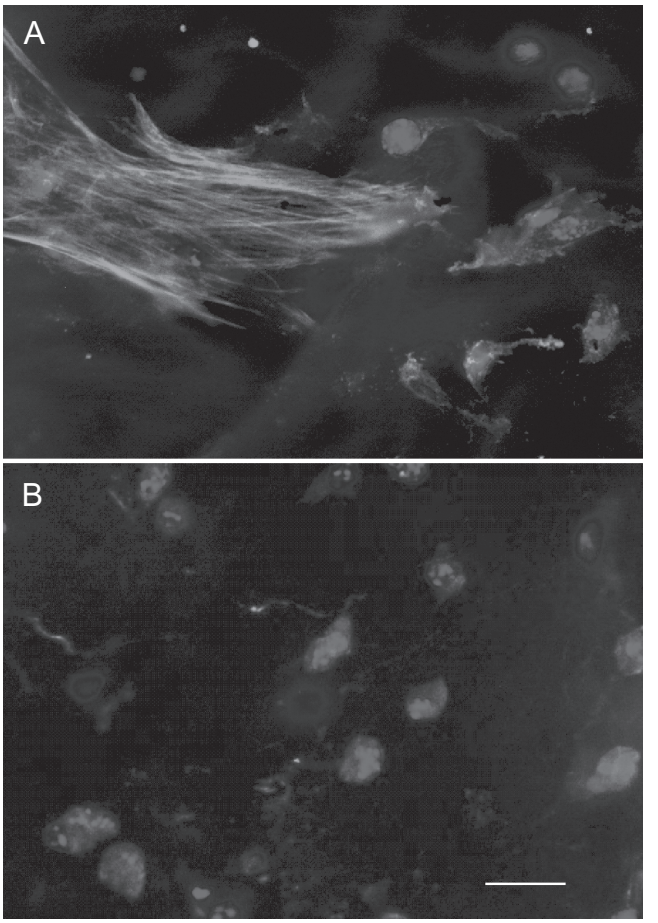
12

BIOMATERIAL ASPECTS OF BIOREGENERATIVE ENGINEERING



Influence of extracellular matrix on the formation of smooth muscle α-actin filaments in CD34-positive bone marrow cells in the mouse. CD34-positive bone marrow cells form smooth muscle α actin filaments (green in color) when cultured on the arterial elastic lamina matrix (A), but not on the arterial collagen (type III) matrix (B). Blue: cell nuclei. Scale: 100 μm. See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

Biomaterials are natural or synthetic materials that can be used to replace partially or completely a disordered tissue and thus to restore or improve the function of the disordered tissue. Several types of material, including synthetic polymers, metals, and ceramic materials, have been developed and used as biomaterials. In addition, natural polymeric materials generated by biological systems, such as collagen fibers, elastic fibers and laminae, and polysaccharides, have been used for the repair, replacement, and regeneration of disordered tissues. These materials are referred to as *biological materials*. Both biomaterials and biological materials can be used for constructing tissue scaffolds, which can be used for facilitating the regeneration of disordered tissues.

Biomaterials and biological materials have been used in a number organ systems for regenerative engineering purposes, including the skeletal, cardiovascular, gastrointestinal, epidermal, and urinary systems. Successful examples include replacement of hip joints and bones with metallic prostheses, replacement of blood vessels with biological and polymeric materials, replacement of the heart valves with biological and metallic materials, repair and regeneration of skin with biological materials, replacement of the heart with artificial cardiac pumps, argumentation of the respiratory function with polymeric artificial lungs, and improvement of the renal function with kidney dialysis devices. With the development of new technologies in biomaterial synthesis and analysis, it is expected that new biomaterials will be developed with improved performance and biocompatibility. In this chapter, several fundamental concepts, including the types, properties, and biocompatibility of biomaterials, are briefly reviewed.

SYNTHETIC POLYMERS AS BIOMATERIALS [12.1]

A synthetic polymer is a long-chain compound derived by bonding together many single-unit molecules. The single-unit molecules that constitute a polymer are known as *monomers*. Examples of synthetic polymers include nonbiodegradable polymers, such as polyethylene, poly(vinyl chloride), Dacron, nylon, and Teflon, and biodegradable polymers, such as polyglycolides, polylactides, polyanhydrides, and polysaccharides. Synthetic polymers possess unique features suitable for biological applications. These materials can be modulated to change their chemical and mechanical properties, and can be tailored into various shapes. In addition, these materials are light, strong, and inert. Thus, synthetic polymeric materials have been widely used for the repair, replacement, and regeneration of injured and disordered tissues. In this section, the classification, structure, and properties of synthetic polymers as well as their application to tissue engineering are discussed.

Classification

Polymers can be classified into two groups according to on the mechanisms of synthesis: addition polymers and condensation polymers. An *addition polymer* is synthesized by connecting monomer units via rearranging chemical bonds. The formation of poly(vinyl chloride) is a typical example of addition polymer. In this case, the double bond of a single vinyl chloride opens up in the presence of an initiator such as a peroxide molecule. The initiator can be activated by increasing temperature or exposure to ultraviolet. The initiator can activate monomers to form free bonds, which connect the monomers together. This process is known as propagation. Common addition polymers include polyethylene, polypropylene, polystyrene, and poly(vinyl chloride).

A *condensation polymer* is synthesized by joining two molecules together with the elimination of a molecule such as water and methanol. Two different types of monomers are usually participating in the polymerization reaction. The resulting product is known as copolymer. Nylon is a typical copolymer. During the polymerization process of nylon, a dicarboxylic acid molecule reacts with a diamine. A water molecule is removed when the two molecules are bonded. Examples of condensation polymers include polysaccharides, proteins, polyesters, polyamides, polyurea, polyurethane, and cellulose.

Based on the degradability of polymeric materials in a biological system, polymers can be classified into non-biodegradable and biodegradable polymers. Nonbiodegradable polymers cannot be degraded, whereas biodegradable polymers can be degraded in a biological system. Examples of nonbiodegradable polymers used as biomaterials include polyethylene, polytetrafluoroethylene, poly(vinyl chloride), and polypropylene. Examples of biodegradable polymers include polyglycolides, polylactides, polysaccharides, and poly(α-hydroxyl acids).

In terms of the type of monomer in a polymer structure, polymers can be classified into homopolymers and heteropolymers (or copolymers). Homopolymers are compounds constituted with one type of repeated monomers. Examples of homopolymers include polyethylene, polytetrafluoroethylene, poly(vinyl chloride), and polypropylene. Copolymers are polymeric compounds composed of two or more types of monomer. The different monomers may be randomly distributed or may alternate in a pattern. Examples of copolymers include poly(glycolide lactide), polyurethane, and poly(glycolide trimethylene carbonate).

General Properties

Polymeric materials exist in several forms, including liquid, elastomer, and plastic. The form of polymeric materials is determined by a number of factors, including the nature of monomers, the size and form of polymer molecules, the type and concentration of catalysts used for polymer synthesis, and curing temperature and duration. By selectively altering these factors, a desired form of polymeric materials can be generated.

An important factor that influences the structure and mechanical properties, such as flexibility and strength, of polymeric materials is the composition of polymer molecules. A substitution of a key atom in a monomer may induce a significant change in the mechanical properties. For instance, the replacement of the carbon atom with oxygen in a polyethylene molecule reduces the rigidity of the polymer.

The molecular size or polymeric chain length is another critical factor that determines the structure and mechanical properties of polymeric materials. Polymers are composed of various numbers of monomers. For the same type of monomer, a longer polymer is more flexible and tangled more easily than a shorter one. For instance, polyethylene and paraffin can be synthesized based on the same type of monomer CH_2CH_2, but with different chain length. The length of a paraffin molecule is much shorter than that of a polyethylene molecule. The long-chained polyethylene molecules are more difficult to crystallize, thus exhibiting higher flexibility, compared to paraffin. The chain length of polymers is one of the factors that determines the rigidity and strength of polymer materials. An increase in the chain length reduces the mobility of polymer molecules, and thus enhances the rigidity and strength.

The form of polymer molecules is a major factor that influences the structure and organization of polymeric materials. Polymer molecules exit in several forms: linear, branched,

and crosslinked forms. A linear polymer molecule is composed of a single chain of monomers with various lengths and molecular weights. Examples of linear polymers include polyvinyls and polyesters. These molecules can be partially crystallized to form so-called semicrystalline polymers. It is usually difficult to completely crystallize these polymers.

A branched polymer molecule is composed of a mainchain and various densities of sidechains. Copolymerization may enhance the formation of branches. Since the sidebranches influence the interaction between the main polymer chains, the introduction of sidebranches may reduce polymer crystallization, yielding more flexible polymers. An increase in the length of the sidechain reduces the melting temperature of the polymer in association with a reduction in crystallization.

Polymer molecules can be crosslinked to form networks. Long-chain polymer molecules are usually linked together with sidechains. Crosslinked polymers are usually difficult to crystallize. An increase in the degree of crosslinking reduces the crystallization capability of polymeric materials. Up to a certain degree of crosslinking, polymer crystallization may be completely prevented. During the crosslinking process, the form of monomers and reaction conditions may influence the structure and mechanical properties of the polymeric material. For instance, the presence of tortuous and curved polymer chains may result in a polymeric material that is elastic and flexible. This type of material can be stretched to a large extent, and can return back to the undeformed length upon the release of the stretching force. Natural rubber (*cis*-polyisoprene) is a typical example of such materials.

In addition to the intrinsic factors of polymeric molecules, environmental factors influence the structure and mechanical properties of polymeric materials. A typical environmental factor is temperature. An increase in temperature often reduces the rigidity of polymeric materials and induces the transformation of polymer from plastic to elastomer and liquid.

Nonbiodegradable Polymers [12.2]

Polytetrafluoroethylene. Polytetrafluoroethylene (PTFE) is a fluorocarbon polymer, also known as Teflon, which is commonly used as a biomaterial for the replacement of soft tissues. Teflon is characterized by high crystallization (about 90% molecules crystallized), a relatively high density (~2 g/cm^3), and low surface tension and friction compared to other types of polymer. Teflon is used primarily for the construction of vascular substitutes. Teflon vascular grafts are sufficiently strong for withstanding stretching forces induced by arterial blood pressure. The mechanical properties of Teflon grafts can be stable for years following implantation. Because Teflon stimulates inflammatory reactions and thrombogenesis, which lead to the development of intimal hyperplasia, it can only be used for the replacement of arteries larger than 4 mm in diameter. Teflon can also be used to construct supporting sheaths for vein grafts, an approach for modulating the diameter of the vein graft, reducing diameter mismatch-induced disturbance of blood flow, and suppressing flow disturbance-induced intimal hyperplasia.

Poly(ethylene terephthalate). Poly(ethylene terephthalate) (PET), known as Dacron, is a polyester material that is characterized by hydrophobicity, resistance to hydrolysis, and high strength and toughness. Dacron has primarily been used for the construction of vascular grafts. Porous grafts can be constructed by weaving PET fibers into mesh-like materials. This type of graft usually facilitates cell integration into the graft wall when

anastomosed into a host artery. Dacron grafts are often used to replace malfunctioned thoracic and abdominal aortae. As other polymeric materials, Dacron induces inflammatory reactions and thrombogenesis. Thus, this type of graft can only be applied to arteries with diameter exceeding 4 mm.

Polyethylene. Polyethylene is a polymer composed of ethylene monomers and can be synthesized into polymeric materials of various densities. A low-density polyethylene material (0.91–0.93 g/cm^3) can be synthesized by using peroxide catalysts at pressure 1000–3000 kg/cm^2 and temperature 300–500°C. Polyethylene materials generated under such conditions are composed of branched polymers that are difficult to crystallize. This type of material is tough and flexible, and is often used to fabricate thin membranes for food packaging and also for manufacturing biomedical supplies, such as tubing and containers.

A high-density polyethylene material (0.945–0.96 g/cm^3) can be synthesized by using metal catalysts at pressure ~10 kg/cm^2 and temperature 60–80°C. This polymer material is composed primarily linear polymers and is highly crystallized with strong bonds between ethylene monomers. The high-density polyethylene material is stronger than the low-density polyethylene material, and has been used for fabricating orthopedic implants, such as load-bearing caps for artificial joints.

Biodegradable Polymers [12.3]

A number of biodegradable polymeric materials have been synthesized and used as biomaterials. These materials belong to several polymer families, including linear aliphatic polyesters, polyorthoesters, polyphosphate esters, poly(ester–ether), polyanhydrides, polyamides, polysaccharides, polyamino acids, and inorganic polyphosphazenes. Some of these polymers have been increasingly used for the regeneration, repair, and replacement of tissues and organs. Because these materials can be gradually degraded and removed through various organ systems such as the liver and kidneys, harmful influences, if any, imposed by these materials can be eliminated. Furthermore, when used for constructing tissue scaffolds, biodegradable polymers with desired shapes can serve as a guidance for cell migration and pattern formation. With the degradation of the polymer scaffold, natural tissues can be gradually established and strengthened, eventually integrating into the host system.

Biodegradable polymeric materials have been applied to biomedical research in primarily two areas: wound healing and drug delivery. Linear aliphatic polyesters, such as polyglycolides, poly(glycolide-L-lactide), poly(ester–ether), and poly(glycolide–trimethylene carbonate), have been successfully used for enhancing wound closure and healing. These polymers have been extensively studied for their structure, material properties, and biological compatibility. Several types of biodegradable polymer have been used for constructing drug delivery carriers. Examples include polyanhydrides and poly(ester–ether). By controlling the rate of degradation, the rate of drug delivery can be regulated. In addition, biodegradable polymers have been investigated for their potential use in tissue regeneration and repair. For the last decade (since the mid-1990s), the synthesis and characterization of biodegradable polymers are among the most active research areas in biomedical engineering. Here, several common types of biodegradable polymers are discussed with a focus on the structure, material properties, and potential applications to bioregenerative engineering.

Linear Aliphatic Polyesters. *Linear aliphatic polyesters* are straight-chain polymers in which monomers are joined by the ester bond —COO—. Commonly used linear aliphatic polyesters include polyglycolide (PG) or poly(glycolic acid) (PGA), polylactide (PL) or poly(lactic acid) (PLA), polycaprolactone (PCL), poly(β-hydroxybutyrate), and poly(glycolide–trimethylene carbonate). These polymers have been successfully used in biomedical research and application. Among these aliphatic polymers, polyglycolide and polylactide are basic molecules that can be used to form copolymers and derive different forms of polymers. These polymers have been frequently used in biomedical research.

Polyglycolides and Polylactides [12.4]. *Polyglycolides* and *polylactides* can be synthesized from glycolic acids and lactic acids, respectively. The direct condensation method can be used to synthesize low-molecular-weight polyglycolides or polylactides (<3000 kDa). Common catalysts for such synthesis include phosphoric acids, *p*-toluene sulfonic acid, and antimony trifluoride. For a polymer larger than 3000 kDa, a process known as *ring-opening polymerization* is necessary for polymer synthesis. Several catalysts, such as stannous chloride dehydrate and aluminum alkoxide, has been used for the synthesis of large polyglycolides and polylactides.

The thermal properties of polymers are described by the melting and glass transition temperature, while the mechanical properties of polymers are expressed by the elastic modulus, tensile strength, and maximal distensibility or strain. A typical polyglycolide material has a melting temperature of ~210ºC and a glass transition temperature of ~36ºC, whereas a polylactide material possesses a melting temperature of ~170ºC and a glass transition temperature of ~56ºC. Mechanically, polylactide-based materials possess elastic moduli ranging from 1200 to 3000 MPa and tensile strength of 28–50 MPa depending on the molecular weight, and can be extended to 2–6% of their original length before reaching the breakpoint.

The rate of polymer biodegradation is an important parameter considered in the design and synthesis of biodegradable polymers. Polymers are usually degraded by hydrolysis of the ester bonds, resulting in a decrease in molecular weight. Several factors are known to influence the rate of polymer degradation. These include the chemical structure and molecular weight of the polymer material as well as environmental conditions. For instance, amorphous polymers can be degrade more easily than crystalline polymers. Polymers with a higher molecular weight may be degraded more slowly than those with a lower molecular weight. Branched polymer molecules may be degraded faster than linear molecules. An increase in temperature facilitates polymer degradation. For a typical semicrystalline polylactide material, weight loss can be detected after 30 weeks in a phosphate buffer at 37ºC.

Glycolides and lactides can form copolymers with each other as well as with other types of esters. The copolymer formed on the basis of glycolides and L-lactides is known as poly(glycolide-L-lactide). The relative contents of various esters in a copolymer may influence the rate of polymer degradation. For instance, the degradation rate of poly(glycolide-L-lactide) is dependent on the relative concentration of lactides. It is interesting to note that the relationship is not linear. In the range of 0–25% of L-lactides, the rate of degradation is inversely proportional to the concentration of L-lactides, whereas in the range of 75–100% of L-lactides, the rate of degradation is directly proportional to the concentration of lactides. Interestingly, in the range of 25–75% of L-lactides, the rate of polymer degradation does not change significantly with an increase in the concentration of lactides. Thus, for the design of a copolymer, the degradation rate should be taken into

account. Ideally, a polymer material should have a degradation rate that is comparable to the rate of native tissue formation.

Polyglycolides, polylactides, and their copolymers have been used for constructing various forms of matrix for biomedical applications such as drug delivery and tissue repair. These polymers can be also injected into target tissues. The biocompatibility and toxicity of these polymers have been tested extensively. The degradation product of polylactides is lactic acid, which is a natural metabolite in mammals and can be removed via physiological metabolism. The metabolite of polyglycolides, glycolic acid, has been shown to be a low-toxicity substance. An increase in acidity near a polymer implant may occur, but such a change can be mitigated by a local pH treatment. In general, these polymers exhibit low toxicity and are safe for in vivo implantation and injection.

Polycaprolactones [12.5]. Polycaprolactone (PCL) are another type of polyester that is used in biomedical research and application. Polycaprolactones can be synthesized by polymerization with anionic, cationic, and coordination catalysts. For the anionic catalytic system, tertiary amines, alkali metal alkoxides, and carboxylates are necessary for the polymerization. For the cationic polymerization system, several catalysts, including protic acids, Lewis acids, acylating agents, and alkylating agents, are often used. The coordination polymerization system is used for synthesizing high-molecular-weight polymers. Catalysts for this type of polymerization include stannous octoate, alikoxides, and metallic elements such as Al, Sn, Mg, and Ti. Polycaprolactone materials usually have the following thermal properties: melting temperature ~60ºC and glass transition temperature ~−60ºC. Mechanically, polycaprolactone materials can be stretched to a strain about 0.3 (~30% elongation) at a yielding stress about 11 MPa. The elastic modulus is about 0.3 GPa. Polycaprolactone materials and polycaprolactone hybrids with other materials, such as hydroxyapatite, poly-L-lactides, and silica, have been used in a number of biomedical applications, such as scaffolding and repairing soft and bone tissues (Fig. 12.1). Polycaprolactone materials can be degraded by hydrolysis. In vivo tests have shown that it takes about 2–4 years to completely degrade a polycaprolactone implant. Copolymerization or blending with glycolides and/or lactides increases the rate of degradation. In vivo animal tests have shown that polycaprolactone materials exhibit low toxicity and do not significantly influence the function of host cells and tissues.

Poly(β-hydroxybutyrate) [12.6]. Poly(β-hydroxybutyrate), or PHB, is a polymer of β-hydroxybutyrate. Poly(β-hydroxybutyrate) can be generated by natural fermentation of the bacterium *Alcaligenes* and can also be synthesized by the plant flax or linum usitatissimum, which is genetically transfected with gene constructs required for the synthesis of poly(β-hydroxybutyrate), or other types of plants such as *Arabidopsis thaliana* and *Brassica napus*. Poly(β-hydroxybutyrate) is an amorphous material with a glass transition temperature of about −40ºC and a melting temperature of ~160ºC. The failure strain (strain when a material is extended to the failure point) of poly(β-hydroxybutyrate) is about 0.15 or 15%. When poly(β-hydroxybutyrate) is blended with other types of polymers, such as hydroxyhexanoate, the failure strain can be increased depending on the relative contents of the polymer components. The poly(β-hydroxybutyrate)-containing copolymer can be fabricated into various forms and used for constructing tissue replacements (Fig. 12.2). The degradation of poly(β-hydroxybutyrate) is induced by hydrolysis. In vitro tests have shown that high-molecular-weight poly(β-hydroxybutyrate) films can be completely degraded at 25ºC in freshwater within 3 weeks.

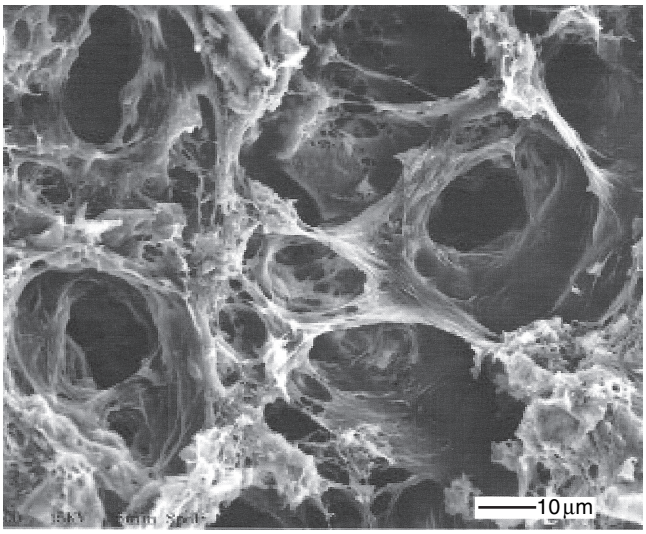


Figure 12.1. Scanning electron microscopic images of freeze fracture hydroxyapatite (HAP)/polycaprolactone (PCL) composite materials. (Reprinted with permission of John Wiley & Sons, Inc. from Verma D et al: Experimental investigation of interfaces in hydroxyapatite/polyacrylic acid/polycaprolactone composites using photoacoustic FTIR spectroscopy, *J Biomed Mater Res A* 77:59–66, copyright 2006.)

Polycarbonates [12.7]. *Polycarbonates* are a group of polyesters, including poly(urethane carbonate), poly(ethylene carbonate), and poly(propylene carbonate). These polymers are synthesized with dihydroxy compounds and carbonyl chloride. For aliphatic poly(urethane carbonate), the glass transition temperature is about −18°C. The tensile failure stress of this polymer is about 50 MPa, and the failure strain is about 416%. Clearly, this is a highly extendable material. The degradation rate varies among different polycarbonate materials. For instance, poly(ethylene carbonate) tablets implanted in the rat can be degraded within about 21 days, poly(propylene carbonate) may last for 60 days, whereas poly(urethane carbonate) is stable for a much longer time. This class of polymeric materials are biocompatible and has been used for cell culture (Fig. 12.3). These materials have also been used as biomaterials for constructing cardiovascular implants (intraaortic balloons, cardiac valves, vascular prostheses, pacemaker leads, ventricular assist devices and artificial heart diaphragms, heart valves, vascular grafts, and urethral catheters) as well as reconstructive implants (wound dressings, mammary prostheses, maxillofacial prostheses).

Polyamides [12.8]. A *polyamide* is a polymer that is formed by joining monomers with an amide bond —CONH—. Natural proteins are amide-based polymers. Amino acids can be used to synthesize artificial polyamides. Typical examples are polyglutamic acid and polylysine. Glutamic acid and lysine can also form copolymers with other types of amino acids. Unlike other synthetic polymers that have been tested in biomedical research, polymers based on amino acids are composed of naturally occurring molecules and thus possess low toxicity. Such a feature renders these polymers promising candidates as biomaterials for tissue repair and regeneration as well as drug delivery. Here, polyglutamic

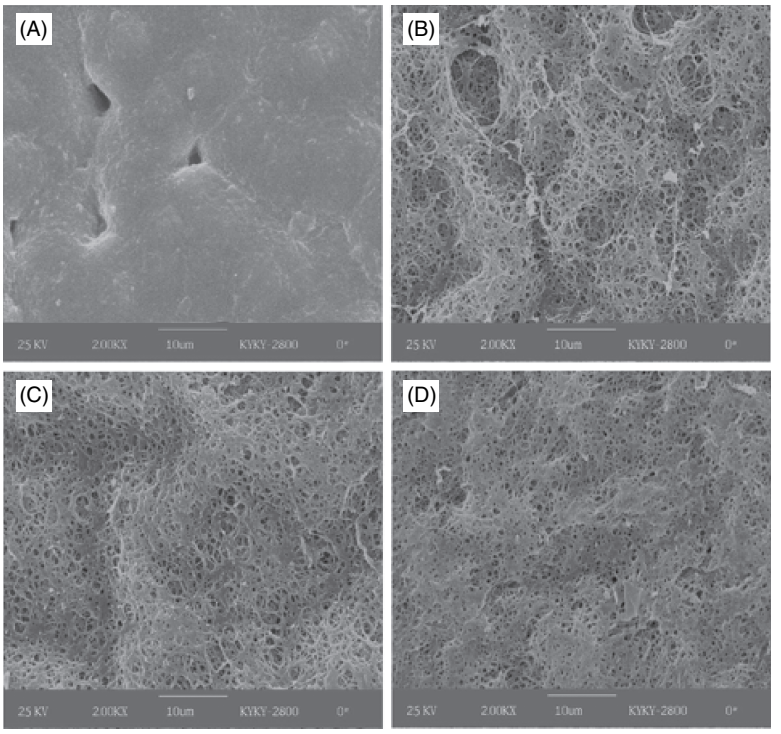


Figure 12.2. Scanning electron microscopic images of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)-based biomaterials: (A) poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) materials; (B) poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) with 5% gelatin blend; (C) poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) with 10% gelatin blend; (D) poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) with 30% gelatin blend. (Reprinted with permission of the American Chemical Society from Wang YW, Wu Q, Chen GQ: Gelatin blending improves the performance of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) films for biomedical application, *Biomacromolecules* 6:566–71, copyright 2005.)

acid is used as an example to demonstrate the principles of the synthesis, material properties, and potential biomedical application of polyamides.

Poly(glutamic acid) can be synthesized from poly(γ-benzyl-L-glutamate) by eliminating the benzyl group by using hydrogen bromide. Poly(glutamic acid) (PGA) can be degraded by enzymatic hydrolysis. In particular, cystein proteases play a critical role in the degradation of PGA. The time course of PGA degradation ranges from several hours to months, depending on the concentration of proteinase, temperature, and the composition of the polymer (with or without additional components). Poly(glutamic acid) exhibits little toxicity when implanted into an animal tissue. Animals can tolerate a single dose of ≤800 mg/kg and an accumulated dose of ≤1.8 g/kg. Polyglutamic acid exhibits little immunogenicity in animal models. This type of materials can be used in various biomedical applications, such as tissue repair and regeneration as well as drug delivery.

Polyphosphazenes [12.9]. Polyphosphazenes are inorganic biodegradable polymers that are constituted with a nitrogen–phosphorus (N═P) backbone. This type of polymer is

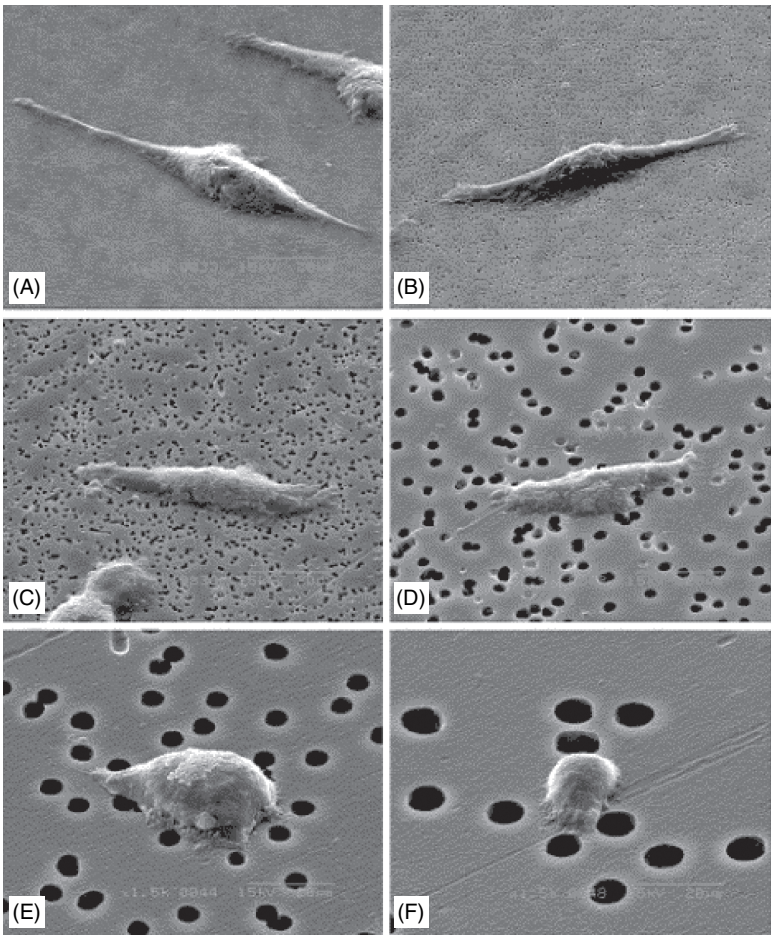


Figure 12.3. Scanning electron microscopic images of MG63 osteoblast-like cells (2-day culture) attached to the polycarbonate membrane surfaces with different micropore sizes: (A) 0.2, (B) 0.4, (C) 1.0, (D) 3.0, (E) 5.0, and (F) 8.0 μm in diameter of micropore sizes. (Reprinted from Lee SJ et al: Response of MG63 osteoblast-like cells onto polycarbonate membrane surfaces with different micropore sizes, *Biomaterials* 25:4699–707, copyright 2004 with permission from Elsevier.)

synthesized via reactions of polydichlorophosphazene with amines or alkoxides in tetrahydrofuran or aromatic hydrocarbone solutions. Polymers with various sidegroups can be synthesized by mixing different components. The material properties of polyphosphazenes are dependent on the composition of the polymer. Biodegradable polyphosphazenes can be generated when amino acid derivatives are used as sidegroups. For instance, the ethylglycinato-derived polymers can be degraded into ammonia, phosphate, ethanol, and glycine. Experimental tests in vitro have demonstrated that amino acid-based polyphosphazenes can be degraded within several months. The rate of degradation is dependent on the type of amino acid selected for the sidegroups.

Polyphosphazenes have been used for constructing various structures, such as matrix scaffolds (Fig. 12.4), hydrogels, and microspheres, for drug delivery. The degradation of

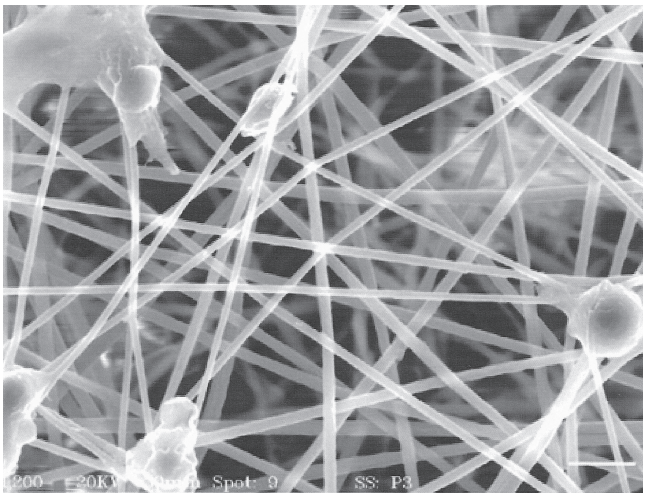


Figure 12.4. Scanning electron micrograph showing electrospun poly[bis(*p*-methylphenoxy) phosphazene fiber matrix with arterial endothelial cells after 24 h of culture. (Reprinted with permission of the American Chemical Society from Lakshmi S et al: Fabrication and optimization of methylphenoxy substituted polyphosphazene nanofibers for biomedical applications, *Biomacromolecules* 5:2212–20, copyright 2004.)

polyphosphazenes is sensitive to changes in temperature. Thus, the degradation rate of these polymers can be regulated by controlling environmental temperature. Such polymers can be used for constructing drug delivery carriers for temperature-related diseases. Other environmental factors, such as pH, may also influence the rate of degradation of polyphosphazenes. For instance, oxybenzoate-containing polyphosphazenes are pH-sensitive. The degradation of this type of polymer can be controlled by altering the content of oxybenzoate within a specified range of pH. Such polymers can be used to deliver drug for diseases that result in a change in pH. In vivo animal tests with subcutaneous implantation have shown that polyphosphazenes materials exhibit low toxicity, induce little inflammatory reactions in host tissues, and are relatively safe for implantation and drug delivery.

Polyanhydrides [12.10]. Polyanhydrides are polymers formed with anhydrides, compounds in which two carbonyl groups are joined with an oxygen atom, RCO—O—COR′, where R and R′ are any organic groups. The polymers are synthesized by reactions of diacids with anhydrides to form acetyl anhydride prepolymers. High-molecular-weight polyanhydrides can be formed from the prepolymers by melt condensation (180ºC for 90 min in vacuo). The addition of coordination catalysts, such as cadmium acetate and metal oxides, can facilitate the polymerization process, increasing the molecular weight of the polymer. Polyanhydrides can be dissolved in organic solvents such as chloroform and dichloromethane. Various components can be copolymerized with anhydrides to alter the solubility. Homopolymers of anhydrides usually exhibit a high level of crystallinity. Copolymerization with different components may reduce the crystallinity, producing more amorphous materials. Copolymerization also influences the mechanical properties of polyanhydrides.

Polyanhydrides are degraded by hydrolytic erosion. A number of factors influence the rate of polyanhydrides degradation. These include pH and copolymerization with different compounds. An increase in pH facilitates polyanhydrides degradation. The incorporation of different aliphatic monomers may facilitate the degradation of the polymer, whereas the addition of methylene groups into the polymer backbone reduces the rate of degradation. Thus, polyanhydrides with various levels of degradation can be synthesized by copolymerization with various compounds. Polyanhydrides can be used to construct various forms of matrix, such as disks and pellets, and can also be injected into target tissues. The injection of mixed polyanhydrides and therapeutic substances is a promising technique for controlled drug delivery. A number of studies have shown that polyanhydrides do not significantly influence the growth of cultured cells and exhibit little toxicity when implanted into target tissues in animal tests.

BIOLOGICAL MATERIALS

Collagen Matrix [12.11]

Collagen matrix is found in mesenchymal and connective tissues, such as the subcutaneous tissue and bone, and the adventitia of tubular organs (blood vessels, airways, esophagus, stomach, and intestines). In mammalian tissues, there exist about 15 types of collagen matrix, namely, collagen types I–XV. Among these types of collagen, types I, II, III, IV, V, IX, XI, and XII are commonly found in connective tissues. Collagen types I, II, III, V, and XI are organized into filamentous structures, known as *collagen fibrils*, with a diameter of ~10–100 nm. These fibrils usually form large collagen bundles as found in subcutaneous tissues and the adventitia of tubular organs. Collagen types I and V are often found in the bone, skin, cornea, tendon, ligament, and internal organs such as the lung, liver, pancreas, and kidney. Collagen types II and XI are found in the cartilage, notochord, and intervertebral disks. Collagen type III is found in blood vessels, skin, and internal organs. Collagen types IX and XII are molecules that link other types of collagen fibril and are known as *fibril-associated collagens.* These types are found in the cartilage, tendons, and ligaments. In contrast to the filamentous collagen molecules, collagen type IV participates in the construction of a membrane-like structure, known as the *basal lamina*, which underlies epithelial and endothelial cells. The structure and biochemical features of collagen molecules are discussed on page 103. These aspects will not be repeated here.

Collagen molecules play important roles in the constitution of mammalian tissues or organs. The collagen matrix serves as a structural framework that supports cells, helps organize cells into various forms of tissues and organs, and protects cells from mechanical injury. In addition, collagen matrix participates in the regulation of cellular activities such as cell survival, adhesion, proliferation, and migration. Collagen molecules can directly interact with cells via the cell membrane collagen receptors, or indirectly via the mediation of fibronectin, a matrix component that binds collagen molecules at one side and cell membrane matrix receptors, known as *integrins*, at the other side. The binding of collagen and fibronectin molecules to the integrin receptors initiate the activation of intracellular signaling pathways that stimulate or activate mitogenic processes, including cell survival, proliferation, and migration.

Given the structural and functional features, collagen matrix has long been used for constructing drug-delivery devices and scaffolds for tissue regeneration. Collagen matrix

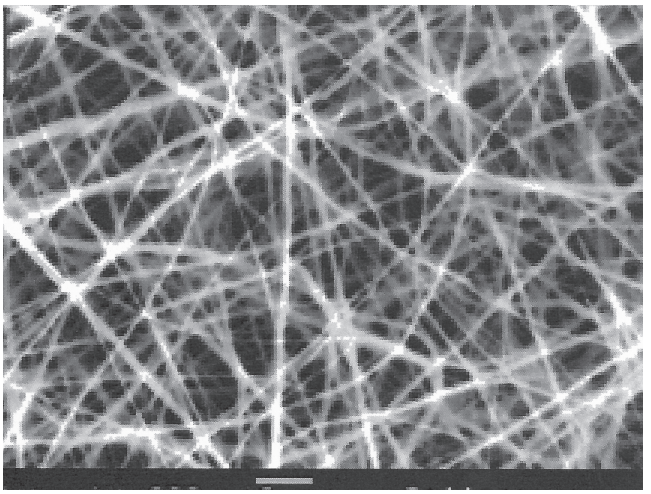


Figure 12.5. Scanning electron micrograph of electrospun collagen matrix. (Reprinted with permission of the American Chemical Society from Zhang YZ et al: Characterization of the surface biocompatibility of the electrospun PCL-collagen nanofibers using fibroblasts, *Biomacromolecules* 6:2583–9, copyright 2005.)

has been used in several forms: collagen gels, meshes, composites with different molecules, and decellularized natural matrix. Collagen gels and meshes are suitable for drug delivery, whereas cell-free natural collagen matrix can be used as scaffolds or grafts for the repair or regeneration of various tissues and organs, such as blood vessels, airways, intestines, and bladder. Collagen gel can be spun into collagen fibers, which can be used to construct collagen scaffolds for tissue repair and regeneration (Fig. 12.5).

Native collagen matrix is a suitable material for the construction of tissue scaffolds. Such a material maintains the natural biological and mechanical characteristics and exhibits superior biocompatibility compared to in vitro crosslinked collagen gels or matrix. To prepare a native collagen matrix, mammalian tissue specimens can be collected from the submucosa of intestines, the adventitia of blood vessels, and the subcutaneous tissue. Cells in these specimens can be removed by various enzymatic and hydrolytic methods. Such treatments eliminate the cellular immunogenicity of allogenic tissues (note that extracellular matrix molecules exhibit neglible immunogenicity). The resulting cell-free collagen matrix can be tailored into a scaffold with a desired form and used for tissue repair or regeneration.

Elastic Fibers and Laminae [12.12]

Elastic fibers and laminae are major extracellular matrix components found in mesenchymal and connective tissues. Elastic fibers are present in the lung, connective tissue, the submucosa of intestines, and the wall of veins, whereas elastic laminae are found primarily in the media of large and medium arteries. Elastic fibers and laminae are composed of several proteins, including elastin, microfibrils, and microfibril-associated proteins. Elastin is the most abundant protein in elastic fibers and laminae. Mature elastin is a highly insoluble and hydrophobic protein, and is formed by crosslinking the 72-kDa elastin

precursor, known as *tropoelastin*. Tropoelastin is produced by several cell types, including the smooth muscle cell, endothelial cell, and fibroblast, and is released into the extracellular space where crosslinking and elastin formation take place. The structure and biochemical features of elastic fibers are discussed on page 109.

Elastic fibers and laminae play an important role in the constitution of tissues and organs as well as in the maintenance of the stability of tissues and organs. For instance, multiple layers of elastic laminae are found in large arteries. These laminae have long been known to contribute to the structural stability and mechanical strength of the arterial wall (44,45). Arteries are subject to extensive mechanical stress induced by arterial blood pressure. Without the support of the elastic laminae, vascular cells may be overstretched under arterial blood pressure. Elastic laminae also contribute to the elasticity of soft tissues, such as connective tissues and arteries. The recoil of the arterial wall is a critical mechanism for the continuation of bloodflow during diastole when cardiac ejection is ceased. Elastic laminae have also been shown to serve as a signaling structure and play a role in regulating arterial morphogenesis and pathogenesis. An important contribution of elastic laminae is to confine smooth muscle cells to the arterial media by inhibiting smooth muscle cell proliferation and migration, thus preventing intimal hyperplasia under physiological conditions. In addition, elastic laminae exhibit antiinflammatory effects and inhibit leukocyte adhesion, activation, and transmigration relative to collagen matrix. These features render elastic laminae a potential material for vascular reconstruction. Furthermore, elastic laminae and elastin-containing structures can be used to prevent inflammatory reactions after surgery.

Polysaccharides [12.13]

Polysaccharides are polymers composed of many monosaccharides bonded together by glycosidic bonds. There are a number of forms of natural polysaccharides, including glycogen, cellulose, alginate, chitosan, starch, and glycosaminoglycan. These polysaccharides are found in animals and plants, and play an important role for the survival and function of animals and plants. Glycogen is a polymer composed of glucose monomers and synthesized in animals for the storage of energy. Alginates are linear polysaccharides composed of β-mannuronic acid and α-guluronic acid, and are found in brown seaweed and in certain bacteria. Starch is a polymer found in plants and synthesized for the storage of energy. Cellulose is found in plants and bacteria. Chitosan is found in the shell of crabs and shrimps. One of the important properties of polysaccharides is their ability to form hydrogel. This property is the basis for polysaccharide-mediated drug delivery. Several types of polysaccharide, such as cellulose, chitosan, and starch, have been used as materials for tissue engineering and drug delivery.

Cellulose. *Cellulose* is a linear polysaccharide composed of D-glucose units jointed together by 1,4-β-glucosidic bonds. In plants, cellulose participates in the constitution of plant skeleton and cell wall. Cotton is a well-known cellulose-containing material. Cellulose molecules are often arranged in parallel, giving cellulose fibers high mechanical strength. Humans cannot use cellulose as an energy source because of the lack of β-glycosidase, which catalyzes the hydrolysis of β-glycosidic bonds (note that mammals have α-glycosidase that catalyzes the hydrolysis of glycogen and starch). Cellulose can also be produced by bacteria. Bacterial cellulose has been often used for tissue engineering and will be the focus here.

Several types of microorganism, including algae (*Vallonia*), fungi (*Saprolegnia*, *Dictystelium discoideum*), and bacteria (*Acetobacter*, *Achromobacter*, *Aerobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium*, *Sarcina*, *Alcaligenes*, *Zoogloea*) can synthesize cellulose. Among these microorganisms, the bacterium Acetobacter xylinum, which is usually found in fruits, vegetables, and alcoholic beverages, has been used to generate cellulose for tissue engineering applications. In a culture medium, this bacterium can produce a network of cellulose fibers. The cellulose fibers can be collected, fabricated into desired forms, and used to construct scaffolds for tissue engineering applications.

The bacterial cellulose synthesized by *Acetobacter xylinum* is similar to the plant cellulose in molecular composition. Both types of cellulose contain D-glucose. However, bacterial cellulose exhibits a higher crystallinity, higher water absorption capacity or lower hydrophobicity, higher mechanical strength, and finer molecular arrangement compared to the plant cellulose. Cellulose and its derivatives, such as cellulose nitrate, cellulose acetate, and cellulose xanthate, can be easily fabricated into desired forms. Unlike other polysaccharides, such as glycogen and starch, cellulose exhibits low water solubility and, therefore, a low rate of degradation when implanted into an animal tissue. A decrease in the crystallinity and hydrophobicity of cellulose usually results in an increase in the biodegradability of cellulose. Given the chemical composition, bacterial cellulose is highly biocompatible and nontoxic to the host. Furthermore, bacterial cellulose is a highly moldable material and can be used to fabricate scaffolds with desired forms. Cellulose-based materials have been used in a number of biomedical applications. These include construction of cellulose membranes for hemodialysis, construction of enzyme carriers for biosensors, drug delivery, construction of scaffolds for the regeneration of various tissue types, such as the bone, cartilage, liver, skin, and blood vessels. These investigations have consistently demonstrated that cellulose-based materials elicit little inflammatory and toxic reactions. Cellulose has been proven a promising material for the construction of tissue regenerating scaffolds.

Alginates [12.14]. *Alginates* are linear polysaccharides composed of β-mannuronic acid and α-guluronic acid. Alginates are found in brown seaweed and in certain bacteria. The content of β-mannuronic acid and α-guluronic acid may vary depending on the plant or bacterial species from which alginates are obtained. Alginates can be used to form hydrogel and matrix. Divalent cations, such as Ca^{2+} and Mn^{2+}, can initiate alginate gelation by linking α-guluronic acid units between different polymer chains. The feature of gelation renders alginates a potential material for tissue engineering applications, such as cell seeding and transplantation, tissue repair, and drug delivery.

Alginate gels with various mechanical properties can be generated under different gelling conditions and by using different crosslinkers. Numerous studies have been conducted to test the elastic and shearing mechanical properties. Under compressive forces, alginate matrices exhibit elastic modulus ranging from 1 to 1000 kPa, depending on gelling and experimental conditions. Similarly, the shear modulus of alginate matrices spreads widely from 0.02 to 40 kPa under different experimental conditions. Under tensile forces, the maximal tensile strength or failure stress of alginate gels ranges from 3 to 35 kPa and the maximal or failure strain is from 0.3 to 1.25, depending on the composition of alginates and the strain rate applied.

Alginate gels crosslinked by Ca^{2+} have been used for a number of biomedical applications. One of the applications is alginate-mediated gene delivery. Alginate microspheres have been fabricated to carry genes of interest. The alginate microspheres can be delivered

to target tissues, where the gene is released. Because of the biodegradability of alginates, genes can be released in a controlled manner with the releasing rate depending on the rate of alginate degradation. Similarly, an alginate-based gel or matrix can be used to mediate controlled protein and drug delivery. In addition, alginate-based materials have been fabricated and used to mediate wound healing. Alginates can form a thin layer of gel when crosslinked by Ca^{2+}. Such a gel layer can be used to cover skin wound to prevent the loss of body fluids and bacterial infection. Alginate-based materials can be used to construct various forms of matrix scaffolds for the repair or regeneration of various tissue types such as the cartilage, liver, and bone. Alginate materials have also been used to construct capsules for cell transplantation. Cells can be encapsulated within alginate capsules and delivered to target tissues (Fig. 12.6). The alginate capsules can partially protect the enclosed cells from inflammation-induced injury.

Chitosan [12.15]. Chitosan is a linear polysaccharide composed of D-glucosamine units jointed by β-1,4-glycosidic bonds with randomly inserted *N*-acetylglucosamine units. Chitosan is a partially deacetylated derivative of chitin, which is a copolymer of randomly distributed *N*-acetylglucosamine and *N*-glucosamine units. A polymer molecule with more than 50% *N*-acetylglucosamine units is known as *chitin*, and that with more than 50% *N*-glucosamine units is called *chitosan*.

Chitosan and chitin are found in the shell of crabs and shrimps and are similar to cellulose in structure. Chitosan and chitin can be collected from these shellfish sources. Chitosan is a semicrystalline molecule and is usually stable. It is insoluble in water, but soluble in acidic solutions (pH ~5). The use of chitosan for tissue engineering relies partially on its gelation ability. Chitosan solutions can be gelled in methanol and under a high pH condition. A dried chitosan structure can be mechanically very strong. Chitosan molecules are usually positively charged and can bind to molecules with negative charges, such as glycosaminoglycans and alginates. A unique feature is that the charge density of chitosan is dependent on pH. Such a feature renders chitosan a candidate material for pH-controlled drug delivery.

When implanted in vivo, chitosan is degraded by lysozyme-catalyzed hydrolysis. Chitosan is disintegrated into oligosaccharides. The rate of chitosan degradation is inversely proportional to the degree of crystallinity. The crystallization of chitosan is regulated by deacetylation. Chitosan molecules with increased deacetylation on the *N*-acetylglucosamine units exhibit increased crystallinity and reduced degradation. In a highly crystal form, it takes several months to degrade chitosan scaffolds in vivo. Amorphous chitosan exhibits more rapid degradation.

Chitosan is a polysaccharide that can be fabricated into various forms of porous matrix. To produce a chitosan matrix, chitosan can be dissolved in acetic acid. The chitosan–acetic acid solution can be frozen and lyophilized to produce chitosan matrix. The freezing process induces the formation of ice crystals. The following lyophilizing process removes the ice crystals, allowing the formation of a porous matrix. The size of the pores can be controlled by altering the rate of ice crystal formation.

Chitosan can be used to make materials with various mechanical properties. A pure chitosan material without apparent pores exhibits elastic modulus ranging from 5 to 7 MPa. However, the introduction of pores reduces the elastic modulus and mechanical strength. Porous chitosan materials could have an elastic modulus as low as 0.1 MPa. The failure strain or maximal strain of chitosan is also dependent on the porosity of the material. Nonporous chitosan materials can be stretched to a strain about 0.3, whereas a porous

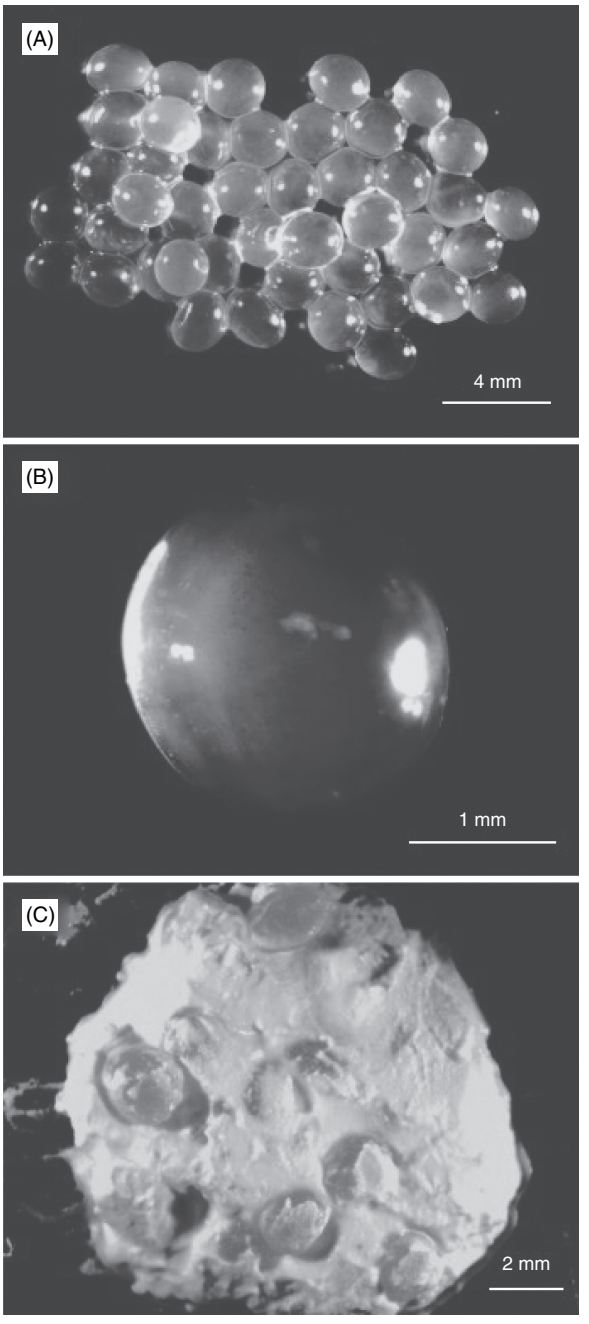


Figure 12.6. Cell-containing alginate beads for cell transplantation. Cells were encapsulated with alginate beads by dropping the cell–alginate mixture into an agitated bath of calcium chloride using a syringe. (A) A-low magnification image of the beads. (B) A higher-magnification image. (C) Cell–alginate beads were mixed into a calcium phosphate cement paste at a 54% volume fraction of alginate beads. (Reprinted with permission of John Wiley & Sons, Inc. from Weir MD et al: Strong calcium phosphate cement-chitosan-mesh construct containing cell-encapsulating hydrogel beads for bone tissue engineering, *J Biomed Mater Res Pt A*, published online Feb 15, 2006.)

chitosan material can be stretched to a strain about 1. Porous chitosan exhibits a nonlinear mechanical behavior, i.e., the mechanical behavior is dependent on the level of strain and stress. The material gains stiffness (with increased elastic modulus) when strain and stress are elevated.

Chemical modifications significantly influence the mechanical properties of chitosan materials. For instance, the coating of a chitosan material with hyaluronic acid significantly increases the tensile strength of the chitosan material. This mechanical reinforcement is due to the formation of tight bonds between positively charged chitosan molecules and negatively charged hyaluronic acid molecules. Such reinforced chitosan materials are suitable for repairing tissues with high mechanical loads such as cartilage. Furthermore, the incorporation of hydroxyapatite or other calcium containing materials into chitosan or chitin can generate composite materials with increased mechanical strength. Such materials can be used for bone repair or regeneration.

Given the molecular structure, mechanical properties, biocompatibility, and the capability of forming various matrix structures, chitosan and chitosan derivatives have been considered candidate materials for the engineering and regeneration of injured tissues and organs. Chitosan materials have been used to construct matrix scaffolds for seeding, culturing, and transplanting cells into target tissues. These materials have also been used as carriers for drug delivery. Several studies have shown that chitosan can serve as a gene transfer carrier. Genes mixed with chitosan-based materials have been successfully delivered to target cells in the knee joints in animal models. Chitosan-mediated gene transfer can also be carried out together with cell transplantation, enhancing therapeutic effects on target diseases.

Numerous investigations have shown consistently that chitosan and chitosan derivatives are relatively nontoxic and biocompatible. In particular, chitosan-based materials do not induce significant fibrous encapsulation around the implants. Although chitosan implantation induces leukocyte infiltration during the early period (within days), chronic inflammation does not occur significantly. The application of chitosan to cartilage repair and regeneration has demonstrated a beneficial effect on the recovery of injured cartilage tissues, such as stimulation of chondrocyte growth and expression of structural proteins. These observations have demonstrated the feasibility of using chitosan and chitosan derivatives as biomaterials for tissue regenerative engineering.

Starch [12.16]. Starch is composed of D-glucose and is a form of polysaccharide for the storage of energy in plants. It can be found in all plant seeds and tubers. There are two forms of starch: amylase and amylopectin. Amylose is a linear polymer with the D-glucose units joined by the α1,4-glycosidic bonds, whereas amylopectin contains branching polymer chains, in which the D-glucose units are joined by the α1,4-glycosidic bonds in the linear portion and those at branching points are joined by the α1,6-glycosidic bonds.

Cornstarch is usually used in biomedical research. Starch can be blended with chemical compounds such as ethylene vinyl alcohol and cellulose acetate to make matrices that can serve as engineering scaffolds or cell seeding/culture substrates. The fabricated matrix can be reinforced by mixing with hydroxyapatite to form a composite material. Polymer matrices of various forms can be prepared by injection molding. Starch and its composites have been used as substrates for cell culture and carriers for cell transplantation. Starch-based materials do not significantly influence the growth and function of cultured cells. These materials have also been used in vivo for several biomedical applications, including

drug delivery and tissue repair and regeneration. While starch may not be mechanically strong, the addition of reinforcement compounds may enhance the mechanical strength. As starch is composed of natural D-glucose, starch-based materials are usually nontoxic and biocompatible. Such features render starch a promising material for tissue regenerative engineering.

Glycosaminoglycans [12.17]. Glycosaminoglycans (GAGs) are linear polysaccharides composed of repeated disaccharide units. Each unit contains an uronic acid and amino sugar molecule. According to the type of the disaccharide unit, GAGs can be classified into several groups, including chondroitin sulfate, hyaluronate, keratan sulfate, and heparan sulfate. A chondroitin sulfate molecule is composed of a glucuronic acid and an *N*-acetylgalactosamine unit with a SO_4^- group on the 4 or 6 carbon position. A hyaluronate molecule contains a glucuronic acid and an *N*-acetylglucosamine unit. A keratan sulfate molecule contains a galactose and an *N*-acetylglucosamine unit with a SO_4^- on carbon position 6. A heparan sulfate molecule contains a D-glucuronic acid and an *N*-acetyl-D-glucosamine unit.

Glycosaminoglycans are found in mammalian connective tissues, such as the subcutaneous tissue, cartilage, and blood vessels. These molecules attach to core proteins and form proteoglycans, major extracellular matrix molecules known as *ground substances.* Heparan sulfate is found on the surface of vascular endothelial cells and is similar in structure and function to heparin, which is a potent anticoagulant.

Glycosaminoglycans are characterized by several general features, including the presence of a high density of negative charges, high hydrophilicity and water solubility, and low crystallinity. However, there are differences in material properties between various GAGs molecules. For instance, hyaluronate is a large molecule and has a high gel-forming capability. These molecules absorb a large amount of water, constituting a major part of the extracellular matrix. Because of the gel-forming capability, hyaluronate is often used as a media for drug-delivery or a material for tissue repair and regeneration. The composition of hyaluronate may be modified to construct materials with various properties. For example, partial esterification of the carboxyl groups of hyaluronate molecules reduces the water solubility of the polymer and increases its viscosity. Extensive esterification generates materials that form water-insoluble films or gels. Thus, hyaluronate gels with desired properties can be prepared by altering chemical compositions.

Compared to hyaluronate, other types of GAGs exhibit poor gel-forming capability in vitro. These GAGs alone have not been used extensively as biomaterials. However, negatively charged GAGs can bind tightly to positively charged molecules, such as chitin and chitosan, and form composite polymeric materials. Such composite materials can be used to form gels with various material properties by altering the relative contents of GAG and/or chitosan.

Glycosaminoglycans and glycosaminoglycan-based composite polymers have been used to construct hydrogels and matrices for various biomedical applications, such as drug delivery, cell seeding and transplantation, tissue repair, and tissue regeneration. Since GAGs are natural molecules, they are biocompatible and do not cause significant toxic and inflammatory reactions. These molecules can be degraded at different rates, depending on the compositions of the materials. For fully esterified hyaluronate membranes, the lifetime is several months. A reduction in esterification increases the rate of degradation.

METALLIC MATERIALS AS BIOMATERIALS [12.18]

Several types of metallic material have been used as biomaterials. These include iron (Fe), chromium (Cr), cobalt (Co), nickel (Ni), titanium (Ti), molybdenum (Mo), and tungsten (W). These materials have also been used to create alloys, providing favorable properties for the fabrication and performance of biomaterials. Typical examples of alloys include Co–Cr and Ti alloys. In addition, stainless steels have been developed and used as biomaterials. Because of their superior strength, elasticity, and endurance, metallic materials are often used for the repair and replacement of bones and joints. For the past several decades, these alloys have been well accepted for their performance, biocompatibility, and stability.

Stainless Steels as Biomaterials

Steels are artificially modified forms of iron with various carbon contents and are characterized by mechanical hardness, elasticity, and strength. Thus, steels are considered candidate materials for the repair of the skeletal system. The mechanical features of steels are dependent on the content of carbon and temperature. The crystal structure of iron, which determines the mechanical characteristics of iron, can be modulated by altering the treatment temperature and carbon concentration. At a relatively cold temperature, say 20ºC, iron atoms are organized into a unit structure with a *body-centered cubic* form. In each unit, eight neighboring atoms are symmetrically localized to the corners of an imaginary cube with one atom at the cube center. An increase in temperature to a certain degree can induce a transformation of the atomic structure from the body-centered cubic form into a unit structure with a *face-centered cubic* form, in which the atoms are localized to the faces of an imaginary cube. Carbon atoms can be integrated more easily into the iron unit structure with the face-centered cubic form than that with the body-centered cubic form. Thus, an appropriate alteration in temperature facilitates the integration of carbon into the iron. Carbon integration enhances the stability, hardness, and strength of the iron. However, the solubility of carbon in iron is relatively low. An excessive level of carbon induces carbon precipitation, a problem influencing the endurance and mechanical properties of the steel. An appropriate concentration of carbon is about 0.03%.

A common problem for using steels as biomaterials is corrosion. To resolve such a problem, chromium has been added to steels, rendering the steels stainless. For the manufacturing of biomaterials, chromium is used at a concentration ranging from 17 to 20%. However, the use of chromium introduces a problem: the mixing of chromium and carbon can form carbides, which enhances carbon precipitation. An approach used to mitigating carbide formation is to add nickel to the steel. Nickel can stabilize the iron structure, prevent carbide formation, and enhance corrosion resistance. Nickel is used at a concentration of 12–14% in steels as biomaterials. The chromium–nickel stainless steel has a high yielding strength (>170 MPa) and is considerably corrosion-resistant. However, corrosion can still occur when steel materials are implanted into the body. Thus, this material is often used to fabricate temporary implants such as fracture plates and nails.

Co–Cr Alloys as Biomaterials

Co–Cr alloys are metallic mixtures containing primarily Co and Cr as well as various amounts of other elements, such as Ni, Mo, Fe, C, Si, Mn, and Ti. There are two types of

Co–Cr alloy that have been fabricated and used as biomaterials: CoCrMo and CoNiCrMo alloys. The CoCrMo alloys are composed of Co 63–68%, Cr 27–30%, and Mo 5–7%. The CoNiCrMo alloys consist of Co 31.5–39%, Ni 33–37%, Cr 19–21%, and Mo 9–10.5%. These alloys possess high yielding strength (~450 MPa for the CoCrMo alloys, and 240–655 MPa for the CoNiCrMo alloys). The CoCrMo alloys can be cast, while the CoNiCrMo alloys can be forged, into implants of desired shapes. Both types of alloy are highly corrosion-resistant. These alloys are suitable materials for the fabrication of artificial bones and joints.

Titanium and Titanium Alloys as Biomaterials

Titanium is a metallic material that is characterized by superior hardness, corrosion resistance, and lightness (4.5 g/cm^3 compared to 7.9 g/cm^3 for iron). Given such features, titanium has been used as a biomaterial for the replacement of bones and joints. Titanium materials usually contain several elements, such as nitrogen, carbon, hydrogen, oxygen, and iron. The contents of these elements are very low, with nitrogen ranging from 0.03–0.05%, carbon about 0.1%, hydrogen about 0.015%, oxygen 0.18–0.40%, and iron 0.2–0.5%.

Titanium has been used to make alloys. A typical titanium alloy is $Ti_6A_{l4}V$, which contains ~6% aluminum, ~4% vanadium, ~90% titanium and low contents of nitrogen, carbon, hydrogen, oxygen, and iron. The addition of aluminum and vanadium increases the strength and corrosion resistance of the titanium alloy. For instance, pure titanium possesses yielding strength ranging from 170 to 485 MPa, whereas $Ti_6A_{l4}V$ exhibits yielding strength ~795 MPa. Other types of titanium alloys have also been created to provide more features suitable for the performance of titanium alloys as biomaterials. Examples include $Ti_{13}V_{11}Cr_3Al$ and $Ti_{13}Nb_{13}Zr$. The $Ti_{13}V_{11}Cr_3Al$ alloy contains ~13% vanadium, ~11% chromium, ~3% aluminum, and ~73% titanium. The addition of these elements enhances the strength of the titanium alloy. The $Ti_{13}Nb_{13}Zr$ alloy is composed of ~13% niobium, ~13% zirconium, and ~74% titanium. The addition of these elements enhances the corrosion resistance of the titanium alloy.

Potential Problems with Metallic Materials

There are two potential problems with the use metallic materials as biomaterials. These are corrosion and bioincompatibility. These problems potentially influence the performance and endurance of metallic biomaterials, especially when these materials are used to replace bones and joints that are subject considerably high mechanical loads.

Corrosion is a process of metal degradation induced by chemical reactions, primarily oxidization. When subject to water-based solutions containing dissolved oxygen and ions such as chloride and hydroxide, metal atoms react with these species and form oxide or hydroxide compounds. These compounds detach from the metal surface and dissolve in the solution. The metal is degraded gradually.

Since the physiological fluids in the human body contain oxidative chemical species, providing a harsh environment for metallic implants, corrosive degradation of metals occur at various rates, depending on the type of the metal and the local environment. Iron and steels can be corroded easily in the presence of water and oxygen, whereas chromium, nickel, and titanium are considerably corrosion-resistant. The concentration of oxygen in the interstitial fluids varies considerably in the different compartments of the body. Such

variations significantly influence the rate of metal corrosion. An increase in oxygen concentration facilitates metal corrosion.

In addition to chemical factors, physical factors such as mechanical loads and friction accelerate metal corrosion. For instance, repetitive deformation of metal implants can induce mechanical fatigue, which facilitates chemical corrosion, a phenomenon known as *fatigue corrosion*. Shearing motions between two implants induces damage of the protective passivation layer, contributing to corrosion, which is known as fretting corrosion. These mechanical factors should be taken into account in the design of metallic implants.

There are several methods that can be used to measure the rate of corrosion. These include the estimation of the number of ions liberated from a metal per unit time, the measurement of the depth of the metal corroded away, and the measurement of the loss of the metal weight due to corrosion per unit time. These are fairly straightforward methods and can be applied to in vitro tests and in vivo tests in animal models.

Several approaches can be used to reduce the rate of metal corrosion. For steel-based implants, the addition of chromium can significantly reduce the rate of implant corrosion, since chromium can form a stable passive chromium oxide film on the steel surface. Modulation of carbon contents may also influence the rate of steel corrosion. Since excessive carbon content induces carbon precipitation, which may facilitate steel corrosion, lowering the carbon content is an effective approach to reduce the rate of steel corrosion. Other metallic materials, such as cobalt and titanium, are considerably resistant to corrosion, since these metals are inert in physiological fluids and can form a passivating oxide film. Alloys based on these metallic materials exhibit improved resistance to corrosion.

Although corrosion-resistant alloys are used, corrosion still occur in artificial metallic bones and joints. Metallic corrosion often causes local swelling and pain, and influences the function of the artificial implants. Corrosion can be detected by x-ray examination. At surgery, inflammatory reactions and metal debris can be found in tissue surrounding the metallic implants. Because corrosion accelerates metal wear and fatigue failure, metallic implants with severe corrosion should be replaced.

CERAMICS AS BIOMATERIALS [12.19]

Ceramics are a group of inorganic, polycrystalline, and refractory materials, including metallic oxides, carbides, silicates, hydrides, and sulfides. Ceramics are characterized by several physical properties, including the hardness, inertness to physiological ionic fluids, and resistance to high compressive stress. Given such properties, ceramics have been used as biomaterials for the replacement of bones and teeth. In terms of their interaction with biological tissues, ceramics can be classified into several types: bioactive, bioinert, and biodegradable ceramics. The characteristics and applications of these ceramics are discussed here.

Bioactive Ceramics

Bioactive ceramics are ceramics that can interact and form bonds with surrounding tissues. Such ceramics can be used as "adhesives" for prostheses, enhancing the attachment of prostheses to adjacent tissue. Given the mechanical strength and hardness, this type of ceramic is often used as adhesive for orthopedic applications such as the repair and replacement of bones and joints. A major type of bioactive ceramic is glass ceramics. This

type of ceramics is constructed with SiO_2, CaO, Na_2O, and P_2O_5. The adhesive properties of glass ceramics are dependent on the formation of a surface layer composed of calcium phosphate and silicon oxide (SiO_2).

Bioactive glass ceramics may not only serve as structural materials, but also play a role in regulating the function of host cells. For instance, silicon–calcium glass ceramics, once implanted into the skeletal system, can release silicon and calcium ions, which stimulate osteoblast growth and differentiation. Such a process involves genes that encode proteins responsible for the regulation of cell mitosis and differentiation. In addition, a controlled release of soluble calcium and silicon from a composite material composed of bioactive glass and resorbable polymer has been shown to enhance the generation of vascularized soft tissues. Thus, by controlling the compositions and releasing rate of silicon and calcium, bioactive glass ceramics can be used to mediate the growth of bone tissues.

Bioactive glass ceramics have been used not only for bone replacement but also for soft tissue regeneration. Recent studies have shown that bioglass-coated polystyrene scaffolds stimulate the proliferation of cultured fibroblasts. Such an influence is dependent on the concentration of the coating bioactive ceramics. An excessive concentration of bioglass induces a reduction in the rate of cell proliferation, in association with a change in cell shape. A limited number of experiments have demonstrated that low concentration of bioglass (0.01%) may stimulate the expression and release of vascular endothelial growth factor. In vivo experiments have shown that bioglass-coated scaffolds can be well tolerated for up to 42 days when implanted subcutaneously in the rat. While fibroblasts actively adhere to poly(glycolic acid) (PGA) meshes, they rarely adhere to the bioglass particles. These observations demonstrate that bioactive glass ceramics can be used as compounds for the fabrication of composite scaffolds for soft tissue engineering.

Bioinert Ceramics

Bioinert ceramics, including alumina, zirconia, and carbons, have been used as biomaterials. These ceramics are generally corrosion-resistant and wear-resistant. They do not cause significant toxic, inflammatory, and allergic reactions and are relatively biocompatible. These ceramics possess common ceramic characteristics such as hardness, low friction, and resistance to compressive stress. Because of these characteristics, bioinert ceramics are often used to fabricate bone plates, screws, femoral heads, and middle ear ossicles.

Alumina, or aluminum oxide (Al_2O_3), is a typical type of bioinert ceramics. Alumina exists in nature as crystal corundum. A crystal form of alumina can be synthesized by applying fine alumina powder to a flame of mixed oxygen and hydrogen. The mechanical strength of synthetic alumina is dependent on the grain size and porosity. Alumina with small grains and low porosity has high strength. A minimum of flexural strength 400 MPa and elastic modulus 380 GPa is required for using alumina as an orthopedic biomaterial. In general, alumina is a material that is characterized by hardness, low friction, inertness to physiological fluid environment, low toxicity, and low immunogenicity. These properties render alumina a suitable orthopedic biomaterial. Alumina has been used to fabricate artificial joints and total hip prostheses.

Biodegradable Ceramics

Biodegradable ceramics are ceramics that can be degraded and absorbed in a biological system. A number of biodegradable ceramics have been developed and used as

biomaterials. These include calcium phosphate, aluminum calcium phosphate, coralline, zinc-calcium-phosphorous oxide, and zinc sulfate–calcium phosphate. Most biodegradable ceramics contain calcium. Biodegradable ceramics are often used for constructing artificial bones and drug delivery carriers, as well as to repair bone damages due to trauma, tumor removal, and pathological disorders. A biodegradable ceramic implant may serve as a temporary frame that guides the formation of the shape of remodeling tissues. The absorbed ceramic material can be replaced by growing tissue, eventually restoring the natural structure and function of the damaged tissue. Thus, biodegradable ceramics are suitable materials for orthopedic tissue regeneration.

Calcium phosphate is a typical biodegradable ceramic and has been used to fabricate artificial bones. Calcium phosphate can be crystallized into a form known as hydroxyapatite. Crystallized calcium phosphate can be very stiff and strong with an elastic modulus up to ~100 GPa. Note that the hardest tissue in our body, such as compact bones, dentin, and dental enamel, is composed of the crystal form of calcium phosphate with structure similar to hydroxyapatite. Thus, calcium phosphate is commonly used in orthopedic regenerative engineering for the replacement and repair of malfunctioned bones. Calcium phosphate-based biomaterials are usually nontoxic and biocompatible.

The biocompatibility of calcium phosphate-based bioceramics has been a topic of research in orthopedic regenerative engineering. Extensive investigations have shown that osteoblasts exhibit normal growth patterns when cultured on calcium phosphate materials. In addition, calcium phosphate biomaterials exert a stimulatory effect on the expression of osteogenic proteins and the proliferation of osteoblasts. These observations demonstrate the suitability of using calcium phosphate compounds as biomaterials for orthopedic regenerative engineering.

Calcium phosphate ceramics can also be used to fabricate drug delivery devices. Drugs, hormones, or growth factors can be packed into biodegradable calcium phosphate ceramics for implantation and delivery into target tissues. With the degradation of the ceramic, drugs or proteins can be gradually released. By controlling the density or compounds of the drug delivery material, the rate of substance release can be regulated. Biodegradable ceramics can be mixed with biodegradable polymers, such as poly *d*,*l*,-lactic acid-polyethyleneglycol copolymer (PLA-PEG) to form composite materials. Such an approach enhances the capability of controlling the rate of substance release. A composite ceramic material can also serve as a scaffold for tissue regeneration. Biological active substances can be integrated into the scaffold for controlled substance release, which enhances the regeneration of injured tissues.

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