received significant attention because of their many applications in biomedical applications. Hydrogels are prepared by cross linking polymer chains by irradiation or chemical methods and then expanding the network of chains by swelling the structure with water. The most widely used hydrogel is cross-linked polyhydroxyethylmethacrylate (PHEMA). The PHEMA structure has a water content similar to living tissue, has resistance to degradation, is not absorbed by the body, withstands heat sterilization without damage, and can be prepared in a variety of shapes and forms. Applications of hydrogels include contact lenses, drug delivery vehicles, wound healing adhesives, sexual organ reconstruction materials, artificial kidney membranes, and vocal chord replacement materials.

Quite a variety of techniques are employed in forming polymer medical devices. The technique depends on several factors such as whether the material is thermosetting or thermoplastic, and if thermoplastic, the temperature at which it softens. Thermosetting polymers must be prepared as a liquid linear polymer and then cured in a mold. They cannot be molded after this step. Thermoplastic polymers can be molded repeatedly (by compression, injection, extrusion, etc.), cast, and formed into fibers or films by extrusion followed by drawing or rolling to improve properties such as strength. Precipitation after being dissolved in a solvent by introduction of a nonsolvent is a way to form porous polymer scaffolds for tissue engineering. Large pores suitable for cellular infiltration can be created by adding particulates with the desired pore size to the polymer/solvent mixtures. Pores are created when the particulates are washed out after solvent removal. Additional methods for creating porous polymer scaffolds are described later in this chapter.

Example Problem 6.4

What material is preferred to produce a blood bag? A dialysis bag? What design parameters are involved?

Solution

PVC has been used for blood bags since the 1950s. Since PVC is naturally brittle, phthalate plasticizers are used to make it flexible. These leach out over time from the plastic bag and into the liquid which they contain. When fed in large quantities to rats, the plasticizers can cause cancer; therefore, other plastics are being investigated. Dialysis bags are made of low-density polyethylene (LDPE). In these examples, materials selection has been governed by the fact that the material must be flexible, chemically stable, and relatively inert. ■

6.2.5 Natural Materials

Natural materials are synthesized by an organism or plant and are typically more chemically and structurally complicated than synthetic materials. Examples of natural

biomaterials currently used in medical devices are listed in Table 6.1. Proteins and polysaccharides are nature's form of polymers and are used in medical devices. The directional bonds within proteins give rise to the very high mechanical properties of natural polymers. For example, the ultimate tensile strength of silk is higher than that of drawn nylon, one of the strongest synthetic polymers. Furthermore, the elastic modulus of silk is nearly thirteen times that of the elastic modulus of nylon. There are also natural ceramic materials. Natural ceramics are typically calcium based, such as calcium phosphate bone crystals or calcium carbonate coral or sea shells. Natural ceramics are typically much tougher (resistant to fracture) than synthetic ceramics due to their highly organized microstructure which prevents crack propagation. Small ceramic crystals are precisely arranged and aligned and are separated by thin sheets of organic matrix material. A crack in the material is forced to follow this tortuous organic matrix path. The category of natural materials also encompasses donor tissue such as bone or skin which may be patient derived (autograft), from another human (allograft), or from a different species such as bovine or porcine (xenograft).

Natural materials exhibit a lower incidence of toxicity and inflammation as compared to synthetic materials; however, it is often expensive to produce or isolate natural materials. There is also variability between lots of natural materials, which makes it difficult to maintain consistency and sometimes prevents widespread commercial use. The isolation or purification steps typically involve the use of solvents to extract the desired component from the rest of the tissue or the use of solvents to remove the undesired components from the tissue and leave the desired natural material intact. Collagen can be prepared by either method. If it is labeled as soluble collagen, it has been removed by pepsin enzymatic treatment from natural tissues such as cock's comb. Fibrillar collagen is prepared from natural tissue such as tendon by salt and lipid and acid extraction steps to remove the noncollagenous proteins and molecules, leaving the collagen fibers intact.

Biopolymers also may be produced by bacteria. Production of polyhydroxybutyrate (PHB) is carried out through a fermentation procedure. The bacteria produce the polymer in granules within their cytoplasm when they are fed a precise combination of glucose and propionic acid. The cells are then disrupted, and the granules are washed and collected by centrifugation and then dried. This polymer has properties similar to polypropylene and polyethylene yet it degrades into natural components found in the body. Because of the desirable environmental characteristics of biopolymers, they are rapidly finding use in several nonmedical niche markets such as biodegradable monofilament fishing nets. The more rapid and widespread introduction of biopolymers has been hindered by their high price (up to 10 times) relative to that of petroleum-based polymers.

Biopolymers also can be produced by chemically polymerizing naturally occurring monomers. Although these polymers are not produced by biological systems, the fact that they are derived from basic biological building blocks makes them biocompatible, nontoxic, and biodegradable. Lactic acid–based polymers have been used widely for many years for medical devices ranging from biodegradable sutures to tissue engineering scaffolds. Lactic acid is found in blood and muscle tissue and is produced

commercially by microbial fermentation of sugars such as glucose or hexose. Polylactides are frequently used in combination with polyglycolic acid.

Example Problem 6.5

What materials are preferred by surgeons for repairing large surgical or traumatic defects in bone? What factors influence this decision? Search the Internet for companies producing bone repair products. Go to the website of the American Academy of Orthopaedic Surgeons and the American Society of Plastic Surgeons.

Solution

Autograft bone is the first choice for surgeons for the repair of bony defects. The tissues are vital and contain living cells and growth factors that are required for bone regeneration. Allograft bone or demineralized allograft bone matrix is the second choice. Demineralized bone has advantages over as-harvested allograft bone because it is flexible and can conform to the defect site, resorbs more rapidly (within months as compared to years for nondemineralized allograft bone), and releases the bone inductive proteins known as bone morphogenetic growth factors originally discovered by Dr. M.R. Urist in the 1950s. ■

6.2.6 Composites

Composite materials consist of two or more distinct parts. Although a pure material may have distinct structural subunits such as grains or molecules, the term *composite* is reserved for materials consisting of two of more chemically distinct constituents that are separated by a distinct interface. Examples of composites used in biomedical applications include carbon fiber reinforced polyethylene and hydroxyapatite particle reinforced polylactic acid polymers for bone healing applications. The discontinuous phase is typically harder and stronger than the continuous phase and is called the reinforcement.

Composites are made by mixing two components and molding, compacting, or chemically reacting them together. The fibers are typically coated or impregnated with the polymer phase so that the composite can be heated and pressurized to densify the assembly. A chemical reaction may be utilized to form composites in which a second phase precipitates or forms upon reaction. There may be a filament winding process if high-strength hollow cylinders are being formed. Composites are well suited for devices that require a combination of properties such as total joint replacements, dental fillings, and bone plates. The advantages of composites are that the properties can be tailored to fit nearly any application; however, it is difficult to make a composite with an ideal structure. There are typically problems with dispersion of the second phase or weak interfacial bonds between the two phases which leads to less than ideal mechanical properties and, hence, poor product performance. However, in many cases the actual performance is still much better than any single component biomaterial and so composites are becoming more widely used in biomedical applications.

Example Problem 6.6

What materials are preferred for reconstructive dental applications? What are some advantages of the composite structure over a monolithic structure?

Solution

Mercury amalgams made of mercury, silver, and tin are the most commonly used filling materials. Aesthetically pleasing tooth-colored filling materials also can be used and are made of filled resins [e.g., large molecule bifunctional methyacrylates (BisGMA) filled with micro- and nanoparticulate silica]. PMMA is the predominant material used for complete and partial dentures. Chrome-based alloys are used for the framework of removable dentures. Crowns and bridges are made of a cast metal frame veneered with tooth-colored porcelain. All-ceramic systems are available as well. Recently, composites made of light curing resins reinforced with glass fibers have been developed for dental bridges. The metallic post typically used to provide structural support for crowns is now being replaced with this type of glass fiber reinforced composite, primarily for better aesthetic results—the polymer post does not show through the porcelain crown like the metallic post does. ■

6.3 LESSONS FROM NATURE ON BIOMATERIAL DESIGN AND SELECTION

6.3.1 An Overview of Natural Tissue Construction

Biomedical engineers are asked to design medical devices or systems that repair, monitor, or assist the functions of the human body. Approaches that mimic or replicate nature's techniques, known as biomimetics, are often at the heart of a successful medical device or therapy. There is an incredible complexity to the genesis of natural tissues and organs which is still far beyond the capacity of scientists to replicate. Furthermore, the precise function of every aspect of the tissues or organs is not known. For these reasons, it is very difficult to theoretically design medical devices, and the field has progressed through a fair amount of trial and error. Nonetheless, there are several general concepts that have emerged from the study of structural biology that provide design strategies and guidance for a biomaterials scientist involved in tissue/organ regeneration.

- Cells are programmed by their genetic code to build the tissues and organs of our bodies.
- Cells produce proteins, polysaccharides, glycoproteins, and lipids that self-assemble into composite extracellular matrices that have multiple diverse forms and serve to support tissue growth.
- Cells communicate via growth factors and their recruitment, and even cellular fate is determined by protein signals.
- Blood vessels play a crucial role in tissue growth by providing nutrients, a means for waste removal, and a supply of additional cells to support further growth.

- The nervous system is responsible for the integration and control of all the body's functions.
- Skeletal tissues are made hard and stiff by the protein-controlled nucleation and growth of small, discrete, nanometer-sized mineral crystals within a collagen matrix microenvironment.

Overall, natural tissue design is hierarchical: that is, a structure within a structure, like a nested set of eggs or the branches of a tree. The same structural motif is repeated at multiple length scales to endow the tissue with strength and efficiency of function. Biomimetic paradigms that have been derived from these basic structural and developmental biology concepts provide a rational starting point for the design and fabrication of biomaterials, especially for regenerative tissue-engineered medical devices.

6.3.2 Cells Build Natural Tissues

There are over 200 different cell types in the human body and approximately 100 trillion cells per person. Bone tissue alone has more than ten types of cells:

- Osteoblasts which make bone
- Osteoclasts which degrade bone
- Osteocytes which maintain bone
- Cells in blood vessels that run through bone [erythrocytes (red blood cells) and immune cells (lymphocytes, monocytes, macrophages, neutrophils, and eosinophils)]
- Lipid cells and mesenchymal stem cells that are not yet differentiated into a specific type of cell, which are both found in bone marrow

The existence of a cell population that has the potential to differentiate into a number of tissue cell types is a key design strategy that our bodies use to repair damaged tissues. Even as adults, we retain a small capacity to generate new tissues through stem cells. Stem cells are discussed further in the chapter on tissue engineering (Chapter 7). Stem cells differentiate into specific cell types in response to contact from neighboring cells and their chemical and physical environment and by mechanical forces transmitted by their support matrix.

Cells not only manufacture the tissue constituents, they also maintain the tissue and adapt the tissue structure to the changed environments, including mechanical load environments. Key elements in the assembly of tissues are:

- *Cell–cell signaling:* Cellular interactions are controlled by complex molecular communication between cells. Cell signaling molecules are typically proteins, known as cytokines and chemokines. Cytokines (also called growth factors) cause proliferation (cell replication) and differentiation. Chemokines induce cell migration. Cells attach to each other by various types of junctions. These cell-to-cell linkages provide additional mechanical strength to the tissue and provide semipermeable barrier layers that regulate cell, fluid, ion, and protein transport.
- *Apoptosis:* As part of the coordinated function of tissue growth and morphogenesis (pattern or shape formation), certain cells are programmed to die in a

process known as apoptosis. This is another design strategy that can be utilized by the biomaterials engineer: initiation of a biological event is controlled, and there is a mechanism for stopping it. In a very tidy manner, the unneeded cells shrink, condense, and then fragment into membrane-bound granules that can be cleaned up by macrophages.

- *Necrosis:* In contrast to apoptosis, another form of cell death due to trauma or toxin is known as necrosis, in which the cell swells and bursts, releasing its intracellular contents. Since cells contain signaling proteins, necrotic cell death leads to unwanted, uncontrolled cell recruitment and activity. Therefore, apoptotic cell death is the typical mode of cell death during normal tissue functioning.
- *Angiogenesis:* Cells cannot survive without a supply of oxygen and nutrients and a way to remove waste products. Blood vessels provide these functions, and the lymphatics assist with additional waste removal. The formation of new blood vessels, or angiogenesis, must occur rapidly during new tissue growth or the newly formed tissue will die or necrose. Angiogenic factors are released from nearby macrophages, platelets, or the extracellular matrix.
- *Neurogenesis:* The nervous system consists of several cell types, neurons that conduct electrical impulses; glial cells that protect, support, and nourish neurons; and Schwann cells that build the fatty insulating material called the myelin sheath. As described in Chapter 3, this system of cells is responsible for the integration and control of all of the body's functions. Newly regenerated tissue must establish connections with the nervous system in order to function.

Example Problem 6.7

Is angiogenesis always necessary and desirable?

Solution

In some situations, such as growth of new tissues, angiogenesis is necessary and desirable. In other situations, blood vessels are not desired because they may bring an oversupply of immune cells, causing autoimmune disorders, or may enhance tumor growth by supplying cancer cells with nutrients. This has led to the development of anti-angiogenic factors by the pharmaceutical industry and their delivery by biomaterials. ■

6.3.3 The Extracellular Matrix: Nature's Biomaterial Scaffold

The extracellular matrix (ECM) provides a physical and chemical support structure for cells. From a biomaterials perspective, it is a complex woven polymeric material (fibrous proteins) with reinforcing struts (other fibrous proteins) and gels to resist compaction (water-swollen proteoglycans). A full description of the components of the ECM and their functions can be found in Chapter 7. Basically, the ECM provides a microenvironment for the cells. It provides a surface for cells to adhere to and to

migrate across, attachment sites for proteoglycans, and a means of controlling the release of the cell signaling proteins. It is a scaffold that interconnects all the cells physically and mechanically. Cells attach to the ECM via specific cell surface receptors that govern proliferation, differentiation, and protein expression. Interactions of the cells with the ECM thus play a crucial role during tissue regeneration.

The ECM is made by spontaneous folding of proteins produced by cells, typically different collagenous proteins (there are more than 10) and large glycoproteins (e.g., fibronectin, laminin, ostepontin). It is dynamic and is constantly being modified by the cells. The spontaneous folding of peptides is known as self-assembly. The collagen molecule self-assembles from three alpha chains into a triple helix. Within the triple helix, glycine must be present as every third amino acid, and proline and hydroxyproline are required to form and stabilize the triple helix. The collagen molecules then assemble together, a few molecules at a time, with a quarter overlap to form a staggered linear array. The linear aggregates then laterally associate into bundles (Fig. 6.12). Hole zones are left open between the collagen molecule terminal groups for subsequent mineralization (as described further in the example on bone biomineralization later in this chapter). The molecular assembly of DNA is somewhat similar to that of collagen: two preexisting complementary DNA chains combine to form a double helix and then the helices assemble. Self-assembly is one of the key design strategies of tissue formation and must be involved in producing highly ordered structures at the molecular level. Biomaterials scientists are now utilizing this principle to fabricate complicated microstructures at the nanometer length scale.

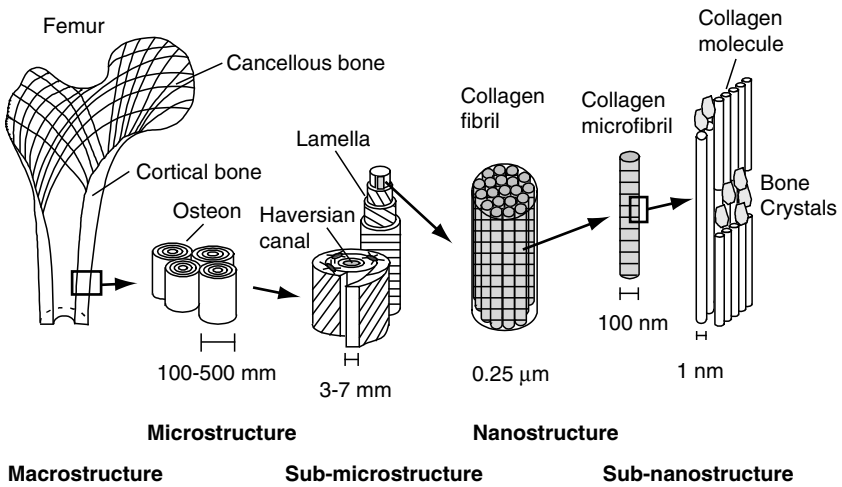

Figure 6.12 The hierarchidal structure of bone. There are at least 5 levels of microarchitecture in bone: (a) the femur, (b) the layers of lamellar bone shown within a cylindrical osteon, that are the building blocks of the cortical bone of (c) fibrils composed of the microfibrils, which form (d) microfibrils with periodic gaps between the ends of the molecules that provide nucleation sites for bone mineral, (e) collagen molecules.

6.3.4 Hierarchical Design

Similar to a nested set of eggs or Russian dolls, in which you open up one just to find another smaller one inside, natural tissues have nested structures. This is known as hierarchical design. The same structural motif is repeated at multiple length scales, and this endows the tissue with efficiency of assembly, properties, and function. During cell-mediated tissue construction, the smallest units self-assemble first, then these units self-assemble to form larger units, and finally the larger units self-assemble. This is how a functional tissue or organ is built. Cells typically become embedded or trapped within the layers of extracellular matrix as the tissue is built. The cells survive and act to maintain the tissue around them. Lamellar bone has similar layered fibrillar structures at the nano, micro, and macro levels. At the smallest level there are the alpha chains that have assembled in a triple helix to form the Type I collagen molecule, the collagen molecules then assemble into microfibrils, microfibrils assemble into larger fibers that assemble by alignment into sheets, and the sheets (lamellae) are layered like plywood in a criss-crossed orientation around blood vessels to form osteons, which have a tubular or large fiber appearance (Fig. 6.12). Skeletal muscle also has a hierarchical structure. The actin and myosin filaments organize into linear constructs known as sarcomeres, which are bundled into myofibrils, which are bundled together to form a muscle fiber (which is the muscle cell with hundreds of nuclei), and multiple muscle fibers form the muscle fascicles we call muscle. A diagram of this can be found in Chapter 3 of this book.

The biological structure of chromosomes is also governed by hierarchical design. Nature efficiently compacts a 7-cm long strand of DNA until it is 10,000 times smaller by twisting and coiling at multiple length scales. At the nanometer level, DNA is a double helix of single-stranded DNA. Subsections of the twisted DNA strand (146 base pairs each) are then subcoiled around protein (histone) cores to form chromatosomes which cause the strand to resemble a beaded necklace. The linked chromatosomes are then coiled to generate a shorter, thicker fiber. The thicker fiber is then coiled upon itself to further shorten its length to form the chromatid, two of which are linked together to form a chromosome.

6.3.5 Biomineralized Tissue Example

Bones, antlers, teeth, coral, eggshells, and seashells are examples of biomineralized tissues. Although they serve different functions and have considerably different external shapes, they are all composed of numerous small, isolated calcium phosphate or calcium carbonate crystals that are held together by a protein matrix. The nucleation and growth of the mineral crystals are regulated by the organic protein component that is secreted by cells and self-assembles to provide a template for the mineral growth and nucleation. Three general biological processing principles governing the composition, architecture, and methods of assembly of a variety of mineralized tissues have been identified that have significant implications for material scientists and engineers:

Biomineralization occurs within specific subunit compartments of microenvironments, which implies stimulation of crystal production at certain functional sites and inhibition or prevention of the process at other sites.

A specific mineral phase is produced with a defined crystal size (frequently in the nanometer range), shape, and orientation.

Macroscopic shape forming is accomplished by packaging many incremental units together, which results in unique net-shaped composites with layered microarchitectures that impart exceptional material properties.

Another powerful feature of biomineralization is that, in most systems, remodeling of the original mineral structure occurs as needed to optimize strength, accommodate organism growth, maintain mineral ion equilibrium, and effect repairs.

In bone, controlled mineral nucleation and growth are accomplished within the microcompartments formed by the collagen matrix. The Type I collagen molecules secreted by osteoblasts self-assemble into microfibrils with a specific tertiary structure having a 67-nm periodicity and 40-nm gaps or holes between the ends of the molecules (Fig. 6.12). The holes localize a microenvironment containing free mineral ions and bound side chain groups from phosphoproteins attached to the collagen. The molecular periodicity of the functional groups serves to nucleate the mineral phase heterogeneously. The nucleation of the thin, platelike apatite crystals of bone occurs within the discrete spaces within the collagen fibrils, thereby limiting the possible primary growth of the mineral crystals and forcing them to be discrete and discontinuous. Only one phase of calcium phosphate is nucleated during normal, nonpathological mineralization processes (carbonated apatite), and the minerals grow with a specific crystalline orientation. In this example on bone tissue formation, the key elements of natural tissue fabrication—cells, an ECM defining a microenvironment for both cells and mineral, protein signaling, and hierarchical design—are all present.

6.4 TISSUE–BIOMATERIAL INTERACTIONS

6.4.1 Interactions with Blood and Proteins

The implantation of a biomaterial often creates a wound, and bleeding generally ensues. Blood thus typically makes first contact with the implanted biomaterial. Blood is a mixture of water, various kinds of cells and cell fragments (platelets), salts, and proteins (plasma). Proteins, the primary group of molecules responsible for making life possible, are built of long chains of only 20 amino acids that are strung together by peptide bonds. Proteins have a myriad of functions in the human body. They can function as enzymes that catalyze thousands of important chemical reactions essential to life. Cell signaling molecules responsible for cell migration and proliferation are made of proteins. Proteins are the building blocks of the supporting extracellular matrix of many tissues. Changes in the levels of proteins or the structure of proteins lead to altered function and are responsible for many diseases. This has led to blood screening for certain proteins that may indicate a diseased state or cancer.

Proteins play an important role in determining the final nature of the tissue–implant interface. Biomaterials can promote cell/tissue attachment and activity by allowing selective protein adsorption or can inhibit tissue interactions by repelling protein. Importantly, changes in microenvironment which can occur after biomaterial implantation, such as pH and ionic strength, can alter the conformation of a nearby protein and hence its function. Proteins also can experience structural alterations during interaction with the solid surfaces of biomaterials and lose some of their biological activity. Albumin is the most common protein in blood, followed by the protective immune system proteins known as immunoglobulins. However, because exchange between absorbed proteins occurs, the final layer of absorbed protein may be fibrinogen, which although less abundant, may have a greater affinity for the biomaterial surface.

Blood coagulation is directed by attachment of the protein clotting factor XII, which is found in blood, to the foreign biomaterial surface. After attachment of this factor, platelets from the blood can and will adhere to the biomaterial, which leads to fibrin clot formation. A cascading chain of cellular reactions that is governed by the initial protein attachment begins. Blood contact provides the cells and cytokines that participate in the biological interaction with the biomaterial. Therefore, every biomaterial that contacts blood will elicit biological responses from the body.

6.4.2 The Wound Healing Response after Biomaterial Implantation

The implantation of a biomaterial creates a disruption of the anatomic continuity of tissue and, as such, creates a wound. The body has a highly developed wound healing response that is immediately triggered by the biomaterial implantation. Much is known about the normal cellular events that transpire after the initiation of a wound (see Chapter 7), and this knowledge provides a foundation to understand and anticipate tissue–biomaterial interactions. From the perspective of tissue–biomaterial interactions, there are four overlapping phases that will always occur (Fig. 6.13):

Hemostasis: Platelet cells control bleeding through coagulation by adhering to the proteins attached to the biomaterial surface and by releasing clot-forming proteins. The clot that is formed acts as a provisional matrix for the initiation of repair tissue and fills the gaps around the implanted biomaterial.

Inflammation: Clot formation induces the production of cell signaling molecules (cytokines) that induce the recruitment of inflammatory cells from a nearby bloodstream. These cells (neutrophils, monocytes, lymphocytes, and macrophages) arrive and attempt to digest tissue debris and the biomaterial by a process known as phagocytosis. The growth factors released at the wound site by the inflammatory cells initiate mitosis (cell replication) of sedentary connective tissue cells at the wound margin.

Proliferation/initial repair: As a result of all the growth factor signaling by the inflammatory cells, there is a proliferation and population of the biomaterial with cells that can recreate the lost or damaged tissue. A nondegrading biomater-

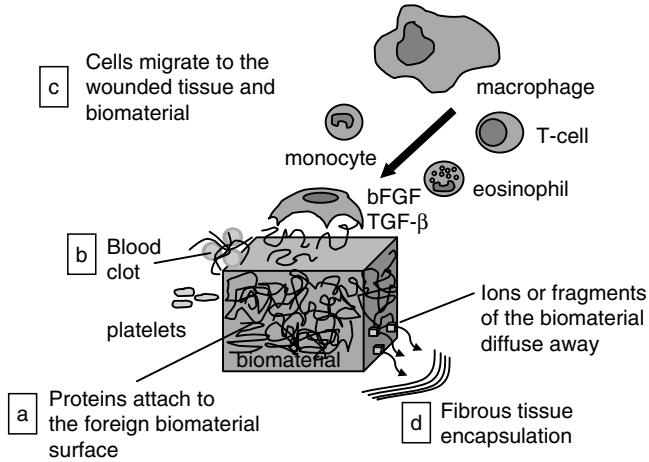

Figure 6.13 Normal tissue–biomaterial interactions involve the four overlapping and interdependent phases of wound healing: hemostasis, inflammation, proliferation/repair, and tissue remodeling. (a) Protein attachment to the biomaterial surface guides cellular interactions. (b) Hemostasis is accomplished by clot formation. (c) Cells found in blood and other inflammatory cells attempt to process the foreign biomaterial and repair adjacent material. (d) The host protects itself from the foreign biomaterial through encapsulation with fibrous tissue.

ial located in the center of the wound typically becomes encapsulated with tight fibrous tissue. The fibrous capsule isolates the material from the biological environment and protects the host. The extent of the inflammatory foreign body response governs the thickness of the fibrous capsule. The chemical characteristics, the shape and physical properties of the biomaterial implant, and the rate of release, accumulation, and bioactivity of released chemicals and corrosion products from implanted materials all also affect the thickness of the fibrous capsule. If the implant is permanent and does not biodegrade, then a small capsule remains throughout the life of the implant, except in bone where there is direct bone apposition on calcium phosphate surfaces without an intervening fibrous tissue layer.

Remodeling: The rapidly formed neotissue will be remodeled by cells into functional tissue more similar to the original tissue, although typically a scar remains.

6.4.3 Metallic Corrosion

There are a number of mechanisms by which metals can corrode, and corrosion resistance is one of the most important properties of metals used for implants. The mechanisms of most significance to implant applications in aqueous saline solutions are galvanic (or mixed metal) corrosion, crevice corrosion, and fretting corrosion.

Galvanic (mixed metal) corrosion results when two dissimilar metals in electrical contact are immersed in an electrolyte. There are four essential components that must

exist for a galvanic reaction to occur: an anode, a cathode, an electrolyte, and an external electrical conductor. The *in vivo* environment contains electrolytes. A patient with two total hip replacements (THRs) made of different alloys is not subject to mixed metal corrosion since there is no electrical connection. However, a THR made of two alloys or a fracture plate of one metal fixed with screws of another metal may be susceptible to mixed metal corrosion.

When two dissimilar metals are connected in an electrochemical cell, one will act as an anode while the other will be the cathode. Metal oxidation will occur at the anode, as shown in Equation 6.4. The reaction at the cathode will depend on the pH of the environment (Equations 6.5 and 6.6). The direction of the reaction can be determined by examining the electromotive force (EMF) series, a short listing of which is shown in Table 6.3. These potentials represent half-cell potentials of metals in equilibrium with 1 molar solutions of their ionic species. The potential for hydrogen is defined as zero. As shown, the standard potential for iron is −0.44 V. If iron is connected to copper with an EMF of +0.34 V, the potential difference is 0.78 V. Since iron is the anode, iron oxidation will occur according to the reaction shown in Eq. 6.4. The reaction at the copper cathode will depend on the pH of the solution, as shown in Eq. 6.5 and 6.6.

$$\text{anodic reaction } Fe \rightarrow Fe^{+2} + 2e- \tag{6.4}$$

$$\text{cathodic reaction (acidic solution) } 2H^{+} + 2e^{-} \rightarrow H_2 \tag{6.5}$$

$$\text{cathodic reaction (neutral or basic) } O_2 + 2H_2O + 4e^{-} \rightarrow 4OH^{-} \tag{6.6}$$

Because the free energy per mole of any dissolved species depends on its concentration, the free energy change and electrode potential of any cell depends on the composition of the electrolyte. Thus, the direction and rate of the reactions also depends on the concentration of the solution. Increasing the concentration of Fe^{+2}

TABLE 6.3 Electromotive Force Series: Standard Reduction Potentials (E^0 V) in Aqueous Solution at 25°C

$K = K^{+} + e^{-}$	−2.93 Active (more anodic)
$Na = Na^{+} + e^{-}$	−2.71
$Al = Al^{+3} + 3e^{-}$	−1.66
$Ti = Ti^{+2} + 2e^{-}$	−1.63
$Zn = Zn^{+2} + 2e^{-}$	−0.76
$Cr = Cr^{+3} + 3e^{-}$	−0.74
$Fe = Fe^{+2} + 2e^{-}$	−0.44
$Co = Co^{+2} + 2e^{-}$	−0.28
$Ni = Ni^{+2} + 2e^{-}$	−0.25
$Sn = Sn^{+2} + 2e^{-}$	−0.14
$H_2 = 2H^{+} + 2e^{-}$	0.000
$Cu = Cu^{+2} + 2e^{-}$	+0.34
$Ag = Ag^{+} + e^{-}$	+0.80
$Pt = Pt^{+2} + 2e^{-}$	+1.20
$Au = Au^{+3} + 3e^{-}$	+1.50 Noble (more cathodic)

in the environment will shift the potential in the positive or noble direction. As the Fe^{+2} concentration increases, the potential difference between the iron and copper will become less as the iron becomes more cathodic. Similarly, the concentration of oxygen at the cathode will affect the EMF of the cell. Increasing O_2 will make it more noble while decreasing O_2 will make it more anodic. In fact, crevice corrosion is initiated by changes in oxygen concentration as is discussed in a following paragraph.

Galvanic cells occur not only with different alloys but also with differences within an alloy. Carbides, grain boundaries, and different phases within an alloy also present differences in EMF and thus the possibility for localized galvanic cells. Cold working also increases the free energy of metal and thus its susceptibility to corrosion. Bending a plate or pounding on a nail head causes localized cold working and makes that area anodic to the rest of the piece.

Galvanic corrosion can also be utilized to prevent corrosion by cathodically polarizing the part to be protected. Steel ships are protected from rusting by the attachment of blocks of zinc. The zinc blocks (“zincs”) serve as a sacrificial anode and protect the steel hull. Metal pumps and other metallic components on ships are also protected with zincs. A power supply can be attached to a part, such as in a steel underground pipeline, to make the pipe cathodic to a replaceable anode. This protects the pipeline.

Electrode size also has an effect on galvanic reaction rates. The classic example is the difference between galvanized and tin-plated iron. As Table 6.3 shows, zinc is anodic to iron. Thus, galvanization results in coating the iron with an anodic material. When the zinc is scratched and the iron is exposed, the small size of the iron cathode limits the reaction, and there is minimal corrosion. In contrast, tin is cathodic to iron. When a tin plate is scratched, the small iron anode is coupled with a large cathode. Anodic corrosion and removal of iron from the scratch results in an increased area of the exposed iron and thus an increase in corrosion rate. This self-accelerating corrosion can be prevented by coating the tin cathode with a nonconductive material such as paint or varnish.

Crevice corrosion can occur in a confined space that is exposed to a chloride solution. The space can be in the form of a gasket-type connection between a metal and a nonmetal or between two pieces of metal bolted or clamped together. Crevice corrosion involves a number of steps that lead to the development of a concentration cell, and it may take 6 months to 2 years to develop. Crevice corrosion has been observed in some implanted devices where metals were in contact, such as in some total hip replacement devices, screws and plates used in fracture fixation, and some orthodontic appliances.

The initial stage is uniform corrosion within the crevice and on the surfaces outside the crevice. Anodic and cathodic reactions occur everywhere, with metal oxidation at the anode and reduction of oxygen and OH-production at the cathode. After a time, the oxygen within the crevice becomes depleted because of the restricted convection of the large oxygen molecule. The cathodic oxygen reduction reaction ceases within the crevice, but the oxidation of the metal within the crevice continues. Metal oxidation within the crevice releases electrons which are conducted through the metal and consumed by the reduction reaction on the free surfaces outside the crevice. This creates an excess positive charge within the crevice that is balanced by an

influx of negatively charged chloride ions. Metal chlorides hydrolyze in water and dissociate into an insoluble metal hydroxide and a free acid (Eq. 6.7). This results in an ever-increasing acid concentration in the crevice and a self-accelerating reaction.

$$M^+Cl^- + H_2O \rightarrow MOH + H^+Cl^- \tag{6.7}$$

The surgical alloys in use today all owe their corrosion resistance to the formation of stable, passive oxide films, a process called passivation. Titanium, which appears as an active metal on the EMF series in Table 6.3, forms a tenacious oxide that prevents further corrosion. Stainless steels and cobalt alloys form chromium oxide films. As indicated in Table 6.3, they are active, but in the environment where this oxide film is formed, they become passive or noble.

To be self-passivating, stainless steels must contain at least 12% chromium. However, carbon has a strong affinity for chromium, and chromium carbides form with the average stoichiometry of $Cr_{23}C_6$. The formation of a carbide results from the migration of chromium atoms from the bulk stainless steel alloy into the carbide. The result is that the carbide has high chromium content while the alloy surrounding the carbide is depleted in chromium. If the chromium content is depleted and drops below 12% Cr, then there is insufficient Cr for effective repassivation, and the stainless steel becomes susceptible to corrosion. As a safety factor, surgical stainless contains 17–19% chromium, and the carbon content in surgical alloys is kept low at $<0.08\%$ or $<0.03\%$.

The problem of carbide formation is especially important with welded stainless steel parts. If steel is heated to the "sensitizing range" of 425°C to 870°C, the chromium can diffuse in the solid and form carbides. At temperatures above 870°C, the carbon is soluble in the atomic lattice. Below 425°C, the mobility is too low for carbide formation. If the peak temperature in the metal away from the weld is in the sensitizing range, carbides can form. This is known as weld decay or corrosion of the sensitized metal on each side of the weld. By heat treating after welding, the carbides can be redissolved, and the metal quickly quenched to avoid reformation.

With the oxide film intact, surgical alloys are passive and noble. If the film is damaged, as with scratching or fretting, the exposed metal is active. Reformation or repassivation results in restoration of the passive condition. Fretting corrosion involves continuous disruption of the film and the associated oxidation of exposed metal. Devices that undergo crevice corrosion are also examples in which fretting corrosion has accelerated crevice corrosion.

Example Problem 6.8

Old cars in the northern United States are often rusted on the bottom of their doors and on their trunk lids, and the tailgates of old pickup trucks are also often rusted. Name and discuss five reasons for this. Would bolting on a zinc block help?

Solution

(1) The edges and bottoms of doors and lids are formed by bending the metal back on itself. This causes cold working at the bend, which makes it anodic to the rest of the

metal. (2) The crimps are then spot welded closed. This creates an area of different microstructure, which leads to a galvanic situation. (3) The roads are salted in the winter, and the saltwater spray gets caught in the crimp. That is the electrolyte. Sand may also get in the crimped space and help maintain a moist environment. (4) Car manufacturers put a decorative strip of chromium-plated steel along the bottom of the lid. The chromium is cathodic to the steel and provides another source of galvanic corrosion. (5) There are potholes in the roads. This causes bouncing of the lid against the frame. This can chip the paint and expose the unprotected metal, or it may be a cause of fretting corrosion. ■

Bolting on a zinc block would not help except for corrosion of metal in the same electrolyte pool. A piece of zinc in the crevice would help slow the corrosion of the crevice. If the car fell into the ocean, then the zinc block would protect the whole car.

Example Problem 6.9

Your grandmother has a stainless steel total hip. Now she needs the other hip replaced, and the doctor wants to use one made of a cobalt chromium alloy. Is that a problem for corrosion?

Solution

No. One of the four essential elements for galvanic corrosion is missing. There is an anode, the stainless steel. There is a cathode, the cobalt alloy. There is an electrolyte, the saltwater of the body. However, there is no electrical connection, so there is no problem. If she were to fall and fracture her pelvis, and the break was repaired with an external fixator, then there might be an electrical connection and a problem. However, these alloys are so corrosion resistant and similar electrochemically that there is probably no need to worry. ■

6.4.4 Biomaterial Degradation and Resorption

Biomaterials may be permanent or degradable. The degradation process may be chemically driven or accomplished by cells. Bioresorbable implants are designed to degrade gradually over time in the biological environment and be replaced with natural tissues. The goal is to meet the requirements of strength and cell support while the regeneration of tissues is occurring. Small changes in biomaterial chemistry and structure may greatly alter the resorption rate, allowing for materials to be tailored for various applications or leading to unexpected product failure. Collagen and the lactic acid and/or glycolic acid polymers (PLLA and PGA or copolymer PLGA) are the most commonly used for resorbable applications. PLLA and PGA degrade through a process of hydrolytic degradation of the polyester bond. At low molecular weights, the implant can disintegrate and produce small fragments that elicit an immune response from macrophages. PLLA and PGA degrade in a time period of 6 months to several years depending on initial molecular weight and crystallinity.

Copolymers of the two typically degrade into fragments in a few months. The lactic acid and glycolic acid fragments are eventually metabolized into carbon dioxide and water. Tricalcium phosphate ceramics degrade through a surface dissolution process into calcium and phosphate salts, which are also present naturally in the body.

Biomaterial degradation may lead to chronic nonhealing wounds that are arrested at one of the normal phases of wound healing. This may happen if a biomaterial degrades too quickly and releases particulate matter that extends the inflammation stage. Persistent inflammation leads to the formation of giant multinucleated cells that continue to attempt to remove the offending material. They are the trademark of a foreign body response and may necessitate surgical removal of the implanted device. If the healing passes through to the fibrous capsule formation stage, there may still be complications. For example, a drug delivery implant may eventually no longer function due to impaired drug release by the fibrous encapsulation in response to the degrading drug delivery implant.

Example Problem 6.10

Is it possible to successfully pass through all four wound healing phases only to have the biomaterial degrade and lead to wound healing reversal? Explain your answer.

Solution

Yes, this has happened in some patients with total joint replacements. Total joint replacements such as artificial hips typically consist of two metallic components that meet at a polymeric bearing surface (typically UHMWPE). During bending of the joint, wear debris is produced as the metal surface rubs against the softer polymeric surface. This leads to the recruitment of macrophages that identify the particles as foreign and attempt to remove them. Since the synthetic particles cannot be degraded by cell enzymes, inflammation continues indefinitely. The excessive production of inflammatory cytokines leads to resorption of the newly healed adjacent bone that supports the implant, resulting in implant loosening. Fortunately, there have been improvements in the processing of polyethylene so that wear debris is no longer generated at the high rate observed in some of the earliest used hip replacements. Ceramic-on-ceramic hip joints also have been developed, which have better wear properties and are not susceptible to corrosion; however, ceramic hip replacements are badly tolerated by elderly osteoporotic bone because the material is very hard. ■

6.4.5 Immunogenicity

Immunogenicity is the tendency for an object to stimulate the immune response. Examples of immunogens are bacteria, pollen from grass or trees, small or absorbable biomaterials, and proteins in food that lead to allergies or inflammation. Basically, our immune system protects us through a combination of physical barriers such as skin,

chemical barriers such as enzymes and antibodies, and cellular barriers such as targeted cytotoxic T lymphocytes (T cells). When a biomaterial is implanted in the body, the immune system associated proteins immediately attach to the surface, thereby directing subsequent cell behavior toward the biomaterial. Once again it must be emphasized that the surface chemistry and structure of the biomaterial play important roles in determining the extent and type of protein attachment and, hence, the tissue–biomaterial interaction. Proteins of all types will be competing for attachment sites on the biomaterial surface. Depending on the conformation of the attached proteins, a variety of messages may be sent to the nearby cells. Methods for modifying the biomaterial surface to control tissue–biomaterial interactions are discussed later in this chapter.

When allogenic (human) graft biomaterials are implanted in another human, acute rejection can occur if the major histocompatibility complex (MHC) groups on the cells in the graft are of different types than the donor's MHCs. MHCs are a class of cell-surface molecules that provide information as to what has been identified as foreign in the past to the cytotoxic T cells. With nonmatching MHC groups, the T cells receive two sets of instructions as to what is foreign, and this causes an extremely vigorous immune response. Tissue typing can reduce this type of rejection, although the patient usually still requires long-term medication to suppress some of the activity of the immune system. Rejection can also occur against cell–biomaterial scaffolds in tissue engineering applications. The implanted cells may be recognized as foreign and be damaged directly by the attacking immune cells such as macrophages or be starved to death by the lack of nutrients passing through a thick fibrous capsule created through the inflammatory process to protect the host. Therefore, in some tissue engineering applications, the implanted cells are protected from the immune system by enclosing the cells in selectively permeable biomaterials (e.g., islet cells that produce insulin in alginate hydrogels).

Corrosion of metallic implants releases metal ions that can cause metal sensitivity or allergic reactions in some individuals. Allergic reactions can lead to slow or inadequate bone fusion or skin dermatitis. Both of these conditions usually require removal of the implant. Once again, this demonstrates that biomaterials are not inert.

Biomaterials are sometimes deliberately designed to enhance the immune system's response. For example, vaccines are typically given with a particulate biomaterial known as adjuvant for enhanced and longer lasting immunity. Vaccine adjuvants have their own immunogenic properties, resulting in a stronger local stimulus to the immune system. Adjuvants can be simple particles that adsorb the weak immunogen, increasing the effective size of the weak immunogen and enhancing phagocytosis of the particle by macrophages. Adjuvants also work like controlled-release vehicles by prolonging the local retention of a weak immunogen and increasing the chance of a local immune response. Vaccine adjuvant selection represents a compromise between a requirement for adjuvanicity and an acceptable low level of side effects. The FDA has approved only three materials for human use (all of which are mineral salts): aluminum phosphate, aluminum hydroxide, and calcium phosphate. Aluminum compounds are often incorrectly identified in the scientific literature as alum. Alum is potassium aluminum sulfate, which is used as the starting solution to precipitate

antigens with either aluminum phosphate or aluminum hydroxide. Other biomaterial adjuvants used in research include oil emulsions, lipopolysaccharide products from bacteria (LPS), and their synthetic derivatives (liposomes).

6.5 GUIDING TISSUE REPAIR WITH BIO-INSPIRED BIOMATERIALS

6.5.1 Surface Chemistry Modifications (1-D)

The interaction of cells and tissues with biomaterial surfaces is critically important to promote new tissue deposition and for healthy integration with the surrounding extracellular matrix. When a biomaterial surface is placed in the body, many different chemical and molecular level events occur in the biomaterial that can affect the cellular response (Fig. 6.14). For example, at the surface of a traditional metal implant, metal and oxide ions diffuse away from the implant surface, while biological ions (Ca, P, Na, Cl) are incorporated. Concurrently, at the implant surface, there is adsorption and desorption of native biological molecules. As discussed in Section 6.4, the adsorption of certain proteins onto the implant surface will initiate a cascade of events such as blood clotting and inflammatory cell recruitment or cell differentiation. In nature, the surface chemistry of every cell and extracellular matrix is carefully controlled to obtain the desired response. Cell–extracellular matrix interactions are dominated by the interaction of cell-adhesion proteins (ligands) bound to the ECM and cell surface receptors (integrins). In medical device applications, the biomaterial replaces the ECM and sends signals to the cells interacting with it through similar mechanisms (Fig. 6.14). Thus, the biomaterial surface plays a very important role in determining tissue–biomaterial interactions, and this concept has become very important to the biomaterials scientist.

One biomimetic approach to controlling cell–surface reactions is to preadsorb proteins on the implant surface that mimics those most involved with cell adhesion. The three-amino-acid sequence of Arg-Gly-Asp (RGD) found in fibronectin and bone sialoprotein is now well known for mediating adhesion of cells to surfaces; therefore, RGD-containing peptides are now being deposited on surfaces to promote cell attachment. Heparin and heparin-sulfate binding peptides that mimic proteoglycan activity also have been found to enhance cell adhesion. Adsorption of other biological molecules such as growth factors to the surfaces of implants can control the tissue–biomaterial interaction and lead to enhanced cell activity and more differentiation than will the cell adhesion molecules alone.

There are many chemical reactions that can be used to attach a biomimetic peptide sequence to a biomaterial. For example, a protein can be immobilized on a surface through a technique known as organosilane chemistry (Fig. 6.15a). The details of the chemical coupling and derivitization processes are beyond the scope of this text. Basically, there are coupling agents such as salines used to create a covalent bond between the biomaterial surface and the protein to be attached. Well-ordered protein attachment results. A wide variety of solid surface modification techniques are available to create the reactive coupling groups, such as photochemical grafting, chemical

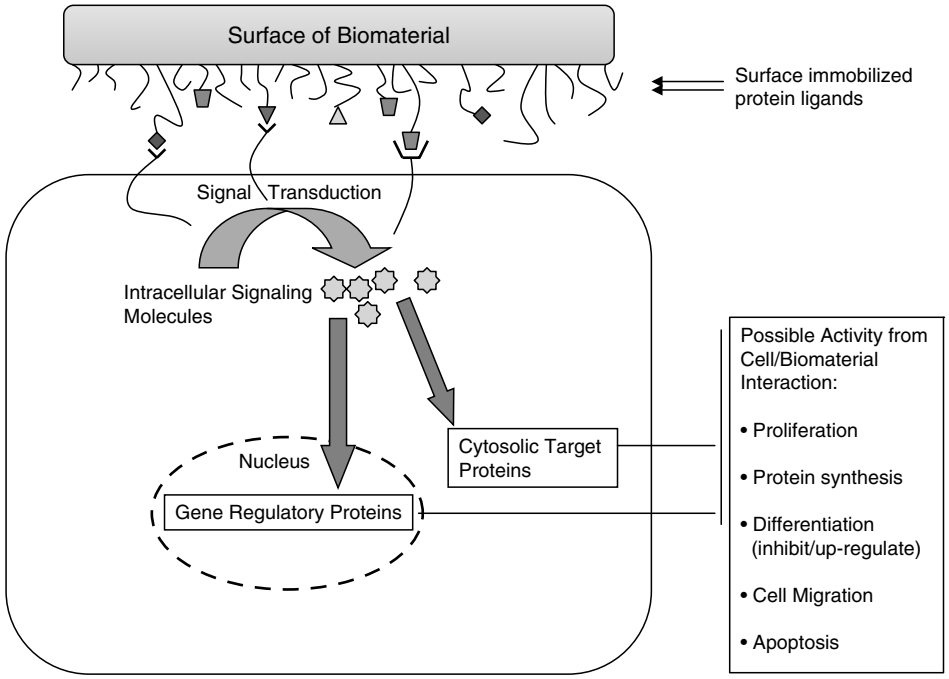

Figure 6.14 Like the extracellular matrix, a protein-covered biomaterial sends signals to the cell interacting with it through ligand-receptor mechanisms. Primary cell signal transduction is facilitated through multiple pathways, leading to the synthesis of various intracellular signaling molecules. Acting both on the genetic regulatory proteins in the nucleus and other cytosolic target proteins, the signaling molecules can induce various phenotypic expressions. Ideally, the biomaterials scientist can engineer the proper surface treatment to elicit the desired cellular activity.

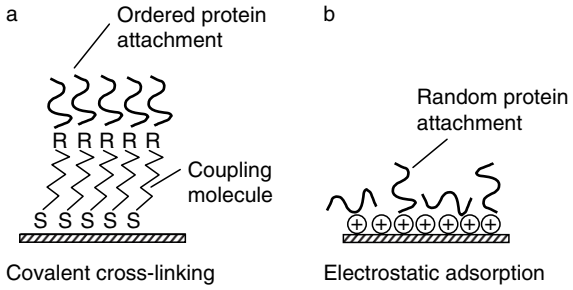

Figure 6.15 Two types of chemical reactions used to modify a biomaterial surface: (a) covalent coupling techniques and (b) physical adsorption methods utilizing electrostatic interactions.

derivation, and plasma gas discharge. Physical adsorption methods utilizing other types of bonding, such as van der Waals and electrostatic binding, can also be used to immobilize proteins (Fig. 6.15b). Physical and electrostatic adsorption is the easiest technique; however, it is the least specific and tends to readily release the adsorbed

molecule. Lipid groups and dye molecules can also be used to immobilize proteins on surfaces.

A critical component of surface modification is the resulting ligand density. If protein adsorption is too low, the addition of more functional groups to a relatively inert polymer can be accomplished by plasma glow discharge treatment. The greater reactivity of the surfaces with higher surface energy after plasma treatment generally leads to increased tissue adhesion.

Surface modification also can be used to produce protein-resistant surfaces that are needed in blood-contacting applications such as vascular grafts. For example, polyethylene oxide has been attached to surfaces to reduce protein adsorption. Cell adhesion was significantly reduced on these treated surfaces. Anticoagulants can also be attached to biomaterial surfaces to decrease unwanted cell attachment. Various hydrophilic biomaterials have been shown to reduce platelet adhesion and thrombus formation. Hydrophilic materials have also been shown to hinder bone healing, so what is appropriate for one biomaterial application does not necessarily apply to another.

Hyaluronan is a biomolecule found in cartilage extracellular matrix. It is responsible for tethering a proteoglycan (aggrecan) to the collagen matrix. Studies have shown that it can guide the differentiation of mesenchymal stem cells to cartilage chondrocytes. In those experiments, hyaluronan was chemically bound to tissue culture dishes, and undifferentiated cells were added. It was found that a specific molecular size (200,000–400,000 daltons) was optimal to initiate cartilage formation. Biomolecules such as enzymes, antibodies, antigens, lipids, cell-surface receptors, nucleic acids, DNA, antibiotics, and anticancer agents can all be immobilized on or within polymeric, ceramic, or metal surfaces.

Surface deposition of calcium ions or the use of calcium-containing biomaterials strongly influences the attachment of bone cells. Hydroxyapatite-coated hip implants show decreased fibrous tissue formation and increased direct bone bonding. Better bone attachment has also been found for hydroxyapatite-coated dental implants and spinal fusion cages. Recent studies have shown that hydroxyapatite ceramics are selective in cell recruitment from the bone marrow. This may be due to an intermediate step of selective protein adsorption.

6.5.2 Surface Topography (2-D)

Surface topography of a biomaterial has been shown to provide cues to cells that elicit a large range of cellular responses, including control of adhesion, cell morphology, apoptosis, and gene regulation. Modification of a biomaterial surface can therefore have dramatic effects on guiding tissue growth. Early total joint replacements had smooth surfaces; however, rough, porous coatings or grooved surfaces are now being used on hip implants to achieve bony ingrowth. The hip and knee implants in Figures 6.5 and 6.6 have rough, porous coatings. The porous coatings on orthopedic implants are achieved by partial fusion of small metallic spheres to the implant surface. The interparticle spaces are the pores. The pores, as well as grooves, seem to encourage bone cells to migrate into or along them. It has been observed that the stretching out

and aligning of the cells along a surface feature (greater than 5 μm) causes them to initiate bone deposition. This leads to a mechanical interlocking between the bone tissue and the implant that increases the bond strength. The directed activity of cells is known as contact guidance, and it has also been used to increase migration of other cell types into tissue engineering scaffolds. In addition to guiding cell migration downwards into a pore, surface topography variations can be used to restrict cell spreading and force it back upon itself. For example, by restricting cell growth to a long narrow path, endothelial cells were induced to grow upwards and form a three-dimensional capillary tube.

Photolithographic techniques can be utilized to micropattern a surface with proteins, molecules, or functional groups (Fig. 6.16). This technique involves using a photoresist layer in which patterns can be created by selectively exposing certain areas to light. The light degrades the exposed portions of photoresist, leaving a bare biomaterial surface. Proteins or molecules can then be selectively attached to these exposed areas. The remaining photoresist is then removed to obtain a biomaterial surface that has protein or molecular patterning. Cell culture studies have demonstrated preferred cell attachment to the chemically modified areas. Other methods for modifying surface topography include surface roughening by laser ablation or wet etching with a corrosive solvent. It has been observed that the roughness must be on a biologically relevant scale (1–10 μm) to affect cell growth and attachment.

Smooth surfaces such as pyrolitic carbon resist protein and cell attachment and are ideal for heart valve applications. Bioprostheses made of bovine or porcine heart valves are even more superior for valve applications and reduce coagulation and embolism by a combination of an ideal surface topography and surface chemistry. However, they typically fail due to calcification, which is enhanced on the natural collagen surface as compared to the smooth pyrolitic carbon.

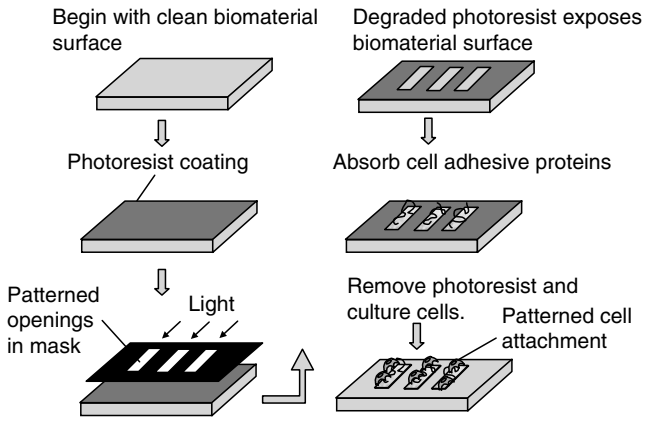

Figure 6.16 Photolithography techniques for micropatterning a biomaterial surface with immobilized proteins or functional molecules.

6.5.3 Scaffolds (3-D)

Tissue repair of large defects is best accomplished by filling the defect space with a scaffold material that can simulate the microenvironment provided by the embryonic extracellular matrix. There are many functions the scaffold must serve: it must provide sites for growth factor attachment, cell migration and attachment, and new tissue deposition. Natural tissues are the ideal choice; however, due to the limited supply of natural donor tissue, there is a large market for synthetic tissue analogs. While metallic or polymeric plates and screws may be useful for temporarily holding the healing tissue together, they do not themselves recreate new tissue. Either natural tissue or analogs to the extracellular matrices of natural tissue and its cells and growth factors are needed to reconstitute a functioning vital tissue. The goal of a scaffold is to recreate important aspects of the cell microenvironment that will allow cell proliferation, differentiation, and synthesis of extracellular matrix. Synthetically produced regenerative materials now available commercially include biomaterial scaffolds (ceramic and polymeric) with or without a tissue stimulating biological molecule and with or without cells. One of the most critical elements of the scaffold biomaterial is that it mimic the ECM scaffold that normally serves to maintain space, support cells, and organize cells into tissues. The section on surface chemistry (Section 6.5.1) gave an example of how mimicking components of the adhesive proteins of the ECM can enhance cell attachment and differentiation. This section focuses on the structural and physical characteristics of the ECM scaffold that appear to be critical to imitate in synthetic scaffolds in order to stimulate cells and lead to the functional regeneration of tissues.

Pore size is a very important parameter of biomaterial scaffolds used for tissue regeneration. Through trial and error, optimal ranges of pore sizes have been determined for different tissues and for different biomaterials. There are now some rules of thumb, such as that the pores must be at least 5–10 μm for a cell to fit through. Successful bone scaffolds typically have pores that traverse the full thickness of the scaffold and are 100–250 μm in size. New blood vessel formation, or neovascularization, has been shown to require pores within polymer scaffolds that are between 0.8–8 μm and to not be possible within polymers with pores less than 0.02 μm. Typically, the acceptable pore size in polymers is smaller than in ceramics or metals, perhaps due to pore size expansion which can occur in the body due to degradation or swelling of the polymer.

The pore size determines many aspects of the scaffold, such as mechanical strength and permeability to gases, fluids, and nutrients, in addition to cell ingrowth. Interconnected porosity is essential for tissue engineering applications requiring nutrient diffusion and tissue ingrowth. A highly porous material degrades more quickly than a solid block of material. The biodegradation rate of a scaffold biomaterial is usually found through prolonged immersion of the biomaterial in a fluid that simulates body fluid.

There are several techniques for producing porous biomaterials. Some methods are better suited for drug delivery or for creating a low-density, stiff reinforcing structure than for cell scaffold tissue engineering applications. One approach to creating pores

within a material is to dissolve the polymer in a solvent and mix in particulate materials that are stable in the solvent but can be dissolved later (porogens). The solvent is allowed to evaporate, leaving a film of polymer containing porogens (like a chocolate bar with nuts). The porogens are then dissolved by washing in a different solvent, such as water, to form pores. This is known as solvent casting/particulate leaching. Related to this technique is coascervation, in which the polymer is dissolved in a solvent, the porogen is added, and then a nonsolvent for the polymer is added. A polymer precipitate forms, entrapping the porogen particulates. The precipitate can be collected and then compacted prior to removal of the porogen. After washing to remove the porogen, a porous structure is revealed, as shown in the composite bone crystal/polyhydroxybutyrate hydroxyvalerate (PHBHV) polymer of Figure 6.17. Organic solvents are used with these procedures, which precludes the possibility of adding pharmaceutical agents to the scaffold during fabrication. Also, the porogen leaching step significantly increases the scaffold preparation time. This has led to the development of other techniques such as gas foaming. In one variation of gas foaming, solid disks of polymer are formed and then exposed to high pressure CO_2 for three days. Pores are created when these gas-containing disks are suddenly returned to atmospheric conditions. The gas forms bubbles of up to 100 μm, and porosities of up to 93% can be obtained. Other methods for gas foaming include adding a foaming agent that chemically produces a gas upon heating. The pores formed by the gas foaming technique are often not interconnected, making cell seeding and cell migration within the foam difficult.

Phase separation/emulsification can also be used to fabricate porous polymer scaffolds. In this technique, a polymer is dissolved in a solvent and then an immiscible

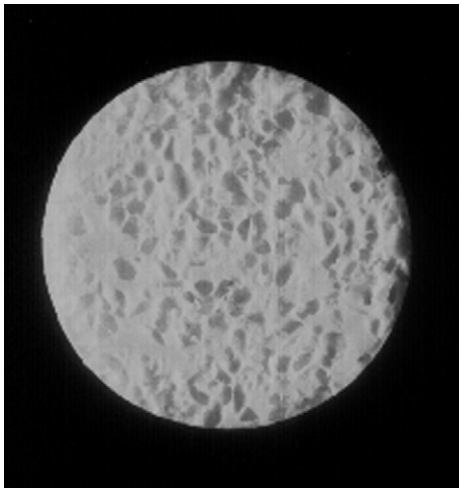

Figure 6.17 A composite biomaterial scaffold made of bone crystals and the resorbable polyhydroxybutyrate hydroxyvalerate (PHBHV) copolymer. The coascervation technique was used to precipitate the polymer from solution to produce this scaffold. Dissolvable porogens were used to create the large pores needed for bone repair applications.

liquid (such as water) is added and mixed to form an emulsion. The polymer/water mixture is cast into a mold, rapidly frozen and then freeze-dried, which is known as lyophilization. The space that was water becomes a pore. Scaffolds with high porosity (up to 95%) have been formed by this method, but the small pore sizes (13–35 μm) are a drawback. Fiber bonding methods in which preformed fibers are layered or woven and then hot melted or glued together by solvent exposure is another technique for forming porous materials. The advantage is that the pore sizes are controllable and interconnected. The drawback is that the pore channels are rectangular and regular, unlike natural extracellular matrix structure. Furthermore, the use of high temperatures and solvents prevents the incorporation of bioactive molecules during processing.

Solid freeform fabrication is also used to make biomaterial scaffolds (in addition to being used for rapid prototyping of automotive parts). Stereolithography utilizes a focused laser that follows a pattern dictated by computer-assisted design drawing to selectively cure only certain areas within a thin layer of liquid polymer. The depth of the liquid is raised around the part being fabricated, and the laser is again sent on a computer-assisted path to form the next layer of the component. This is repeated over and over again to produce a complex three-dimensional shape. Similarly, a technique known as 3-D printing can be used to create porous scaffolds or complex shapes layer by layer, this time using a print head to deposit "glue" over computer-specified areas of a powder bed. After all of the layers have been "printed," the final part is picked up, and the unbonded particles fall away, revealing the three-dimensional component. Pieces of replacement bone have been made using these techniques with computer assisted tomography (CAT) scans of x-ray images. The features of the scaffolds are limited to 10–1000 μm with these techniques which have been used to prepare polymer and ceramic scaffolds.

6.6 SAFETY TESTING AND REGULATION OF BIOMATERIALS

6.6.1 Product Characterization

Ensuring product purity and identity is one of the first steps in developing a safe product. There are extensive data documenting the safety of various biomaterials; however, since processing methods may include additives and the final sterilization step may alter the biomaterial, it is crucial to always verify the end product purity and identity. The American Society for Testing and Materials (ASTM, www.astm.org) has developed many standards that manufacturers of medical device products can use as guidelines to evaluate product purity and identity, as well as safety. Under ASTM specifications for metals used in medical devices, there are restrictions on the composition, microstructure, phase and grain size, inclusion size, defect size, and macro- and microporosity to help ensure safety. There are ASTM standards for ceramic materials that specify chemical composition, phase determination, grain size, and impurities such as sintering aids, which may decrease fatigue resistance. New standards are being written by ASTM to help ensure quality and reliability of tissue

engineering scaffold materials. In addition to specifying the correct tests and techniques to determine the chemical identity of the tissue engineering scaffold materials, standardized tests for measuring porosity and permeability have also been developed. ASTM also has standards for dissolution testing, degradation testing, and stability testing. ASTM standards are written through a consensus process and represent the best available knowledge from a wide cross section of manufacturers, users, and general interest groups.

There are many techniques for evaluating the composition and structure of biomaterials. Unfortunately, no single technique is capable of providing all of the needed information. Thorough characterization requires the use of multiple analytical methods. For example, if the material is crystalline, x-ray diffraction can be used for bulk product identification (typically, powdered samples are analyzed). The x-ray beam is diffracted as it goes through the rows of atoms, and a detector measures the reflected intensities as a function of angle from the surface. The intensities at various angles provide a unique signature of the material structure and can be compared to existing data files for product identification purposes.

Fourier transform infrared spectroscopy is a method that complements the structural information gained by x-ray diffraction by providing information about the chemical groups found within the structure. The material does not need to be crystalline and can be gas, liquid, or solid. The sample is exposed to infrared radiation, and the molecular vibrations induced by the radiation are observed. Radiation at frequencies matching the fundamental modes of vibration is absorbed, causing oscillating dipoles perpendicular to the surface. These are detected as a function of wavelength and provide a chemical "fingerprint" that can be compared to existing databases to identify the material. Most characterization methods capable of identifying an unknown substance involve bombarding the material with some type of energy, quantitating the interaction with the material, and then searching a database for similar results. Other techniques utilizing this basic procedure include secondary ion mass spectroscopy (SIMS) and x-ray photoelectron spectroscopy (XPS), both of which are well suited for identifying the surface chemistry of a biomaterial.

Scanning electron microscopy (SEM) is very useful for characterizing the two-dimensional surface topography of a biomaterial. In SEM, a beam of high-energy electrons is scanned across the sample, causing the material to emit secondary electrons. The intensity of the secondary electrons primarily depends on the topography of the surface. An image can be recreated by recording the intensity of the current generated from the secondary electrons. The resolution of an SEM allows magnifications of up to 100,000×. When greater resolution of a material surface is needed, atomic force microscopy (AFM) can be used. In AFM, an atomically sharp tip attached to a cantilever is dragged across the surface of a material but actually does not touch the material. The interactions of the atoms of the material being analyzed with the tip cause either repulsion or attraction. The height adjustments or changes in interatomic forces are recorded and used to construct images of the surface topography. Under proper conditions, images showing individual atoms can be obtained.

Images in three dimensions can be obtained using computer-aided x-ray tomography (micro-CT) or nuclear magnetic resonance (NMR) imaging. An image obtained

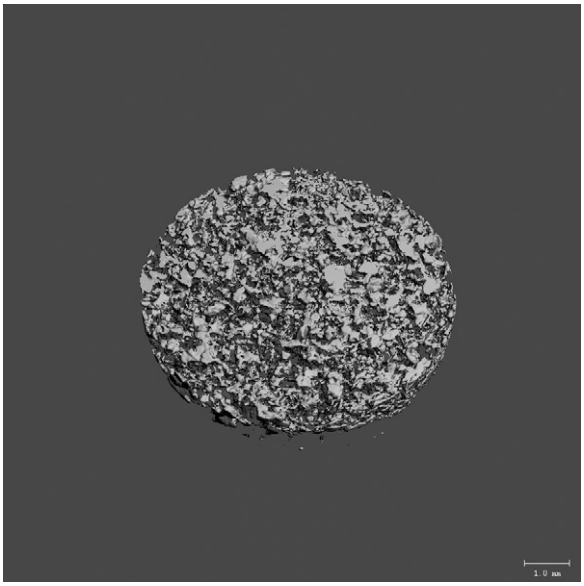

Figure 6.18 The scaffold of Figure 6.17 imaged by computer-aided x-ray tomography (micro-CT). (Courtesy of Douglas J. Adams, Ph.D., Micro-CT Facility, University of Connecticut Health Center.)

by micro-CT of a biodegradable porous scaffold is shown in Figure 6.18. In both techniques, the samples are scanned in all directions, and then the image is created mathematically by a merging of all the directional information. Solid or porosity volume or volume fraction can be measured nondestructively. Direct measurement of solid or pore characteristic dimensions (width, diameter, thickness) and spacing (or period) of repeating structure can be made. In micro-CT, image contrast is achieved via attenuation of X-radiation; thus, polymers (or other scaffold substrates) with relatively higher attenuation coefficients will provide higher-contrast images and improved computational segmentation of scaffolds versus the background. Commercial desktop micro-CT instruments are available with a spatial resolution of approximately 5 μm.

6.6.2 Methods for Testing and Evaluating Safety and Biocompatibility

It has been emphasized repeatedly in this chapter that the body can affect the biomaterial and the biomaterial can affect the body. Therefore, both of these aspects must be investigated prior to biomaterial implantation in the human body. This is known as biocompatibility and safety testing. Before initiating biocompatibility and safety testing, the structure and chemistry of the material should be fully characterized by a combination of techniques as described in Section 6.6.1. This is necessary to confirm the purity and identity of the biomaterial and to ensure that no unintended foreign substances have been introduced during the synthesis, manufacturing, and sterilization procedures.

Biocompatibility and safety tests include *in vitro* assays (using cells and tissues), *in vivo* models (in animals), and, finally, human clinical trials. Several guidelines and procedures have been developed by the standards organizations of the world [ASTM and the International Standards Organization (ISO)] and federal regulatory agencies (e.g., Food and Drug Administration). ASTM Standard F-748 and ISO 10993 provide detailed methods for completing adequate safety and biocompatibility testing and are followed by all implant and medical device manufacturers. The tests are separated into various categories based on intended use. For example, there is a matrix of tests appropriate for surface devices, external communicating devices, or implanted devices. The recommended tests are further categorized based on contact duration (short term, prolonged contact, permanent). The basic tests include cytotoxicity, sensitization, irritation or intracutaneous reactivity, acute systemic toxicity, subacute systemic toxicity, genotoxicity, implantation, hemocompatibility, chronic toxicity, and carcinogenicity. The preferred test sample is the intact medical device that has been processed and sterilized in the same manner as the medical device that will be used in humans. However, it is not always practical to use the intact medical device due to the constraints of the biological tests. Therefore, an extract of the leachable components or the degradation components of the implant are often tested first in the *in vitro* assays (cytotoxicity) and also in the preliminary *in vivo* tests (sensitization and both systemic toxicity tests). Completion of the preclinical tests described in F-748 and ISO 10993 typically takes up to 2 years, even if a qualified and experienced facility is conducting the testing.

It is difficult to correlate *in vitro* testing to *in vivo* testing because the *in vivo* system is much more complex and involves many more variables. Typically, cell culture assays are more sensitive than *in vivo* tests; however, demonstration of cytotoxicity *in vitro* may not necessarily mean that the material cannot be used *in vivo*. Both false negatives and false positives can be obtained by cell culture testing; therefore, animal testing is a required step in understanding safety and biocompatibility, and also for an initial evaluation of the product's performance and effectiveness. There are, however, variations in response to biomaterials and drugs among species of animals. The guinea pig has been found to be the most sensitive animal for assessing delayed immune hypersensitivity (the sensitization test). The rabbit has been found to be the most sensitive animal model for detecting pyrogens *in vivo*. Although animal testing does provide a useful screen for restricting the implantation of most toxic components in humans, the final and ultimate biocompatibility and safety testing occurs during human clinical trials. In some cases, products that demonstrate efficacy in a mouse or dog model may not always perform as well in humans, particularly in the case of a new drug. The effective dose sometimes varies greatly between species, as well as between two humans. Therefore dose escalation schemes are incorporated into biocompatibility and safety and efficacy testing, and large numbers of patients must be used in clinical trials.

6.6.3 The Regulatory Process

Regulatory approval by the Food and Drug Administration (FDA) is required in the United States prior to administering a new drug or biologic or implanting a new

medical device in a human and also prior to marketing the new product. The FDA is currently divided into six individual centers that regulate devices and radiological health (CDRH), drugs (CDER), biologics (CBER), food and cosmetics, veterinary medicine, and toxicology. An assessment must be made as to which mode of action—drug, device, or biological—contributes the most to the therapeutic benefits of the overall product. Based on this criterion, the FDA decides which center will take the lead on the regulatory review. Each FDA center has different procedures and requirements that must be completed and met to gain FDA approval. Some products are a combination of a biologic and a device or a drug and a device, and, therefore, FDA requirements for two or more centers must be met before the product is granted approval. Tissue-engineered medical products are examples of combination products. Combination products are typically the most difficult to regulate and take the longest to reach the market. Biomaterial scaffolds alone without cells or growth factors are typically regulated by the FDA as a device and are under CDRH jurisdiction.

The regulatory procedures are sufficiently complicated that typically a specialist in this area is hired to manage this aspect of new product development. However, it is important to have some idea of product regulation early on, since it, along with good research and development, is needed to bring a medical product to clinical success. Toward that purpose, the procedures utilized by the CDRH branch of the FDA will now be described.

The CDRH branch of the FDA utilizes classification of medical devices to assist with determining the requirements for approval and the extent of regulatory control (http://www.fda.gov/cdrh/devadvice/). Medical devices are placed into one of three classes. Class I devices are those that have limited body contact and essentially pose no significant risk. Class II devices require special controls and must usually meet some performance standards to provide some assurance of safety. A device is placed in Class III if there is insufficient information to determine that general or special controls are sufficient to provide reasonable assurance of its safety and effectiveness. A new device that is not substantially equivalent to a device on the market will automatically be placed in Class III. Examples of Class I medical devices include dental floss, a tongue depressor, a surgeon's glove, and a clinical chemistry test system such as a pregnancy test. Examples of Class II medical devices include a blood pressure cuff, an oxygen mask, dental impression material, and an electrocardiograph. Examples of Class III medical devices include a heart valve, an automated blood warming device, and a silicone inflatable or gel-filled breast prosthesis. Classification is an important step of the FDA approval process since it will determine the extent of the testing required prior to use in humans and when the device can be sold.

At least 90 days prior to commercially distributing a new or substantially modified device, a manufacturer must submit a premarket notification to the FDA. More than 99% of the applications received by CDRH are cleared for marketing through the 510(k) Premarket Notification process. The goal of this process is to demonstrate to the FDA that the new device is substantially equivalent to an already approved predicate device. This is accomplished through careful characterization by several complementary methods that confirm the identity and purity of the substances involved, followed by completion of an abbreviated form of the ASTM F-748 and/or

ISO 10993 test protocols and adherence to quality system regulations. Quality system regulations include compiling a Device Master Record and Design History File. Together these two files contain documentation of the procurement process, the manufacturing details, all testing results—including assay verification tests—and the details of the design rationale and design verification testing.

If a device does not qualify for 510(k) approval, then a full premarket application (PMA) must be submitted containing all the required information on the safety and the effectiveness of the device as determined through preclinical and clinical testing. There is a decision tree in ISO 10993 that helps define which biocompatibility and safety testing is necessary based on length of contact with the body and/or blood. Clinical trials are highly regulated so that human subjects are not exposed to significant risks without their knowledge. Carefully documented and successfully completed *in vitro* and *in vivo* animal safety testing is required at the time of application to begin a clinical trial. Human clinical trials typically are divided into four phases: safety testing, efficacy testing, blinded efficacy compared to a clinically acceptable alternative (these three take approximately 5 years to complete), and finally, post-market surveillance (gathered from product use by the general public after FDA approval). Prior to initiating a clinical trial, a manufacturer must obtain an investigational device exemption (IDE) that includes all manufacturing and quality control procedures, the plan for the clinical study, and the lists of the review boards that have reviewed the proposed plan (see Chapter 2). The FDA has 30 days to approve or disapprove the IDE. If the PMA application is considered complete, the FDA has 180 days to approve or disapprove the application. If approved, the product can be marketed for human use for the purposes declared in the application, which are to be described in the product labeling. Another FDA submission for review is required prior to legally marketing the product for a new use (off-label use). For those interested in further information on regulation by CDRH or any of the other FDA centers, FDA guidance documents that cover all aspects of regulatory approval are readily available online (www.fda.gov).

6.7 APPLICATION-SPECIFIC STRATEGIES FOR THE DESIGN AND SELECTION OF BIOMATERIALS

6.7.1 Musculoskeletal Repair

The design and selection of the biomaterials components for an implant should be based on restoring the biological function of the damaged or diseased tissue. The principal function of musculoskeletal tissues is to provide a framework to support the organs and to provide a means of locomotion. Bone, cartilage, tendons, ligaments, and muscles are all part of the group of musculoskeletal tissues; however, they have different functions and different biological properties. Each one must be considered individually in terms of implant design and biomaterials selection. Bone is the only tissue capable of undergoing spontaneous regeneration. It is constantly in a state of remodeling, always optimizing its structure to best meet the needs of the body. This

ongoing cellular activity is why astronauts rapidly lose bone mass during zero gravity conditions. Unlike bone, cartilage is acellular and has a very limited capacity for repair. Therefore, damage to cartilage is often permanent and often progressive. Cartilage provides an articulating surface enabling low-friction movements between opposing bone surfaces. Ligaments are not simply passive joint restraints; they also provide electromechanical signals for joint stabilizing muscle contractions.

Replacement of damaged or diseased tissues or organs is best accomplished by autograft or allograft donor tissue, but they have limited availability, and a biomimetic synthetic substitute is the next best alternative. Biomimetic calcium phosphate materials (hydroxyapatite) have been shown clearly to enhance bone cell activity and are either used alone or in combination with collagen or other polymers as bone graft substitutes. Hydroxyapatite not only influences bone cell attachment, but it also appears to control the differentiation of stem cells to bone forming cells. This is particularly important for tissue engineering approaches that aim to not only restore function, but also to restore the actual biological tissue. In tissue engineering product development, materials selection is more complicated than it is in traditional approaches; typically, a biomaterial must have sufficient mechanical strength and also have a surface chemistry conducive to cell attachment and proliferation, must also perhaps serve as a drug delivery vehicle and release growth factors, and must also resorb or biodegrade once the new tissue has been formed.

Autografting osteochondral tissue from a healthy portion of a joint to a cleared defect is a current strategy for articular cartilage repair. Another approach involves filling the bulk of the defect with cells that can facilitate the growth of appropriate cartilage and/or bone tissue. This approach recently has been approved by the FDA and utilizes chondrocytes from the patients themselves. The chondrocytes are harvested, are expanded *in vitro*, and then injected into a surgically created compartment over the defect site. The cartilaginous tissue created by this process has been found to degrade faster than cartilage formed during fetal development. Perhaps a vital cell signaling message has been left out during this artificially stimulated biological process.

Further research is being conducted to improve tissue-engineered cartilage reconstruction. Chondrocytes or stem cells are being seeded on soft tissue scaffolds such as collagen, fibrin, and polylactic acid and precultured in bioreactors containing exogenous growth factors to form neocartilage. This approach may offer an improvement over the cell-only approaches, mainly due to the use of a biomaterial scaffold. As an example, a mixture of mesenchymal stem cells suspended in hyaluronan is being developed for direct injection to a damaged knee meniscus. Similar to how hydroxyapatite stimulates differentiation of stem cells to bone cells and is well suited for bony applications, hyaluronan (found in embryonic extracellular matrix) has a chondroinductive and antiangiogenic potential and shows promise as a biomaterial scaffold for cartilaginous tissues.

Biomimetic scaffold materials are clearly more than an inert structural support for cells. When properly selected for the given application they provide a receptive framework and lead to induction of a cell down a specific differentiation pathway (Fig. 6.14). Since mechanical forces do play a role in most tissue function,

it may also be that certain scaffolds are superior due to their biomimetic mechanical properties.

Traditionally, the load-bearing requirements of long bone repair applications have been accomplished by metallic biomaterials. Eventually, scientists and engineers will learn how to engineer total joints and all of the tissues they comprise, but for now, the best option is a total prosthesis that has femoral, tibial, and/or patellar components made of traditional metals and polymers or ceramics (Figs. 6.5, 6.6, and 6.10). Based on the trends of successful current research, most musculoskeletal injuries or defects in the future will probably be treated at an earlier, smaller stage. Resorbable composites delivering cells and growth factors will probably become the material of choice for not only bone, but also ligament and cartilage. The role of the traditional metallic load-bearing material may be reduced in the future to serving as a cast or brace that provides protection from biomechanical loading until the new tissue has fully regenerated internally.

6.7.2 Skin Regeneration

As the largest organ in human physiology, the skin plays a vital role in maintaining homeostasis, providing immunity, and supporting sensory feedback. Not surprisingly, wounding skin compromises its ability to maintain these critical functions and makes dehydration and infection much more of a threat, burns being an extreme example of the latter. Traditionally, medicine has used allogenic skin grafts as a primary means of treatment for most skin injuries. Because of additional wounding that results from skin grafts, considerable research has been devoted to the development of a skin equivalent. In this context, biomaterials improve the capabilities of skin substitutes to truly act as replacements for the original.

Macroscopically, skin is a dual-layer organ consisting of the dermis and epidermis, the latter of which has its own secondary hierarchy (Fig. 6.19). The dermis, owing to its collagen component, is mostly responsible for the structural integrity of the skin. In addition, dermal cells, specifically fibroblasts, produce chemical factors that are essential for the proper proliferation and differentiation of the epidermis. Between the dermal and epidermal layers lies a well-defined basal lamina. A proteinaceous extracellular layer, the basal lamina serves as the substrate upon which basal cells of the epidermis adhere and proliferate. Indeed, one common way to evaluate artificial skin is to assay for the presence of the basal lamina, as this would indicate dermal–epidermal integration. Basal keratinocyte cells form the first layer adjacent to the basal lamina. These cells undergo a program of proliferation and differentiation to form the full epidermal layer. The differentiated forms of epidermal cells from inner- to outermost include basal, spinous, and granular keratinocytes. The stratum corneum, a highly cross-linked layer of packed keratinocytes, serves as the outermost barrier and provides the ultimate protection against desiccation.

In the biomimetic tradition, it has been thought that effective skin substitutes should mimic natural skin structure. In addition, well-designed skin substitutes should possess the proper physical integrity to withstand the environment and, at the same time, support the growth of cells. A schematic of artificial skin is shown in

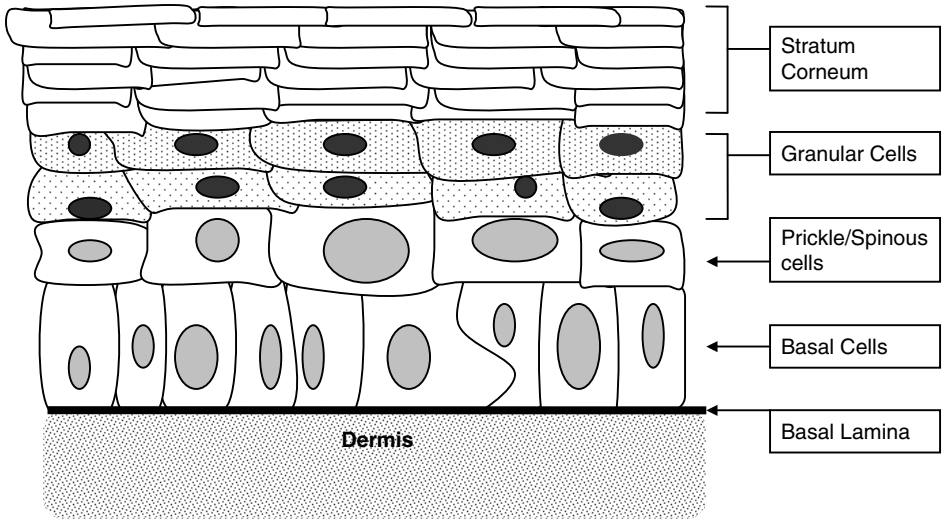

Figure 6.19 Skin is a dual-layer organ consisting of the dermis and epidermis. The main cellular components of the dermis are fibroblasts, and the main cellular components of the epidermis are the keratinocytes that are at various stages of differentiation. (Illustration drawn by Venkatasu Seetharaman.)

Figure 6.20. Biomaterials, in addition to providing the proper substrates for cellular growth, are excellent sources of mechanical strength. Collagen, a cross-linked polymer, is found as the primary dermal component of most skin substitutes. When cross-linked, collagen provides a mechanically sound matrix upon which to grow an epidermal layer and can even be impregnated with fibroblasts to provide chemical factors for proper epidermal differentiation and proliferation.

Type I bovine-derived collagen, typically used for most skin substitutes, is optimized for physiological conditions by altering pH values and moisture content. Once

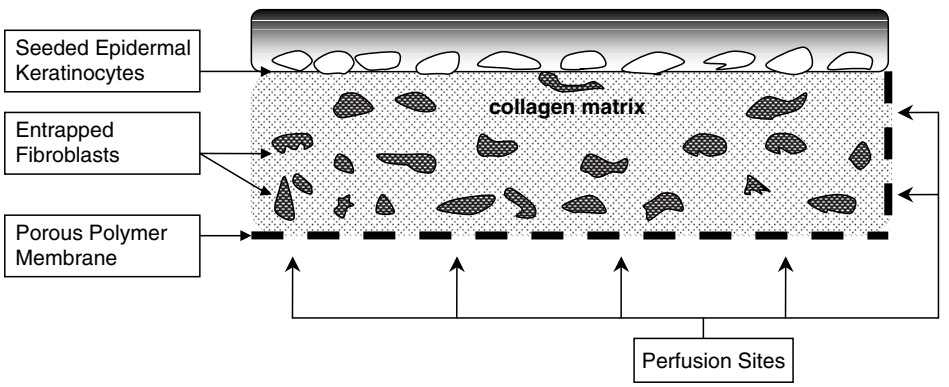

Figure 6.20 A schematic of artificial skin illustrating the similarities to natural skin. (Illustration drawn by Venkatasu Seetharaman.)

prepared, the liquid solution is poured into a specimen plate and allowed to polymerize, thereby taking on a more gel-like consistency. Polymerization shrinks the collagen by way of cross-linking. Depending on how the collagen is prepared and the length of polymerization, it is possible to control the gel consistency. It is important to note that the collagen gel or sponge is quite permeable to most molecules and permits diffusion of essential chemical factors. These can be provided to the epidermis by seeding the collagen with dermal fibroblasts, essentially trapping them within the cross-linked collagen structure. To complete the dual-layer skin substitute, epidermal keratinocytes are seeded on the dermis and allowed to differentiate into the proper epithelial cellular arrangement. Other skin substitutes actually use a thin layer of silicone as a functional epithelial barrier while the dermal layer integrates into the wound site. A subsequent procedure replaces the silicone with an epidermal matrix, thus completing the skin. Both collagen and silicone are examples of how biomaterials can be used to recreate the functionality of normal skin.

6.7.3 Cardiovascular Devices

The primary requirements for biomaterials used for blood-contacting circulatory applications such as heart valves and blood vessel replacements or stents are resistance to platelet and thrombus deposition, biomechanical strength and durability, and biocompatibility and nontoxicity. These key requirements were identified by studying the primary mode of action of the tissue to be replaced. For example, arteries consist of three layers that perform various biological functions: the intima, media, and adventitia. The intima is on the blood vessel interior and has a nonthrombogenic surface—it prevents blood contact with the thrombogenic media tissue. The cells of the intima produce a myriad of biomolecules, including growth factors, vasoactive molecules, and adhesion molecules. The media or parenchymal tissue is the middle muscular layer that provides the required strength while remaining viscoelastic. The media layer is made up of multiple layers of aligned smooth muscle cells. The outer adventitia layer acts as a stiff sheath that protects the smooth muscle media layer from biomechanical overload or overdistention.

As with most tissues, autograft is the preferred biomaterial for vascular tissue replacement. For example, one of the patient's own veins can be harvested and used to replace a clogged artery. Vein grafts have a failure rate as high as 20% in one year. Since vein grafts from the patient are unavailable and unsuitable in approximately 30% of all patients, synthetic graft materials have been developed. The observation that the intact lining of blood vessels (intima) does not induce coagulation has led scientists and engineers to produce more blood-compatible biomaterials by mimicking certain properties of the endothelium. For example, very smooth materials, surfaces with negative charges, and hydrophilic biomaterials are now used with limited success in blood-contacting applications. Knitted Dacron® (polyethylene terephthalate) and Gortex [polytetrafluoroethylene (PTFE)] vascular grafts are commonly used. The use of synthetic grafts has resulted in reasonable degrees of success (approximately 40% experience thrombosis at 6 months when synthetic grafts are used to bypass arteries that are smaller than 6 mm in diameter). Improved

vascular products have incorporated anticoagulants such as heparin on the blood-contacting surfaces.

As discussed in the section on wound healing (Section 6.4.2), most biomaterials are recognized by the body as foreign and lead to platelet deposition and thrombus or coagulation (blood clot formation). This limits their use in blood-contacting applications. Therefore the tissue engineering approach of growing and implanting a living multilayered cell construct holds great promise for cardiovascular applications. It is only recently that the cell culture conditions and scaffold material selection that will promote the smooth muscle cell alignment and tight endothelial cell packing of blood vessels have been identified. A great deal of additional research must be completed prior to commercial availability of a functional tissue-engineered artery. For example, the mechanical burst strength of the highly cellular tissue-engineered arteries is not yet sufficient to withstand what the heart can generate, although these artificial arteries are nonthrombogenic.

Diseased human heart valves can currently be replaced with mechanical prostheses (synthetic biomaterials) or bioprostheses (made of biological tissue). The two mechanical heart valves shown in Figure 6.21 have four essential components: an occluder such as a disc or ball; a seating ring against which the occluder sits when the valve is closed; a capture mechanism, such as a cage, that constrains the occluder when the valve is open; and a sewing ring that permits attachment of the valve to the heart. The occluder bounces back and forth from the seating ring to the capture mechanism with each heartbeat. The more promptly it moves, the more efficient the opening and closing. Thus, weight, or mass, of the occluder and wear resistance of the occluder material are critical features. Early materials that were used included lightweight plastics or hollow metal balls. Silicone rubber proved very effective as a ball. The advent of low-profile disc valves for the mitral position set a new material constraint, namely stiffness. Silicone rubber was too soft for the disc designs. Polyoxymethylene (POM) or poly acetal is stronger and stiffer than silicone rubber, and therefore was used in early disc designs. However, it had a problem with wear. The discs were supposed to be free to spin in the cage, so they could distribute wear evenly around the edge of the disc. However, as the disc moved up and down in the cage, wear tracks developed on the edge, preventing spinning and leading to the development of deep wear tracks and valve failure. Due to its high fatigue strength and wear resistance, pyrolytic carbon was selected as one of the prime materials for the occluder.

Figure 6.21 Three types of artificial valves that are used to replace diseased or malformed human valves. The tissue valve generally contains valve leaflets from pigs; the other two types are composed entirely of manufactured materials.

The capture mechanism and seating ring required strength and stiffness to maintain their shape. Furthermore, they had to be made of a material that could be sprung open for insertion of the occluders. Metal has typically been used, either as machined parts or as separate parts that are welded together. Early designs used the cobalt alloys due to their strength and corrosion resistance. More recently, titanium alloys have been used, due in part to their being lighter than cobalt alloys. Concerns of allergic reactions to cobalt alloy cages have also been expressed over the years.

Both the occluder and containment system are in direct contact with blood. Contact with foreign materials can cause blood to clot and can lead to platelet attachment and clotting. If the design results in eddy flow or stagnation, the clotting factors can accumulate and lead to thrombus formation. Therefore, both the material selection and device design must consider problems of blood contact and blood flow. Since these problems have not been solved, patients with mechanical heart valves receive medication to reduce their tendency to form blood clots.

Biological heart valves resist thrombo-embolism much better than synthetic materials do; however, they are less durable. The valves are harvested from 7- to 12-month-old pigs and preserved with gluteraldehyde fixation. The gluteraldehyde fixation slowly leads to unwanted calcification of the biological valves and eventual failure. Tissue engineering approaches are now being developed to synthesize living heart valves, but durability remains a large concern. The muscle of the heart has never withstood grafting; however, stem cells might be able to rebuild the damaged tissue *in situ*. Recent results from experiments in which mesenchymal stem cells have been injected directly into the ventricle wall have shown that the cells incorporate in the heart muscle and initiate regeneration of damaged heart muscle. Who knows what this will do to the life expectancy of future generations?

6.7.4 Drug Delivery

Biomaterials play an important role as delivery vehicles for pharmaceuticals and biomolecules. The pharmaceutical industry has long made use of powdered biomaterials such as talc and calcium carbonate to form pills and tablets containing a drug. The goal of drug delivery research is to prepare formulations that will result in sustained active drug levels in the body, leading to improved drug efficacy. Controlled-release formulations accomplish this by various techniques that involve conjugation of the drug to a biomaterial. For example, by delivering basic fibroblast growth factor bound to heparin, the blood circulation time (as measured by a half-life) is increased by a factor of three. Conjugation to polyethylene glycol (PEG) is a well-established approach for *in vivo* protein stabilization.

Liposomal drug formulations also exhibit extended circulating half-lives after intravenous injection. Liposomes are made from phospholipids that form hydrophobic and hydrophilic compartments within an aqueous environment. A unilamellar liposome has spherical lipid bilayers that surround an aqueous core. Water soluble drugs can be entrapped in the core and lipid soluble drugs can be dissolved in the bilayer. The drug concentration in plasma over time is elevated three to ten times when incorporated in liposomes.

Polymers are widely used in drug delivery systems. Nondegradable hydrophobic polymers (silicone elastomers) have been used most extensively as semipermeable membranes around drug reservoirs. Alternative formulations involve mixtures of drug and resorbable polymer that release drug when the polymer degrades (Fig. 6.22). Increasing the loading of the protein or drug within the matrix increases the release rate of the compound. As a rule of thumb, as the average molecular weight of the polymer in the matrix increases, the rate of protein/drug release decreases. Polymer microspheres can be formed around drug solutions, thereby encapsulating the drug as another means of preparing a controlled-release formulation. Polymers that undergo bulk erosion tend to have burst release rates as compared to polymers that undergo surface erosion. The targeting of a drug to a particular cell type can be accomplished by using cell-specific ligands. Microparticles that have been modified with ligands do not diffuse uniformly throughout the body. Instead, the drug is delivered directly to only certain cell types through a receptor–ligand interaction. For example, liposomes have been prepared with folic acid ligands that are preferentially taken up by cancer cells because cancer cells express more folate receptors than do other cell types.

Direct injection of the drug into the targeted tissue is another means of obtaining high local drug concentrations. Controlled release of the drug at the site is accomplished through conjugation of the drug to a biomaterial drug delivery vehicle. This is particularly useful when the drug has toxic side effects that can be minimized by exposing only the tissue of interest to high drug levels. With local drug delivery, the toxic peak blood levels of drug are reduced, and there are sustained levels of active drug over a longer time period, leading to better efficacy (Fig. 6.23). As an alternative to direct injection, chemotherapy-carrying magnetic particles (magnetoliposomes)

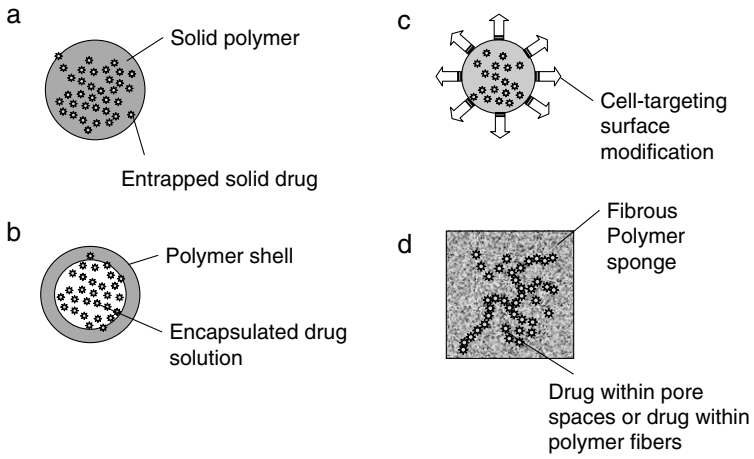

Figure 6.22 Polymers provide a versatile matrix for controlled release of drugs and biomolecules. Pharmaceutical agents can be contained within microparticles, microcapsules, or porous polymer blocks or conjugated to single chains of polymer. Drug targeting can be accomplished through the use of cell-specific ligands.

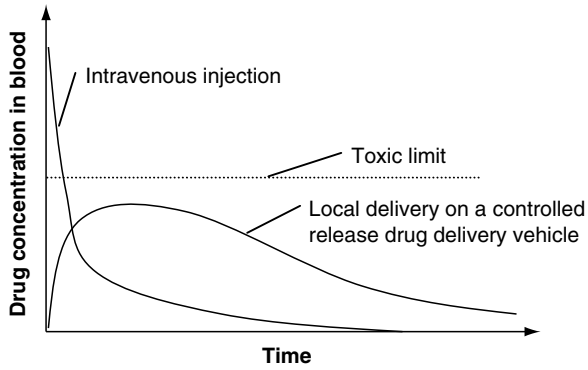

Figure 6.23 The primary advantage of local delivery is a reduction of the systemic blood levels of the drug to below toxic levels. High local levels at the site of injection or implantation allow for increased drug efficacy and reduced side effects. This technology is particularly applicable for chemotherapy drugs and therapeutic hormones.

also have been developed. After systemic injection, the drug-loaded particles are then localized to the cancer site by guidance with magnets.

Some drugs do not need to be solubilized to initiate biological activity. For example, nerve growth factor is effective when immobilized on a surface. Even higher levels of activity may be obtained after immobilization of a protein onto a surface due to conformational changes that occur upon immobilization. The active portions of the protein may be better exposed after immobilization. Alternatively, all biological activity may be lost after immobilization, so it is always necessary to conduct separate tests to confirm drug activity after immobilization or conjugation to a biomaterial surface.

Collagen is commonly used for the delivery of bone growth factors. The protein growth factors are typically not covalently immobilized on the collagen surface, leading to rapid release. Hydroxyapatite materials also are used for delivery of bone growth factors. The drug or protein often attaches quite strongly to the hydroxyapatite surface and release is greatly delayed as compared to a collagen drug delivery vehicle. Depending on the drug and its mode of action, longer release times may or may not be desired.

Rather than attempting to design a controlled-release drug delivery vehicle with the perfect release profile, drug delivery chips that can be programmed to open up drug compartments by an external signal are being developed. The ultimate smart drug delivery vehicle is the cell. Unlike a passive drug delivery device that acts independently, cells produce cytokines, growth factors, and extracellular matrix materials based on the signals from the *in vivo* environment. Attempts are being made to exploit this with the implantation of encapsulated xenograft pancreatic islet cells. Pancreatic islet cells produce insulin in response to the circulating blood levels. The xenograft cells need to be encapsulated within a biomaterial (typically alginate) to evade immune surveillance activity that can be toxic to the cells. Current research in this area is focused on varying the properties of the alginate to maintain

sufficient permeability to keep the cells vital, yet protected from immune cell toxins, while still allowing diffusion of insulin out of the device—a tall order for one material. Materials selection for medical devices will always involve this type of balancing act between properties.

EXERCISES

1. List and briefly describe the five basic categories of biomaterials described in this chapter.
2. Describe which type of biomaterial you would select for the construction of the following implantable devices. Explain which properties will be important and why. More than one material can be used in the same device.
   - **a.** Skin substitute
   - **b.** Guidance tube for nerve regeneration
   - **c.** Hip replacement stem
   - **d.** Dental braces
   - **e.** Urinary catheter
   - **f.** Tissue-engineered bone
3. List three biomaterials commonly used for the following applications: (a) sutures, (b) heart valves, (c) endosseous dental root implants, (d) contact lenses, and (e) hip prosthesis. Provide the specific chemical name.
4. When selecting a biomaterial to be used as an orthopedic implant, what are some of the properties or characteristics of the material that should be considered?
5. What would happen to the mechanical functionality of bone if the carbonated apatite crystals were not discontinuous or discrete and instead were long fibers similar to a fiber composite? The modulus of bone mineral is 114–130 GPa. The modulus of cortical (normal) bone is 19–20 GPa.
6. Discuss three advantages and three disadvantages of natural biomaterials for medical devices. What is the most commonly used natural biomaterial? List three medical applications of the most commonly used natural material.
7. The type of implant–tissue response that occurs at the site of implantation is a major predictor for the success and stability of the device. List four types of implant–tissue responses that can occur, beginning with what happens if the material is toxic.
8. What is the purpose of a suture? What are some of the important properties that must be considered when selecting a biomaterial for use as a suture? List four biomaterials that are commonly used for sutures.
9. Define calcification. What type of application is at the most risk for failure due to biomaterial calcification?
10. What is a bioactive material? What is a biomimetic material?
11. In the past, an implanted biomaterial was considered biocompatible if it became encapsulated with fibrous tissue and did not elicit a further response from the host. Why has this definition of biocompatibility changed?

12. If a protein is attached to a biomaterial surface, specifically a growth factor in order to attract cells once implanted, why would it be important to know the structure of the protein? Think about how a growth factor would work to attract the cell to the surface and how these proteins could be anchored to the surface so that this functionality is not compromised.

13. Many cell types can be found adjacent to a biomaterial implant. What type of cells would you expect to find depositing bone adjacent to an orthopedic implant? What type of cells would you expect to find clearing the site of biomaterial debris?

14. There is a well-defined wound healing response following the implantation of a biomaterial. Briefly describe the four phases of wound healing.

15. You have recently designed a new implantable biomaterial and have conducted an *in vivo* implantation study. Subsequent extraction of the sample reveals a thin, fibrous capsule surrounding your material. What does this experiment reveal regarding the biocompatibility of this device, specifically regarding the inflammation response?

16. Is the growth of a fibrous tissue layer around an implanted material a positive aspect in all applications? Why or why not? In what applications is a fibrous tissue layer not desired?

17. Describe two methods for making a biomaterial porous.

18. What biomimetic polymer appears to be particularly well suited for the differentiation of cartilage cells and why?

19. What biomaterial leads to direct bone bonding without an intervening fibrous tissue layer?

20. Describe a way in which a drug-carrying microparticle or nanoparticle can be modified to make it targeted to a specific cell type.

21. Classify the following biomedical devices according to the FDA definitions (www.fda.gov) for Class I, II, and III devices: (a) intraocular lens, (b) heart valve, (c) preformed tooth crown, (d) oxygen mask, (e) stethoscope, (f) dental amalgam.

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