3

BIOMATERIALS FOR TISSUE ENGINEERING

Learning Objectives

After completing this chapter, students should be able to:

1. Provide a definition for biomaterials.
2. Describe a process flow sheet for biomaterial development and describe the following terms as they relate to biomaterial development: biocompatibility, mechanical properties, biomimetic properties, and material degradation.
3. Discuss the historical development of biomaterials, including examples of dental implants and prosthetic implants.
4. Describe the tensile properties of a material, including sample preparation, components of a mechanical testing system, and components of the stress–strain curve.
5. Describe methods that can be used to improve the tensile properties of biomaterials.
6. Discuss the role of biodegradation in tissue engineering.
7. Describe the role of biocompatibility in tissue engineering.
8. Define biomimetic activity as it relates to biomaterials and tissue fabrication.

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9. Describe the differences between natural and synthetic materials, degradable and nondegradable materials, and metals, ceramics, and polymers.
10. Discuss the following biomaterial platforms: hydrogels, acellular scaffolds, polymeric scaffolds, and self-organization strategies.
11. Explain the concept of smart materials and give examples of smart materials in tissue engineering.
12. Describe the composition and function of the mammalian extracellular matrix and how it relates to the tissue fabrication process.
13. Explain the concept of an idealized biomaterial.

CHAPTER OVERVIEW

In this chapter, we will study the role of biomaterials in tissue engineering. We begin this chapter by providing a definition of biomaterials and then provide a general scheme for the development of biomaterials to support the tissue fabrication process. We then provide a brief description of the historical relevance of biomaterials and the way in which biomaterials have been used over the centuries. After these introductory sections, we provide a discussion of biomaterial properties that are important for tissue engineering: tensile properties, degradation kinetics, biocompatibility, and biomimetic properties. We then provide a classification scheme for biomaterials using three categories: natural vs. synthetic, degradable vs. nondegradable, and metals, ceramics, or polymers. The next section is devoted to a discussion of biomaterial platforms, which include polymeric scaffolds, biodegradable hydrogels, acellular matrices, and scaffold-free platforms. We next focus our discussion on the evolution of smart materials and the development of these materials for tissue engineering. We end this chapter with a discussion of the mammalian ECM and provide insights for the development of an idealized biomaterial.

3.1 DEFINITION OF BIOMATERIALS

In this section, we will look at several widely used definitions of biomaterials (1–6). We will then use this information to formulate a definition of biomaterials that is tailored to tissue engineering and tissue fabrication processes.

According to the National Institutes of Health (NIH), a biomaterial is defined as (7) "*any substance (other than a drug) or combination of substances synthetic or natural in origin, which can be used for any period of time, as a whole or part of a system which treats, augments, or replaces tissue, organ, or function of the body*." There are two important components of this definition that require further explanation. First, a biomaterial may be "*synthetic or natural in origin*", thereby providing a broad classification of the sources of biomaterials. This provides a simple classification scheme that is discussed later in this chapter. However, at this stage, it can be appreciated that biomaterials can either be synthetic, which means that the biomaterial is synthetized in the laboratory using controlled conditions; or the biomaterial

can be natural, which means that it is extracted from tissue specimens like collagen, which is extracted from the tail of rats (referred to as rat tail collagen). The second component of the definition—"*treats, augments, or replaces tissue, organ, or function of the body*"—provides a direct statement of the potential application of the biomaterial. This statement illustrates the potential application of the biomaterial in the medical field as a therapeutic option. The definition provided by NIH suggests that the biomaterial can be used as a whole or as a part of a system for therapeutic purposes. The utilization of a biomaterial as a whole for medical applications means that the biomaterial itself is the therapeutic agent; an example of this application is when biomaterials are sutured onto left ventricular tissue after a myocardial infarction to limit cardiac hypertrophy and support functional remodeling. The second application of a biomaterial is as part of a system; this application refers to biomaterials that are used the fabrication of devices like stents or pacemakers. The use of biomaterials as part of a system applies to tissue engineering, where biomaterials are used for fabrication of 3D artificial tissue.

Additional definitions for biomaterials have been proposed. Clemson University has played a significant role in development of the field of biomaterials. During one of the annual biomaterials symposia at Clemson University, the Sixth Annual International Biomaterials Symposium in April 20–24th 1974, Clemson's Advisory Board for Biomaterials provided the following definition (8): "*a biomaterial is a systematically, pharmacological inert substance designed for implantation within or incorporation with a living system*." Similar to the NIH definition, this one provided by Clemson's Advisory Board can be broken down into two components. The first part of the definition—"*systematically, pharmacological inert substance*"—refers to a specific property of biomaterials, inertness of the material, which is important for any given biological application. The second part of the definition—"*designed for implantation within or incorporation with a living system*"—refers to the application of the material for *in vivo* applications as a direct therapeutic modality. This definition was conceived in 1974, and at the time, the field of tissue engineering was not very well-developed, and therefore, the definition does not discuss the incorporation of materials for artificial tissue fabrication.

We have looked at two definitions of biomaterials: one provided by NIH and one provided by Clemson's Advisory Board for Biomaterials. If we compare the two definitions, there is a common theme—the definition always comprises two parts, the first part focused on material classification or property, and the second part focused on application of the biomaterial.

A third definition is provided by J. Black in a 1982 publication (9): "*a biomaterial is any pharmacological inert material, viable or non-viable, natural product or man-made, that is a part of or is capable of interacting in a beneficial way within a living organism*." The definition provided by J. Black has also been broken into two components, just as the case with the definition provided by NIH and by Clemson's Advisory Board on Biomaterials. The first part of the definition—"*is any pharmacological inert material, viable or non-viable, natural product or man-made*"—talks about biomaterial properties and classification schemes. The second

part of the definition describes potential applications of the biomaterial by stating that it is "*capable of interacting in a beneficial way within a living organism*."

Continuing with this discussion o the definition of biomaterials, let us look at one final definition provided by CP Sharma (8): "*biomaterials are materials designed for interfacing and/or interacting with a living system, inducing no adverse reaction at the site of implantation in vivo or ex vivo and systematically*." As we have seen with the three definitions of biomaterials that have been presented before, the definition by CP Sharma is also composed of two parts, though in this case the application is stated first and is followed by the material properties. In his definition, CP Sharma states that the applications of biomaterials are "*materials designed for interfacing and/or interacting with a living system*." This part of the definition alludes to the use of biomaterials as a therapeutic modality. In the second part of the definition, CP Sharma states an important property of biomaterials: "*inducing no adverse reaction*." This phrase alludes to the biocompatibility of the material and is indeed an important property for any biomaterial.

We have looked at four definitions of biomaterials and seen a general theme. All four definitions can be viewed as two-part definitions, the first of which is focused on a specific material property or classification scheme and the second part focused on *in vivo* application or interfacing with living systems. However, none of these definitions adequately represent the use of biomaterials for tissue engineering and for the tissue fabrication process. In the field of tissue engineering, biomaterials are used specifically to support fabrication, culture, and maturation of 3D artificial tissue by providing functional integration at the cell-material interface. Keeping with the theme of the biomaterial definitions provided by experts in the field, we present our definition of a biomaterial as it applies to tissue engineering and the tissue fabrication process (Figure 3.1): *a biomaterial is any substance that simulates the extracellular matrix by functionally interacting with isolated cells to support fabrication and maturation of 3D artificial tissue*.

Let us discuss this definition. The first point to note is the elimination of any classification schemes and any reference to material properties. We do believe that classification schemes and materials properties are critical in the development of biomaterials for any application; however, we do not believe this information has to be incorporated within the definition. Rather, we will discuss biomaterial classification schemes and biomaterial properties in a later section. Our definition is focused on the application of biomaterials to interface with isolated cells in the context of artificial tissue fabrication: "*a biomaterial is any substance which simulates the extracellular matrix*." In this sense, the biomaterial simulates the roles of the mammalian extracellular matrix. This is an important criterion for any biomaterial used for fabrication of 3D artificial tissue; the role of biomaterials is indeed to simulate properties of the extracellular matrix.

We stated that a biomaterial is any substance that stimulates the extracellular matrix. Implied in this definition is that biomaterials will functionally interact with isolated cells based on specific cell-matrix interactions mediated between cell surface integrins and specific binding sites on the surface of biomaterials. Cell-matrix interactions are known to initiate a complex set of intracellular signaling pathways,

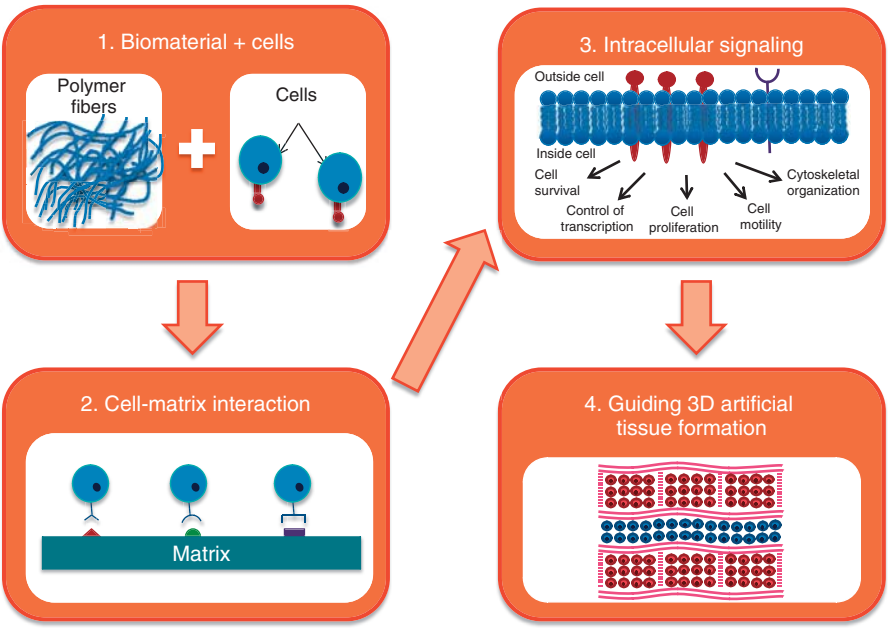

Figure 3.1 Definition of Biomaterials—The definition of biomaterials is presented in four parts. The first part shows a biomaterial that has specific binding sites for integrins, along with cell surface integrins. The second part shows functional coupling between biomaterials and cells, known as cell-matrix interaction. The specific cell-matrix interaction leads to a sequence of intracellular signaling events that support growth, remodeling, and health of cells and the tissue, as shown in the third part of the figure. Finally, fabrication of functional 3D artificial tissue is a result of intracellular signaling events, as can be seen in the fourth and final part of the definition.

which modulate cellular and molecular behavior and phenotype. This is embedded in the second part of our definition of a biomaterial: "*functionally interacting with isolated cells*."

The third and final part of our definition of a biomaterial is: "*support the fabrication and maturation artificial 3-dimensional tissue*." The objective of tissue engineering is to fabricate 3D artificial tissue. Specific functional interactions at the cell-matrix interface will support the fabrication of 3-dimensional tissue, and this interrelationship between biomaterials and isolated cells will continue as artificial tissue matures during controlled *in vitro* culture.

3.2 SCHEME FOR BIOMATERIAL DEVELOPMENT

Figure 3.2 shows a generic scheme for biomaterial development for applications in tissue engineering.

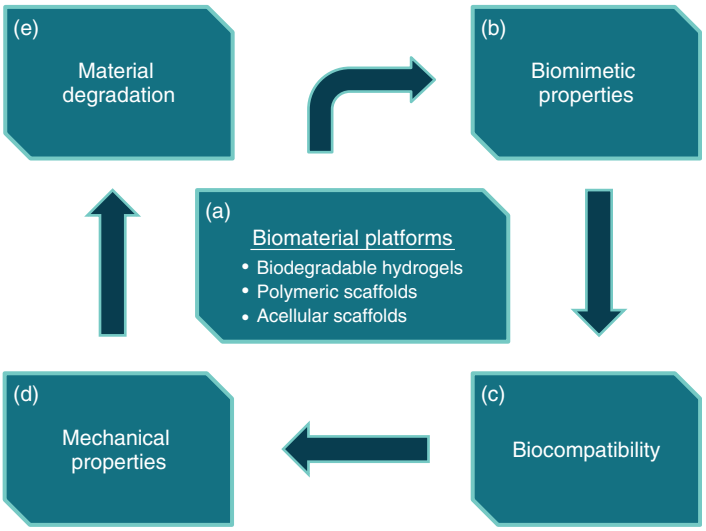

Figure 3.2 Biomaterial Development for Tissue Engineering—(a) Biomaterial Platforms—The first step in biomaterial development involves selection of a suitable platform. Options for biomaterial platforms include biodegradable hydrogels, polymeric scaffolds, and acellular scaffolds. (b) Biomimetic Properties—The second step in the process refers to biomimetic properties, cell-matrix interactions between specific binding sites on the surface of biomaterials and cell surface integrins. (c) Biocompatibility—Biomaterials elicit a foreign body response upon implantation, and biocompatibility refers to the host response to the biomaterial. (d) Mechanical Properties—Depending on application, biomaterials will be exposed to different mechanical conditions. The mechanical properties of biomaterials need to be optimized to match the requirements for any given application. (e) Degradation Kinetics—The rate of material degradation is an important variable that needs to be controlled and optimized when working with degradable biomaterials.

Researchers are often confronted with the difficult task of selecting a biomaterial platform for their specific application, and for sake of simplicity, we can say they have three platforms to choose from: polymeric scaffolds, biodegradable hydrogels, and acellular scaffolds (Figure 3.2a). Once a biomaterial platform has been selected, there are several processing conditions that need to be optimized, and these conditions depend on the platform selected. Fabrication of each of the three platforms requires optimization of processing conditions that vary based on the platform selected.

The second stage of biomaterial development is the incorporation of biomimetic functionality to support cell-matrix interactions (Figure 3.2b). Cells have specific cell surface receptors known as integrins, which bind to specific binding sites on the extracellular matrix, like the RGD site on fibronectin; this process is known as cell-matrix interaction. By definition, in order for a material to be considered a biomaterial, it must have biomimetic activity; however, the material properties may need to be modified to improve the number of binding sites, distribution of these

sites, and binding strength at the cell-material interface, along with a host of other variables.

The third stage of biomaterial development is biocompatibility (Figure 3.2c), which refers to the ability of the biomaterial and/or artificial tissue fabricated using the biomaterial to maintain functionality upon implantation. When a biomaterial or 3D artificial tissue is implanted *in vivo*, the host's immune system undergoes a foreign body reaction to minimize detrimental effects of this foreign body to the host. This process involves infiltration of neutrophils and macrophages to the site of implantation, followed by formation of a fibrotic capsule to seal the foreign body from host cells. Many of the biomaterials utilized for tissue engineering are based on synthetic polymers and, upon implantation, elicit a foreign body response. In order to prevent this, properties of the biomaterial need to be modified to minimize host rejection upon implantation.

The fourth stage in biomaterial development is assessment and optimization of mechanical properties (Figure 3.2d). Depending on the application, artificial tissue will be subjected to various biomechanical forces, and biomaterials need to withstand these forces. The mechanical properties of a biomaterial are commonly characterized based on tensile properties, which are measured by stretching the material to the point of failure. The tensile properties of a material are used to plot a stress–strain graph, which is then utilized to obtain several variables that are used to assess tensile properties of the material.

The fifth and final stage of the biomaterial development process is degradation kinetics (Figure 3.2e). There are many applications in which the biomaterial acts as a temporary scaffold to support the initial homing of cells, which then synthesizes the extracellular matrix. As cells produce ECM, the biomaterial is degraded, and the rate of material degradation is controlled to match the rate of new tissue formation. As such, the degradation properties of the biomaterial need to be characterized and optimized. Often, the rate of degradation of a material can be controlled by changing the material synthesis process; for example, the addition of a cross-linking agent during polymer synthesis can stabilize the scaffold and delay the rate of material degradation.

In this section we presented a generic scheme for biomaterial development as it applies to tissue engineering for the fabrication of 3D artificial tissue. We introduced the role of the biomaterial platform, biomimetic properties, biocompatibility, mechanical properties, and material degradation for biomaterial development. This section serves to familiarize the reader with these concepts, and in subsequent sections in the chapter, we will provide additional details on each of these topics.

3.3 HISTORICAL PERSPECTIVE ON BIOMATERIALS

Biomaterial science has advanced to a well-defined scientific discipline with broad applications for surgical reconstruction. However, the use of materials to restore lost functionality is not a new concept, and specific cases have been cited hundreds and even thousands of years ago (10–13). Some relevant examples can be seen in the

history of dental implants, which dates back thousands of years, and in the history of prosthetics, which dates back hundreds of years. In this section, we provide a brief historical overview of the materials used in dental implants, prosthetics, and other significant developments in the field.

One of the earliest documented cases of a dental implant took place about 3000 years ago (1000 BC), where a copper stud was implanted into a patient in Egypt, using nails to secure the implant (14–16). Another early example of a dental implant dates back about 1400 years ago (600 AD), where pieces of shell were implanted into sockets of three incisor teeth of a young woman. This incident happened in the Mayan civilization, in what is today known as Honduras. In addition to copper and shells, ivory was often used as a dental implant in both Egyptian and South American cultures (14–16). Gold and platinum were also used as dental implants, with early use dating back to 1809 and 1887, respectively. In 1937, vitallium, an alloy consisting of cobalt, chromium, and molybdenum, was used in patients at Harvard University (17,18). More recently, in 1965, titanium was first used as a dental implant in a patient in Sweden (19). Copper, shells, ivory, gold, platinum, and titanium are all examples of early biomaterials that were used for reconstructive applications in humans. These materials were not developed for dental applications, but rather used due to accessibility. The success rate was limited, with immune tolerance being low and likelihood of rejection being very high. Nonetheless, these early examples serve to illustrate the utility of biomaterials to restore lost functionality in humans.

Artificial limbs have a long history filled with several innovations spanning several centuries (20–24). Although limb prosthetics are commonly used in cases of birth defects, accidents, and in cases of amputation required due to cancer or infection the long-standing history of prosthetic limbs has been brought about by the loss of limbs during warfare. The earliest documentation of the use of a prosthetic limb was about 5500 years ago (3500 BC) in India, where Queen Vishpla used an iron limb in battle after losing her own leg. Another early documentation of the use of a prosthetic limb was about 2500 years ago (484 BC), when a Persian solder lost his leg while escaping from enemy captivity and later used a wooden support as a prosthetic. The earliest prosthetic limb that has been discovered was from about 2300 years ago (300 BC) in Capau Italy and was fabricated from copper and wood, while more recent prosthetic limbs in the 15th and 16th centuries were fabricated from iron. Notably, in the 19th century, prosthetic limbs were primarily fabricated from wood, likely due to their light weight compared with the heavier metallic compounds.

The advent of modern biomaterial science can be traced back to early work conducted by Sir Nicholas Harold Lloyd Ridley in the development of intraocular lenses to restore vision in patients affected by cataracts (25–30). Under normal conditions, the eye has a specialized structure known as the crystalline lens, which together with the cornea, refracts light to the retina leading to the formation of an image and, therefore, vision. A cataract is a medical condition that results in clouding of the crystalline lens and alteration of the ability to refract light on the retina and leads to total loss of vision. Intraocular lenses were developed to replace

crystalline lenses that were affected by cataracts. This work was pioneered by Sir Nicholas Harold Lloyd Ridley and has been instrumental in defining the field of biomaterial science. Sir Nicholas Harold Lloyd Ridley was an English ophthalmologist who was working with the Royal Air Force treating patients during World War II. He found splinters of acrylic plastic from aircraft cockpit canopies in the eyes of wounded pilots and made the observation that the material did not trigger any host immune response in the eyes of these pilots. This led him to conclude that the material that was used to fabricate the canopies, polymethyl methacrylate (PMMA), would serve as a good biomaterial for fabrication of intraocular lenses for treatment of cataracts, as it is not rejected by the host. He went on to develop intraocular lenses, and the first artificial lens was implanted in a patient on November 29th, 1949, at St. Thomas Hospital in London. This technology proved to be very successful, and intraocular lens are now implanted in over 10 million patients per year worldwide as a treatment modality for cataracts. The work conducted by Sir Nicholas Harold Lloyd Ridley pioneered artificial intraocular lens implantation as a corrective surgery for patients with cataracts This laid the foundation for biomaterial science as we know it today.

The history of Biomaterials as a scientific discipline can be traced back to 1969 when Clemson University hosted the first symposium of Biomaterials, which was later known as the Annual International Biomaterials Symposium (7). At this inaugural meeting, 17 research papers were presented and there were approximately 100 participants. There were two hallmarks of this meeting that provided the foundation for the evolution of Biomaterials as a scientific discipline. First, the participants of this meeting represented an interdisciplinary group of scientists and physicians, thereby actively engaging a broad spectrum of thoughts and opinions from basic material design, fabrication, and properties all the way to potential applications in very specific clinical situations. Second, discussions at this inaugural meeting provided the impetus for the formation of the Society of Biomaterials, the leading authority in the field, which was founded in 1975.

The field of biomaterials has been a part of mankind for thousands of years and has slowly evolved into a knowledge-based scientific discipline. Although the field had modest beginnings, biomaterial science has grown at an exponential rate during the last few decades, and there are entire academic departments in major research universities dedicated to the advancement of this field. Our understanding of biomaterials, including design considerations, functional assessment, and host response upon implantation has been greatly improved over the last couple of years. Some of this information will be presented in subsequent sections of this chapter.

3.4 TENSILE PROPERTIES

The mechanical properties of a material are important in determining function in any given application and are commonly assessed based on tensile properties. The tensile properties of a material are used very frequently in engineering design as

an important criterion for material selection. The tensile properties of a material provide information about the strength of the material, its ability to withstand a particular load, and information about elastic properties (31–33). All of these properties are extremely important for material selection during tissue fabrication, and depending on the application, certain properties will be desirable: material strength is an important design criterion for load-bearing applications like bone tissue engineering, while elasticity becomes important in valve tissue engineering.

We begin with a description of tensile testing of biomaterials and follow this with a discussion of strategies to change the tensile properties of biomaterials. For the first section—tensile properties of biomaterials—we will discuss sample preparation, mechanics of tensile testing, and data acquisition and interpretation.

Introduction to Tensile Testing—The deformation of a material in response to a load provides information about tensile properties (31–33). The tensile properties of a material are extrapolated from a stress–strain plot, which is obtained after a load-deflection test (also known as a stress–strain test or tensile test). The test material is clamped in a mechanical testing system with one end being held stationary while the other end is subjected to a load, which stretches the material to the point of failure. The force applied to the material, along with material deformation, is recorded and used to plot a stress (force per unit area) versus strain (deformation) curve; the stress–strain plot is used to obtain specific variables that provide information about the mechanical properties.

Sample Preparation for Tensile Testing—Sample preparation is very specific for tensile testing; the material is prepared in a dog bone shape, as shown in Figure 3.3a. The ends of the materials are referred to as the shoulder and are thicker compared to the rest of the material to allow gripping during tensile testing. The gage length is the center region of the material; it is thinner than the shoulder and is part of the material for which tensile properties are determined.

Testing Apparatus—Tensile testing is conducted in a specialized apparatus known as a mechanical testing system (MTS) (34,35), which consists of the following major components: a fixed member, a movable member, a set of grips, a drive mechanism, a load indicator, a crosshead extension indicator, and an extensometer. The fixed and movable members position the grips in place, and the specimen is secured to these grips by attachment at the shoulder region of the sample. The drive mechanism applies a uniform, controlled velocity to the movable member resulting in a tensile load on the sample. The load indicator shows the total tensile load carried by the test specimen, while the extensometer is used to determine the distance between two designated points on the test specimen. A crosshead extension indicator shows change in the separation of the grips. ASTM International, which develops international standards for materials, products, systems, and services used in construction, manufacturing, and transportation, has defined the specific requirements for the testing apparatus.

The Tensile Test—The specimen is secured between the two grips, and the movable grip applies a tensile load that causes deformation of the material. Tensile load is applied until the material fails (Figure 3.3b), and material failure is accompanied by a change in length and diameter (Figure 3.3c).

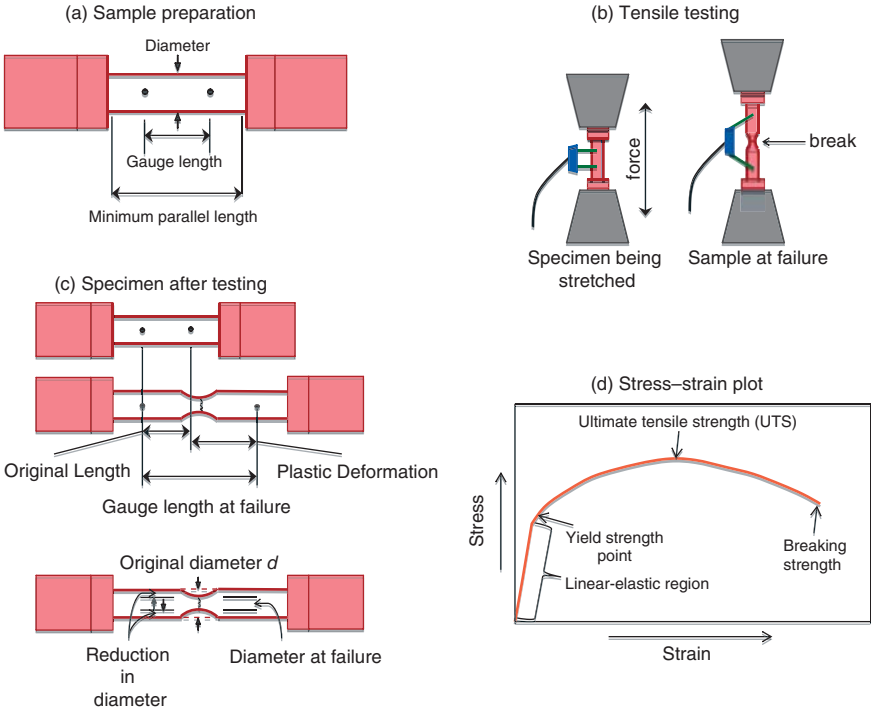

Figure 3.3 Tensile Properties of Materials—(a) Sample Preparation—Dog bone-shaped materials are used for tensile testing. The edges of the material are known as the shoulder and are designed for ease of gripping in a mechanical testing system. The gauge length is the region in the center of the material. **(b) Tensile Testing**—During tensile testing, the specimen is secured between two grips, and a tensile load is applied to the specimen until the material reaches a point of failure. **(c) Specimen after Testing**—As a result of tensile testing, the material undergoes a change in geometry, becomes elongated, and is accompanied by a change in diameter. **(d) Stress–strain Plot**—The slope of the linear region of the stress–strain curve is known as the elastic modulus. The yield strength is the point at which the material transitions from the linear region to plastic deformation. The tensile strength of a material is the maximum force that the material can withstand without failure.

Stress–strain Curve—The stress versus strain curve for most materials is divided into two components; a linear region and a region in which the material exhibits plastic deformation (Figure 3.3d). The linear region is characterized by a linear relationship between strain and stress, and for every unit increase in stress, there is one unit increase in strain or deformation of the material. This behavior is characterized by Hooke's law, and the slope of the linear region, obtained by dividing tensile stress by tensile strain, is known as Young's modulus, also known as elastic modulus or modulus of elasticity (36–39). Many materials, known as brittle, fracture upon application of any additional stress beyond this linear region.

However, ductile materials do not fracture and exhibit plastic deformation, which means that application of additional stress results in greater material deformation. The material deformation is not fully reversible, as the material will not return to its original state upon removing external stresses. The point at which a ductile material transitions from linear stress–strain behavior to plastic deformation is known as yield strength. The toughness (of either brittle or ductile materials) is obtained by the area under the stress–strain curve, and is a measure of the work required to deform a material until it reaches a point of failure. Finally, tensile strength, also known as ultimate tensile strength, is an important material property and measures the maximum force the material can withstand without failure. The tensile strength can be obtained from the stress versus strain curve by determining the maximum value of stress on this curve.

Tensile Properties and Tissue Engineering—We have seen that the tensile property of a material is an important criterion used to aide material selection in any engineering design problem; this argument also applies to the tissue fabrication process (40–44). During tissue fabrication, the function of a biomaterial is to support formation of 3D artificial tissue and guide tissue development and maturation. In order to satisfy this requirement, the biomaterial needs to satisfy a set of design constraints, which include constraints on tensile properties. For any given tissue engineering application, mechanical properties of the artificial tissue need to match mechanical properties of mammalian tissue. The tensile properties of most mammalian tissue have been published, and values can be obtained from the literature. During the tissue fabrication process, the objective is to fabricate 3D artificial tissue that has the same or similar tensile properties to mammalian tissue. While this may sound like a trivial task, this is not one that has been accomplished with a high degree of success in the recent tissue engineering studies. This is due to the inherent lack of mechanical compatibility between engineered naturals and those found in nature. In order to address this limitation, numerous strategies have been implemented to improve tensile properties of engineered materials, some of which are discussed in the following section.

3.5 MODULATION OF TENSILE PROPERTIES

In this section we examine strategies to modulate tensile properties of biomaterials. As we saw in the concluding paragraph of the previous section, for any tissue engineering application, it is important for the tensile properties of biomaterials to match those of mammalian tissue. However, this similarity is not always the case, and various strategies have been developed to change and improve the tensile properties of biomaterials to bridge the gap with mammalian tissue. Let us look at some of these strategies in this section.

Polylactic acid (PLA) has been used extensively in tissue engineering for many different applications, including the development of bone and heart muscle. For bone tissue engineering applications, the tensile properties of PLA do not match

that of normal bone tissue. This incongruence can be seen by looking at one particular metric, the Young's modulus, also known as the modulus of elasticity or the elastic modulus. As we have seen in the previous section, the elastic modulus is a measure of the deformation of the material in response to stress and is measured by the slope of the stress–strain curve in the linear region. For bone tissue engineering, the Young's modulus should be in the range of 3–30 GPa, while for PLA, the Young's modulus has been reported to be in the range 2–7 GPa. Therefore, the tensile properties of PLA are not suitable for bone tissue engineering, and strategies need to be developed to bridge this gap. *How exactly can this be achieved?*

In one study, hydroxyapatite (HA) was used to increase the Young's modulus of PLA fibers (45). HA is a naturally occurring mineral that is present in human bone and teeth and has calcium and phosphate as components. The hypothesis is that addition of HA to PLA during the fabrication process will enhance the tensile properties of the scaffold, thereby making it suitable for bone tissue engineering. In this study, PLA fibers were fabricated with the addition of varying amounts of HA, from 0 to 70 wt. % (45). There was a linear relationship between HA percentage and Young's modulus, which increases to approximately 12 GPa with incorporation of 70% HA (45).

This study showed a clear relationship between the composition of HA and tensile properties of PLA fibers. Utilization of an additive is commonly used to modulate the tensile properties of biomaterials, and has been used extensively for tissue engineering applications. The choice of additive and composition at which it is used, are important design variables and must becarefully chosen.

In the previous example, we looked at the use of additives to modulate tensile properties of PLA fibers. Another commonly used strategy to improve tensile properties of biomaterials is the use of cross-linking agents to stabilize fibers. Let us look at this from a conceptual standpoint first; then we will provide a specific example. Cross-linking is the process by which polymer chains are linked together by ionic or covalent bonds, or by the use of specific probes which form a bridge between polymer chains. Irrespective of the method used for cross-linking, the objective is always the same—to stabilize the material. Intuitively, this would translate to an increase in tensile properties of the material, as stabilization of polymer chains would result in an increase in mechanical properties. Glutaraldehyde is a commonly used cross-linking agent and has been used extensively in biological sciences for cross-linking of many proteins, including collagen.

Cross-linking of protein molecules has been used as a strategy to improve tensile properties of biomaterials. In one study, glutaraldehyde was used as cross-linking agent to stabilize gelatin molecules in an attempt to improve tensile properties of the scaffold (46). Gelatin films were fabricated in a petri dish by solvent evaporation followed by air-drying. Different concentrations of glutaraldehyde solution were used, in the range 0.125–2.5 (w/w), to cross-link gelatin films, and tensile properties were evaluated (46). Glutaraldehyde cross-linking proved to be an effective strategy to increase the Young's modulus of gelatin films (46).

Cross-linking of polymer fibers has been used extensively to improve the tensile properties of biomaterials and proves to be an effective method. During the design

process, the choice of cross-linking agent, the concentration of cross-linking agent, and processing conditions necessary for efficient cross-linking of polymer fibers need to be addressed and optimized.

In the two previous examples, tensile properties were modulated by use of an additive or cross-linking agent. In both cases, properties of an existing material were modified. However, a completely different and novel approach is to engineer custom materials by polymerization of predefined monomer units under optimized processing condition. This strategy is very different from the first two, as this strategy involves custom fabrication of the biomaterial, which allows greater flexibility for tuning tensile and other properties.

In one study, researchers set out to fabricate titin-mimicking artificial elastomeric proteins or, in other words, artificial proteins that mimic the properties of the naturally occurring protein titin (47). Titin is one of the largest proteins known; it can be found in muscle tissue of humans, and it provides passive elasticity and acts as a molecular spring and scaffolding protein. In order to develop artificial equivalents of titin, protein domains GB1 and resilin were used to mimic titin immunoglobulin domains, and the resulting polymer was fabricated into a ring configuration (47). Based on the proportion of the two components used for biomaterial synthesis, Young's modulus could be changed. Utilization of urea as a denaturing agent also had a significant impact on tensile properties, as can be seen by changes in Young's modulus (47).

The third strategy for the synthesis and modulation of tensile properties is very complex; only highly specialized research laboratories have the necessary technological capabilities to undertake such an endeavor. However, the strategy of building tailor-made biomaterials is novel and provides great promise for the future of biomaterials for tissue engineering.

3.6 MATERIAL DEGRADATION

Introduction—Material degradation refers to the loss of integrity and molecular organization, which in turn affects function of the material for any given application (48–52). Most, if not all, materials are subjected to degradation, although in some cases the rate of degradation may be too slow to be observed or measured in any meaningful way. We can think about material degradation from an engineering standpoint, where degradation of the material can have a catastrophic effect and lead to structural failure. Loss of functionality of materials like stainless steel can lead to instability in major structures like bridges. In engineering design, material degradation is a negative result; it is something that needs to be reduced, eliminated, or managed. Another important application of material degradation can be found in human physiology. Turnaround of proteins in the human body is a normal part of homeostasis, and based on the physiological state of the person, proteins are either degraded or synthesized; this is important for normal human function. Material degradation during the tissue fabrication process is an important property of the biomaterial. The purpose of the biomaterial is to support culture and remodeling of

isolated cells to form functional 3D artificial tissue. Degradation of the biomaterial during early stages of tissue fabrication, or any stage, as a matter of fact, will have a significant impact on functional performance of artificial tissue.

Definition of Material Degradation—The definition of material degradation varies depending on the application and context in which it is used (53–57). The word "biodegradation" has often been used to refer to degradation that occurs in a biological environment and has been defined as "*gradual breakdown of a material mediated by a specific biological activity*." Protein degradation in the human body will fall within this category and can be referred to as biodegradation. Material degradation from an engineering standpoint has a completely different meaning and has been defined as "*a simple definition of materials degradation is that it is the consequence of a wide range of physical processes; it is almost universal in occurrence and is a major engineering problem*." When we compare the two definitions, we can easily see that from a biological perspective, degradation is considered to be a natural process and the objective is not to control or regulate the process, but to understand it. From an engineering standpoint, material degradation is an undesirable outcome, and the objective is to develop countermeasures to limit any accompanying adverse effects. Applied to tissue engineering, neither of the two definitions is adequate; the definition of biodegradation only provides a platform to start with. Biodegradation has been defined as the "*gradual breakdown of a material mediated by a specific biological activity*." This process restricts material breakdown to a specific biological activity, which is the case during human physiology but not always the case in tissue engineering. For tissue engineering and the tissue fabrication process, degradation can be defined as "*gradual breakdown of a biomaterial mediated in a controlled manner to support the fabrication of 3-dimensional artificial tissue*." This definition removes any restriction of material degradation by biological activity and expands the scope to include 3D tissue fabrication.

Biomaterial Degradation and Tissue Engineering—Many materials used in tissue engineering, particularly for soft tissue applications (for example, in the cardiovascularsystem), are biodegradable. Biodegradable materials are those materials that undergo a significant change in chemical structure under specific conditions, and these changes result in a loss of physical and mechanical properties (58–62). The rationale for using a biodegradable material for tissue engineering is the ability to provide a temporary scaffold to support tissue fabrication and remodeling. During the early stages of 3D tissue formation, the biodegradable scaffold acts as the extracellular matrix, providing structural and functional support during tissue formation (Figure 3.4).

As the 3D tissue develops and matures, extracellular matrix components are generated by cells, and the temporary scaffold is no longer required. At this stage, it is desirable for the biomaterial to be completely degraded (Figure 3.4). In order for a biodegradable material to be suitable for tissue engineering, two requirements need to be met. First, the degradation kinetics needs to be tunable, so that the rate of biomaterial degradation is balanced by the rate of extracellular matrix formation by cells. Second, the degradation products need to be nontoxic to the cells, and if

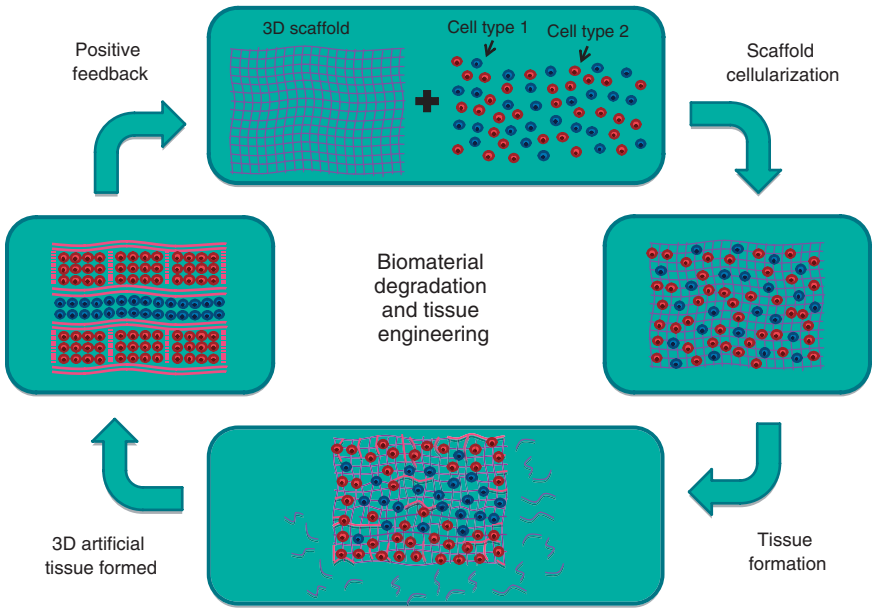

Figure 3.4 Biomaterial Biodegradation—During initial stages of tissue fabrication, the 3D scaffold provides structural support. Gradually, cells produce their own extracellular matrix, and as this process continues, the cells are less dependent on the scaffold for structural support; scaffold degradation can be initiated. The process of scaffold degradation and extracellular matrix production continues until the entire biomaterial has been degraded and replaced by extracellular matrix produced by the cells. This results in the formation of 3D artificial tissue.

the material is used *in vivo*, the degradation products need to be safely eliminated from the body.

Mode of Action for Biomaterial Degradation—Material degradation can occur as a result of physical processes, chemical reactions, or biological activity. Physical degradation refers to material degradation, which occurs in response to physical processes like heat and does not require any chemical reaction or biological intervention (63). Chemical degradation occurs in response to a chemical reaction like oxidation and results in an organized change in the 3D architecture of the biomaterial (64–66). Chemical processes that lead to material degradation include hydrolysis (mediated by water) or enzymatic activity. In addition to the mode of action, the chemical degradation of materials varies based on the mechanism of degradation, cleave of crosslinks between polymer chains, transformation/cleavage of side chains, and/or cleave of backbone linkages between polymer repeating units. Degradation due to biological activity is caused by enzymatic reactions involving specific protein interactions and highly orchestrated sequences of events (67–70). Examples of material degradation by physical processes, chemical reactions, and biological processes are as follows:

- *Physical*—heat, wear, fracture and fatigue, impact fracture, creep, radioactivity, sorption, swelling, softening, dissolution, mineralization, extraction, crystallization, decrystallization, stress cracking
- *Chemical*—aqueous corrosion, solvation by liquid metals, reaction with organic solvents, thermolysis, oxidation, solvolysis, photolysis, radiolysis, fracture-induced radical reactions
- *Biological*—enzymatic

Selection Criteria for Degradation Strategy—The choice of degradation strategy must be carefully selected to support fabrication of artificial tissue and must satisfy the following design requirements: 1) the rate of degradation should be tunable and user-defined on requirements of the specific application. The rate of degradation can vary from days to months, depending on application. 2) The mode of action of degradation should not be harmful to cells. While there are many options for material degradation, some of these will not be suitable for tissue engineering, as they can induce damage to the cells. Degradation strategies that involve fracture, fatigue and corrosion may not be suitable for tissue engineering studies. 3) The degradation products should not be harmful to cells. As polymers degrade, they are broken down into simpler monomer units; these monomer units should not damage the cells in any way. The degradation products will be affected by the composition of the polymer itself along with the degradation strategy employed.

3.7 BIOCOMPATIBILITY

Introduction—Under normal physiological conditions, the human body has a host of defense mechanisms that work in tandem to protect against a variety of treats from the environment. The foreign body response, complement activation, and thrombosis are examples of host defense systems that work to protect against foreign bodies in the environment. These systems are very sophisticated and remarkable in their ability to provide a host defense mechanism. These systems also come into play when a foreign body is implanted for therapeutic purposes, which can be in a medical device, a biomaterial, isolated cells during cell transplantation, or 3D artificial tissue (71). The human body considers these therapeutic agents to be foreign bodies and unleashes its defenses to limit the effect of these agents on human physiology. This reaction to therapeutic agents, in turn, limits the therapeutic benefit from the implanted agent and negates any intended beneficial effects. In order for any implanted biomaterial or artificial tissue to have a beneficial effect, it has to interface with the host immune system in a way that allows it to be accepted by the host. Functional integration needs to take place at the host-implant interface. In general terms, the property of an implanted agent to be accepted by the host immune system is referred to as biocompatibility.

Definition of Biocompatibility—As we have seen throughout this book, a specific definition that relates biocompatibility and tissue engineering does not exist.

While many definitions have been presented in the literature, a commonly utilized one is "*biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation*" (72). Unfortunately, this definition is very general and does little to relate or provide a specific interpretation of biocompatibility to tissue engineering. As we have done in several instances throughout this book, not by choice but rather by need, we will develop a working definition of biocompatibility as it relates to tissue engineering. Prior to providing a definition of biocompatibility, let us take a step back and revisit the tissue fabrication process. In tissue engineering, our objective is to fabricate 3D artificial tissue, which, upon implantation, serves to augment, repair, and/or restore lost tissue function. In order for artificial tissue to function in the host environment, it has to be accepted and tolerated by the host immune system, which includes a large number of defense mechanisms, including the foreign body reaction, complement system, and thrombosis. The argument that we have just presented will form the basis for biocompatibility as it applies to tissue engineering—"*The ability of 3-dimensional artificial tissue to be accepted by host defense mechanisms upon implantation, while maintaining functional capacity, is known as biocompatibility*." This definition focuses on tissue engineering and refers to the host defense mechanisms in their entirety, rather than specifying one particular mechanism. The definition also refers to the functional capacity of artificial tissue, which is necessary for the implanted tissue to serve as a therapeutic agent to recover and/or restore lost functionality of host tissue.

Biocompatibility and Tissue Engineering—From a tissue engineering or tissue fabrication standpoint, biocompatibility refers to the ability of the implanted tissue to be accepted by host defense mechanisms, which include the foreign body reaction, complement activation, and the coagulation pathway (67–70,72,73) (Figure 3.5).

In subsequent sections, we provide a brief description of these pathways and show how they function to protect against foreign pathogens. A similar response takes place when artificial tissue is implanted, and biocompatibility refers to the ability to design artificial tissue that minimizes these reactions.

Foreign Body Reaction—When a foreign body is implanted, it elicits the foreign body response which consists of the following steps (71,74–77): 1) injury, 2) acute inflammation, 3) chronic inflammation, 4) granulation tissue, 5) foreign body reaction, and 6) fibrosis.

Injury—In order to implant any biomaterial at a functional site, invasive procedures are often required, which result in perturbation of the host tissue, thereby leading to some degree of injury (71). Implantation of a biomaterial requires severing skin tissue, disturbing existing musculature, and excising vasculature, all of which lead to some form of tissue injury. In addition, the physical positioning of 3D artificial tissue at the site of injury requires physical contact with host tissue, particularly to secure the implanted tissue using surgical sutures. All steps in the implantation cascade can lead to tissue injury. In response to injury, a cascade of molecular events is triggered; this cascade begins with acute inflammation and can

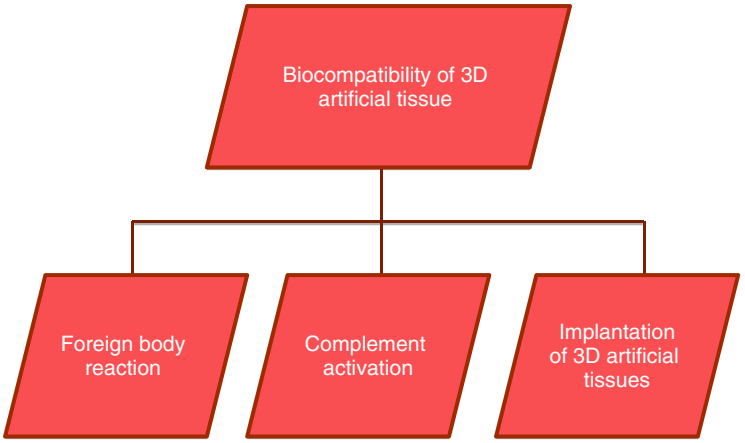

Figure 3.5 Biomaterial Biocompatibility—Biocompatibility refers to the ability of 3D artificial tissue to functionally interface with host defense mechanisms, which include foreign body reactions, complement systems, and coagulation pathways.

lead to fibrotic encapsulation of the implanted graft. While under physiological conditions, the tissue response to injury is designed to contain the injury, limit infection, and reduce the adverse effects on tissue function This response can reduce functionality of artificial tissue. Therefore, strategies need to be designed to minimize these effects. The host response to injury is dependent upon the extent of injury and the properties of the implanted biomaterial or artificial tissue.

Acute Inflammation—Inflammation is defined as the reaction of vascularized living tissue to local injury and is designed to contain, neutralize or dilute the injurious agent or process (71,78–80). Acute inflammation occurs within the first few hours or the first few days of tissue injury and is characterized by leukocyte accumulation at the site of injury. Neutrophils are generally the first cells to be found at the site of injury and are then replaced by monocytes, which later differentiate to form macrophages. The primary function of the neutrophils is to phagocytize microorganisms and foreign materials.

Chronic Inflammation—Chronic inflammation occurs over time, with a time horizon ranging from weeks to years. Although multiple cell types mediate the chronic inflammatory response, macrophages are central due to the large number of compounds that can be secreted by these cells, including neutral proteases, chemotactic factors, arachidonic acid metabolites, reactive oxygen metabolites, complement components, coagulation factors, growth-promoting factors, and cytokines (71,81–83). Some of the tissue responses associated with chronic inflammation include the proliferation of fibroblasts, vascularization of the injured tissue, and regeneration of epithelial cells.

Granulation Tissue—The time frame for the onset of granulation tissue depends on the extent of injury, and upon biomaterial implantation, it can be seen within 3–5 days. Granulation tissue consists of connective tissue and cells like fibroblasts that

are known to produce extracellular matrix components (71,84). Granulation tissue is also vascularized and contains vascular cells like endothelial cells. Due to the presence of vasculature, granulation tissue has a pinkish color. Granulation tissue serves as a protective barrier, preventing the infiltration of pathogens at the site of injury.

Foreign Body Reaction—Foreign body giant cells are the products of macrophage fusion and are a hallmark of the foreign body reaction. When macrophages encounter a foreign object too large to be phagocytized, such as an implant, it is thought that the macrophages experience "frustrated phagocytosis." They fuse to form larger foreign body giant cells composed of up to a few dozen individual macrophages (71,85,86). Giant cells secrete degradative agents such as superoxides and free radicals, causing localized damage to implants and other foreign bodies. Currently, little is known of the role of foreign body giant cells and it is hard to say whether they are "more or less inflammatory" than a collection of macrophages. Macrophages and foreign body giant cells tend to remain at the surface of an implant for the duration of its residence.

Fibrosis—Fibrosis is the final stage of the host response to a foreign material, like an implanted biomaterial graft or artificial tissue. Fibrosis involves encapsulating the biomaterial with fibrous tissue, which is about 50–200 μm thick and consists of an abundance of collagen (71,87). The primary rationale for the fibrotic response is to separate the foreign body from the host and minimize any adverse effects to the host.

Complement Activation—The complement system is a part of our immune system and provides a defense mechanism against a host of pathogens that we are constantly exposed to, including bacteria, viruses, and fungi (88–90). While the complement system can act alone and serve to recognize and destroy pathogens that would otherwise have adverse effects on health, it can also serve as an intermediary to tag pathogens for phagocytosis; the latter process is known as opsonization. There is more than one molecular pathway by which our complement system works, one of which is the classical pathway. There is a cascade of events that takes place in the classical pathway. The trigger for initiation of the classical pathway is a foreign antigen on a microbe, followed by binding of host antibody to the foreign antigen (88–90). Formation of this antigen-antibody complex is followed by activation of the protein C1, which then leads to cleavage of the protein C4 to form C4a and C4b, with C4b binding to the surface of the pathogen. This is followed by cleavage of C2 to form C2a and C2b, with C2a binding to C4b on the pathogen surface and C2b acting on C3 to form C3b and C3a. Formation of C3b can have one of two possible outcomes. First, C3b can bind to cell surface receptors on macrophages, promoting opsonization. Second, C3b can bind to the C4b and C2a complex, leading to formation of a C5 convertase; this in turn can lead to formation of C5b, which results in the formation of a membrane attack complex (MAC) (91,92). The MAC is formed in the cell membrane of pathogens, acting as a transmembrane channel leading to disruption of cell activity and function and eventually leading to cell death. Therefore, the classical pathway of the complement system can lead to direct destruction of pathogens by formation of the MAC or can act as an intermediary

by formation of C3b to promote recognition and binding by macrophages followed by phagocytosis.

Platelet Activation and Blood Coagulation—Thrombosis is the process by which platelets, in combination with fibrin, form a blood clot designed to plug injured blood vessels, thereby limiting the loss of blood and containing the site of injury (93–95). This is a very important hemostasis mechanism during normal function and serves to regulate blood loss after injury. When a blood vessel is damaged or injured, collagen in the endothelium layer is exposed and serves as a trigger for platelet adhesion and activation. Activation of platelets leads to a cascade of signaling events culminating in the conversion of prothombin to thrombin, which then acts to convert fibrinogen to fibrin and promotes formation of a blood clot that plugs the injured vessel.

3.8 BIOMIMETIC BIOMATERIAL

Introduction—Naturally occurring biomaterials like collagen have functional sites that support cellular interactions, leading to enhanced cell-material interactions. On the other hand, synthetic polymers like PLA and PGA do not possess functional interaction sites for cells; these materials can be modified by introducing functional sites within the polymeric structure. Proteins and peptides are commonly used as linking agents to modify the biomaterial properties, and enzymes, antibodies, antibiotics, and cell adhesion molecules are commonly employed. While covalent bonding has been extensively used to link functional molecules to polymer chains, physical methods like adsorption or electrostatic attractions have also been employed. Biomimetic activity refers to the "cell-friendliness" of the biomaterial, and in order for a biomaterial to be cell-friendly, it must possess functional binding sites for cell surface integrins (96–107).

Definition of Biomimetic Biomaterial—A two-part definition of biomimetic biomaterials has been provided in a recent article (108):

1. *The development of biomaterials for tissue engineering applications has recently focused on the design of biomimetic materials that are able to interact with surrounding tissues by biomolecular recognition.*
2. *The design of biomimetic materials is an attempt to make the materials such that they are capable of eliciting specific cellular responses and directing new tissue formation mediated by specific interactions, which can be manipulated by altering design parameters; instead of by non-specifically adsorbed ECM proteins.*

In the article, the two-part definition was used as a discussion point to describe the nature of biomimetic biomaterials, rather than using the statements as a definition. However, the explanation provided in the article fits into the role of defining biomimetic biomaterials and has therefore been used in this manner and will be retained in this book. Simply stated, a biomimetic biomaterial is one that elicits

specific cell-matrix interactions that guide intracellular signaling pathways thereby regulating cellular and molecular responses; this in turn dictates 3D tissue formation and function. Another way to state this is: biomimetic biomaterials are designed to resemble mammalian extracellular matrix, which itself functionally interacts with cells to support 3D remodeling and dynamic tissue formation.

Fabrication of Biomimetic Biomaterials—There is an abundance of examples of biomimetic biomaterials in nature and almost all, if not all, components of the extracellular matrix are considered to be biomimetic biomaterials. The objective of tissue engineering is to replicate biomimetic properties of naturally occurring biomaterials to support fabrication of functional 3D artificial tissue. *How can this be achieved?* Let us take a closer look at naturally occurring biomaterials using a commonly utilized one in tissue engineering, fibronectin (109–113). Fibronectin is a large extracellular glycoprotein with a very complex 3D architecture; however, a sequence of three amino acids, Arg-Gly-Asp (RGD) is known to be critical for cell adhesion (Figure 3.6). Cells interface with the RGD sequence on fibronectin through specific cell surface integrins, $\alpha_5\beta_1$. The $\alpha_5\beta_1$-RGD interaction is an example of a specific cell-matrix interaction and is one of the factors responsible

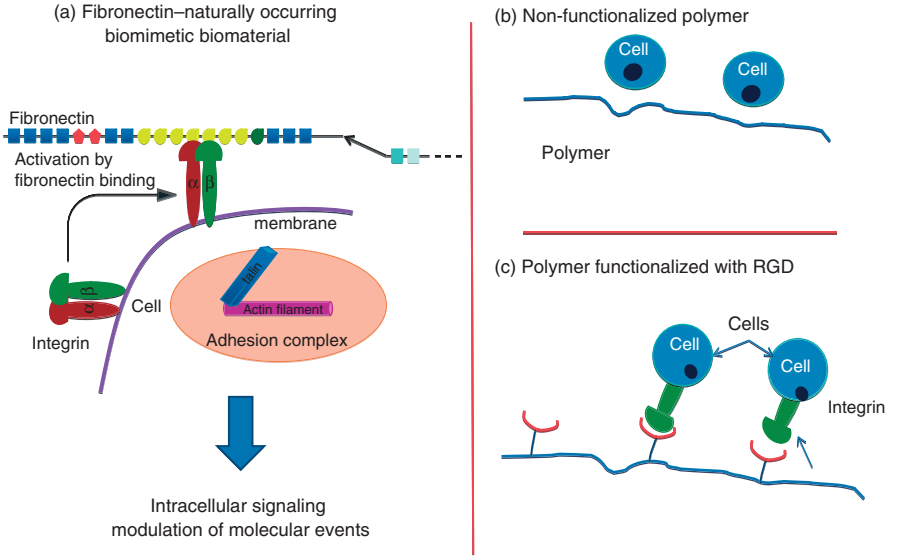

Figure 3.6 Biomimetic Biomaterials—(a) Fibronectin (FN)—Naturally Occurring biomimetic biomaterial—FN has a specific sequence of three amino acids that bind to specific alpha-5 beta-1 integrins on the surface of cells. Binding of alpha-5 beta-1 to the RGD site on FN leads to a cascade of intracellular signaling events that result in cellular and molecular changes. (b) Non-Functionalized Polymer—Cells passively interact with a polymer that does not possess any functional sites to support cell-matrix interaction. (c) Polymer Functionalized with RGD—A RGD sequence can be linked to a polymer that can then support functional interaction with alpha5beta1 integrins on the surface of cells.

for the biomimetic activity of fibronectin; this in turn leads to specific intracellular signaling events that regulate molecular and cellular behavior.

How do we use this information in the design of synthetic biomimetic biomaterials? Biomaterials have been synthesized by functionalizing the RGD sequence, chemically linking this sequence to the polymer backbone, thereby providing a binding site for cells that express the $\alpha_5\beta_1$ integrin. Linking the RGD sequence to polymer backbones has shown to significantly increase cell adhesion and functionality of 3D artificial tissue.

3.9 CLASSIFICATION OF BIOMATERIALS

Several schemes have been used to classify biomaterials, and the most common ones are discussed here. Biomaterials are frequently classified based on source (natural and synthetic), based on degradation (biodegradable and non-biodegradable), and based on interatomic bonding forces (metals, polymers, and ceramics) (114–116), as seen in Figure 3.7. These terms appear frequently throughout this chapter and the remainder of this book; therefore, we take a moment to discuss their meaning and relevance to tissue engineering.

Natural versus Synthetic Materials—This classification scheme is based on the source of the material. It is very simple and self-explanatory, and its relevance to tissue engineering is paramount; therefore, frequent discussion of this topic can be found in many scientific forums. Simply stated, naturally derived materials are obtained from natural sources, while synthetic materials are synthesized in the laboratory. One common example of a naturally occurring material is collagen, which is frequently extracted from rat tails and used extensively for tissue engineering and other medical applications. As example of synthetic material used for tissue engineering and other medical applications is the aliphatic polymer poly(glycolic acid), PGA. PGA has found extensive application in resorbable sutures and as scaffolding material to support the fabrication of 3-dimensional tissue constructs.

There are clear advantages of natural or synthetic materials in any given tissue engineering application. Natural materials have anatomically matched 3-dimensional architecture and are biologically active, thereby supporting functional interaction with isolated cells. However, the main disadvantage of natural materials is batch variability inherently due to differences in isolation efficiency. Synthetic materials have the advantage of reproducibility, as synthesis is tightly regulated, and tenability, as materials of different properties and functionality can be fabricated. However, the main disadvantage of synthetic materials is the lack of biological functionality, as many synthetic materials do not have functional binding sites for isolated cells.

Degradable versus Nondegradable—The degradation kinetics of a material define the rate at which the material disintegrates or loses structural stability as a function of time. On the surface, material degradation may appear to be a nondesirable material property, as loss of structural integrity can lead to catastrophic effects. This is indeed the case for numerous medical applications, as

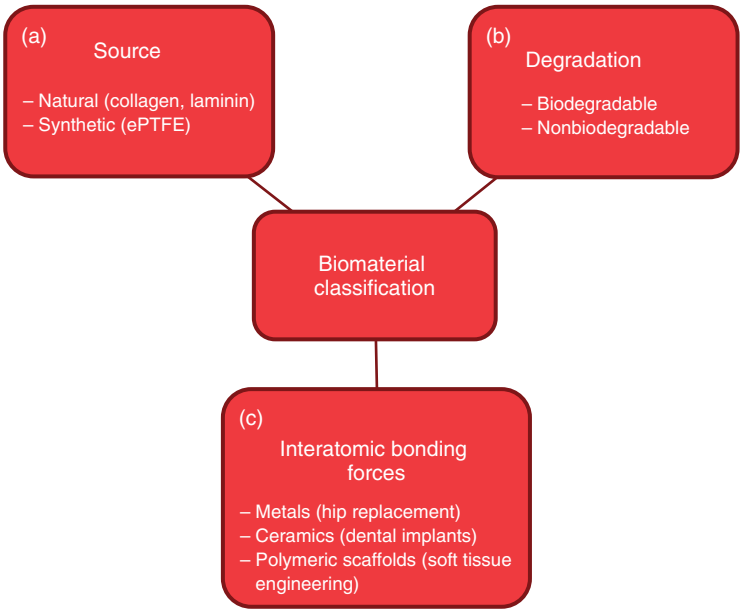

Figure 3.7 Classification of Biomaterials—(a) Natural versus Synthetic—Natural biomaterials are found in nature and are extracted from mammalian tissue to support fabrication of 3D artificial tissue. Examples of naturally occurring biomaterials include collagen, fibronectin, and laminin. Synthetic biomaterials are synthesized in the laboratory under controlled reaction conditions. (b) Degradable versus Nondegradable—Degradable materials have a measurable change in weight over a specific time frame while nondegradable materials maintain a constant weight over any given time frame. (c) Metals, Ceramics, or Polymers—Biomaterials are classified as metals, ceramics, or polymers, each of which has very different applications in tissue engineering.

in the case of knee and hip replacements, which are often fabricated using metallic components like stainless steel, and in which longevity of the implant is a critical functional determinant. Metallic materials fall into the category of nondegradable materials where long-term structural stability is essential for function. In the case of tissue engineering, a biodegradable material is molded into a scaffold and then populated with isolated cells. The cells use the scaffold as a temporary support matrix, and during the culture period, extracellular matrix components are fabricated by cells. During the tissue fabrication process, the material degrades and is replaced by extracellular matrix fabricated by the cells. In this case, material degradation is an important property required to support 3D tissue formation. The degradation kinetics of scaffolds is an important material property and is the focus of many tissue engineering studies.

Metals versus Ceramics versus Polymers—Metals, ceramics, and polymers represent a large group of materials that are used frequently for medical and tissue engineering applications. Some examples of metals used as implants include titanium

and its alloys, and stainless steel. These materials have been used in hip and knee replacement implants and in bone applications, including bone plates, screws, pins, and rods. Titanium screws or posts are also used in dental implants to anchor prosthetic teeth. The titanium implant is placed in the bone socket of the missing tooth, and within a couple of weeks, the jawbone and implant form a functional bond. An abutment is then attached to the titanium implant as an intermediary between the implant and prosthetic tooth. Artificial teeth, also known as dentures, are fabricated from acrylic resins like PMMA—polymethyl methacrylate. In addition to PMMA, ceramics like porcelain are also used in dental implants and have also found applications in hip and joint replacement implants. Metals and ceramics have traditionally been used for hard tissue applications like orthopedic and dental, and most materials in these categories are known to be nondegradable. Metals are naturally occurring, while ceramics are synthetic. Polymers have been used extensively for soft tissue engineering and can be derived from natural sources or can be synthesized in the laboratory; degradable and nondegradable polymers are both used in tissue engineering, although the former are more common.

3.10 BIOMATERIAL PLATFORMS

One of the early decisions that need to be made when considering the use of biomaterials for any tissue engineering application is the biomaterial platform to be implemented. There are four platforms that have been widely used for tissue engineering applications: polymeric scaffolds, biodegradable hydrogels, acellular matrices, and self-organization strategies (Figure 3.8). In this section, we provide an overview of these platforms, describe their properties, identify advantages/disadvantages of each, and provide examples of tissue engineering applications where each platform has been successfully utilized.

Decellularized Matrices—This strategy is based on the utilization of naturally occurring extracellular matrix as the scaffolding material for 3D tissue formation (117–122). Tissue specimens are obtained from cadaveric or xenogeneic sources, and cells are completely removed using one of several potential strategies. Removal of cellular components from tissue specimens is known as decellularization, and the material that is obtained after removal of the cells is known as an acellular scaffold. Removal of cells does not distort the extracellular matrix components in any significant manner. The composition and 3-dimensional organization of individual components of the extracellular matrix remains intact. As a result, the acellular scaffold retains the physiological architecture of the extracellular matrix and therefore can serve as a scaffold for tissue engineering. In addition, removal of cells reduces the immunogenicity of the scaffold, as the immunogenic response is primarily a cellular response. Some examples of tissue engineering applications where decellularized matrices have been used include heart valves, blood vessels, skeletal muscle, skin, nerves, tendons, and ligaments.

Several approaches have been implemented to decellularize tissue specimens. These approaches have been categorized as physical, chemical, and enzymatic, with

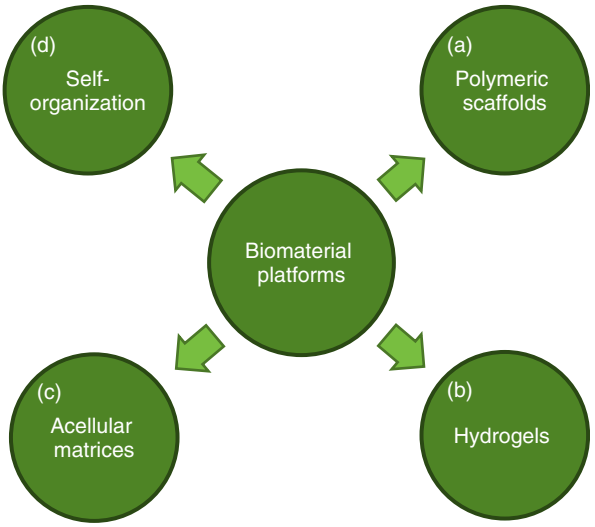

Figure 3.8 Biomaterial Platforms—(a) Polymeric Scaffolds—Polymerization of individual monomer units leads to formation of long chain structures known as polymers. Polymer chains can be fabricated into 3D scaffolds that can then be used to support tissue fabrication. (b) Hydrogels—Materials that have a high water content are known as hydrogels and are used extensively in tissue engineering. Many materials can be formed into 3D hydrogels, including collagen, chitosan, and fibrinogen. (c) Acellular Matrices—Mammalian tissue is subjected to a decellularization protocol that completely removes all cellular components leaving an intact ECM that can be used to support 3D tissue fabrication. (d) Self-Organization—Refers to scaffold-free technology to fabricate 3D artificial tissue; extracellular matrix is produced by the cells and used to support the fabrication of 3D artificial tissue.

some protocols based on a combination of these three broad classifications. Decellularization methods that rely on physical techniques for cell removal include the use of repeated freeze thaw cycles or the use of pressure, sonication, and/or mechanical agitation, all of which function to disrupt the cell membrane, with subsequent washing required to remove cellular components. The primary advantage of physical treatment methods is the ease of implementation. However, physical treatment methods are not always sufficient for complete removal of cellular components and can lead to damage of the extracellular matrix.

A wide variety of chemical compounds have been used to decellularize tissue specimens. Alkalinic and acidic solutions, detergents like Triton X-100 and sodium dodecyl sulfate (SDS), hypertonic/hypertonic solutions, and chelating agents like ethylene glycol tetraacetic acid (EGTA) or ethylenediaminetetraacetic acid (EDTA) have all been used. The mechanism by which these compounds act is very different. Alkalinic/acidic solutions are known to solubilize cytoplasmic components of the cells, detergents can disrupt the cell membrane and denature proteins, and hypertonic/hypertonic solutions result in changes in osmotic gradients causing cells to

swell and eventually burst. In addition, chelating agents like EGTA/EDTA disrupt cellular interactions with other cells and the extracellular matrix by binding divalent ions like calcium. Chemical methods have been very efficient in the removal of cells with limited disruption of the extracellular matrix.

Enzymatic methods have commonly employed the use of trypsin, a protein known to cleave peptide chains at the carboxyl side of lysine and arginine by a process known as trypsin proteolysis. In addition, endonucleases and exonucleases, which degrade DNA and RNA, have been used in decellularization protocols. While the efficiency of cell removal is high with enzymatic methods, degradation of extracellular matrix can occur.

Hydrogels—The term hydrogel is composed of "hydro" (water) and "gel," and refers to aqueous (water-containing) gels; to be more precise, it refers to polymer networks that are insoluble in water; they swell to an equilibrium volume but retain their shapes. Specifically, the water content of hydrogels is greater than 30% on a weight basis. Polymers that form hydrogels are known to have specific chemical residues within their 3-dimensional lattice structure, some of which include hydroxylic (−OH), carboxylic (−COOH), amidic (−CONH−), primary amidic ($-CONH_2$), and sulfonic ($-SO_3H$) groups.

Hydrogels are classified using several different schemes, although two are commonly seen in tissue engineering: natural or synthetic and biodegradable or nonbiodegradable. Natural occurring hydrogels are based on polymers that are found in nature. These polymers include agarose, alginate, chitosan, collagen, fibrin, gelatin, and hyaluronic acid (123–128). Collagen in particular has been used extensively in tissue engineering due to its prominent role in modulating mammalian physiology. Synthetic hydrogels are formed using polymers that are synthesized in the laboratory and include compounds like poly(ethyleneoxide) (PEO), poly(vinyl alcohol) (PVA), poly(acrylicacid) (PAA), poly(propylene furmarate-co-ethylene glycol) (P(PF-co-EG)). Some hydrogels retain structural stability over time and are known as nondegradable, while other hydrogels degrade over time, result in loss of structurally stability, and are known as degradable.

The interaction of water with the polymer chains plays an integral role in hydrogel formation and subsequent functionality. The water content of hydrogels is divided into two components: the total bound water and free or bulk water. The total bound water is the amount of water that is functionally interacting with hydrophilic and hydrophobic groups in the polymer lattice. The free or bulk water is any additional amount of water present within the hydrogel lattice occupying spaces between polymer chains.

Hydrogels have been used extensively for tissue engineering, for example in the fabrication of blood vessels, heart muscle, tri-leaflet heart valves, and skeletal muscle (123–128). The advantages of hydrogels include the aqueous environment that spatially separates polymer fibers, thereby increasing the material porosity and supporting nutrient delivery throughout the 3D structure. The main disadvantage is the lack of mechanical strength due to the high porosity and water content, making hydrogels difficult to use for load bearing applications.

Polymeric Scaffolds—Polymers have been used extensively for tissue engineering applications. Before proceeding to discuss the role of polymers in tissue engineering, we introduce the definition of a polymer in terms of its constitutional units and macromolecules, followed by the definition of polymerization in terms of converting monomer molecules (129).

- A constitutional unit is an atom or group of atoms (with pendant atoms or groups, if any) comprising a part of the essential structure of a macromolecule.
- A macromolecule is a molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetitions of units derived, actually or conceptually, from molecules of low relative molecular mass.
- A polymer is a substance composed of macromolecules.
- Polymerization is the process of converting a monomer or a mixture of monomers into a polymer.
- A monomer molecule is a molecule that can undergo polymerization, thereby contributing constitutional units to the essential structure of a macromolecule.
- A monomer is a substance composed of monomer molecules.

While it is important to use the right terminology to define polymers, the definition presented can be conceptually simplified, and polymers can be viewed as molecules of a high molecular weight that are composed of repeating monomer units.

Some of the earliest work in the field of tissue engineering was based on polymeric scaffolds. As discussed in Chapter 1, 3D liver constructs were fabricated by culturing primary hepatocytes on three different polymeric scaffolds. Since this initial application, utilization of polymeric scaffolds has increased considerably to support different tissue engineering applications, including applications in the cardiovascular space. Although there are several reasons for this exponential growth, the ability to synthesize polymers with different properties makes it possible to customize materials to suit different tissue engineering applications. The bioactivity, degradation kinetics, mechanical stretch, immunogenicity, and surface properties can be controlled by varying the polymer composition and processing conditions. This provides a very high degree of freedom and has been one of the reasons for such a high degree of interest in polymers (130–136).

Polymers used for tissue engineering applications are conveniently classified based on their source as natural or synthetic polymers. Natural polymers are derived from nature, and some common examples include the polysaccharides alginic acid, hyaluronic acid, chitin, chitosan, and collagen. The primary advantage of using naturally occurring polymers is the physiological role these polymers play in mammalian function. For example, collagen is the most abundant protein in humans and provides structural support for tissue formation and maturation; therefore, the rationale for using collagen in tissue engineering is to mimic its anatomical role. However, the main disadvantage of using natural polymers is the

high degree of variability between independent batches, thereby making process control and scale-up challenging. Synthetic polymers are synthesized in the laboratory by polymerization of monomer units, some examples of which include poly(methyl methacrylate), poly(ethylene terephthalate), poly(dimethylsiloxane), poly(tetrafluoroethylene), polyethylene, and polyurethane. The primary advantage of using synthetic polymers is the reproducibility by which the polymers can be synthesized in the laboratory and the ability to tune material properties, allowing functional modifications to match the requirements for any given tissue engineering application. However, synthetic polymers do have some disadvantages, as they are not anatomically matched and may not exhibit biomimetic activity and may be rejected by the host upon implantation.

Self-Organization Strategies—Self-organization is prevalent in biological systems; it involves the physical interaction of molecules in a steady-state structure (137–142). In a broad sense, self-organization can be viewed as a process that occurs in the absence of any constraining conditions, thereby providing a greater degree of freedom and flexibility. Prior to evaluating the role of self-organization strategies in the development of 3D tissue constructs, we provide a working definition, along with specific requirements that need to be satisfied in order for a system to be considered self-organized. Self-organization in the context of cell biology can be defined as the capacity of a macromolecular complex or organelle to determine its own structure based on functional interactions of its components. In a self-organizing system, the interactions of its molecular parts determine its architectural and functional features. The processes that occur within a self-organized structure are not underpinned by a rigid architectural framework; rather, the components of the structure define its organization. For self-organization to act on macroscopic cellular structures, three requirements must be fulfilled: the cellular structure must be dynamic, material must be continuously exchanged, and an overall stable configuration must be generated from dynamic components.

Self-organization strategies are focused on the assembly of structures based on internal dynamics without significant external control. It is important to explore this phenomenon from a biomaterials and tissue engineering standpoint. Thus far, we have evaluated the role of acellular matrices, hydrogels, and polymeric scaffolds in the fabrication of 3D tissue constructs. The concept of self-organization is somewhat tangential and one may question its relevance to tissue engineering. Tissue engineering strategies are focused on the fabrication of 3D tissue, often by culturing cells within a support matrix; this process was introduced in Chapter 1 and is common in tissue engineering studies. However, there is some interest in exploring the ability of isolated cells to self-organize into functional tissue constructs without the need of external scaffolding. This viewpoint is based on the assumption that cells have all the information required for tissue formation; isolated cells interact with other biological components, like other cells or the extracellular matrix, in order to support tissue formation under normal mammalian function.

Based on the requirements put forth for self-organization to occur, cells must be dynamic, material must be exchanged, and an overall stable configuration must be the end result of the process. These requirements are satisfied by isolated cells, and

therefore, these cells can be said to have the properties required to participate in a self-organization process. Using self-organization strategies, isolated cells participate in 3D tissue formation by interacting with other cells and extracellular matrices that are generated by the cells; the process of tissue formation is independent of any external scaffolding material. The ability to form 3D tissue without the need for any external scaffolding is central to the self-organization process.

The main advantage of self-organization strategies is the ability to minimize external constraints and therefore allowing the cells to govern the process of 3D tissue formation. With less constraints and a high degree of freedom, the probability of fabricating functionally and anatomically matched constructs increases. Culturing isolated cells on a prefabricated scaffold defines boundaries for tissue formation and growth, while self-organization processes remove or reduce these boundaries and allow cells to determine the best course of action. While self-organization strategies have advantages, they are also faced with challenges. Self-organization strategies can require long culture times and can be difficult processes to control.

3.11 SMART MATERIALS

Tissue engineering has traditionally focused on the use of scaffolds to support 3D tissue formation. The role of the scaffold has been to provide temporary structural support for cells. During tissue formation, extracellular matrix components are generated by cells, and as this happens, the temporary scaffold is degraded and replaced by newly formed extracellular matrix. The result is a 3D tissue construct that only consists of biological components. The first generation of scaffolds used for tissue fabrication was designed to provide passive support during tissue fabrication. The next generation of biomaterials was designed to be "cell-friendly" or biomimetic; these materials were fabricated by incorporation of biological activity within the scaffold, which provided functional attachment sites for cells (143–150). Covalent linking of the amino acid sequence RGD is one example used extensively to support fabrication of 3D tissue constructs. The most recent generation of biomaterials has been designed to respond to changes in the cellular environment; these materials, known as smart materials, are receptive to changes in the physiological environment and are adaptive to changes in the degree of tissue maturation (143–150).

We can illustrate the concept of smart biomaterials with one specific hypothetical example. In this case, the biomaterial is fabricated to deliver a specific target to mammalian tissue; the smart biomaterial is fabricated with specifically targeted cell attachment sites and targets for cleavage by changes in physiological environment, all linked to a polymer backbone containing internalized growth factors. The objective is to utilize the cell attachment site to deliver biomaterials to specific targets, while changes in the physiological state of cells promote the release of growth factors in the local environment. Although this concept is at an early stage of development, there have been several examples of the development of smart materials using several different stimuli like compression, oxidation state, pH, and MMP cleavage activity.

In one example, VEGF was embedded within a 3D alginate gel, and the rate of release was regulated by application of compressive loads (using a mechanical testing system), both during *in vitro* culture and upon subcutaneous implantation into the dorsal region of severe combined immunodeficient (SCID) mice (151). Application of compressive load resulted in an increase in the rate of release of VEGF (determined by a radioactive tracer) during *in vitro* culture; during *in vivo* culture, this led to an increase in neovascularization, as determined by vessel count. Compressive loads are commonly observed during normal mammalian bone function, and using this stimulus to regulate the functionality of biomaterials provides an excellent platform for novel tissue engineering therapies for development of bone grafts.

In another example, changes in oxidation state were used to modify material properties, leading to release of embedded biomolecules. A tri-block copolymer (ABA) was fabricated with the A block consisting of hydrophilic poly(ethylene glycol) and B block consisting of hydrophobic poly(propylene sulfide) (PPS) (152). Upon exposure of the ABA copolymer to an oxidative environment, the sulfide moieties of the PPS were oxidized to sulfoxides and then to sulfones, which changed the properties of the PPS from hydrophobic to hydrophilic. This resulted in destabilization of the copolymer material, and as a result, embedded biomolecules were released within the culture environment (153,154).

A similar concept has been developed by utilization of changes in pH upon cellular endocytosis of biomaterials (155). A polymer was designed by conjugating a biomolecule to a PEG copolymer using disulphide bonds and then forming a complex between the PEG copolymer (conjugated with a biomolecule) to a backbone or carrier polymer using pH-sensitive acetyl linkers. When the biomaterial is implanted, it is internalized within the cell endosome via endocytosis. The pH within the endosome is acidic; this acidity provides a mechanism to cleave pH-sensitive acetyl linkers, thereby separating the PEG copolymer from the carrier polymer (155). The biomolecule that is bound to the PEG copolymer is separated from the PEG copolymer and released from the endosome to the cytoplasm. In the cytoplasm, the biomolecule can perform its biological function.

The ability of matrix metalloproteinases (MMPs) to recognize and cleave specific amino acid sequences makes them a suitable mechanism for functionality of smart biomaterials. In one example, a polymer was synthesized by cross-linking PEG copolymers with the peptide gly-pro-gln-gly-lle-trp-gly-gln, a substrate which contains a cleavage site for MMP-2 (156). The polymer was embedded with VEGF and used to support 2D culture of endothelial cells. MMP-2- mediated release of VEGF during 2D culture was identified using a fluorescent tag; the release resulted in an increase in the rate of endothelial cell proliferation.

3.12 THE DYNAMIC EXTRACELLULAR MATRIX

Introduction—Mammalian tissue consists of two components—cells and the extracellular matrix. Cells provide functionality, while the extracellular matrix

provides structural support. In the case of heart muscle tissue, cardiac myocytes are the functional cells, and cytoskeletal proteins within the myocytes generate contractile force in response to elevated intracellular intracellular calcium; this in turn results in pumping capacity of the heart. Heart muscle tissue also contains the extracellular matrix, which anchors cardiac myocytes and provides mechanical support during continuous contractions of the heart. The extracellular matrix has numerous functions and plays a critical role in tissue formation and function. In terms of tissue engineering, tissue fabrication technology consists of coupling functional cells with biomaterials, and the purpose of the biomaterial is to mimic functionality of the extracellular matrix. It is important to gain an appreciation of the composition, organization, and function of the extracellular matrix and the way in which extracellular components interact with cells to support tissue formation and function. In this section, we will look at the role of the ECM in the formation and function of mammalian tissue.

Components of the ECM—The extracellular matrix consists of proteins, glycoproteins, glycosaminoglycans (GAGs), and proteoglycans (157,158). Some examples of proteins in the extracellular matrix are collagen, laminin, fibronectin, and elastin, each of which has very distinct functions within the tissue, including mechanical and tensile properties and binding specificity for cells. GAGs are long chain polysaccharides, some examples of which are chondroitin sulfates, dermatan sulfates, heparan sulfates, heparin, keratan sulfates, and hyaluronic acid. Glycoproteins are proteins that are covalently linked to a carbohydrate; they serve many functions, including stabilizing protein molecules. Proteoglycans are glycosylated proteins, which mean that proteins are conjugated to specific types of GAGs; examples of proteoglycans based on the GAG chondroitin sulfate are decorin and versican. In mammalian tissue, every component of the extracellular matrix has a specific role in providing structural support or functional interaction with cells. The composition of ECM components varies from one tissue to another and changes during development and in response to change in the physiological or pathological state of the tissue. Hence, the ECM is considered to be dynamic in nature as it constantly changes in composition in response to its environment.

Functions of the ECM—The ECM has many diverse functions, ranging from mechanical support for tissue formation and function to regulation of cell behavior by specific cell matrix interaction. Functions of the ECM include (157,158): 1) *Structural integrity for 3D tissue*—The ECM provides scaffolding to confer strength to the tissue, and mechanical properties of tissue are a direct result of properties of ECM components. 2) *Attachment sites for cells*—Extracellular matrix proteins like fibronectin, laminin, and collagen have specific binding sites for cell surface integrins. The binding of cell surface integrins with ECM proteins is known as cell-matrix interaction and is responsible for initiating a cascade of intracellular signaling events that support cell proliferation, viability, and functionality. 3) *Binding site for growth factor*—The ECM binds to growth factors like BMPs and FGFs and acts as a reservoir of these factors. 4) *Serve as mechanosensitive receptors*—The ECM responds to changes in the biomechanical environment, including changes in the stretch profile, via mechanosensitive receptors. These

signals are then transmitted to the intracellular environment, initiating a cascade of signaling events that allow cells to modify specific molecular events to accommodate and adjust to the changes in external biomechanical environment.

ECM and Tissue Engineering—The ECM is a complex structure consisting of proteins and many other molecules. It supports 3D tissue fabrication, organization, and function. During the tissue fabrication process, the properties of the extracellular matrix are replicated by biomaterials. The composition of the ECM depends on the tissue system under consideration, and biomaterials are designed to match these tissue-specific properties. Once fabricated, the properties of the biomaterials are compared with those of the mammalian extracellular matrix by assessing the tensile properties, biocompatibility, and biomimetic function. The objective in tissue engineering is to fabricate biomaterials that closely replicate the properties of the mammalian extracellular matrix.

3.13 IDEALIZED BIOMATERIAL

An idealized biomaterial needs to replicate properties of the mammalian extracellular matrix both in terms of form and function. Many of the biomaterials currently under development are uniform in composition, which means the entire scaffold has the same properties. On the other hand, mammalian ECM consists of a diverse array of proteins and related compounds that support many functions of the ECM. Therefore, the first requirement of an idealized biomaterial is that it must have fiber composition that mimics the composition of mammalian ECM. We looked at several classification schemes earlier in this chapter and studied natural versus synthetic biomaterials. An idealized biomaterial will need to resolve problems associated with both naturally occurring (batch variability) and synthetic biomaterials (lack of biological activity) which necessities synthetic fabrication strategies leading to biologically active biomaterials. This means that an idealized biomaterial will need to replicate many, if not all, properties of mammalian ECM, and the process for material fabrication would need to be carefully optimized and regulated. This is depicted in Figure 3.9a.

The second component of our idealized biomaterial, shown in Figure 3.9b, is the ability to selectively bind specific cell types using specific binding sites or receptors for selectivity. As we have seen before, cell-matrix interactions are critical for tissue formation and function, and in the case of mammalian tissue, cell surface integrins recognize specific amino acid sequences on ECM proteins (like RGD for fibronectin). Cell surface integrins bind amino acid sequences, and this leads to a series of intracellular signaling events that regulate cellular and molecular events and tissue function. In our idealized biomaterial, we have specific binding sequences for selectively binding specific cell types. In Figure 3.9b, four binding sites are shown with specificity for functional cells, cells that provide structural support, cells for vasculature formation, and stem cells. If we relate this general scheme to heart muscle, the four cell types would be cardiac myocytes, fibroblasts, endothelial cells, and cardiac stem cells.

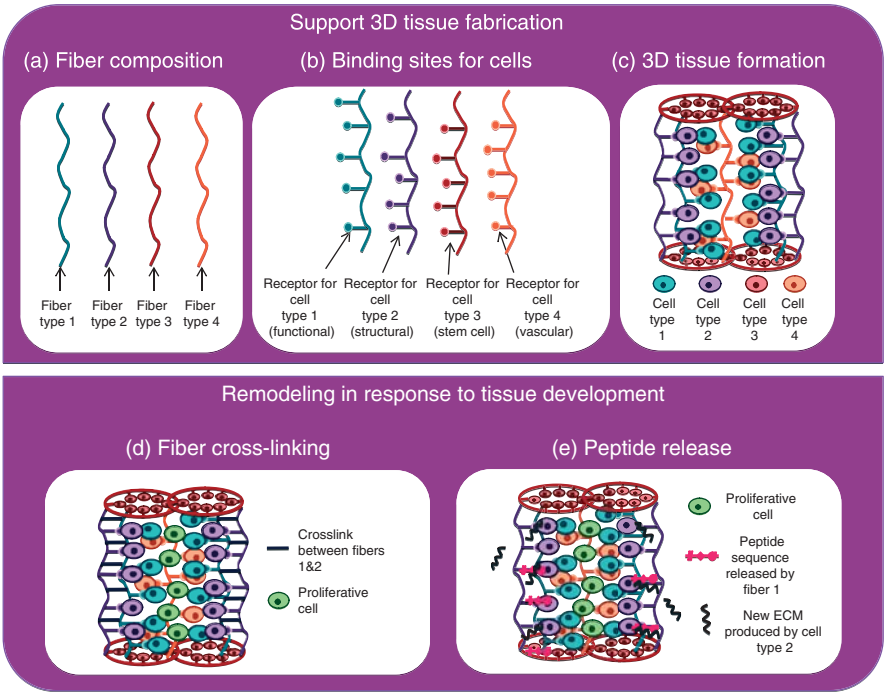

Figure 3.9 Idealized Biomaterial—(a) Fiber Composition—An idealized biomaterial will be composed of multiple fiber types, each with distinct properties. In the example presented here, the idealized biomaterial is shown to consist of 4 different fiber types. **(b) Binding Sites for Cells**—Individual fibers within the idealized biomaterial will have specific binding sites for cell surface integrins. **(c) 3D Tissue Formation**—Cells attach to specific binding sites on fibers of the idealized biomaterial to support 3D formation. **(d) Fiber Cross-Linking**—In response to cell proliferation, an idealized biomaterial will promote cross-linking between individual fibers to increase stability of the scaffold. **(e) Peptide Release**—In response to an increase in cell proliferation, peptide sequences can be cleaved from fibers within an idealized biomaterials. The peptide sequences act on structural cells, increasing the rate of ECM productions by these cells. This in turn has the effect of stabilizing the scaffold to support new tissue formation.

The third phase during the development of an idealized biomaterial is scaffold fabrication, as illustrated in Figure 3.9c. Important parameters in scaffold design and fabrication include fiber orientation and alignment, selective attachment of specific cell types to promote 3D tissue formation, and the ability to support functional organization of vasculature. As shown in Figure 3.9c, in order to support 3D tissue formation, the functional and structural cells are organized and aligned in a specific manner that allows cell-cell interaction. In the case of 3D heart muscle, functional coupling of cardiac myocytes is a prerequisite for tissue formation and can be achieved by aligning the fibers longitudinally; for another tissue system, a different organization of fibers may be optimal. Similarly, the organization of

structural cells like fibroblasts is an important determinant of tissue function, and in our idealized biomaterials, the structural cells have been organized in parallel with functional cells; again, the specific organization will vary from application to application. However, the idealized biomaterial will have the capacity to selectively position structural cells in any orientation relative to functional cells. Finally, specific fibers within the idealized biomaterial have been fabricated in a circular pattern to support adhesion of vascular cells (endothelial cells) to promote capillary formation.

Collectively, we have discussed the ability of our idealized biomaterial to support 3D tissue formation by replicating many of the properties of mammalian ECM. These properties include composition and alignment of proteins and other related molecules, presence of cell specific binding sites, and the ability to orient fibers of the idealized biomaterials in customized patterns to support localization of specific cell types. The second function of the idealized biomaterial is to support maturation and development of 3D artificial tissue and modulate material properties in response to the phenotypic state of the tissue.

During the development and maturation of artificial tissue, traditional materials play a passive role; they do not respond to changes in cell behavior and/or phenotype. Cell proliferation is an important prerequisite for development of 3D tissue, and during normal mammalian tissue growth, this is accompanied by changes in the ECM to support increase in cell number. Many of the materials used in tissue engineering do not respond to these changes in cell number. However, in an idealized case, biomaterials will have the capacity to respond to changes in tissue growth, particularly an increase in cell number, as shown in Figure 3.9d–e. In the first case, an increase in cell proliferation leads to conformational changes in ECM proteins, which in turn results in cross-bridge formation between neighboring protein molecules. This in turn leads to stabilization of the biomaterial, providing additional structural stability. In the second case, an increase in cell proliferation leads to cleavage of a specific amino acid sequence from the biomaterial fibers. This amino acid sequence acts on structural cells, stimulating production of new extracellular matrix components. The newly formed ECM integrates with existing fibers to strengthen, stabilize, and expand the scaffold; this integration in turn serves to support the increase in cell number and tissue development and maturation.

In the two examples, the biomaterial responds to changes in the physiological state of cells. During early stages of tissue fabrication, the idealized biomaterial serves to support attachment of cells. This attachment leads to the formation of artificial 3D tissue. During later stages of tissue development, the idealized biomaterial responds to changes in the cellular environment to accommodate tissue maturation.

SUMMARY

Current State of the Art—Biomaterials are integral for the fabrication of artificial tissue and serve several functions during the tissue fabrication process. The field of

biomaterial science is very well developed and extremely mature. It has provided the impetus for the development of biomaterials that can be used to support 3D artificial tissue. There are three biomaterial classification schemes: natural versus synthetic, degradable versus nondegradable, and metals, ceramics, and polymers. There are four biomaterial platforms: polymeric scaffolds, biodegradable hydrogels, acellular matrices, and scaffold-free models. Several properties of biomaterials are important during tissue fabrication: mechanical properties, degradation kinetics, biocompatibility, and biomimetic properties.

Thoughts for Future Research—One area of research that needs to be developed further is the use of scaffold-free technologies to fabricate 3D artificial tissue. Scaffold-free technologies are based on the premise that cells can generate their own ECM and that this ECM will be closer in form and function to mammalian ECM than synthetic biomaterials. In addition, it is hypothesized that ECM generated by cells will be superior to synthetic biomaterials. In order to achieve this objective, strategies need to be optimized to guide ECM production by cells by regulating the microenvironment and delivering controlled physiological stimuli. The development of scaffold-free technologies to support tissue fabrication is challenging; there are several hurdles that need to be overcome. The two most significant technological hurdles are: 1) guidance to regulate ECM production by cells, and 2) guidance to regulate tissue fabrication using newly synthesized ECM.

PRACTICE QUESTIONS

1. Based on your understanding of biomaterials, provide a general discussion of the role of biomaterials in tissue engineering. How are biomaterials used during the tissue fabrication process? What are the functions of the biomaterial during the fabrication of 3D artificial tissue?

2. Provide a definition for a biomaterial.

3. Provide a general scheme for biomaterial development. Explain the following terms: biomaterial platforms, biomimetic properties, biocompatibility, and mechanical properties.

4. During our discussion of biomaterial development, the following concepts were described: biomaterial platforms, biomimetic properties, biocompatibility, and mechanical properties. Pick any tissue fabrication application and explain how these concepts apply to the selected application.

5. Describe historical applications of biomaterials. Provide examples that are not discussed in the chapter.

6. Explain what the tensile properties of a material refer to. Draw the stress–strain curve and explain what different regions of the curve mean.

7. Why are the tensile properties of a biomaterial important for tissue engineering?

8. Describe one method that can be used to modify tensile properties of a biomaterial. Explain the impact of this modification on the biomaterial using the stress–strain curve.

9. Why are the degradation kinetics of biomaterials important for tissue engineering?

10. Pick any tissue engineering application. For your selected application, do you believe that a degradable or nondegradable biomaterial would be more suitable? Explain your selection.

11. Define biomaterial biocompatibility.

12. Explain why the biocompatibility of biomaterials is important for tissue engineering.

13. Describe the foreign body response, complement activation, and the coagulation pathway.

14. Define biomimetic biomaterials.

15. Explain why the biomimetic properties of a biomaterial are important for tissue engineering.

16. Provide one example of how you would synthesize a biomimetic biomaterial to fabricate 3D artificial heart muscle.

17. There are several classification schemes for biomaterials: natural versus synthetic, degradable versus nondegradable, and metals, ceramics and polymers. Explain these classification schemes and discuss the relative advantages and disadvantages of each.

18. As described in Question 17, there are several classification schemes for biomaterials. Pick any tissue engineering application and explain which group of biomaterials would be most suited for the selected application.

19. Scaffold-free methods rely upon cells to generate ECM to support tissue fabrication. Explain the concept of scaffold-free technology. Do you think that it is suitable for tissue engineering? Identify important design considerations for the development of scaffold-free technology.

20. Acellular matrices have received significant attention in recent literature. Explain the concept of acellular matrices. How is an acellular matrix fabricated? What are the relative advantages and disadvantages of acellular matrices? Do you believe that acellular matrices can be used clinically in patients?

21. We discussed four biomaterial platforms: polymeric scaffolds, biodegradable hydrogels, acellular tissue, and scaffold-free technology. Explain these classification schemes and discuss the relative advantages and disadvantages of each.

22. In Question 21, we discussed four biomaterial platforms. Which one of these four is best suited to bioengineer artificial heart muscle and why?

23. In Question 21, we discussed four biomaterial platforms. Pick any one tissue engineering application. Which one of these four is best suited for the selected application and why?

24. Describe the concept of smart materials—what exactly is a smart material? Pick any one tissue engineering application and design a smart material for the selected application.

25. What is the most significant challenge in the development of biomaterials to support the fabrication of artificial tissue? What steps will you take to overcome this challenge?

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