

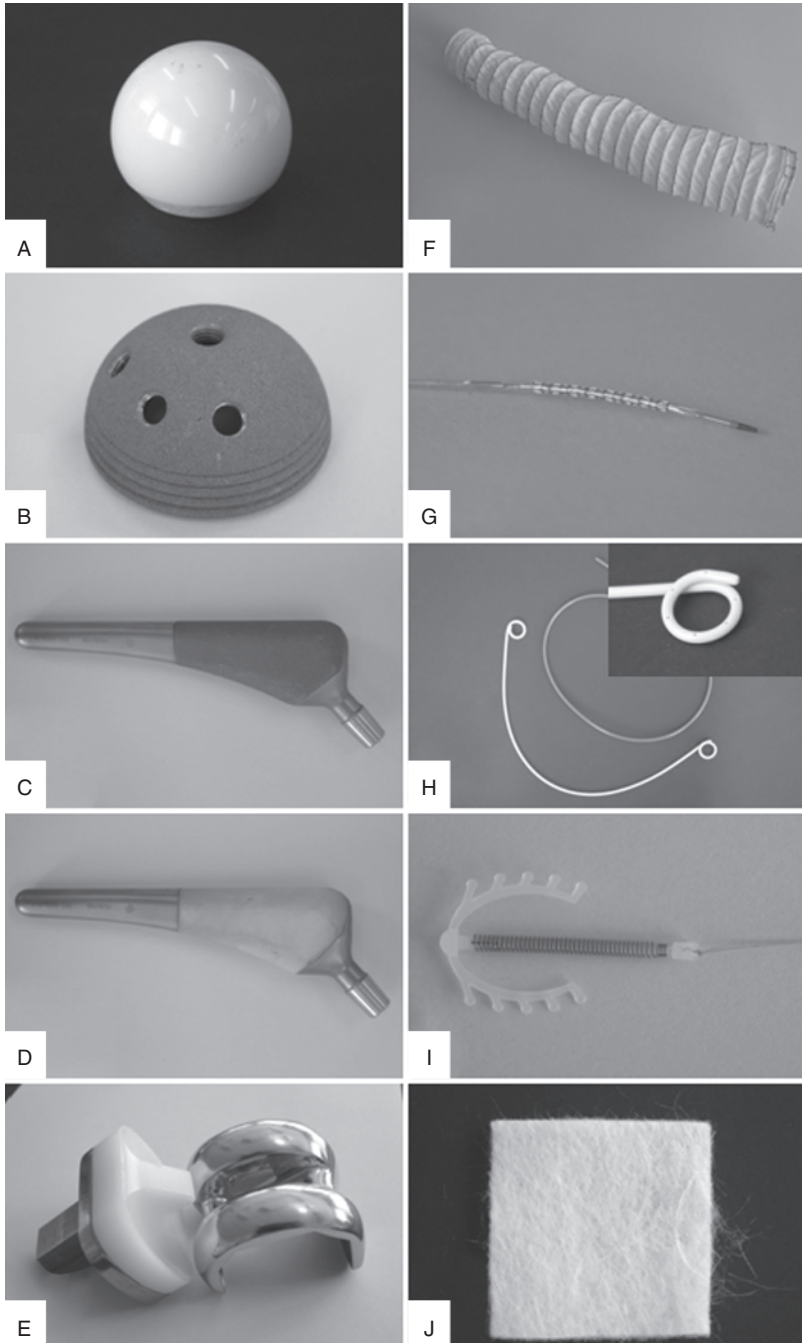
# HISTORY OF BIOMIMETIC, BIOACTIVE, AND BIORESPONSIVE BIOMATERIALS

Matteo Santin and Gary Phillips

## 1.1 THE FIRST GENERATION OF BIOMATERIALS: THE SEARCH FOR “THE BIOINERT”

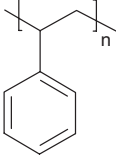
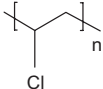
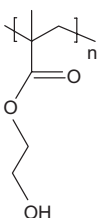
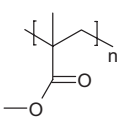
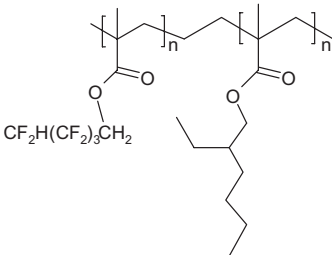
Since it was first perceived that artificial and natural materials could be used to replace damaged parts of the human body, an “off-the-shelf” materials selection approach has been followed. These materials, now referred to as “first-generation” biomaterials, tended to be “borrowed” from other disciplines rather than being specifically designed for biomedical applications, and were selected on the basis of three main criteria: (1) their ability to mimic the mechanical performances of the tissue to be replaced, (2) their lack of toxicity, and (3) their inertness toward the body’s host response [Hench & Polack 2002].

Following this approach, pioneers developed a relatively large range of implants and devices, using a number of synthetic and natural materials including polymers, metals, and ceramics, based on occasional earlier observations and innovative approaches by clinicians. Indeed, many of these devices are still in use today (Figure 1.1A–J). A typical example of this often serendipitous development process was the use of poly(methyl methacrylate) (PMMA) to manufacture intraocular and contact lenses. This material (Table 1.1) was selected following observations made by the clinician Sir Harold Ridley that fragments of the PMMA cockpit that had penetrated into



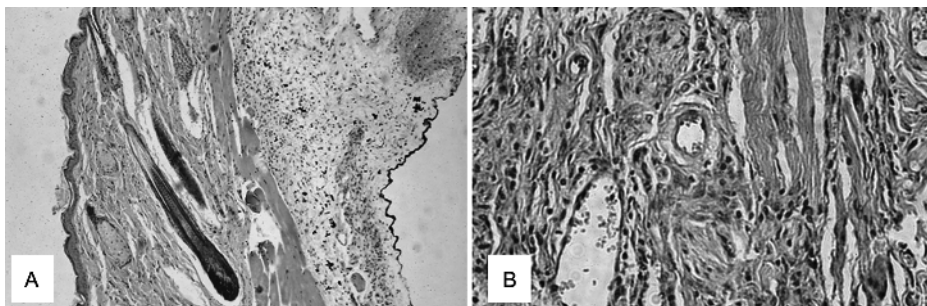
**Figure 1.1.** Examples of medical implants and their components. (A–F) Orthopedic implant components: (A) femor head, (B) hip socket, (C) titanium stent coated with porous titanium foam, (D) titanium stent coated with hydroxapatite coating, (E) knee implant components. (F–J) Other types of biomedical implants: (F) vascular graft, (G) coronary stent, (H) ureteral stent (insert shows detail of the device pig-tail end), (I) intrauterine device, (J) wound dressing.

TABLE 1.1. Chemical Structure of Typical Polymeric Biomaterials

Polymer	Structure
Polystyrene	
Poly(vinyl chloride)	
Poly(2-hydroxyethyl methacrylate)	
Poly(methyl methacrylate)	
Chronoflex 80A poly(urethane)	-(-PHECD-DesmW-BD-)- <sub>n</sub> Polyethylene glycol polyurethane
Hydrothane poly(urethane)	
PFPM/PEHA75/25	
Poly(octofluoropentyl methacrylate/ethylhexyl methacrylate)	

the eyes of World War II pilots induced a very low immune response (see Section 1.3.1) [Williams 2001].

Also against the backdrop of the Second World War, a young Dutch physician named Willem Kolff pioneered the development of renal replacement therapies by taking advantage of a cellophane membrane used as sausage skin to allow the dialysis of blood from his uremic patients against a bath of cleansing fluid [Kolff 1993]. Later, in the early 1960s, John Charnley, learning about the progress materials science had made in obtaining mechanically resistant metals and plastics, designed the first hip joint prosthesis able to perform satisfactorily in the human body [Charnley 1961]. These are typical examples of how early implant materials were selected; however, it was soon



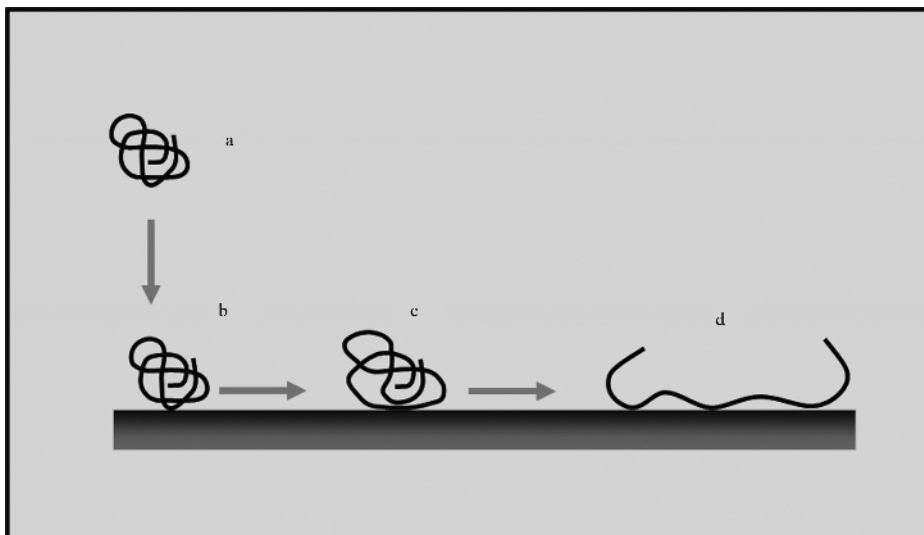
**Figure 1.2.** Fibrotic capsule formed around a commercial polyurethane biomaterial: (A) general view, (B) high magnification showing histological details of the fibrotic tissue.

recognized that the performance of these materials was often limited by the host response toward the implant, which often resulted in inflammation, the formation of a fibrotic capsules around the implant, and poor integration with the surrounding tissue (Figure 1.2A,B) [Anderson 2001].

The poor acceptance and performance of early biomaterials indicated that their interaction with body tissues was a complex problem that required the development of more sophisticated products. As a result, it was realized that inertness was not a guarantee of biocompatibility. Indeed, in 1986, the Consensus Conference of the European Society for Biomaterials put in place widely accepted definitions of both biomaterials and biocompatibility, which took into account the interaction between an implanted material and biological systems. According to these definitions, a biomaterial was “a nonviable material used in a medical device intended to interact with biological systems,” whereas biocompatibility was defined as “the ability of a material to perform with an appropriate host response in a specific application” [Williams 1987]. Perhaps for the first time, materials scientists and clinicians had an agreement on what their materials should achieve. However, as you will see later in this chapter and throughout this book, the ever-expanding fields of biomaterials science and tissue engineering call for newer and more specific definitions.

### 1.1.1 Bioinert: Myth, Reality, or Utopia?

In the 1980s, the formation of a fibrotic capsule “walling off” many biomedical implants from the surrounding tissue triggered biophysical and immunological studies that identified the molecular, biochemical, and cellular bases of the host response that caused the formation of this interposed and pathological tissue [Williams 1987; Anderson 1988]. In particular, many studies highlighted that this host response could not be avoided due to the immediate deposition of proteins onto the material surfaces and their change of conformation [Norde 1986]. The material surface-induced conformational changes transformed the host proteins into foreign molecules, antigens, which were capable of eliciting a foreign body response by the host (Figure 1.3).



**Figure 1.3.** Schematic representation of protein denaturation upon adsorption on biomaterial surface: (a) soluble protein approaches biomaterial surface, (b) protein adsorbs to material surface, (c) protein starts to unfold through interactions with material surface, (d) protein acquires an antigenic conformation.

Biomaterial surfaces contacted by blood, saliva, urine, cerebrospinal and peritoneal fluids, or tears cannot avoid interactions with proteins and other molecules that are naturally contained in the overlying body fluid [Santin et al. 1997]. As a consequence, the implant surface is rapidly covered by a biofilm that masks the material surface and dictates the host response. It is clear, therefore, that as a result of these processes, no biomaterial can be considered to be totally inert. However, although they are subjected to a continuous turnover, it is a fact that proteins (and more broadly, all tissue macromolecules) retain their native conformation during the different phases of tissue formation and remodeling. Hence, for the past two decades the scientific community has striven for the development and synthesis of a new generation of biomaterials that are able to control protein adsorption processes and/or tissue regeneration around the implant.

## **1.2 THE SECOND GENERATION OF BIOMATERIALS: BIOMIMETIC, BIORESPONSIVE, BIOACTIVE**

In conjunction with the findings regarding the biochemical and cellular bases of host response toward implants, material scientists began their search for biomimetic, bioresponsive, and bioactive materials capable of controlling interactions with the surrounding biological environment and that could participate in tissue regeneration processes.

The move toward the synthesis and engineering of this type of biomaterial was initiated by the discovery of ceramic biomaterials that were proven to favor the integration of bony tissue in dental and orthopedic applications [Clarke et al. 1990], as well as by the use of synthetic or natural polymers [Raghunath et al. 1980; Giusti et al. 1995]. Second-generation metals also emerged that were able to improve the integration with the surrounding tissue.

### **1.2.1 Hydroxyapatite (HA) and Bioglass®: Cell Adhesion and Stimulation**

The ability of HA, and Bioglass to integrate with the surrounding bone in orthopedic and dental applications strictly depends on the physicochemical properties of these two types of ceramics, which will be described in Chapter 7. Here, it has to be mentioned that since their early discovery and use in surgery, the good integration of these biomaterials with bone, the osteointegration, depends on mechanisms of different nature that have triggered new concepts/definitions and new technological targets among scientists.

Although HA osteointegration can intuitively be attributed to their ability to mimic the bone mineral phase (see Chapter 3), the mechanisms underlying Bioglass-induced bone formation are not as clearly identifiable. It is known that HA favors the deposition of new bone on its surface by supporting osteoblast adhesion and by promoting the chemical bonding with the bone mineral phase [Takeshita et al. 1997]. Furthermore, the ability of HA to induce bone formation when implanted intramuscularly in animals, allegedly via the differentiation of progenitor cells, clearly shows their osteoinductive potential; indeed, osteoinductivity is defined as the ability of a biomaterial to form ectopic bone. Conversely, Bioglass osteoinductivity seems to be intrinsic to its degradation process whereby (1) growth factors remain trapped within the gel phase formed during the degradation of the material and, consequently, released to the cells upon complete material dissolution; (2) structural proteins of the extracellular matrix (ECM) such as fibronectin form strong bonds with particles of the degrading material; and (3) silicon ions stimulate osteoblast (and allegedly progenitor cell) differentiation and, subsequently, the production of new bone [Xynos et al. 2000]. Regardless of the type of ceramic used, it is now widely recognized that the topographical features of these types of biomaterials are also fundamental to their bioactivity. For example, the absence of porosity or porosity of different sizes may lead to no osteointegration or to only poor bone formation [Hing et al. 2004].

### **1.2.2 Collagen, Fibrin Glue, and Hyaluronic Acid Hydrogels: Presenting the ECM**

The use of collagen, fibrin, and hyaluronan, which are all natural components of the ECM, was born from scientists' intuition that tissue cells recognize these biopolymers as natural substrates to form new tissue.

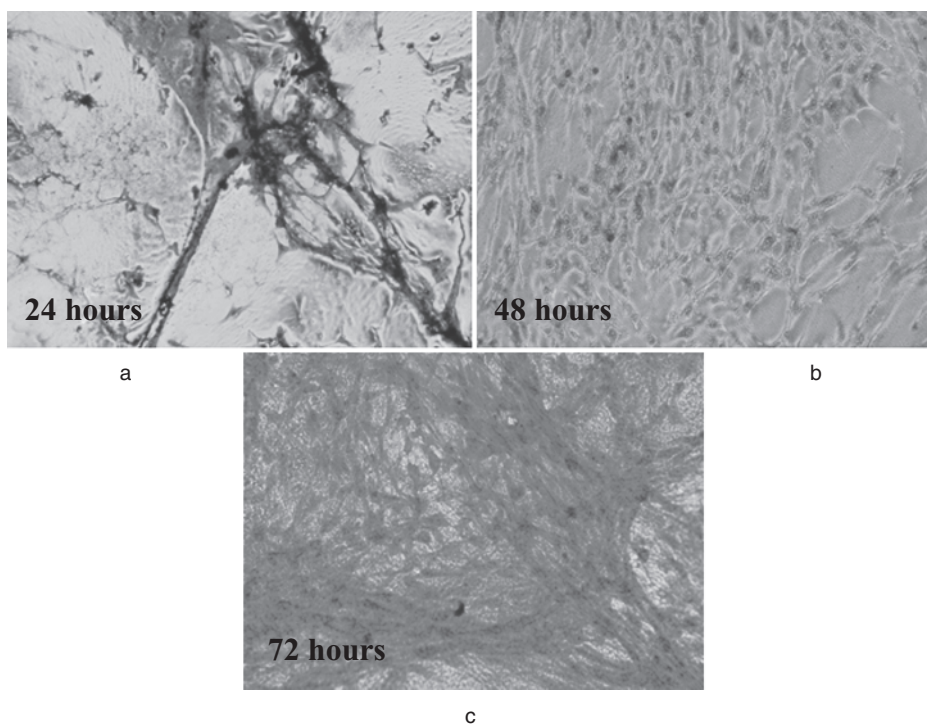
Fundamental to the application of these biological materials was an appreciation of their physicochemical and biological properties. Collagen is the most ubiquitous

structural protein in the human body and the principal constituent of ECM in connective tissues [Rivier & Sadoc 2006]. It consists of a tightly packed structure composed of three polypeptide chains that wind together to form a triple helix [Rivier & Sadoc 2006]. These collagen molecules then associate to form collagen fibrils. A number of reviews are available on the structure of the different types of collagen found throughout the body [Engel & Bachinger 2005; Rivier & Sadoc 2006]. Collagen plays a key role in the wound healing process and the development of cartilage and tendons, and it is known that collagen can favor the formation of HA on its structure, thus inducing bone mineralization [Zhai & Cui 2006]. As part of the ECM, collagen provides a suitable milieu for cell proliferation, migration, and differentiation during the production of new tissue via its biodegradation and tissue remodeling. Collagen is, therefore, a natural biomaterial whose inherent potential has been exploited by biomaterials scientists in ligament replacement and other tissue engineering applications [Rothenburger 2001; Gentleman et al. 2003, 2006; Boccafroschi 2005; Kutschka et al. 2006], and collagen types I and IV have been commercialized as dermal substitutes [Jones et al. 2006].

The use of fibrin as a biomaterial was founded on the fact that fibrin clots are self-assembling networks with biological and physicochemical attributes that have the potential to be used in a number of biomedical applications. Three-dimensional (3D) porous fibrin networks are formed through a series of events during the blood coagulation cascade, resulting in the formation of a biopolymer gel material. The structure of the gel is determined by the thrombin-mediated conversion of fibrinogen to fibrin and the subsequent self-assembly of the fiber network [Helgersson et al. 2004]. Fibrin glue saw its first application as a surgical adhesive, but in the emerging era of tissue engineering, it has been suggested by many scientists as a suitable gel for cell encapsulation (Figure 1.4a–c) [Bach et al. 2001]. This is due to the fact that fibrin clots provide a structural scaffold that allows the adhesion, proliferation, and migration of cells important in the wound healing process and, when associated with proteins as a clot, has intrinsic biological properties that support and control, to some extent, cell differentiation. Fibrin-based biomaterials also benefit from the fact that they are naturally remodeled and resorbed as part of the fibrinolytic processes associated with the cellular deposition of a new ECM as part of the normal wound healing processes [Helgersson et al. 2004] (see Section 1.3.1).

Hyaluronan, one of the main components of cartilage (see Chapter 3), has been chemically modified and commercialized to favor cartilage and skin regeneration (see Chapter 6) [Barbucci et al. 1993]. Hyaluronan consists of a single polysaccharide chain with no peptide in its primary structure, and it has a molecular weight that reaches millions of Daltons [Fraser et al. 1997]. The biological properties of this molecule are imparted by specific hyaluronan binding sites present in other ECM molecules and on the surface of cells [Fraser et al. 1997]. A number of proteins exist—the hyaladherins—that have the ability to recognize hyaluronan and result in the binding of hyaluronan molecules with proteoglycans to reinforce the structure of the ECM [Fraser et al. 1997; Day & Prestwich 2002]. At the molecular and cellular levels, it is now known that these biomolecules are able to support tissue regeneration because of the presence of specific bioligands that are able to recognize receptors on the cell membrane which, in turn, stimulates cell functions [Turley et al. 2002] (see Section 1.3.1.1).





**Figure 1.4.** Collagen deposition by osteoblasts encapsulated in a fibrin hydrogel. Incubation times: (a) 24 hours, (b) 48 hours, and (c) 72 hours.

Furthermore, the physicochemical and biochemical properties of the three molecules discussed here can favor the interaction with other tissue components, forming organized macromolecular structures capable of conferring on tissues their specific mechanical properties (see Chapter 2). As previously mentioned, it is known that collagen can favor the formation of HA on its structure, thus inducing bone mineralization [Zhai & Cui 2006], and that hyaluronan is capable of interacting with other proteins to form macromolecular structures that are able to retain a relatively high water content. This high water content thus acts as an effective shock absorber in cartilage and ocular tissues [Fraser et al. 1997]. However, these substrates have shown some drawbacks and limitations. Although they provided the regenerating tissue with some important properties, some others were missing. As mentioned above, one of the main benefits of using these biopolymers in clinical applications is that they promote biorecognition. However, in most cases, this biorecognition is not specific for the type of cells that need to be targeted to induce tissue regeneration. For example, collagen and fibrin, as well as other important ECM proteins (e.g., fibronectin), present in their structure the arginine–glycine–aspartic acid (RGD) sequence that is recognized by most tissue cells as well as by inflammatory cells such as monocytes or macrophages [Phillips & Kao 2005]. As a result of this relatively broad spectrum of cell recognition, collagen-based bioma-



terials have been shown to induce an immune response in patients, which often leads to the formation of fibrotic tissue (see Section 1.1). In addition, collagen-based implants, either extracted from mammalian sources or from recombinant bacteria, may not represent the composition of the real ECM and miss some components required to regulate the process of tissue regeneration. For example, it has been proven that physiological skin ECM collagen presents, on its surface, proteins such as  $\alpha 1$ -microglobulin, which is capable of modulating the activity of resident macrophages [Santin & Cannas 1999]. The absence of this protein in pathological tissues (e.g., scar tissue) and collagen implants seems to lead to a collagen-induced activation of immunocompetent cells. The modulating action of the  $\alpha 1$ -microglobulin is likely to be only one aspect of a multifaceted process leading to the regulation of the immunocompetent cell activity in connective tissues. Therefore, collagen-based implants, although representing a step forward in developing biomaterials for tissue regeneration, address the problem in a relatively simplistic manner. A plethora of immunomodulators are present in physiological tissues, which may need to be taken into account to improve the performance of the collagen-based biomaterials.

Similarly, hyaluronan is recognized by cell receptors such as CD44, which are present on the membrane of both tissue and inflammatory cells. The role of this polysaccharide in nature is tuned by its molecular weight [Mytar et al. 2001; Teder et al. 2002]. It has been proven that low molecular weight hyaluronan is fundamentally proinflammatory and angiogenic, thus promoting the formation of granular tissue. Conversely, relatively high molecular weight hyaluronan seems to prevent angiogenesis and inflammation. Thus far, at the clinical level, relatively high molecular weight hyaluronan and its ester derivatives have been used, but not enough information has been collected to optimize the molecular weight of this polysaccharide. More accurate studies may be able to define the appropriate physicochemical characteristics of hyaluronan-based biomaterials to encourage some degree of vascularization and inflammation, which are required for a physiological regeneration.

Finally, although the use of fibrin glue as an adhesive material in surgery is widespread and successful, the tissue regeneration potential of this natural hydrogel has been proven to be limited unless key growth factors are loaded in its mesh. As for collagen and hyaluronan, this is not surprising considering that the main function of fibrin is to stop the bleeding and provide the damaged tissue with a temporary scaffold for its repair.

Each of the biopolymers mentioned in this section have reached the market and provided good, although not always satisfactory, clinical performances. Nevertheless, the use of these materials in clinics has opened the door to the development of biomimetic biomaterials able to mimic the structure, biochemistry, and biofunctionality of tissue components.

### 1.2.3 Chitosan and Alginate: Replacing the ECM

As previously mentioned, the ECM is a structural, 3D network consisting of a number of macromolecules and polyelectrolytes including fibronectin, proteoglycan, collagen, laminin, and glycosaminoglycans. This macromolecular architecture mediates the

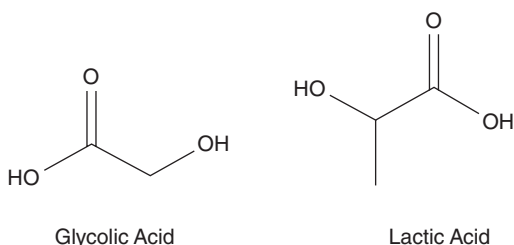
interaction of cells with the substrate and provides a scaffold for cell migration and proliferation [Zaidel-Bar et al. 2004]. In addition to using molecules that naturally occur as components of the ECM, a number of attempts have been made to replace this scaffold using polymers from other natural sources either individually or in polyelectrolyte complexes to form hydrogels or solid porous constructs [Hayashi 1994; Madhally & Matthew 1999]. Two of the principal macromolecules used for these applications are chitosan, the deacetylated product of chitin from the exoskeleton of shellfish and alginate, derived from brown algae, both of which have been used as biodegradable materials for wound healing, tissue reconstruction, cell encapsulation, and drug delivery [Tomihata & Ikada 1997; Madhally & Matthew 1999; Jayakumar et al. 2006; Roughley et al. 2006]. The use of these polymers, either individually or in combination with others to support and reinforce the regenerating tissue, have underpinned the ever-expanding discipline of tissue engineering [Minuth et al. 1998; Madhally & Matthew 1999].

However, although generally accepted to have favorable biocompatibility and toxicity profiles (Rao & Sharma 1997), it has been reported that chitosan polymers used as soluble polymeric carriers for intravenous administration or following particulate degradation may induce cellular toxicity (Carreño-Gómez & Duncan 1997). More recent studies have suggested that hydrogel scaffolds containing collagen, chitosan, and HA elicit a severe inflammatory response associated with an inadequate ingrowth of neovascularization from the surrounding host tissue when implanted in dorsal skinfold chambers of mice [Rücker et al. 2006]. It is apparent, therefore, that as the applications for these materials are explored, their biocompatibility may be altered, depending on the situation.

Like chitosan, alginate is a natural polymer that can be prepared on its own into a number of physical forms, including beads for cell encapsulation and porous sponges suitable for cell ingrowth and neovascularization. The materials produced are relatively nontoxic and noninflammatory, although their applications tend to be limited due to poor mechanical properties and cell performance. These shortcomings have been addressed by combining the alginate with other materials including chitosan (Rosca et al. 2005).

#### **1.2.4 Poly(Lactic/Glycolic) Acid Copolymers: Encouraging Tissue Remodeling by Safe Biodegradation**

The development of a second generation of biomaterials found its inspiration in nature, not only by trying to mimic the biochemical and structural features of natural tissue, but also by taking into account its ability to undergo resorption during the physiological turnover typical of the tissue remodeling processes (see Chapters 2 and 3). In the 1980s, materials scientists recognized the importance of biodegradation in allowing tissue ingrowth. Thus far, biodegradable biomaterials, although ideal for tissue regeneration, have been confined to the manufacture of implants not requiring load-bearing capabilities. In an attempt to synthesize polymers that are able to biodegrade at a rate tuned with tissue regeneration and to ensure the release of degradation by-products that are not toxic for the host, biomaterials based on natural molecules such as lactic and glycolic acid have been developed. Materials scientists have exploited methods of syn-



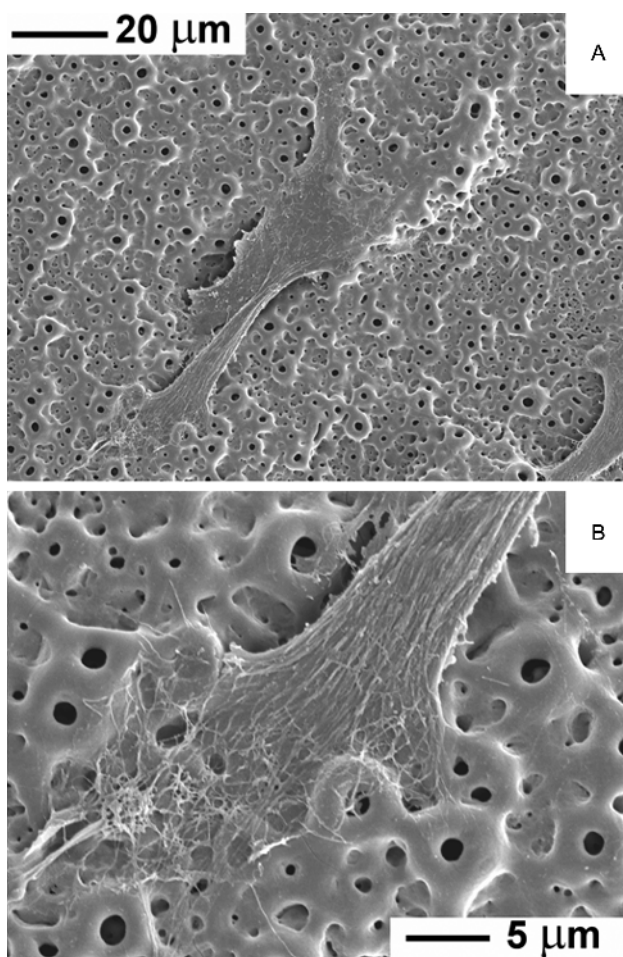
**Figure 1.5.** L-lactic and L-glycolic acid monomers utilized for the synthesis of poly(lactic/glycolic) acid copolymers.

thetic chemistry to produce polymers of these natural molecules and combinations of the two in the form of copolymers (Figure 1.5) [Grayson et al. 2004]. It has since been demonstrated that these polymers can degrade into very basic molecular species such as CO<sub>2</sub> and H<sub>2</sub>O, thus ruling out the formation of any toxic by-product. In addition, the combination of the two monomers in different proportions in copolymer formulations allows the tuning of their degradation rate, depending on the required biomedical application. However, relatively recent studies have demonstrated that before complete dissolution, fragments of these polymers elicit an inflammatory response, thus altering tissue regeneration [Grayson et al. 2004].

### 1.2.5 Porous Metals: Favoring Mechanical Integration

The transition that took place in the 1980s with the movement toward second-generation biomaterials also involved metals. During the past four decades and, indeed, to the present day, materials scientists and biomedical companies have been facing the need to provide clinicians with implants that are able to sustain relatively high and protracted biomechanical stresses. In most cases, these stresses cannot be sustained unless metals are used in the implant manufacture. However, ensuring the integration of metal implants into tissues remains a significant challenge. A major step forward toward this objective has been the improvement of both device design and surface properties, the former leading to biomechanically performing implants, the latter to mechanical integration with the surrounding tissue [Takemoto et al. 2005]. Indeed, the improved distribution of mechanical loads transferred to orthopedic and dental implants has reduced mechanical stresses on the tissue/implant interface and the consequent failure of the implants caused by stress-induced bone fractures. The introduction of surface porosity has led to an enhanced grip of the tissue during its growth around metal implants. At the cellular level, it has been proven that a rough surface can improve cell adhesion to metal implants and, as a consequence, their colonization of the implant surface (Figure 1.6) [Sandrini et al. 2005].

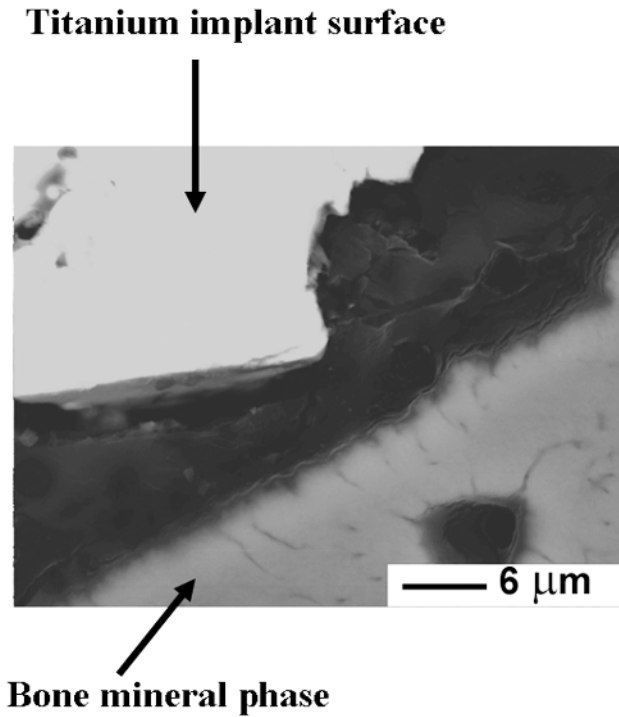
As a result of the development of improved metal implants, the thick fibrotic capsule that typically formed around the first generation of metal orthopedic and dental



**Figure 1.6.** Osteoblast adhesion on rough implant surface: (A) adhering osteoblast, (B) osteoblast focal adhesion establishing contact with rough surface and secretion of collagen fibrils.

implants has been reduced to a thin layer of soft tissue interposed between the metal surface and the mineralized tissue (Figure 1.7) [Steflik et al. 1998]. The integration of these implants has thus been significantly improved and their clinical life extended, but mechanical failure is still the destiny of most of these medical devices.

Chapters 3 and 6 of this book will demonstrate how the introduction of biomimetic, bioactive, and bioresponsive functionalization methods promises to lead to metal implants with improved biological performances under biomechanical loads.



**Figure 1.7.** Bone regeneration around a porous titanium implant. Back-scattered scanning electron micrograph showing the implant surface separated from bone by a nonmineralized area.

### 1.3 THE THIRD-GENERATION BIOMATERIALS: BIOMIMICKING NATURAL BIOACTIVE AND BIORESPONSIVE PROCESSES

In the 1990s, it was evident that a third-generation of biomaterials was required that was capable of improving the clinical performance of implants by harnessing their potential to interact with surrounding tissues. As a consequence, new technological advances were advocated that would fulfill the ambition of abandoning the clinical approach of tissue replacement and achieving tissue regeneration. Tissue regeneration is, therefore, a requirement for both the integration of permanent implants and for a complete tissue regeneration supported by biodegradable biomaterials. It was envisaged that the bioactivity of ceramics and natural polymers could be mimicked by the synthesis of new biomaterials, simultaneously offering adequate physicochemical properties, biointegration potential, and ease of handling during surgical procedures.

Third-generation biomaterials have been designed to modulate processes that are fundamental to tissue regeneration, including cell adhesion, proliferation, and differentiation through the activation of particular genes [Hench & Polack 2002]. Biomimetic

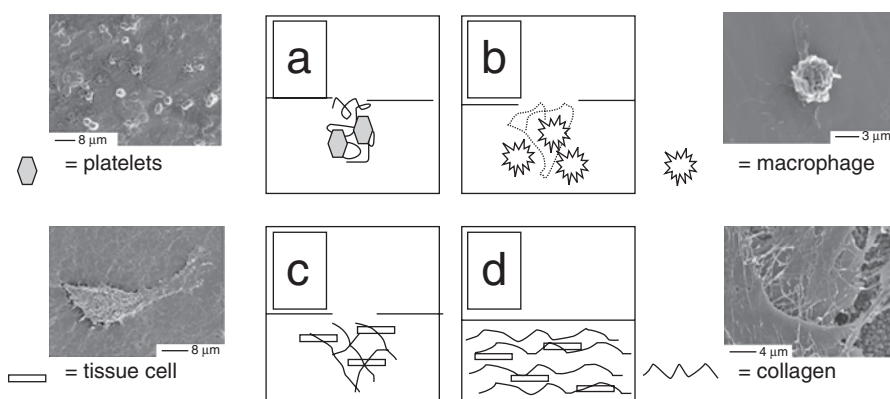
and bioactive biomaterials have been synthesized, which are able to target specific mechanisms of cell adhesion with the ultimate goal of regenerating tissues such as bone, cartilage, vascular tissue, and nerves. Their mechanisms of action can only be understood by learning the basics of the biological processes that they mimic.

### 1.3.1 Principal Phases of Tissue Regeneration

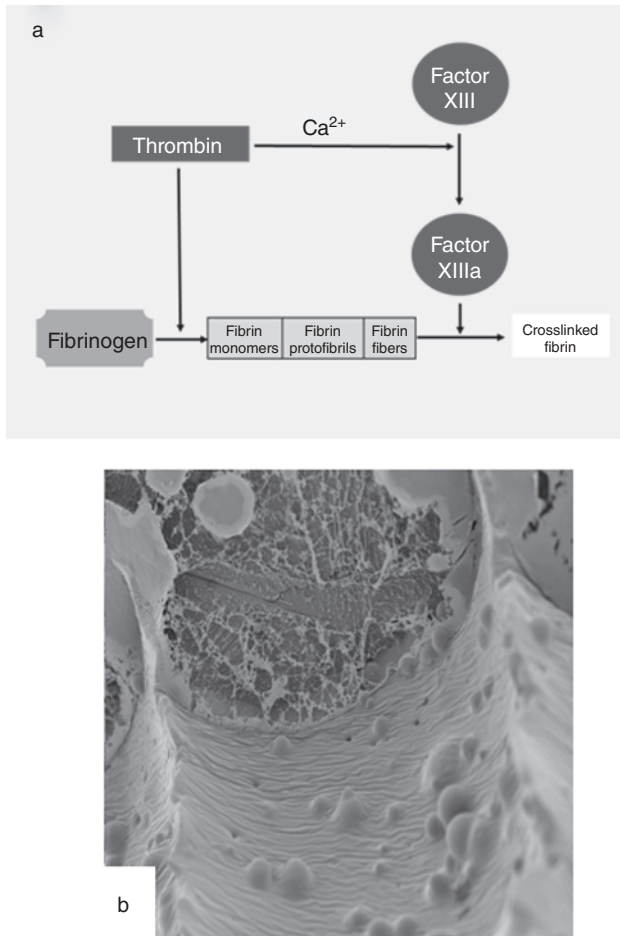
Tissue regeneration takes place through four main phases (Figure 1.8a–d) [Martin & Leibovich 2005]:

1. Clot formation
2. Inflammatory response
3. Cell migration/proliferation
4. New ECM deposition

The formation of a clot is required to stop bleeding following trauma, whether accidental or as a consequence of a surgical procedure. The clot is formed through the activation of a blood plasma protein (fibrinogen), which is transformed into a polymeric form (fibrin) by an enzyme called thrombin and cross-linked by a transglutaminase enzyme called Factor XIII (Figures 1.8a and 1.9a). Platelets, which are responsible for fibrinogen activation, complete the plug by being entrapped in the fibrin mesh (Figure 1.9b). Platelets soon degranulate, releasing a series of biochemical signals that recruit tissue and inflammatory cells to the site of injury. Gradually, the fibrin mesh is invaded by inflammatory cells such as the neutrophils (or polymorphonucleate granulocytes) and, later, by monocytes/macrophages, which clear tissue debris and infiltrated bacteria from the site, and begin to digest the fibrin clot (Figure 1.8b). Monocytes/macrophages



**Figure 1.8.** Principal phases of tissue regeneration: (a) clot formation, (b) clot infiltration by inflammatory cells, (c) tissue cell migration, (d) ECM deposition. Inserts show micrographs of typical cell morphologies.

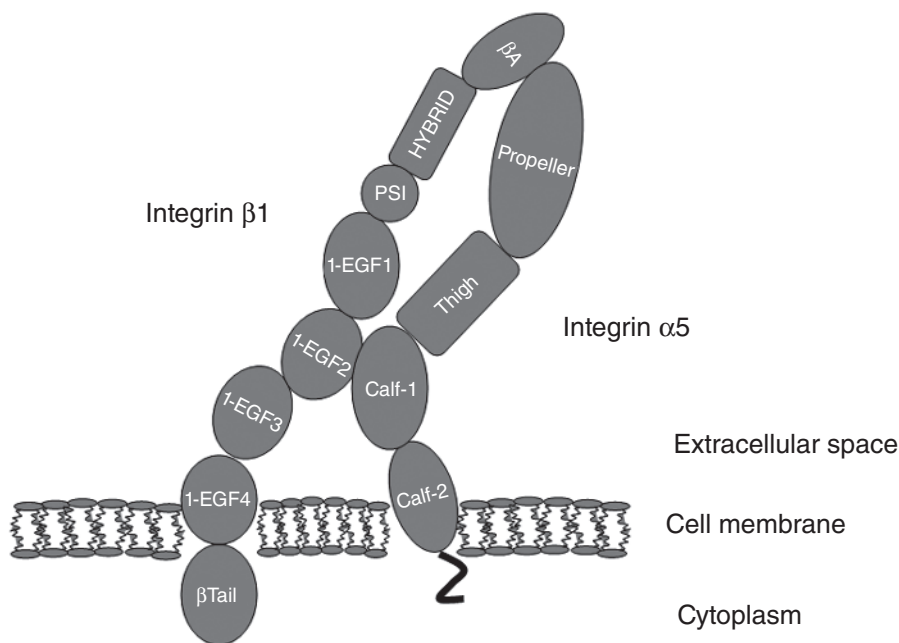


**Figure 1.9.** Fibrin clot formation: (a) schematic representation of the biochemical pathway, (b) scanning electron micrograph of a clot formed on the surface of a vascular graft.

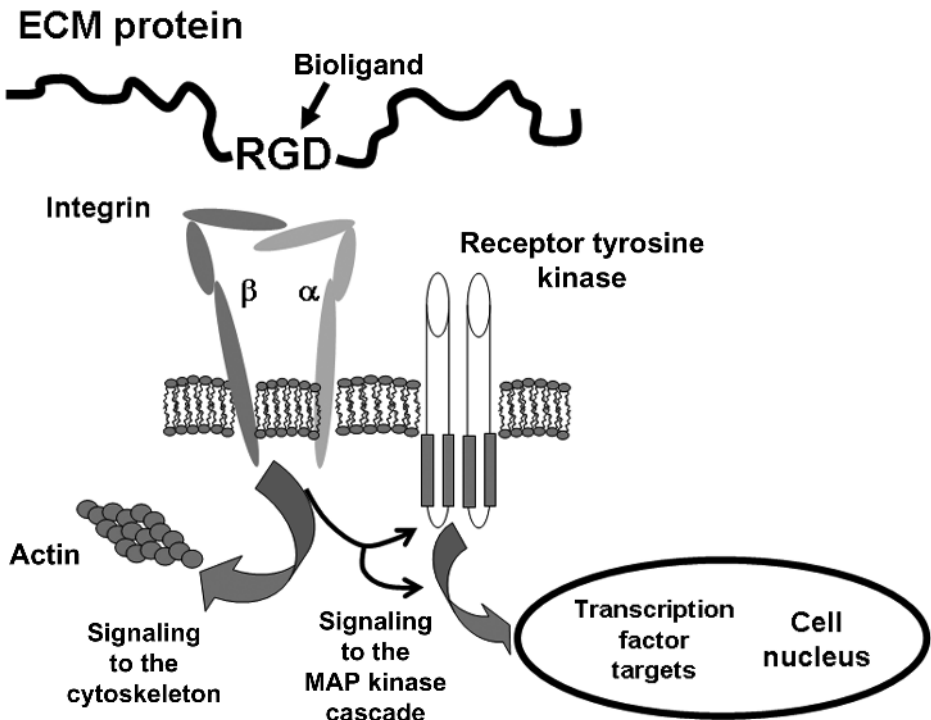
also play a central role in tissue regeneration by releasing important biochemical signals called cytokines and growth factors, which modulate the inflammatory response (see Section 1.1.) as well as the formation of new tissue. This new tissue formation takes place via an early phase of cell migration and proliferation (Figure 1.8c) followed by the synthesis and deposition of a new ECM composed mainly of structural proteins (e.g., collagen, fibronectin, and laminin) and glycosaminoglycans (Figure 1.8d) [Turley 2001]. All of these phases take place with some degree of overlap and are ultimately dependent on the activation of cells and their constant interaction with the surrounding matrix and other cells. Indeed, cell activation is modulated not only by the biochemical signaling constituted by cytokines and growth factors but also through the adhesion to components of the ECM.



**1.3.1.1 Cell Adhesion: The Cornerstone of Tissue Regeneration.** During the very early phases of their activation, tissue cells synthesize and secrete hyaluronan into the pericellular space [Zaidel-Bar et al. 2004]. This proteoglycan-based halo fills the gap that exists between the cell membrane surface and the components of the damaged ECM. Because of its physicochemical properties, hyaluronan can establish interactions with other ECM components while being recognized by the cell through a specific class of membrane receptors called CD44 [Fraser et al. 1997]. This type of cell adhesion, also called “soft contact” [Zaidel-Bar et al. 2004], occurs as soon as the cell faces the new environment. It is replaced after a few seconds by a more stable contact mediated by a network of anchor proteins on the cell membrane. These proteins cluster together in various arrays to form anchoring patterns: focal adhesion, fibrillar adhesion, and focal complexes. These anchoring patterns are very important as they dictate cell motility and, therefore, its migration to the wound site. The anchoring proteins responsible for the adhesion of cells to the pericellular matrix and to neighboring cells are a class of membrane receptors called integrins. These are heterodimeric proteins composed of an  $\alpha$  subunit associated through noncovalent interactions to a  $\beta$  subunit [Stefansson et al. 2004] (Figure 1.10). The two types of subunits are separate families of proteins: The  $\alpha$  subunit includes 18 different members, while the  $\beta$  subunit accounts for 8 different proteins. Both the subunits span across the cell membrane and can, therefore, be schematically divided into three main domains (Figure 1.10):



**Figure 1.10.** Schematic representation of a typical integrin showing the  $\alpha$  and  $\beta$  subunits and their localization across the cell membrane.



**Figure 1.11.** Schematic representation of the relationship between ECM bioligands (i.e., RGD), integrin, and intracellular signaling.

- (1) N-terminal extracellular domain
- (2) Transmembrane domain
- (3) Cytoplasmic domain

Integrins are important, not only for cell anchoring to the surrounding extracellular environment through their interaction with specific bioligands, but also because of their ability to generate intracellular signaling (Figure 1.11). This signaling modulates the main cell activities related to tissue regeneration such as cell migration, proliferation, and differentiation. These activities have been defined as “outside-in signaling,” which is the ligand-induced signaling mechanism and “inside-out signaling,” which includes a series of mechanisms that activate the integrins.

Integrin activation is determined by conformational changes and clustering of the subunits and of their respective domains. Each one of these modifications seems to lead to particular cell activation pathways. In particular, the activation of the transmembrane domain is believed to contribute to signaling in both directions: outside-in and inside-out. The interactions of bioligands exposed on the ECM structure with integrins leads to the activation of the so-called focal adhesion kinase (FAK) and of other not fully characterized tyrosine kinases. Once a cell has adhered to ECM components via

bioligand/integrin interactions, the autophosphorylation of these kinases takes place by integrin clustering and interactions between the cytoplasmic domain of the  $\beta$  subunits and the actin filaments [Juliano et al. 2004; Li et al. 2004]. The interactions with the actin filaments take place through the recruitment of cytoplasmic proteins and by a plethora of downstream responses, which leads to the remodeling of the actin filaments and ultimately to cell motility [Juliano et al. 2004]. Because of these interactions, FAK, their downstream targets, and phosphatidylinositol 3-kinase have been shown to be important mediators of cell migration [Armulik et al. 2004]. Indeed, cell migration can be considered as one of the earliest events in tissue regeneration by which cells try to colonize the remodeling or damaged tissue to synthesize and deposit new ECM (Figure 1.8c). However, cell migration is also favored by the secretion of particular classes of enzymes, the metalloproteinases, which cleave specific peptide sequences in the ECM mesh, thus opening the space to the migrating cell [Buhling et al. 2006].

FAK phosphorylation in turn leads to the activation of another enzyme, the mitosis activation phosphorylase kinase (MAPK). Mitosis is the process by which cells divide and proliferate. Therefore, the activation of MAPK through integrins leads to increased cell proliferation, a key event in tissue regeneration. The effect of integrins on cell proliferation also includes their ability to undergo partner assembling with growth factor receptors. Increasing evidence suggests that the combined actions of integrins and receptor protein tyrosine kinases transduce proliferative signals in cells through an enzymatic cascade (Figure 1.11) [Cabodi et al. 2004; Juliano et al. 2004].

The effects of integrins are not limited to cell migration but also involve their proliferation and differentiation processes as well as the organization of ECM components [Velling et al. 2002; Boulter & van Obberghen-Schilling 2006]. In fact, it has been demonstrated that the polymerization and assembly of ECM proteins such as fibronectin, laminin, and collagens type I and III are enhanced by their interactions with integrins such as  $\alpha_{11}\beta_1$  and  $\alpha_2\beta_1$  and by cytoskeleton-associated tensions.

The interactions mediated by the integrins rely mainly on the presence of a specific amino acid sequence, the RGD sequence, in the structure of many ECM proteins [Takagi 2004]. This ligand is present in fibrinogen, fibronectin, vitronectin, and other ECM glycoproteins with a relatively high molecular weight.

Recently, the increased expression of integrin  $\alpha_v\beta_5$  has been shown to induce the differentiation of dermal fibroblasts, which is driven by the colocalization of specific growth factor receptors such as the TGF- $\beta$ 1 receptor on the cell membrane [Asano et al. 2006]. Conversely, the fibronectin receptor integrin  $\beta_1\alpha_5$  has been shown to be downregulated during the differentiation of mesenchymal stem cells into chondrocytes [Goessler et al. 2006]. These data seem to suggest that the role of integrins in cell differentiation is very complex and depends on the types of integrin, cell, and differentiation stage analyzed.

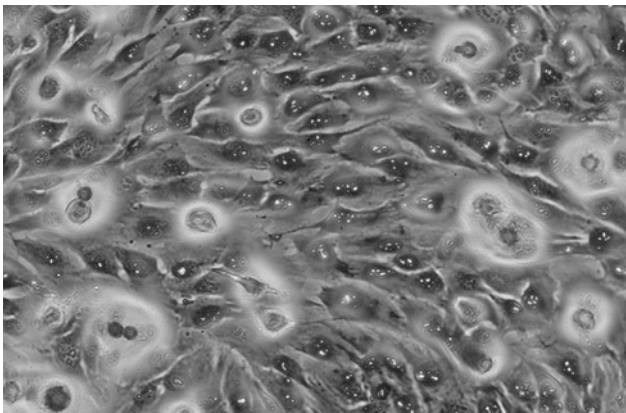
However, ECM modulates cell behavior by a plethora of bioligands that extend beyond hyaluronan and the RGD domain. Cell–cell and cell–ECM interactions also take place through lectins, a class of proteins able to bind carbohydrates both specifically and noncovalently [Sharon & Lis 1993]. Among them, the family of the galectins has been shown to be involved in a number of cellular processes. These span from cell adhesion [Mahanthappa et al. 1994] and the regulation of the cell cycle [Wells & Mal-

luchi 1991] to cell differentiation [Goldrin et al. 2001], and endothelial cell motility and angiogenesis [Fukushi et al. 2004]. Other important peptide sequences have been found to modulate specific cell types and their relative functions. Table 1.2 lists those sequences more relevant to biomimetic biomaterials (see Section 1.4.1).

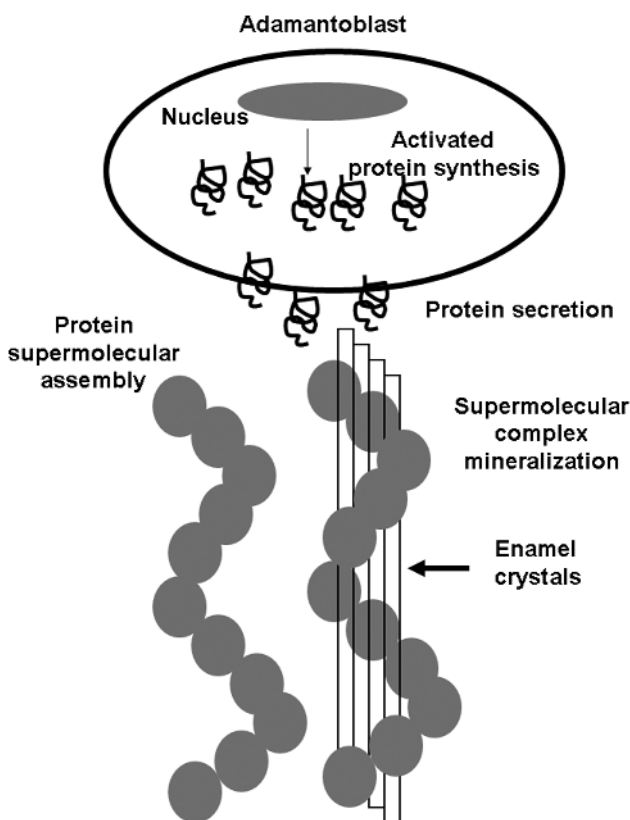
**1.3.1.2 Mechanisms of Tissue Mineralization.** Bone and cartilage have developed their ability to sustain biomechanical stresses by evolving as mineralized structures. The mineralization of these tissues is also driven by cell activation and is, therefore, strictly dependent on the mechanisms described in Section 1.3.1.1. For example, adhering and differentiating osteoblasts express enzymes (such as alkaline phosphatase), which facilitate the formation of a mineral phase. In addition, they secrete collagen and other calcium-binding proteins, and send signals to sister osteoblasts (autocrine signaling) and to other cells such as the osteoclasts (paracrine signaling) (Figure 1.12). The modulation of osteoblasts is key factor in the process of tissue resorption during bone remodeling [Buckwalter et al. 1995].

TABLE 1.2. Main Cell Binding Domains Utilized to Functionalize Biomimetic Biomaterials (Rezania & Healy 1999)

RGD	Cell adhesion domain of most ECM proteins
FHRRKA	Osteoblast migration domain
YIGSR	Laminin cell binding domain
IKVAV	Laminin cell binding domain
REDRV	Fibronectin cell binding domain
LDV	Fibronectin cell binding domain
DGEA	Collagen type I cell binding domain



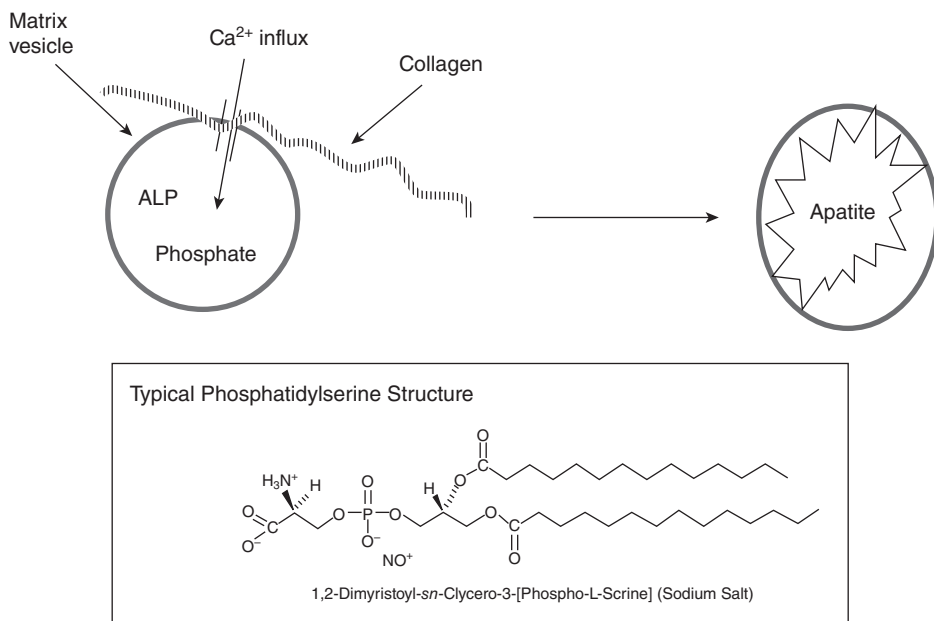
**Figure 1.12.** Typical plurinucleated osteoclasts differentiated from blood mononuclear cells under osteoblast-secreted stimuli. Osteoclast cells are identified as large, multinucleated cells within a prevalent osteoblast population.



**Figure 1.13.** Schematic representation of amelogenin synthesis, secretion, supramolecular assembling, and mineralization. Adamantoblasts are cells producing enamel in teeth.

At the molecular level, the formation of an apatite-rich mineral phase depends on specific functional groups exposed on the surface of ECM components. Collagen and other calcium-binding proteins associated with the collagenic template are able to catalyze tissue mineralization [Camacho et al. 1999]. Amelogenins—globular proteins of tooth enamel—are capable of self-assembling into spiral-like structures, exposing their core calcium-binding functional groups and directing crystal growth to form one of the strongest mineral tissues in nature (Figure 1.13) [Fincham & Simmer 1997].

Another natural approach toward tissue mineralization is the phospholipid-mediated nucleation of apatite crystals [Boskey 1978]. Matrix vesicles (MVs) have been found in developmental bone and cartilage as well as in regenerating bone. The nature of MVs is controversial as it is not clear whether they are membrane fragments derived from apoptotic cell ghosts or if they are produced by osteoblasts through a specific mechanism. Regardless of their origin, it is known that MVs can facilitate the influx of calcium into their interior through calcium channels usually present in the cell membrane. Once inside the MV, calcium can complex with phosphorus ions through the catalytic



**Figure 1.14.** Schematic representation of the mechanism of apatite crystal formation in MV. Insert highlights the PS zwitterionic group catalyzing the crystal seeding process.

action of the zwitterion moiety of phosphatidylserine (PS), a membrane phospholipid (Figure 1.14).

The physicochemical mechanism by which these molecules induce mineralization of tissues is a strategy common to all mineralized tissue throughout the animal kingdom [Mann 1988] (Figure 1.15). This mechanism relies on the ability of certain biomolecules to expose functional groups with a high binding capacity but lower binding affinity for calcium ions. Because of their physicochemical properties, these groups allow the formation of a calcium coordination sphere (the anionic sphere) on the molecule surface and make it available to interact with phosphate ions (the cationic sphere). The presence of moieties with high calcium binding capacity and low binding affinity is indeed essential for the organization of the counterion clusters and subsequent apatite crystal formation. Conversely, a high calcium binding affinity would subtract calcium from phosphorus, making the reaction unfavorable. Once ion clusters are formed, crystals nucleate through the formation of amorphous calcium phosphate and eventually loose water molecules to become mature and water-insoluble apatite crystals.

## 1.4 PRINCIPLES OF BIOMIMESIS AND BIOACTIVITY

The third-generation biomaterials set the stage for a new era in biomedicine; a paradigm shift was seen where the concept of tissue replacement has gradually been replaced by

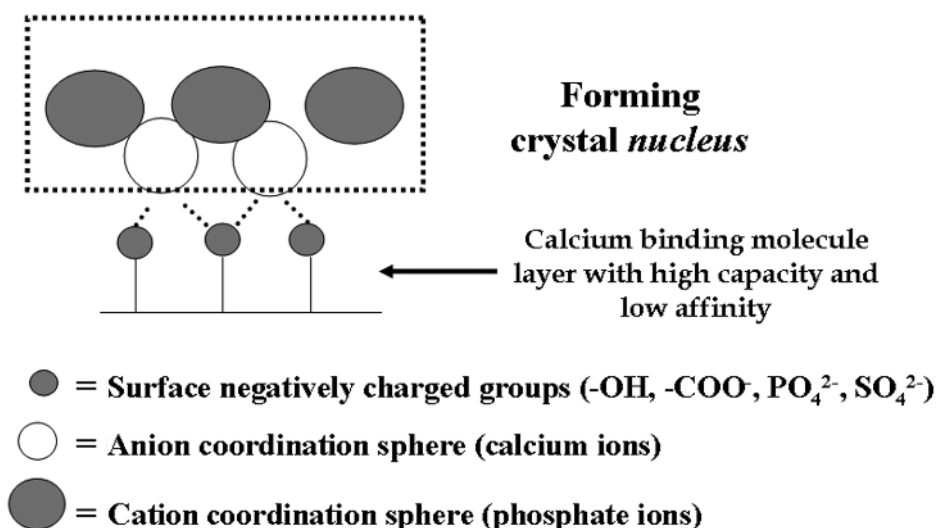


Figure 1.15. Schematic representation of biomineralization mechanisms in nature.

that of tissue regeneration achieved by either “in situ regeneration” or “tissue engineering” strategies [Hench & Polack 2002]. The former approach aims to use biomaterials that can be implanted into the damaged tissue to stimulate tissue regeneration through the activation of specific cell mechanisms. The second approach is based on the encapsulation of cells into porous biomaterial scaffolds to induce tissue regeneration either in a bioreactor system or upon implantation in the damaged tissue. In both cases, it is advocated that the biomaterial used will be able to mimic the components and signaling of natural tissues.

This book aims to explore the progress made in the field of biomaterials with biomimetic and/or bioactive properties and to assess the performances of those that have reached the clinical application stage. The particular scope of the next section of this chapter is to introduce the rationales underpinning biomimicry in the field of biomaterials.

### 1.4.1 Biomimicking of the ECM

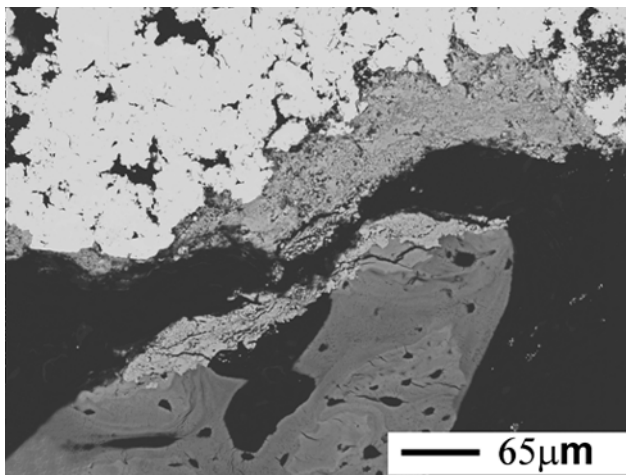
Capitalizing on the performances of second-generation biomaterials and with a greater understanding of tissue regeneration processes, scientists have tried to synthesize novel biomaterials that mimic components of the ECM. In particular, polymers and metals have been functionalized with amino acid sequences involved in the cell–ECM recognition processes described in Section 1.3.1.1 (Figure 1.11). Biomaterials such as polyethylene glycol (PEG) hydrogels have been rendered bioactive through the inclusion of cell adhesion motifs (e.g., RGD) in their structure and bioresponsive by insertion of peptide sequences that are involved in enzyme-catalyzed reactions [Sakiyama-Elbert



& Hubbell 2001; Sanborn et al. 2002]. Substrates for Factor XIII have been exploited to induce the immediate cross-linking of hydrogels when in contact with blood [Sanborn et al. 2002]. Likewise, hydrogels have been synthesized that include peptidic substrates for the matrix metalloproteinase (MMP) enzyme secreted by tissue cells during their migration. This type of hydrogel is, therefore, able to respond to cell-released stimuli to change its structure and provide a route into the mesh for the migrating cell [Sakiyama-Elbert & Hubbell 2001]. Unlike second-generation biodegradable biomaterials (see Section 1.2.4), the MMP-functionalized hydrogels have transferred the control of biomaterial degradation from spontaneous hydrolytic processes to biologically controlled events, thus tuning it to tissue regeneration.

Other biomimetic peptide sequences have been used to functionalize biomaterials and are listed in Table 1.2. These sequences are characterized by their affinity for specific cell types and their capacity to modulate specific cell functions (e.g., migration).

Although HA and Bioglass have set new standards in the development of biomaterials for bone regeneration, their mechanical properties significantly limit their surgical applications. These biomaterials are brittle, especially when porous, and are therefore difficult to adapt to the tissue defect during an operation. Also, when used as coatings, they tend to delaminate from the metal substrate, thus causing implant mobilization (Figure 1.16). In addition, the HA made available for clinical application has a degree of crystallinity significantly higher than bone apatite and, as a consequence, it cannot participate in the bone remodeling process (see Chapter 3). More recently, tricalcium phosphates have been introduced that can be used as bone cements, offering an alternative to the traditional PMMA-based materials. The relatively low degree of crystallinity makes these cements resorbable during bone remodeling, thus encouraging complete tissue regeneration. Progress has recently been made to make these biomaterials injectable and therefore suitable for different clinical applications where noninvasive surgery



**Figure 1.16.** Ceramic coating delamination upon implantation. Micrograph shows bone integration with a delaminating coating.

is sought [Delgado et al. 2005]. Chapters 5 and 6 give an overview of the technological advances made in the attempt to mimic the texture of the ECM on the surface of metals and ceramics. Many investigations have been focused on establishing the optimal degree of surface roughness required to encourage cell adhesion. Anodization methods have been applied on titanium oxide surfaces, and optimal porosity has been sought in porous 3D ceramic scaffolds (see Chapters 5 and 6).

### 1.4.2 Biomimicking of Cell Membrane Components

More recently, biomimesis has been extended to the mimicking of the cell surface. The cell membrane (also called “plasmalemma”) consists of a phospholipid bilayer that not only separates the cell from the extracellular space but also regulates interactions with the surrounding environment. These interactions are regulated by plasmalemma proteins such as the integrins (see Section 1.3.1.1), other ion channels, and glycoproteins that render the cell surface relatively hydrophilic (the glycocalyx). Chapter 4 illustrates typical examples of biomaterial surface functionalization that attempt to mimic these components. Polymers presenting phosphatidylcholine (PC) groups have been developed and used for different biomedical applications, while gels of PS have been obtained to induce surface mineralization of implants. Synthetic polymers such as poly(vinyl alcohol) and poly(2-hydroxyethyl methacrylate) have been modified by grafting sugar moieties such as dextran and galactose onto their surface in the attempt to simulate the cell glycocalyx (see Chapter 4).

### 1.4.3 Biomimicking Cell Signaling Pathways

Although fundamental to the control of tissue regeneration, cell-to-cell and cell-to-ECM recognition processes are complemented by an intricate network of autocrine and paracrine signaling (see Section 1.3.1.1). Cytokines and growth factors are secreted by immunocompetent and tissue cells during the different phases of healing to coordinate the activities required for the formation of new tissue [Martin & Leibovich 2005]. The efficacy of the signaling primarily depends on five factors:

1. Diffusion of the relevant bioactive molecules toward the target cell
2. Interaction with ECM components
3. Biorecognition of the relative cell receptor
4. Timeliness of the delivery
5. Composition of the bioactive molecule cocktail

Many of the third-generation biomaterials have tried to fulfill these goals by loading relevant growth factors in polymeric matrices, porous ceramics, and metal surfaces by entrapment or grafting [Hubbell 1999]. Such biomaterials are generally classified as bioactive biomaterials. Table 1.3 summarizes the growth factors commonly used in the field of biomaterials. Although promising results have been obtained (see Chapters 5, 6, and 7), none of the bioactive biomaterials so far engineered are able to fulfill all five criteria required to control tissue regeneration.

TABLE 1.3. Main Growth Factors Families Used in Bioactive Biomaterials

- 
- Transforming growth factor superfamily
    - Transforming growth factor- $\beta$ 1
    - Bone morphogenetic proteins/osteopontins
  - Growth differentiation factors
  - Vascular endothelial growth factor
  - Platelet-derived growth factor
  - Fibroblast growth factor
  - Epithelial growth factor
  - Nerve growth factor
- 

Thus far, biomaterials loaded with growth factors have tried to fulfill only the requirements of points 1 and 2 since they have been optimized to deliver specific growth factors in a controlled manner. This delivery is not tuned with the expression of the relevant receptors on the cell surface, which depends on cell phenotype and tissue regeneration phase (points 3 and 4). Moreover, most of the bioactive biomaterials tested at the research and clinical level focus on the delivery of only one type of growth factor, thus neglecting the importance of combined release, which leads to a physiological tissue regeneration (point 5).

In general, the optimization of growth factor delivery has been pursued by following traditional drug delivery approaches: The bioactive components are loaded into the biomaterial carrier or grafted on its surface through chemical bonding that is prone to cleavage in biological media. Kinetic release studies are usually performed by traditional pharmacological methods and bioactivity studied by *in vitro* cell experiments or by animal models. In this manner, the delivery of growth factors to promote cell proliferation (TGF- $\beta$ 1), osteoblast differentiation (BMP-2), vascularization (angiogenic factors), and nerve regeneration (neuronal survival and differentiation factors) has been optimized in different biomaterial carriers [Hubbell 1999]. The ability of growth factors to bind components of the ECM has been exploited by materials scientists to control the delivery of the bioactive molecules to cells resident within tissue engineering constructs or in the surrounding tissue. For example, collagen has been functionalized with heparin to utilize the ability of TGF- $\beta$ 1 to bind to heparin domains [Schroeder-Tefft et al. 1997].

**1.4.3.1 Modulation of the Growth Factor Signaling by Gene Expression: Bioactive Gene Delivery Systems.** In an alternative approach, bioactivity of biomaterials has been pursued by gene-delivery strategies. Plasmid DNA presented to cells by a biomaterial has been successfully employed to enhance the gene expression and synthesis of growth factors important to tissue regeneration. This approach has been applied to suture materials as well as to implants to enhance the regeneration of cardiovascular and bony tissues [Hubbell 1999]. Synthetic biomimetic polymers have also been designed that present peptides to the cells that are usually exposed on viral coats, thus facilitating their penetration into the cell by endocytosis [Hoffman et al. 2002]. These biomimetic biomaterials have been loaded with plasmid DNA grafted

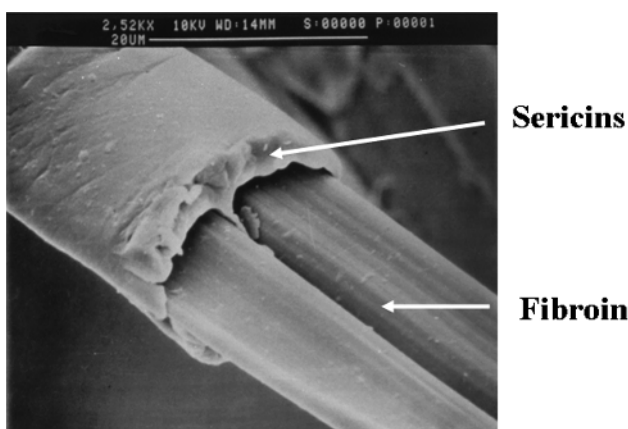
onto the synthetic polymers via bonds that are sensitive to the relatively low pH of the endosomes, the vesicles used by the cell to engulf material from the extracellular space. The cleavage of the bonding in the intracellular space thus leads to the release of the plasmid DNA. The benefit of the gene-delivery approach is the prolonged effect on the synthesis of growth factor and therefore, its sustained production throughout the tissue regeneration process.

## 1.5 BIOACTIVE BIOMATERIALS FROM DIFFERENT NATURAL SOURCES

Nature has evolved common mechanisms to regulate biological processes. Biomolecular recognition, cell interactions, mineralization and, more broadly, bioactivity, share similar pathways across organisms, which have triggered the development of new bioactive biomaterials. Beyond the poorly clarified hemostatic properties of alginate, new biomaterials have recently emerged that appear to be able to participate in the tissue regeneration processes in an active manner. In this section, two main examples are given: silk fibroin- and soybean-based biomaterials.

### 1.5.1 Silk Fibroin

Silk is secreted from the silkworm gland as fibers composed by an inner core made of a protein called fibroin, which is coated by a second protein called sericin (Figure 1.17). The excellent mechanical properties of silk have been long been exploited to produce suturing material. However, in the 1980s, some investigations highlighted the adverse reaction elicited by virgin silk when in contact with healing ocular tissues. Conversely,

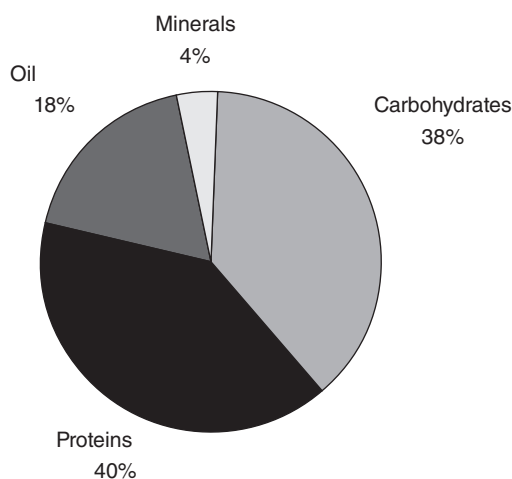


**Figure 1.17.** Scanning electron micrograph of a typical silkworm silk fiber showing the fibroin bavellae emerging from the sericin coating. (Photo by Dr. A Motta, University of Trento, Italy).

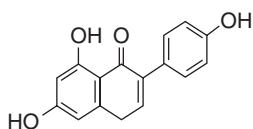
it was noticed that when silk sutures deprived of the sericin coating were used, these adverse reaction no longer occurred [Soong & Kenyon 1984]. The ability to remove the sericin coat from virgin silk, as well as the possibility of engineering fibroin in the form of films and membranes, triggered interest in developing fibroin-based biomaterials. However, no clear assessment of the immunological properties of this material was made until recently when Santin et al. (1999) proved that engineered fibroin films elicited a significantly lower immune response *in vitro* than other conventional polymers. Later studies confirmed these early findings and offered more insights about the degree of interactions that silk fibroin can establish with the host environment depending on its native or denatured conformation [Motta et al. 2002; Santin et al. 2002; Meinel et al. 2005]. These investigations showed that when in fiber form, silk fibroin can elicit the activation of inflammatory cells and exhibit procoagulant activity by interacting with the polymerizing fibrin (see Section 1.3.1). Beyond the low immunogenicity, denatured fibroin biomaterials have been shown to stimulate cell proliferation *in vitro* and support bone regeneration in rabbit models [Motta et al. 2004; Fini et al. 2005]. Although it has been postulated that the cell proliferation stimulus may be derived from specific peptides present in the fibroin structure, no sequence has yet been identified to prove this hypothesis. It is not clear whether the different levels of biointeraction are elicited by the transition from the  $\beta$ -sheet structure of the fiber to the amorphous conformation of the denatured protein films. These conformational changes may lead either to different surface physicochemical properties or to an altered exposure of specific bioligands.

### 1.5.2 Soybean-Based Biomaterials

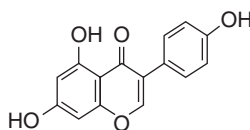
Soybean is one of the most commonly used foods throughout the world. The bean is composed of proteins (40%), carbohydrates (38%), lipids (18%), and minerals (4%) (Figure 1.18). Plant estrogens called isoflavones are also included, which are present in both a glycosylated and nonglycosylated form [Murkies et al. 1998]. The glycosylated forms genistin and daidzin (Figure 1.19a,b) are the most abundant forms and are accompanied by the nonglycosylated genistein and daidzein (Figure 1.19c,d). Glycosylated isoflavones can be transformed by spontaneous- or enzyme-driven hydrolysis into the respective nonglycosylated forms [Walle et al. 2005]. It has been proven that the nonglycosylated form can exert different effects on eukaryotic cells [Middleton et al. 2000]. In general, they can reduce the activation of immunocompetent cells and tissue cell proliferation. Isoflavones can inhibit tyrosine kinase receptors on the immunocompetent cell plasmalemma or act as anti-redox compounds able to buffer free radicals produced by inflammatory cells. It has also been proven that isoflavones can penetrate the cell membrane and interact with the estrogen receptor  $\beta$  of the nuclear membrane. The binding of isoflavones to this receptor leads to an inhibition of the cell cycle in its G1/M phase, thus reducing the cell proliferation rate. Simultaneously, the isoflavones are responsible for an increased cell differentiation, which leads to an induced collagen synthesis both *in vitro* and *in vivo*. In the case of osteoblasts, isoflavones seem to stimulate the differentiation of the cells by inducing the synthesis of ALP and BMP-2 [Zhou et al. 2003; Morris et al. 2006]. Indeed, it is believed that the low incidence of



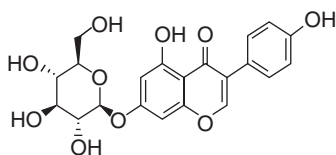
**Figure 1.18.** Soybean composition. A novel class of biomaterials has recently been developed by thermosetting the defatted soybean curd (Santin et al. 2001).



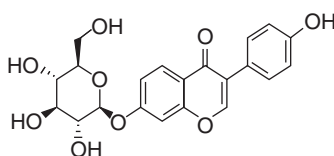
(a) Genistein



(b) Daidzein



(c) Genistin

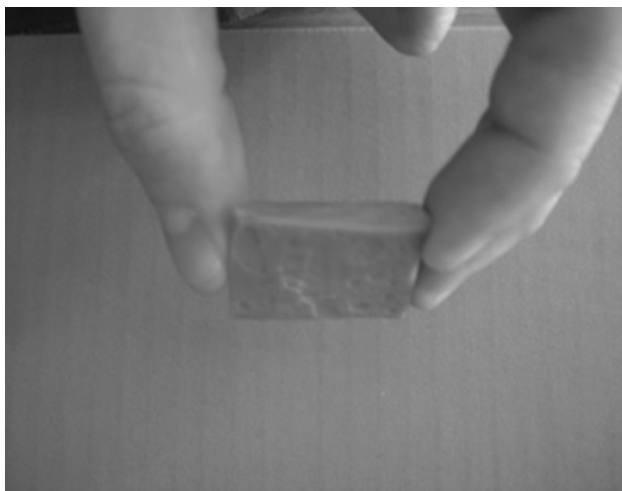


(d) Daidzin

**Figure 1.19.** Structure of the main soy isoflavones: (a) genistin, (b) daidzin, (c) genistein, (d) daidzein.

both breast and prostate cancers, and osteoporosis in populations with a soy-rich diet, can be attributed to the isoflavone bioactivity [Middleton et al. 2000].

Soybeans have long been proposed as sources of biodegradable materials for many applications. Henry Ford invested money in research to make car body parts from soy. Glues, fibers, and other objects can be fabricated from the soybean fraction. Soy protein has also been used to make biodegradable biomaterials.



**Figure 1.20.** Soybean-based biodegradable membrane for surgical application.

More recently, a novel class of biodegradable biomaterials has been introduced that is produced by thermosetting defatted soy curd [Santin et al. 2001] (Figure 1.20). The advantage of this class of soy-based biomaterials is the preservation of the protein, carbohydrate, and isoflavone components that are able to modulate the inflammatory response and tissue regeneration while degrading. These biomaterials are easy to handle during surgical procedures, and animal models have been shown to favor the regeneration of both soft and bony tissues while ruling out the formation of a fibrotic capsule. Histocytochemical analysis seems to suggest that soy's ability to regenerate tissue without forming fibroses is due to its maintenance of the inflammatory response in its acute phase rather than triggering chronic inflammation. The tissues surrounding the soy implants are indeed characterized by a prevalence of neutrophils rather than monocytes/macrophages (see Section 1.3.1). The isoflavone-driven induction of collagen synthesis may also be a key factor in the achievement of tissue regeneration at the implantation site.

Future insights into the biological pathways driven by soybean-based biomaterials may lead to the development of new biomaterials capable of simultaneously modulating both biochemical signaling and ECM components.

## 1.6 SCOPE OF THIS BOOK

This book is aimed at professionals already working in the field of biomedical materials and as a text suitable for students at the graduate and postgraduate levels. In the following chapters we aim to offer a comprehensive overview that highlights the effects of different types of biomaterials in the context of tissue regeneration and/or tissue



functionality. Specifically, the links between tissue functionality and model clinical applications to chemical and engineering solutions will be demonstrated. Each chapter includes problem sets and questions to allow revision of the different topics covered.

## REFERENCES

- Anderson JM (1988) Inflammatory response to implants. *ASAIO* 11: 101–107.
- Anderson JM (2001) Biological responses to materials. *Annual Review of Materials Research* 31: 81–110.
- Armulik A, Velling T, Johansson S (2004) The integrin  $\beta 1$  subunit transmembrane domain regulates phosphatidylinositol 3-kinase-dependent tyrosine phosphorylation of Crk-associated substrate. *Molecular Biology of the Cell* 15: 2558–2567.
- Asano Y, Ihn H, Yamane K, Jinnin M, Tamaki K (2006) Increased expression of integrin  $\alpha v \beta 5$  induces the myofibroblastic differentiation of dermal fibroblasts. *American Journal of Pathology* 168: 499–510.
- Bach AD, Bannasch H, Galla TJ, Bittner KM, Stark GB (2001) Fibrin glue as matrix for cultured autologous urothelial cells in urethral reconstruction. *Tissue Engineering* 7(1): 45–53.
- Barbucci R, Magnani A, Bazkin A, Dacosta ML, Bauser H, Hellwig G, Martuscelli E, Cimmino S (1993) Physicochemical surface characterization of hyaluronic-acid derivatives as a new class of biomaterials. *Journal of Biomaterials Science. Polymer Edition* 4: 245–273.
- Boccafroschi F (2005) Biological performances of collagen-based scaffolds for vascular tissue engineering. *Biomaterials* 26: 7410.
- Boskey AL (1978) Role of calcium-phospholipid-phosphate complexes in tissue mineralization. *Metabolic Bone Disease & Related Research* 1: 137–142.
- Boulter E, van Obberghen-Schilling E (2006) Integrin-linked kinase and its partners: a modular platform regulating cell-matrix adhesion dynamics and cytoskeletal organization. *European Journal of Cell Biology* 85: 255–263.
- Buckwalter JA, Glimcher MJ, Cooper RR, Recker R (1995) Bone biology: Part I. Structure, blood supply, cells, matrix, and mineralization. *The Journal of Bone and Joint Surgery* 77-A: 1256–1289.
- Buhling F, Groneberg D, Welte T (2006) Proteases and their role in chronic inflammatory lung diseases. *Current Drug Targets* 7: 751–759.
- Cabodi S, Moro L, Bergatto E, Boeri Erba E, Di Stefano P, Turco E, Tarone G, De Filippi P (2004) Integrin regulation of epidermal growth factor (EGF) receptor and of EGF-dependent responses. *Biochemical Society Transactions* 32: 438–442.
- Camacho NP, Rinnerthaler S, Paschalis EP, Mendelsohn R, Boskey AL, Fratzl P (1999) Complementary information on bone ultrastructure from scanning small angle X-ray scattering and Fourier-transform infrared microspectroscopy. *Bone* 25: 287–293.
- Carreño-Gómez B, Duncan R (1997) Evaluation of the biological properties of soluble chitosan and chitosan microspheres. *International Journal of Pharmaceutics* 148(2): 231–240.
- Charnley J (1961) Arthroplasty of the hip—a new operation. *Lancet* 1: 1129–1123.
- Clarke HJ, Jinnah RH, Lennox D (1990) Osteointegration of bone-graft in porous-coated total hip arthroplasty. *Clinical Orthopaedics and Related Research* 258: 160–167.

- Day AJ, Prestwich GD (2002) Hyaluronan-binding proteins: tying up the giant. *The Journal of Biological Chemistry* 277: 4585–4588.
- Delgado JA, Harr I, Almirall A, del Valle S, Planell JA, Ginebra MP (2005) Injectability of a macroporous calcium phosphate cement. *Key Engineering Materials* 284-286: 157–160.
- Engel J, Bachinger HP (2005) Structure, stability and folding of the collagen triple helix. *Collagen Topics in Current Chemistry* 247: 7–33.
- Fincham AG, Simmer JP (1997) Amelogenin proteins of developing dental enamel. In: *Dental enamel*. Ciba Foundation Symposium 118–134.
- Fini M, Motta A, Torricelli P, Glavaresi G, Aldini NN, Tschon M, Giardino R, Migliaresi C (2005) The healing of confined critical size cancellous defects in the presence of silk fibroin hydrogel. *Biomaterials* 26: 3527–3536.
- Fraser JRE, Laurent TC, Laurent UBG (1997) Hyaluronan: its nature, distribution, functions and turnover. *Journal of Internal Medicine* 242: 27–33.
- Fukushi J, Makagiansar IT, Stallcup WB (2004) NG2 proteoglycan promotes endothelial cell motility and angiogenesis via engagement of galectin-3 and  $\alpha 3\beta 1$  integrin. *Molecular Biology of the Cell* 15: 3580–3590.
- Gentleman E, Lay AN, Dickerson DA, Nauman EA, Livesay GA, Dee KC (2003) Mechanical characterisation of collagen fibres and scaffolds for tissue engineering. *Biomaterials* 24: 3805–3813.
- Gentleman E, Livesay GA, Dee KC, Nauman EA (2006) Development of ligament-like structural organization and properties in cell-seeded collagen scaffolds in vitro. *Annals of Biomedical Engineering* 34(5): 726–736.
- Giusti P, Lazzeri L, Cascone MG, Seggiani M (1995) Blends of synthetic and natural polymers as special performance materials. *Macromolecular Symposia* 100: 81–87.
- Goessler UR, Bieback K, Bugert P, Heller T, Sadick H, Hormann K, Riedel F (2006) In vitro analysis of integrin expression during chondrogenic differentiation of mesenchymal stem cells and chondrocytes upon dedifferentiation in cell culture. *International Journal of Molecular Medicine* 17: 301–307.
- Goldrin K, Jones GE, Thiagarajah R, Watt DJ (2001) The effect of galectin-1 on the differentiation of fibroblasts and myoblasts in vitro. *Journal of Cell Science* 115: 355–366.
- Grayson ACR, Voskerician G, Lynn A, Anderson JM, Cima MJ, Langer R (2004) Differential degradation rates in vivo and in vitro of biocompatible poly(lactic acid) and poly(glycolic acid) homo- and co-polymers for a polymeric drug-delivery microchip. *Journal of Biomaterials Science. Polymer Edition* 15: 1281–1304.
- Hayashi T (1994) Biodegradable polymers for biomedical uses. *Progress in Polymer Science* 9: 663–702.
- Helgersson SL, Seelich T, DiOrio JP, Tawil B, Bittner K, Spaethe R (2004) Fibrin. In: *Encyclopedia of Biomedical Engineering*, 603–610. New York: Marcel Dekker Inc.
- Hench LL, Polack J (2002) Third-generation biomedical materials. *Science* 295: 1014–1017.
- Hing KA, Best SM, Tanner KE, Bonfield W, Ravell PA (2004) Mediation of bone ingrowth in porous hydroxyapatite bone graft substitutes. *Journal of Biomedical Materials Research. Part A* 68A: 187–200.
- Hoffman AS, Stayton PS, Press O, Murthy N, Lackey CA, Cheung C, Black F, Campbell J, Fausto N, Kyriakides TR, Bornstein P (2002) Design of “smart” polymers that can direct intracellular drug delivery. *Polymers for Advanced Technologies* 13: 992–999.
- Hubbell JA (1999) Bioactive biomaterials. *Current Opinion in Biotechnology* 10: 123–129.

- Jayakumar R, Reis RL, Mano JF (2006) Phosphorous containing chitosan beads for controlled oral drug delivery. *Journal of Bioactive and Biocompatible Polymers* 21(4): 327–340.
- Jones V, Grey JE, Harding KG (2006) Wound dressings. *British Medical Journal* 332: 777–780.
- Juliano RL, Redding P, Alahari S, Edin M, Howe A, Aplin A (2004) Integrin regulation of cell signalling and motility. *Biochemical Society Transactions* 32: 443–446.
- Kolff WJ (1993) The beginning of the artificial kidney. *Artificial Organs* 17(5): 293–299.
- Kutschka I, Chen IY, Kofidis T, Arai T, von Degenfeld G, Sheikh AY, Hendry SL, Pearl J, Hoyt G, Sista R, Yang PC, Blau HM, Gambhir SS, Robbins RC (2006) Collagen matrices enhance survival of transplanted cardiomyoblasts and contribute to functional improvement of ischemic rat hearts. *Circulation* 114(Suppl. 1): I167–I173.
- Li R, Bennett JS, DeGrado WF (2004) Structural basis for integrin  $\alpha\text{IIb}\beta_3$  clustering. *Biochemical Society Transactions* 32: 412–415.
- Madhally SV, Matthew HWT (1999) Porous chitosan scaffolds for tissue engineering. *Biomaterials* 20: 1133–1142.
- Mahanthappa NK, Cooper DN, Barondes SH, Schwarting GA (1994) Rat olfactory neurons can utilize the endogenous lectin, L-14, in a novel adhesion mechanism. *Development* 120: 1373–1384.
- Mann S (1988) Molecular recognition in biomineralization. *Nature* 332: 119–124.
- Martin P, Leibovich SJ (2005) Inflammatory cells during wound, repair: the good, the bad and the ugly. *Trends in Cell Biology* 15: 599–607.
- Meinel L, Hofmann S, Karageorgiou V, Kirker-Head C, McCool J, Gronowicz G, Zichner L, Langer R, Vunjak-Novakovic G, Kaplan DL (2005) The inflammatory responses to silk films in vitro and in vivo. *Biomaterials* 26: 147–155.
- Middleton E, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: implication for inflammation, heart disease, and cancer. *Pharmacological Reviews* 52: 673–751.
- Minuth WW, Sittering M, Kloth S (1998) Tissue engineering: generation of differentiated artificial tissues for biomedical applications. *Cell and Tissue Research* 291: 1–11.
- Morris C, Thorpe J, Ambrosio L, Santin M (2006) The soybean isoflavone genistein induces differentiation of MG63 human osteosarcoma osteoblasts. *The Journal of Nutrition* 136: 1166–1170.
- Motta A, Migliaresi C, Lloyd AW, Denyer SP, Santin M (2002) Serum protein absorption on silk fibroin fibers and films: surface opsonization and binding strength. *Journal of Bioactive and Compatible Polymers* 17: 23–35.
- Motta A, Migliaresi C, Faccioni F, Torricelli P, Fini M, Giardino R (2004) Fibroin hydrogels for biomedical applications: preparation, characterization and in vitro cell culture studies. *Journal of Biomaterials Science. Polymer Edition* 15: 851–864.
- Murkies AL, Wilcox G, Davies SR (1998) Phytoestrogens. *The Journal of Clinical Endocrinology and Metabolism* 83: 297–303.
- Mytar B, Diedlar M, Woloszyn M, Colizzi V, Zembala M (2001) Cross-talk between human monocytes and cancer cells during reactive oxygen intermediates generation: the essential role of hyaluronan. *International Journal of Cancer* 94: 727–732.
- Norde W (1986) Adsorption of proteins from solution at the solid-liquid interface. *Advances in Colloid and Interface Science* 25: 267–340.

- Phillips JM, Kao WJ (2005) Macrophage adhesion on gelatine-based interpenetrating networks grafted with PEGylated RGD. *Tissue Engineering* 11: 964–973.
- Raghunath K, Rao KP, Joseph KT (1980) Chemically modified natural polymers as biomaterials: 1. Polysaccharide-gelatin conjugates. *Polymer Bulletin* 2: 477–483.
- Rao SB, Sharma CP (1997) Use of chitosan as a biomaterial: studies on its safety and hemostatic potential. *Journal of Biomedical Materials Research* 34: 21–28.
- Rezania A, Healy KE (1999) Biomimetic peptide surfaces that regulate adhesion, spreading, cytoskeletal organization, and mineralization of the matrix deposited by osteoblast-like cells. *Biotechnology Progress* 15: 19–32.
- Rivier N, Sadoc JF (2006) The transverse structure of collagen. *Philosophical Magazine* 86(6–8): 1075–1083.
- Rosca C, Popa MI, Chitanu GC, Popa M (2005) Interaction of chitosan with natural or synthetic anionic polyelectrolytes: II. The chitosan-alginate complex. *Cellulose Chemistry and Technology* 39(5–6): 415–422.
- Rothenburger M (2001) In vitro modelling of tissue using isolated vascular cells on a synthetic collagen matrix as a substitute for heart valves. *The Thoracic and Cardiovascular Surgeon* 49: 204.
- Roughley P, Hoemann C, DesRosiers E, Mwale F, Antoniou J, Alini M (2006) The potential of chitosan-based gels containing intervertebral disc cells for nucleus pulposus supplementation. *Biomaterials* 27: 388–396.
- Rücker M, Laschke MW, Junker D, Carvalho C, Schramm A, Mülhaupt R, Gellrich N, Menger MD (2006) Angiogenic and inflammatory response to biodegradable scaffolds in dorsal skin-fold chambers of mice. *Biomaterials* 27(29): 5027–5038.
- Sakiyama-Elbert SE, Hubbell JA (2001) Functional biomaterials: design of novel biomaterials. *Annual Review of Materials Research* 31: 183–201.
- Sanborn TJ, Messersmith PB, Barron AE (2002) In situ crosslinking of a biomimetic peptide-PEG hydrogel via thermally triggered activation of factor XIII. *Biomaterials* 23: 2703–2710.
- Sandrini E, Morris C, Chiesa R, Cigada A, Santin M (2005) In vitro assessment of the osteointegrative potential of a novel multiphase anodic spark deposition coating for orthopaedic and dental implants. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 73(2): 392–399.
- Santin M, Cannas M (1999) Collagen-bound alpha(1)-microglobulin in normal and healed tissues and its effects on immunocompetent cells. *Scandinavian Journal of Immunology* 50: 289–295.
- Santin M, Wassall MA, Peluso G, Denyer SP (1997) Adsorption of  $\alpha$ -1-microglobulin from biological fluids onto polymer surfaces. *Biomaterials* 18: 823–827.
- Santin M, Motta A, Freddi G, Cannas M (1999) In vitro evaluation of the inflammatory potential of the silk fibroin. *Journal of Biomedical Materials Research* 46: 382–389.
- Santin M, Nicolais L, Ambrosio L (2001) Soybean-based biomaterials PCT/GB01/03464.
- Santin M, Lloyd AW, Denyer SP, Motta A (2002) Domain-driven binding of fibrin(ogen) to silk fibroin. *Journal of Bioactive and Compatible Polymers* 17: 195–208.
- Schroeder-Tefft JA, Bentz H, Estridge TD (1997) Collagen and heparin matrices for growth factor delivery. *Journal of Controlled Release* 49: 291–298.
- Sharon N, Lis H (1993) Carbohydrates in cell recognition. *Scientific American January Issue*: 73–81.
- Soong HK, Kenyon KR (1984) Adverse reactions to virgin silk sutures in cataract surgery. *Ophthalmology* 91: 479–483.

- Stefansson A, Armulik A, Nilsson IM, von Heijne G, Johansson S (2004) Determination of N- and C-terminal borders of the transmembrane domain of integrin subunits. *The Journal of Biological Chemistry* 279: 21200–21205.
- Steflik DE, Corpe RS, Lake FT, Young RT, Sisk AL, Parr GR, Hanes PJ, Berkery DJ (1998) Ultrastructural analyses of the attachment (bonding) zone between bone and implanted biomaterials. *Journal of Biomedical Materials Research* 39: 611–620.
- Takagi J (2004) Structural basis for ligand recognition by RGD (Arg-Gly-Asp)-dependent integrins. *Biochemical Society Transactions* 32: 403–406.
- Takemoto M, Fuhibayashi S, Neo M, Suzukin J, Kokubo T, Nakamura T (2005) Mechanical properties and osteoconductivity of porous bioactive titanium. *Biomaterials* 26: 6014–6023.
- Takeshita F, Ihama S, Ayukawa Y, Akedo H, Suetsugu T (1997) Study of bone formation around dense hydroxyapatite implants using light microscopy, image processing and confocal laser scanning microscopy. *Biomaterials* 18: 317–322.
- Teder P, Vandivier RW, Jiang DH, Liang JR, Cohn L, Pure E, Henson PM, Noble PW (2002) Resolution of lung inflammation by CD44. *Science* 296: 155–158.
- Tomihata K, Ikada Y (1997) In vitro and in vivo degradation of films of chitin and its deacetylated derivatives. *Biomaterials* 18: 567–575.
- Turley EA, Noble PW, Bourguignon LYW (2002) Signalling properties of hyaluronan receptors. *The Journal of Biological Chemistry* 277: 4589–4592.
- Turley EA (2001) Extracellular matrix remodelling: multiple paradigms in vascular disease. *Circulation Research* 88: 2–4.
- Velling T, Risteli J, Wennerberg K, Mosher DF, Johansson S (2002) Polymerization of type I and III collagens is dependent on fibronectin and enhanced by integrins  $\alpha_{11}\beta_1$  and  $\alpha_2\beta_1$ . *The Journal of Biological Chemistry* 277: 37377–37381.
- Walle T, Browning AM, Steed LL, Reed SG, Walle UK (2005) Flavonoids glucosides are hydrolyzed and thus activated in the oral cavity in humans. *The Journal of Nutrition* 135: 48–52.
- Wells V, Malluchi L (1991) Identification of an autocrine negative growth factor: murine beta-galactoside-binding protein is a cytostatic factor and cell regulator. *Cell* 64: 91–97.
- Williams DF, ed. (1987) Definitions in biomaterials. In: *Proceedings of a Consensus Conference of the European Society for Biomaterials*, Chester, England, 3–5 March 1986, *Progress in Biomedical Engineering* Vol. 4, Elsevier, Amsterdam.
- Williams HP (2001) Sir Harold Ridley's Vision. *The British Journal of Ophthalmology* 85: 1022–1023.
- Xynos ID, Edgar AJ, Buttery LDK, Hench LL, Polak JM (2000) Ionic products of bioactive glass dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor II mRNA expression and protein synthesis. *Biochemical and Biophysical Research Communications* 276: 461–465.
- Zaidel-Bar R, Cohen M, Addadi L, Geiger B (2004) Hierarchical assembly of cell-matrix adhesion complexes. *Biochemical Society Transactions* 32: 416–420.
- Zhai Y, Cui FZ (2006) Recombinant human-like collagen directed growth of hydroxyapatite neocrystals. *Journal of Crystal Growth* 291: 202–206.
- Zhou S, Turgeman G, Harris SE, Leitman DC, Komm BS, Bodine PVN, Gazit D (2003) Estrogens activate bone morphogenetic protein-2 gene transcription in mouse mesenchymal stem cells. *Molecular Endocrinology* 17: 56–66.