

# Degradation of Biomaterials

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## LEARNING OBJECTIVES

- To understand what defines degradable biomaterials and why they are needed
- To identify the most important degradation mechanisms of biomaterials
- To understand the effect of tissue response on the (in vivo) degradation rate
- To understand the requirements for a degradable biomaterial to be used as a cell scaffold for tissue engineering
- To learn why degradable bioceramics are used in bone regeneration
- To learn how degradable polymers can be synthesized
- To recognize examples of degradable polymers broadly used in tissue engineering

## 6.1 DEGRADABLE BIO CERAMICS

Inorganic nonmetallic solids can be classified as ceramic, glass, or glass-ceramics.

The word *ceramic* is derived from the Greek word *keramikos*, “having to do with pottery.” The American Society for Testing and Materials defines a ceramic article as “an article having a...body of crystalline or partly crystalline structure, or of glass, which body is produced from essentially inorganic, nonmetallic substances and either is formed from a molten mass which solidifies on cooling, or is formed and simultaneously or subsequently matured by the action of the heat.”

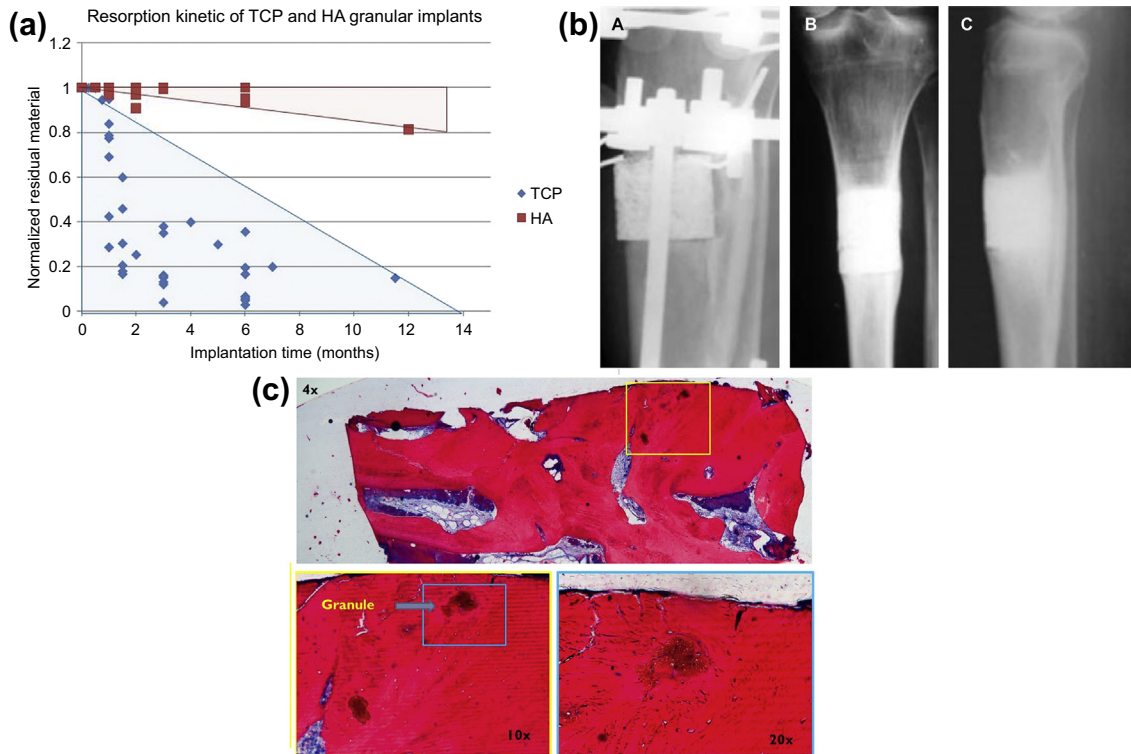
For several decades, bioceramics (i.e., calcium phosphates (CaPs) and bioactive glasses (BGs)) have been used clinically as bone-regenerative materials,

particularly in nonload-bearing dental and orthopedic applications since they are not mechanically strong (Temenoff and Mikos, 2008). Current biomedical applications of bioceramics include repair for periodontal disease, maxillofacial reconstruction, augmentation and stabilization of the jawbone, spinal fusion, and bone fillers after tumor surgery. On the horizon, bioceramics show promise as effective carriers for drug delivery, growth factors, bioactive peptides, and various types of cells for tissue engineering purposes (Williams, 2014). Because of their excellent biocompatibility and ability to regenerate bone, integration of these bioceramics with stronger biomaterials (e.g., polymers) is another research focus to improve the mechanical properties of bone substitutes. For example, a titanium hip implant can be coated with bioceramics to aid integration with native bone or bioceramic particles can be blended with polymers to create composites with enhanced mechanical properties (Dorozhkin, 2011).

Since 1920, clinicians have used CaPs as bone graft substitutes with success. Over the past two decades, CaPs consisting of hydroxyapatite (HA) and  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) phases have been commonly used as bone void fillers because they are both osteoconductive, i.e., able to support bone formation directly at their surface (Dorozhkin, 2011). However, because of their different crystalline structure, HA and  $\beta$ -TCP degrade at significantly different rates in physiologic solution given by their thermodynamic solubility constant ( $K_s$ ):  $6.62 \times 10^{-126}$  and  $2.07 \times 10^{-33}$  for HA and  $\beta$ -TCP, respectively (Elliot, 1994). Hence, HA bioceramics are considered to be nondegradable and  $\beta$ -TCP bioceramics are considered to be degradable. Their distinct degradation profiles are apparent in preclinical studies, where HA and  $\beta$ -TCP were implanted in bony locations and analyzed over time (Figure 6.1). Similarly in clinical studies,  $\beta$ -TCP ceramics can totally degrade, while HA ceramics remain almost unaltered several years after implantation (Horch et al., 2006; Mastrogiacomo et al., 2005).

One way to modulate the degradation profile of CaP bioceramics is by mixing less soluble HA and highly soluble  $\beta$ -TCP, or incorporating other biocompatible CaP phases. In addition, other CaPs with various solubilities and degradation kinetics are also used clinically to regenerate bone, such as dicalcium phosphate dihydrate (DCPD) and amorphous CaP (ACP). Table 6.1 summarizes CaPs of current biological and clinical interest.

BGs represent another class of degradable bioceramics. Glass is an amorphous inorganic solid. Glass results from the very rapid cooling of a viscous molten material to a solid state without crystallization. Silica ( $\text{SiO}_2$ ) is the common base for glass. A slower, more controlled cooling protocol can induce the formation of glass-ceramics, which are partly crystalline and partly glassy. An important change in the glass microstructure, which usually precedes crystallization, is the glass-in-glass phase separation. After phase separation, the glass

**FIGURE 6.1**

(a) Degradation kinetics of HA and  $\beta$ -TCP ceramics implanted in preclinical studies. Residual material was determined by histological analysis on undecalcified sections. Material was normalized across publications: the starting amount of material at the time of implantation corresponds to 1; no residual material corresponds to 0 (Nyangoga et al., 2010; Walsh et al., 2008; Hing, 2005; Von Doernberg et al., 2006; Jensen et al., 2009; Wiltfang et al., 2002; Bodde et al., 2007; Gonda et al., 2009; Peltola et al., 2001; Peltola et al., 2003; Liu et al., 2000); (b) Tibia repair by a tissue engineering approach in a human subject. Radiographs obtained immediately post (A) 18 months and (B) 5.5 years after surgery (Mastrogiacomo et al., 2005); (c) Histological evidence of  $\beta$ -TCP resorption after 1 year implantation (de Ruijter et al., 2013).

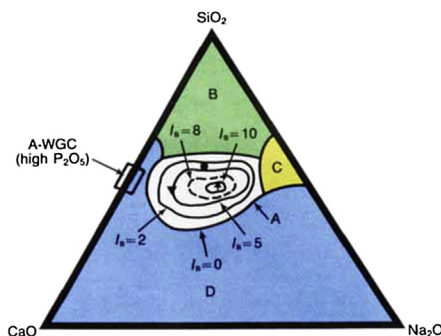
**Table 6.1** Calcium Phosphates of Biological Interest

Name	Formula	Abbreviation	Ca/P
Dicalcium phosphate anhydrate	$\text{CaHPO}_4$	DCPA	1.00
Dicalcium phosphate dihydrate	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	DCPD	1.00
Octacalcium phosphate	$\text{Ca}_8(\text{PO}_4)_4(\text{HPO}_4)_2 \cdot 5\text{H}_2\text{O}$	OCP	1.33
Tricalcium phosphate	$\text{Ca}_3(\text{PO}_4)_2$	TCP	1.50
Hydroxyapatite	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	OHAp	1.67

no longer has a homogenous composition, but rather consists of two or more immiscible glassy phases of different chemical constituents. Unlike crystallization, phase separation in glass might not be visible by optical microscopy and in most cases can be detected only by electron microscopy.

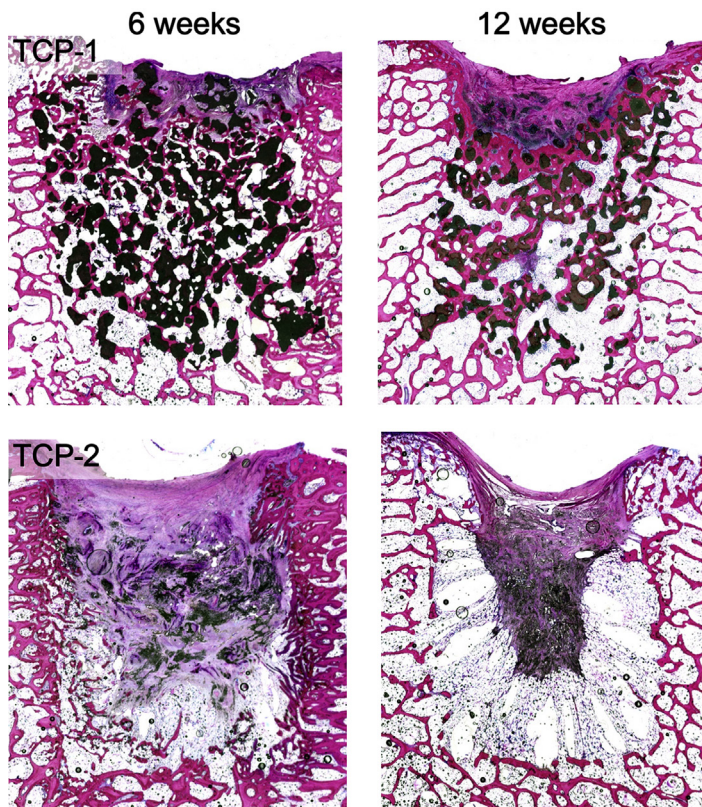
BGs consist of  $\text{SiO}_2$ ,  $\text{CaO}$ ,  $\text{Na}_2\text{O}$ , and  $\text{P}_2\text{O}_5$  in specific proportions. These components form a two-dimensional glass network in which silica ( $\text{SiO}_2$ ) forms the network and the alkali metal (e.g., Na, K, Ca, Mg) modifies it (Ducheyne et al., 1994). For instance, the ratio of silica network former to metal network modifier in a BG determines its solubility in physiological solutions, and hence its bioactivity and resorbability. For example, BG45S5, the first tested BG composition contains (in weight percentage, wt%) 45% of  $\text{SiO}_2$ , 24.5%  $\text{CaO}$ , 24.5%  $\text{Na}_2\text{O}$ , and 6%  $\text{P}_2\text{O}_5$ . This glass was first studied in the late 1960s and early 1970s (Hench, 1991). Later, glass-ceramics were developed in order to increase the mechanical properties of these BGs. To this end, BGs are subjected to thermal treatments that alter the material's microstructure. Although mechanisms were not understood, it was suggested that this glass type formed a chemical bond with bone. Since then, it has been established that glasses do not bond to bone if the Ca/P ratio is substantially lower than 5:1, as seen in Figure 6.2, relating to its degradability.

The degradation kinetics of bioceramics is an important factor in bone-regenerative medicine. First, the degradation profile orchestrates the available space to allow new bone to grow. To illustrate this, the implantation of two macroporous  $\beta$ -TCP bioceramics (TCP-1 and TCP-2) of identical composition but made by different synthesis methods demonstrated significantly different in vivo degradation profiles and consequently significantly different



**FIGURE 6.2**

Compositional dependence (in wt%) of bone and soft tissue bonding of BGs and glass-ceramics. All compositions in region A are bioactive and bond to bone. Compositions in region B are bioinert and lead to formation of a nonadherent fibrous capsule. Compositions in region C are resorbable. Region D is restricted by technical factors. Region E (soft tissue bonding) is inside the dashed line (Hench, 1991).



**FIGURE 6.3**

Light microscopy pictures of macroporous CaPs implanted in goat's femoral condyle (magnification: 2 $\times$ ). This figure represents the tunable nature between degradation and bone formation of two  $\beta$ -TCP ceramics with similar composition but made by different synthesis methods. TCP-1 degrades slower than TCP-2 and supports continuous bone ingrowth from the host bone to the center of the implant, reflecting full bone regeneration in the defect. TCP-2 degrades too fast and does not support bone ingrowth; instead, the defect is filled with fibrous tissue. *Courtesy of Dr. Huipin Yuan.*

bone-regenerative potential in the femoral condyle of goats (Figure 6.3). TCP-2, which was resorbed too rapidly, produced only fibrous tissue growth in the bone defect, while TCP-1, which was resorbed much slower, produced significant bone growth in the defect. In addition to the kinetics of degradation, it is theorized that the biodegradation mechanisms and products have a direct (positive or negative) impact on cells and tissues, thus directing tissue formation.

### 6.1.1 Degradation Mechanisms of Bioceramics

Ceramic materials are held together by either ionic or covalent bonds. They can be crystalline or amorphous. The crystalline and amorphous states are typical solid



states which represent the degree of order between ions, atoms, or molecules. The *crystalline state* is characterized by a well-ordered, defined internal molecular structure. This structure is periodically repeated in a specific tri-dimensional pattern (a). The cohesion in crystals is insured by the binding energy between the atoms in the case of covalent solids, or by electrostatic forces existing between anions and cations in the case of ionic solids. On the contrary, *amorphous state* is characterized by no order between ions, atoms, or molecules (b); therefore the values of binding energy between entities greatly vary within the solid. The dissolution of ceramics is strongly dependent on these binding energies.

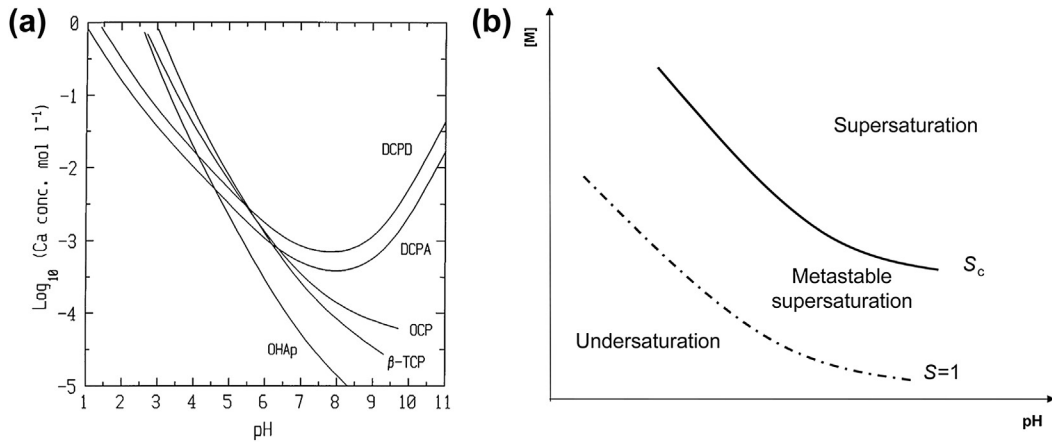
In physiologic and artificial aqueous environments, bioceramics can degrade via multiple mechanisms: (1) physico-chemical dissolution accompanied by possible phase transformation, (2) cellular degradation mediated by multi-nucleated cells (MNC), and (3) mechanical fragmentation due to a loss of structural integrity resulting from mechanisms (1) and (2). In biological systems, degradation of bioceramics reflects nonequilibrium processes that occur simultaneously or in competition with each other. Because bioceramics alone are not used in load-bearing conditions, the mechanical degradation mechanism will not be addressed in this chapter.

#### **6.1.1.1 Physico-Chemical Degradation of Bioceramics**

The physico-chemical degradation of bioceramics can be described as a dissolution–reprecipitation phase transformation process, which is the result of ion exchange at the solid–liquid interface. The kinetics of this degradation process and the surface transformations resulting from ionic exchange depend on the intrinsic properties of the bioceramics and the nature of the aqueous environment.

From a thermodynamic point of view, CaPs are sparingly soluble in water but readily dissolve in acids. To study and compare the dissolution kinetics of CaPs in vitro, physiologic saline solutions at different pH are generally used; in these simplified solutions, solubility kinetics correlate with the thermodynamic solubility constant of the CaP. However, more complex supersaturated solutions can be used to more closely mimic the in vivo environment. Supersaturated solutions, such as blood, contain higher concentrations of ions than could be dissolved in water under equilibrium conditions; thus, the supersaturation state is inherently unstable. Figure 6.4(a) shows the solubility of various CaP phases as a function of  $\text{Ca}^{2+}$  concentration of the solution and pH under equilibrium conditions at 37°C. In supersaturation conditions, crystal growth has not yet occurred, but this can be initiated by introducing seeds, i.e., CaP substrates (Figure 6.4(b)). Supersaturated solutions frequently used to mimic physiologic solution include cell culture media supplemented with serum or simulated body fluid (SBF) (Bohner and Lemaître, 2009).

Although not always the case, it can be generally stated that the physico-chemical dissolution behavior of CaP ceramics in vitro and in vivo follows



**FIGURE 6.4**

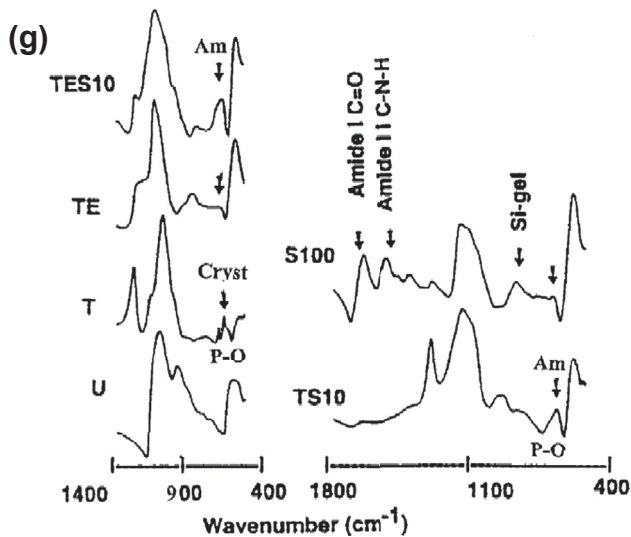
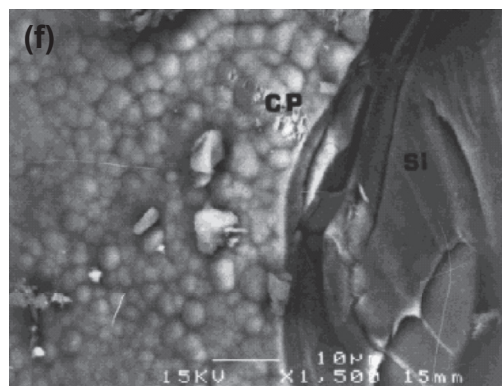
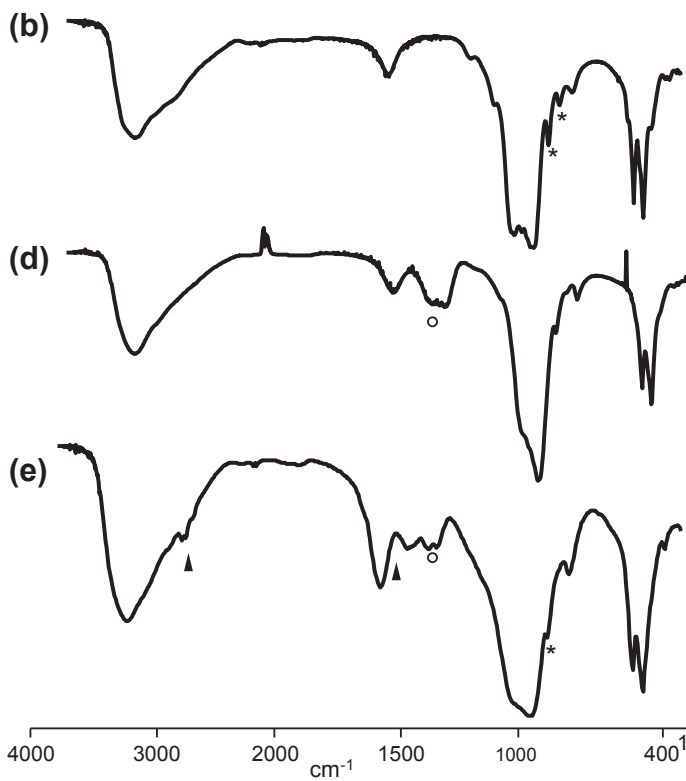
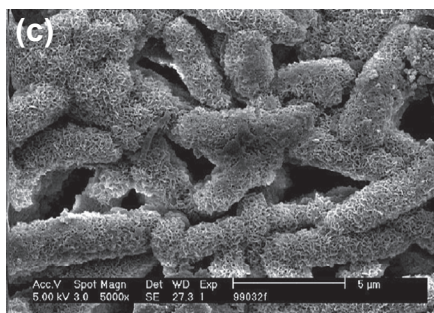
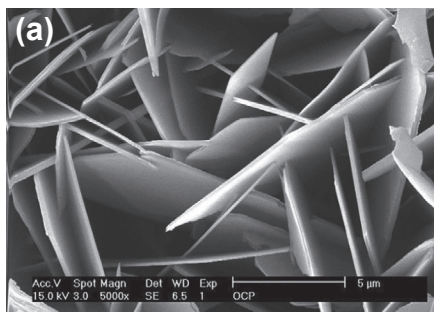
(a) Solubility isotherms of calcium phosphates at 37°C (Elliot, 1994). (b) Solubility isotherms of salts in solution as a function of the pH. This diagram represents the three different saturation states of an ionic solution susceptible to precipitation.

their *thermodynamic solubility*, i.e.,  $HA < TCP < OCP < DCPD$ , from least soluble to the most soluble. Still, additional factors can also affect the physico-chemical dissolution kinetics:

- *The crystalline features.* The higher the crystallinity, the lower the degradation kinetics. Eventually, amorphous biomaterials dissolve faster than crystalline ones.
- *The presence of additives.* The presence of some additives of mineral origins within the CaP structure can affect the crystal lattice, and therefore can accelerate the dissolution, e.g., carbonate, silicate, or strontium added to HA.
- *The physical structure and geometry (e.g., porosity, granules vs blocks).* The larger the exposed surface to the solution environment, the faster the biomaterial dissolves, simply because more exchanges can take place.

*Crystalline solids as bioceramics* are different from the theoretical crystals. First, these crystals generally contain imperfections: (1) additional entities are inserted in the crystal lattice (c), or (2) some entities can be absent in the crystal, i.e., vacancies (d). Second, crystalline solids are composed of several crystals packed together in a roughly organized bulk structure. These imperfections result from the materials' processing (e.g., impurities, sintering) and can affect their solubility.

From a surface reactivity point of view, physico-chemical dissolution can be seen as ionic transfer from the solid phase to the aqueous liquid via surface hydration of  $Ca^{2+}$  and phosphate species ( $PO_4^{3-}$ ,  $HPO_4^{2-}$ ,  $H_2PO_4^-$ ) as bioceramic material interacts with other ions, proteins, and sugars present in the biological fluid. On the one hand, this dissolution process is highly dependent on the nature of the





CaP bioceramics and on the other hand, it is dependent on the nature of the environment, i.e., chemical composition, supersaturation, and pH. Whether the environment is *in vivo*, in culture medium, or in SBF, the nature of the precipitate is a poorly crystallized carbonated apatite. When organic compounds are present in the surrounding milieu, they generally slow down the formation of this new apatitic layer, and they can be incorporated therein. However, not all CaP ceramics without known bone-regenerative properties *in vivo* allow the growth of such a newly formed apatite layer *in vitro* (Bohner and Lemaître, 2009).

The physico-chemical degradation of BGs can be generalized as three processes including leaching, dissolution, and precipitation. Briefly, sodium ions leach from the surface and are replaced by hydrogen ions through an ion-exchange reaction. This depletion of sodium leads to the formation of a silica-rich layer. An ACP layer forms on the silica-rich layer. Calcium is present in the solid state, but is equally drawn from solution. In simple electrolyte solutions (which are not good simulations of *in vivo* behavior), the amorphous layer crystallizes to form carbonated HA (c-Ap) with properties akin to the mineral phase of bone. This simple reaction sequence that brings about the deposition of c-Ap was first proposed by Hench (1971).

Hench described five reaction stages:

- Stage 1: Leaching and formation of silanols
- Stage 2: Loss of soluble silica and formation of silanols
- Stage 3: Polycondensation of silanols to form a hydrated silica gel
- Stage 4: Formation of an amorphous calcium phosphate layer
- Stage 5: Crystallization of c-Ap layer (Figure 6.5(a)).

## FIGURE 6.5

Morphology and structure of an octacalcium phosphate (OCP) coating (a and b) and the subsequent changes due to the dissolution/ reprecipitation process *in vitro* after immersion in culture medium for 2 weeks (c and d), and *in vivo* after 4 weeks of subcutaneous implantation in rat (e). The morphology of the substrate is shown in scanning electronic microscopy (SEM) (a and c). The structure is characterized by Fourier Transform InfraRed (FTIR) spectroscopy (b, d, and e). The initial OCP substrate is composed of sharp elongated crystals (a) and its crystal structure is characterized by sharp phosphate (P–O) bands between 1100 and 1000  $\text{cm}^{-1}$  and at  $\sim 600$  and 560  $\text{cm}^{-1}$  (b). Two typical OCP bands can be noted at 906 and 852  $\text{cm}^{-1}$  (\*). *In vitro*, the substrate exhibits smaller crystals that seem to grow along the initial OCP crystals (c), the FTIR structure of these crystals have lost their OCP features and exhibit broader and less defined bands (d). In addition, new bands typical of carbonate groups at 1460 and 1416  $\text{cm}^{-1}$  (O) have appeared. This FTIR spectrum indicates a carbonate apatitic structure (d). *In vivo* (subcutaneous implantation in rats for 4 weeks), the FTIR structure of the coating has evolved in a similar way as *in vivo*: the coating transformed into a carbonated apatite (e). In addition, organic compounds ( $\blacktriangle$ ) were found in the transformed substrate. Phase transformations of bioglass (BG) *in vitro* (f). SEM of a fractured granule showing the Ca–P (CP) surface layer and underlying silica (Si) (Radin et al., 2000) (g). FTIR spectra of unreacted BG 45S5 prior to (U) and after immersion for 3 h into 0.05 M Tris buffer (T), T+plasma electrolyte (TE), TE+10% serum (TES-10), T+10% serum (TS-10), and 100% serum (S-100). Notice the appearance of a split P–O bend indicating formation of a crystalline phase (Cryst) after immersion in T and undivided P–O bend indicating amorphous (Am) phosphate phase after immersion in other solutions (Radin et al., 2000).

The dissolution of BGs has been studied for over 40 years. Many different factors influence the mechanism and rate of the dissolution of BGs, such as glass composition, glass particle size, and glass powder type.

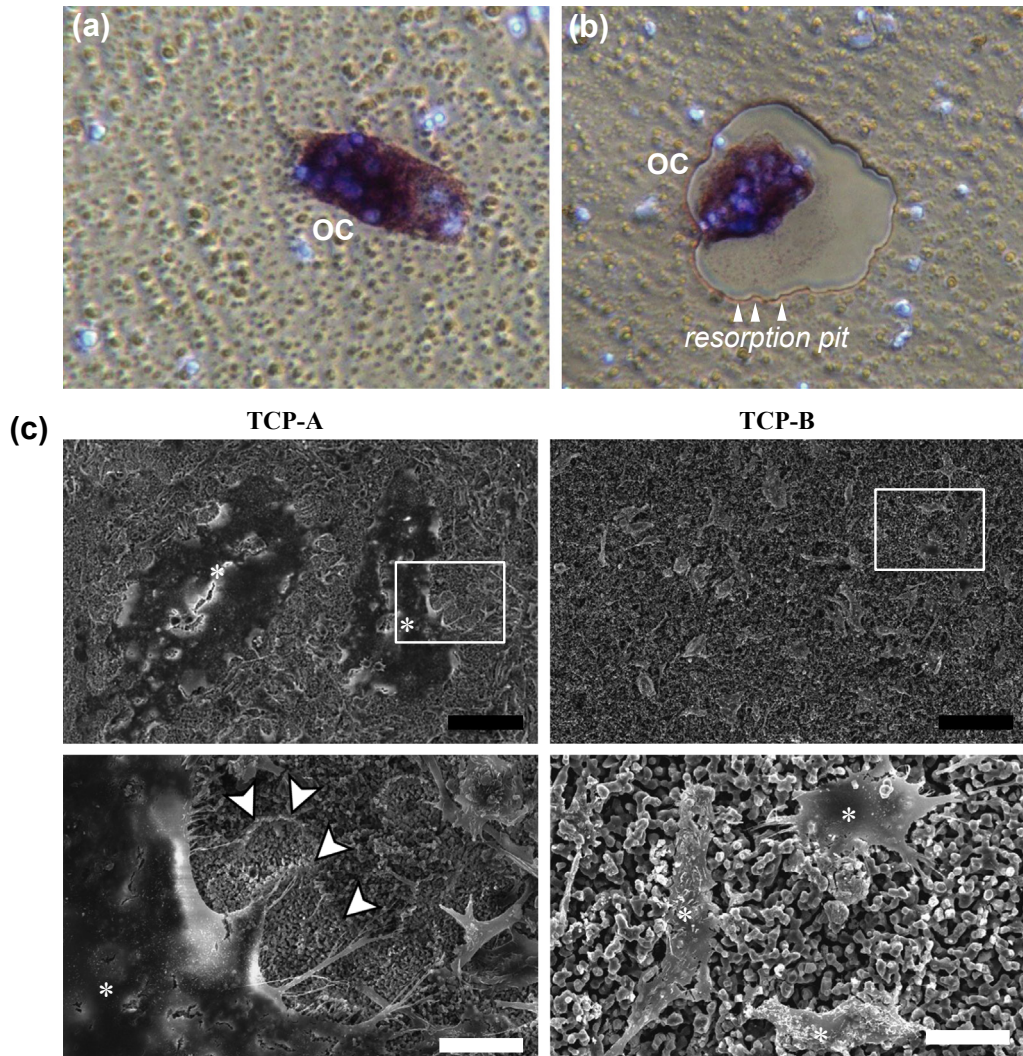
### 6.1.1.2 Cellular Degradation of Bioceramics

The cellular degradation of bioceramics is mediated by MNC, which resorb the material surface and phagocytose degraded material particulate. Osteoclasts are specialized MNC uniquely responsible for the resorption of bone through the acid dissolution of bone mineral and enzymatic cleavage of bone protein matrix. Osteoclasts also play a critical role in regulating new bone formation through bone coupling mechanisms. Bone coupling describes the tendency of osteoblasts to lay down new bone precisely where it was first resorbed by osteoclasts in tight synchronicity.

In vitro and in vivo, it has been observed that osteoclasts can degrade CaPs in a similar way as bone mineral: osteoclasts adhere to the CaP surface and create resorption pits, presumably through acid dissolution of the sealed micro-environment formed between the osteoclast and the material substrate (the “sealing zone”). In vitro, osteoclasts readily adhere and resorb several CaP ceramics including HA and TCP. In vivo, multinucleated osteoclast-like cells form microconcavities on the ceramic surface adjacent to newly formed bone and degraded material particulate (Figure 6.6.). In vitro experiments identified specific physico-chemical parameters beyond the primary CaP composition that influence cellular degradation.

- *The physico-chemical dissolution kinetics of the CaP ceramics.* Depending on the synthesis methods, dissolution kinetics can vary widely among CaPs with the same chemical composition. The release of  $\text{Ca}^{2+}$  influences osteoclastic activity; for instance, above a certain  $\text{Ca}^{2+}$  concentration, osteoclastic resorption is inhibited. As previously discussed, CaP structure and crystallinity also play a role in dissolution kinetics and therefore may also dictate osteoclast activity.
- *The presence of additives.* The incorporation of carbonate or other ions in CaP may stimulate the carbonic anhydrase activity known to promote the osteoclastic acidic secretion in vitro. On the other hand, the presence of zinc and fluoride in CaP can inhibit osteoclastic resorption in vitro and in vivo.
- *The surface features.* Surface energy was found to modulate the osteoclastic adhesion in vitro. Surface roughness and microporosity appear to enhance osteoclastic attachment and activity (Barrère et al., 2006).

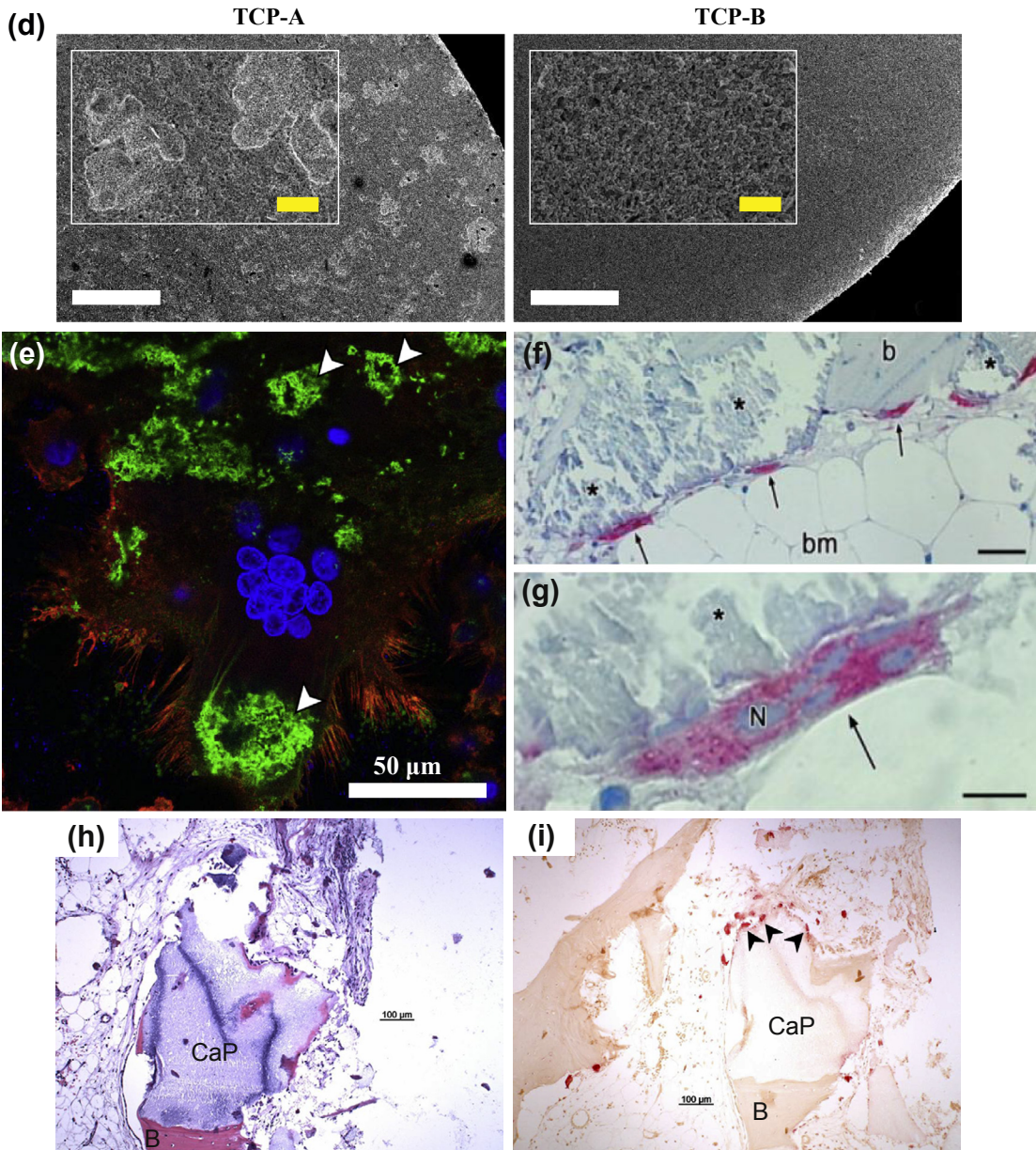
Osteoclast resorption of BGs has not been extensively studied. Both in vivo and in vitro studies demonstrate that active osteoclasts are only detected on the



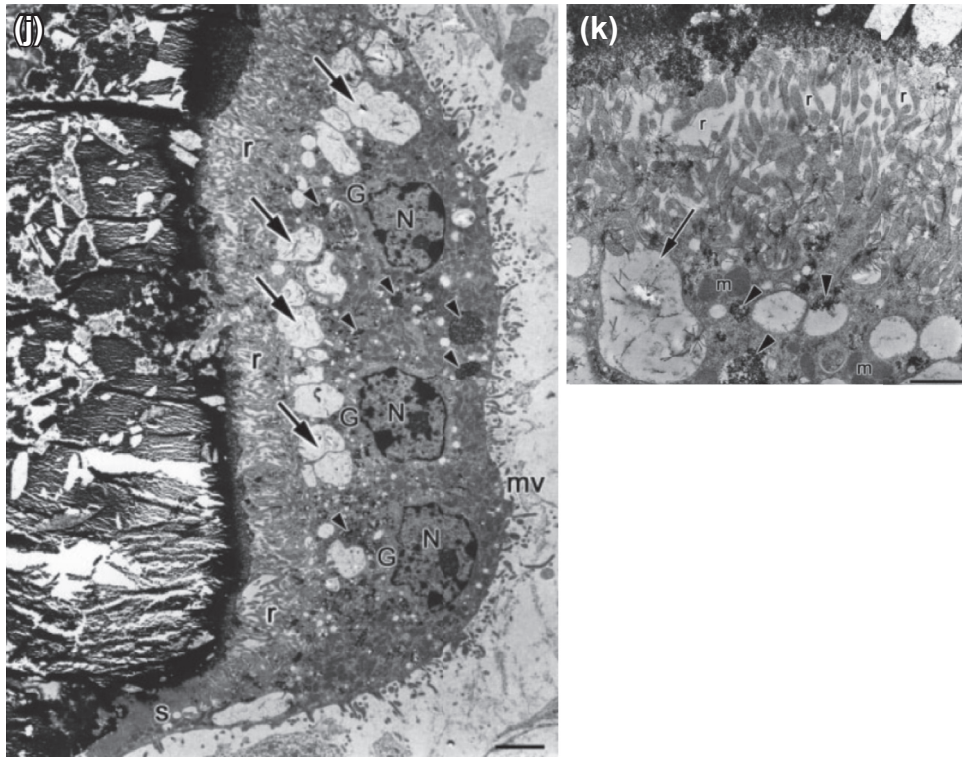
**FIGURE 6.6**

Osteoclasts (OCs) on CaPs in vitro (a–e) and in vivo (f–m). OCs stain positive for tartrate-resistant acid phosphatase (TRAP) (red) in both (a) nonresorbing and (b) resorbing states. Nuclei staining (blue) shows that OCs are multinucleated. (Courtesy Dr. S.G. Perez.) (c) OC (white arrows) formation occurs differently on two TCP ceramics with different surface structure (black scale bars = 100  $\mu\text{m}$ ; white scale bars = 20  $\mu\text{m}$ ). After removing OCs, extensive resorption pits were evident on TCP-A (lighter areas) but not on TCP-B (d) (white scale bars = 500  $\mu\text{m}$ ; yellow scale bars = 50  $\mu\text{m}$ ). (e) Fluorescent microscopy of an OC resorbing TCP. Actin rings (white arrows)—where osteoclast resorption takes places—are evident near the cell membrane border (Green = F-actin, Red = cell membrane, Blue = cell nuclei). (f and g) TRAP-positive OCs ( $\rightarrow$ , stained red) resorbing a CaP implant (\*) and bone (B), next to bone marrow (BM). At high magnification, many nuclei (N) in one resorbing OC can be seen ((f), scale bar = 50  $\mu\text{m}$ ; (g), 10  $\mu\text{m}$ ) (Wenisch et al., 2003). (h and i) Consecutive histological sections from a biopsy after 6 months implantation of TCP granules for sinus lift augmentation: (h) CaP granules in contact with bone (B) are surrounded by OCs ( $\rightarrow$ ) stained positively for TRAP (red). (Courtesy of Dr. J.W. Hoekstra.) (j) Transmission electronic micrograph of an OC that is closely associated with a ceramic surface. Several cell features are noted: the osteoclast nuclei (N), ruffled border (r), sealing zone (s), dorsal microvilli (mv), golgi complexes (G), vacuoles filled with longer slender CaP crystals ( $\rightarrow$ ), and denser particles ( $\blacktriangledown$ ) (scale bar = 2  $\mu\text{m}$ ) (Wenisch et al., 2003) (k). Ultrastructural details of the osteoclastic ruffled border at the implant surface showing internalized CaP particles in the cellular vacuoles (scale bar = 1  $\mu\text{m}$ ).





**FIGURE 6.6** *Continued*



**FIGURE 6.6** *Continued*

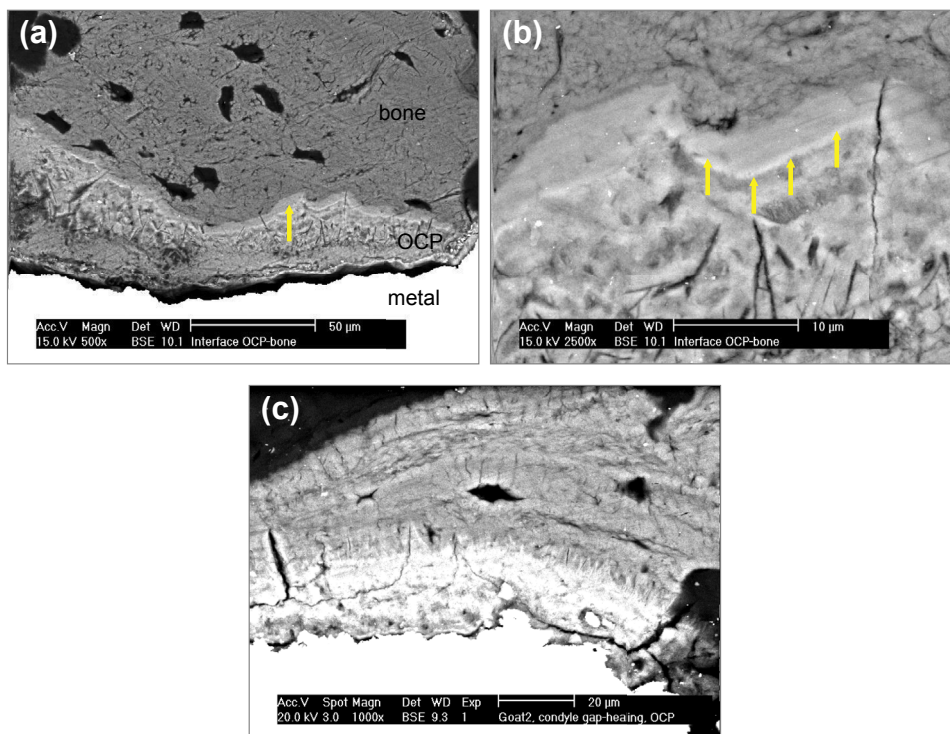
CaP layer that can precipitate on the BG surface. Osteoclasts may be responsible for resorbing this formed CaP layer; however, there is no direct evidence of cellular degradation of silicon. Regardless of the mode of degradation, the silicon content of BG can be safely excreted in urine when implanted in vivo (Lai et al., 2005).

## 6.1.2 Translation of Degradable Bioceramics to Bone Tissue Engineering Systems

### 6.1.2.1 Bone Bonding and Stimulation of Bone Formation

The dissolution/precipitation phenomena are associated with bioactivity related to bone bonding, i.e., the formation of an interfacial mineralized layer between bioceramics and bone tissue that ensures their cohesion. Structurally, this layer is comparable to the films grown in vitro by dissolution-precipitation mechanisms, i.e., nanocrystals of carbonated apatite in SBF that mimic the mineral composition of blood plasma (Figure 6.7). When formed in the presence of osteogenic cells, this mineralized layer growing on CaP ceramics is comparable to the cement lines present in bone. In vitro, modification of  $\text{Ca}^{2+}$

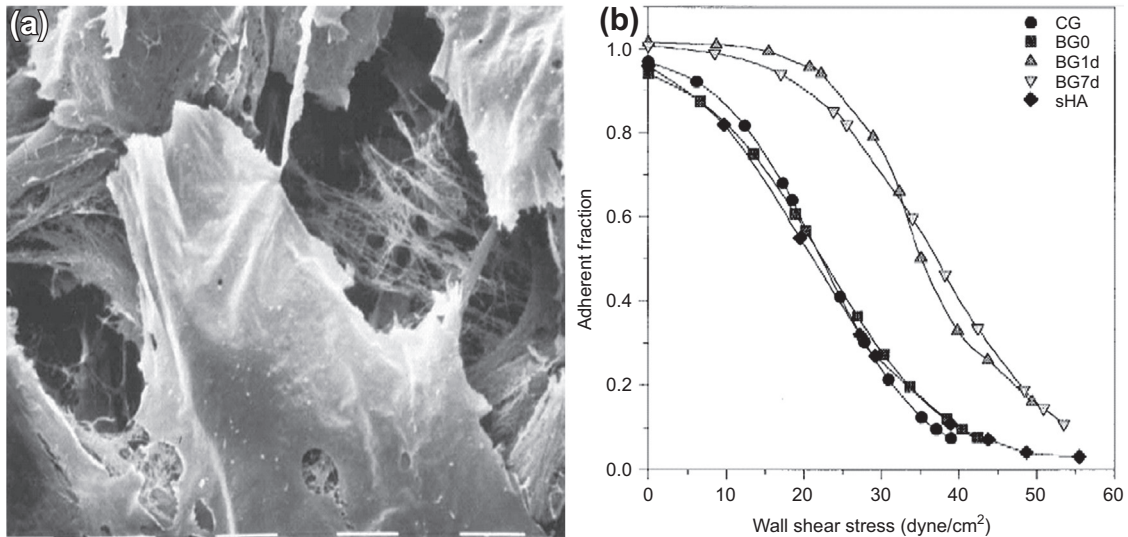




**FIGURE 6.7**

Back-scattering electronic microscopy picture of an octacalcium phosphate (OCP) coating on a metallic porous scaffold implanted for 12 weeks in the femoral condyle (goat) at different magnification. (a and b) Between the OCP coating and the newly formed bone, an interfacial phase (arrow) that can be attributed to superficial phase transformation is clearly visible. However, on the same implant, this interfacial layer is not always visible (c).

and inorganic phosphate ( $P_i$ ) concentrations in cell culture medium due to incubation with CaPs are reported to affect cell proliferation, differentiation, and may even induce cell death. In the absence of CaPs, increasing or decreasing  $Ca^{2+}$  and  $P_i$  concentrations in culture medium directly affect osteoblast activity (Barrère et al., 2008). Human mesenchymal stem cells (hMSC) cultured on various biphasic calcium phosphate (BCP) ceramics, ranging from 100% HA to 100%  $\beta$ -TCP, showed higher osteogenic differentiation on the ceramics composed of more  $\beta$ -TCP (i.e., 20% HA/80%  $\beta$ -TCP, “20/80 HA/ $\beta$ -TCP”) potentially due to increased  $Ca^{2+}/P_i$  release from  $\beta$ -TCP dissolution. In a mouse model of ectopic bone formation, hMSC cultured on the same range of BCP also induced more bone formation and at a faster rate on the 20% HA/80%  $\beta$ -TCP (20/80 HA/ $\beta$ -TCP) versus the other, more stable formulations (e.g., 100% HA, 76/24, 63/37, and 56/44 HA/ $\beta$ -TCP), or 100% TCP, which may have degraded too quickly (Arinzeh et al., 2005). Degradable  $\beta$ -TCP had



**FIGURE 6.8**

(a) Scanning electron micrograph of a BG surface cultured with rat calvaria osteoblasts for 1 week. The glass surface was covered by collagen fibrils and bone-like tissue (magnification = 1000 $\times$ ) (Ducheyne et al., 1994); (b) cell detachment profiles for substrates coated with 0.1 mg/mL fibronectin ( $10 \text{ dyne cm}^{-2} = 1 \text{ N m}^{-2}$ ). Cell adhesion was significantly higher on reacted bioactive (BG1d, BG7d) than on unreacted bioactive (BG0) and control (CG) glasses and stoichiometric hydroxyapatite (sHA) (García et al., 1998).

a positive influence on osteogenic differentiation of hMSC compared to the nondegradable HA (Barradas et al., 2013).

From in vitro cell culture studies, BGs have shown stimulatory effects on the differentiation of bone-forming osteoblasts from precursor cells. Ducheyne et al. (1994) reported the characteristics of the BG substrate that augment extracellular matrix secretion by osteoblast lineage cells. After 1 week in culture with calvarial osteoblasts, SEM (Figure 6.8(a)) shows that the BG surface was covered by collagen fibrils and bone-like tissue. This and many other studies showed and confirmed the ability of BGs to stimulate the commitment and bone formation of osteoblasts and their precursors. It is thought that the dissolution products of BGs play an important role in stimulating osteogenesis (Xynos et al., 2000); thus the degradation of BG is likely to be crucially important to its performance.

### 6.1.2.2 Osteoinduction and Degradable Bioceramics

Osteoinductive biomaterials have intrinsic ability to induce ectopic bone formation in nonosseous environments, i.e., where bone cells are initially absent, such as muscle or subcutis. Osteoinductive bioceramics perform better in bone defects than those that are only osteoconductive in preclinical models (Yuan et al, 2010). They may be regarded as the most simple but effective bone tissue engineering approach because an osteoinductive material can induce

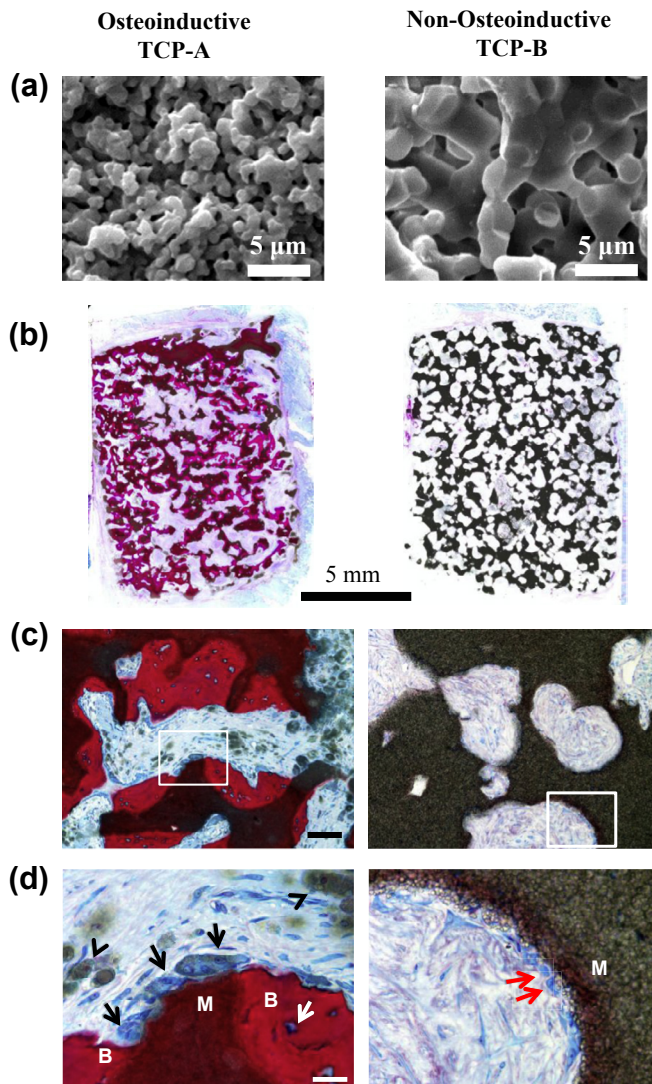
stem cell differentiation and tissue formation in situ without the need to seed and culture cells on the material or add growth factors prior to implantation. Although the performance of osteoinductive biomaterials has been reported for over 20 years, the biological mechanism of how an osteoinductive biomaterial stimulates de novo bone formation is largely unknown. Three-dimensional architecture, concavities, interconnected pores, and surface microstructure have all been shown to promote bone formation. Increased ion and protein reactivity resulting from larger specific surface area are thought to play a role in this enhanced bone-forming capacity. For instance, increased specific surface area may lead to increased precipitation/release of  $\text{Ca}^{2+}/\text{P}_i$  or increased absorption of bone morphogenetic proteins (BMPs) (Barradas et al., 2011). Therefore the degradation rate of these ceramics may be crucial for their performance.

Osteoclasts that resorb CaPs may also contribute to osteoinduction by CaPs, again underlining the importance of bioceramic degradation and functionality. For instance, osteoclasts have been found to precede de novo bone formation by osteoinductive  $\beta$ -TCP in the dorsal muscle of dogs by 4 weeks (Kondo et al., 2006), and osteoclast inhibitors have been shown to block osteoinduction by CaP (Tanaka et al., 2010). Though osteoclasts are normally only found in osseous sites or in pathologies related to heterotopic ossification, the natural inflammatory response following the implantation of a bioceramic is thought to provide all of the necessary components for osteoclastogenesis—both osteoclast precursors and osteoclast differentiating cytokines. This potential link between cellular degradation of CaPs and osteoinduction is illustrated in Figure 6.9.

## 6.2 BIODEGRADABLE POLYMERS

Scaffolds for tissue engineering have been prepared from biologically derived polymers, such as collagen, hyaluronic acid, and chitosan. However, the number of natural polymers that can be used, as well as the possible modifications to improve their mechanical properties and degradability, are limited. Natural polymers are often difficult to process, and pathogenic risks may be associated with materials of animal or human origin. Alternatively, the use of synthetic polymers of different chemistries enables the design of scaffolds with specific mechanical and biological properties, and degradation rates. Synthetic polymers can be produced cheaply and reproducibly, and can be easily processed into devices of virtually any shape and form.

Much of the research on degradable polymers in tissue engineering has been focused on hybrid cell/scaffold constructs using degradable polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers (PLGA), which are well known in the medical field. These polymers degrade via hydrolysis of the main chain ester bonds and are resorbable in vivo as their



**FIGURE 6.9**

Osteoinductive and resorbable  $\beta$ -TCP (TCP). TCP with different surface microstructures were synthesized and implanted in the dorsal muscle of dogs for 12 weeks. (a) Scanning electron micrographs show the different surface structures of TCP-A and TCP-B. (b) Histological sections were stained with basic fuchsin and methylene blue for bone. Overview images show ectopic bone formation (bright red/pink) by TCP-A (reddish brown) and not by TCP-B (brown). (c) In TCP-A, extensive bone formation is bonded to the material and degraded material particulate is present in the loose connective tissue space (light brown spots), but no bone or material particulate is apparent in TCP-B (scale bar = 100  $\mu\text{m}$ ). (d) At high magnification, osteoclast-like cells (black arrows) resorb the TCP-A material (M) surface between stretches of mature bone (B) containing osteocytes (white arrow) in characteristic lacunae. In the loose connective tissue, material particulate has been internalized by other cells (arrow heads). In contrast, mononuclear cells (red arrows) are mainly found on TCP-B and no material particulate is evident in the loose connective tissue space (bottom row: scale bar = 25  $\mu\text{m}$ ). Adapted from Davison et al. (2014).





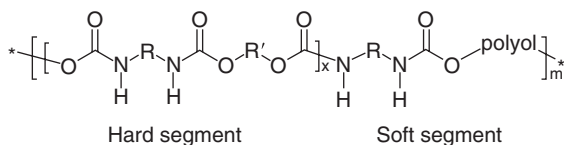


Depending on the intended application, polymeric materials with widely differing physical properties (e.g., thermal properties, mechanical properties, and hydrophilicity) and degradation characteristics are required (Ratner et al., 2013). Parameters that often need to be optimized are the melting and glass transition temperatures, the tensile strength, the elastic modulus or stiffness, and the (surface) hydrophilicity. Therefore, copolymerization, i.e., the preparation of polymers from two or more types of monomers, is often employed to tune the material properties (O dian, 2004).

Copolymers can be classified as *random-*, *alternating-*, *block-* or *graft copolymers*. *Random copolymers* have a statistical arrangement of different monomer units in their backbone. In *alternating copolymers*, the different monomer units are arranged in such a way that they alternate along the polymer chain. The physical and degradation properties depend strongly on the nature and composition of monomers. *Block copolymers* are composed of connected polymeric or oligomeric segments built up of different monomer units. Examples are diblock copolymers, triblock copolymers, and multiblock copolymers. In *graft copolymers*, a comb-like structure is obtained by attaching (co)polymer segments to a polymer backbone at various places. Several types of block copolymer architectures can be synthesized for use as biomaterials.

An important class of block copolymers is that of phase-separated multiblock copolymers containing alternating soft- and hard segments. Frequently, a hard phase (with high melting temperature or glass transition temperature) dispersed in a continuous soft phase (with a glass transition temperature below room temperature) can be distinguished. These hard segments form physical cross-linkages between the polymeric chains adding strength and toughness to a flexible and elastic thermoplastic elastomeric material.

Well-known examples of multiblock copolymers are segmented poly(urethane)s. Building blocks of segmented poly(urethane)s include diisocyanates, polyols, and chain extenders. Usually the hard segment originates from the diisocyanate and chain extender molecules, and the soft segment contains the polyol (a hydroxyl group terminated polymer or oligomer) moiety. A general structure of such a poly(urethane) is shown in Figure 6.10.



**FIGURE 6.10**

General structure of a segmented poly(urethane) prepared from a diisocyanate,  $\text{OCN-R-NCO}$ ; chain extender,  $\text{HO-R'-OH}$ ; and polyol building blocks.

### 6.2.1.3 Chemically Cross-Linked Polymer Networks

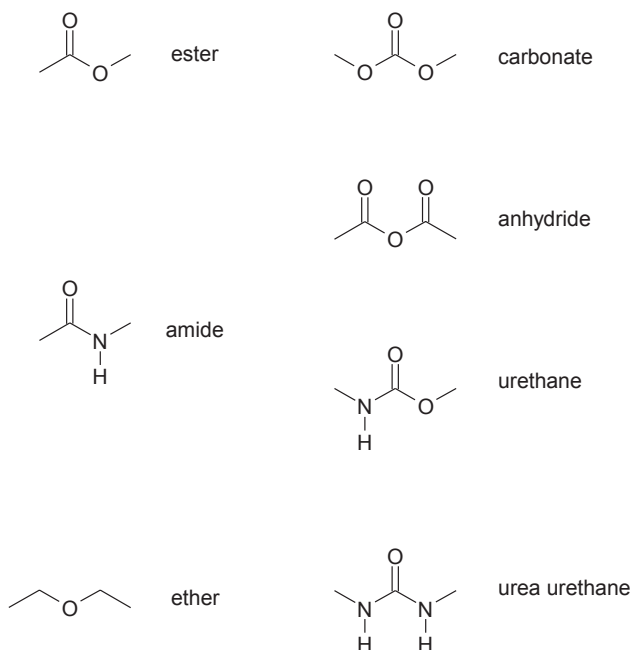
Besides the *physical* cross-linkages between the polymeric chains as described earlier, polymer networks can be obtained by *chemical* cross-linking. Bifunctional monomers such as methyl methacrylate and lactic acid yield linear polymers (which are often soluble in solvents and can be processed at elevated temperatures above their glass transition temperature or melting temperature). Monomers with a functionality greater than 2, e.g., glycerol which contains three reactive hydroxyl groups, can be used to prepare branched polymers and cross-linked network structures when polymerized with a diacid monomer.

In chemically cross-linked polymers a network structure is created by the formation of covalent linkages between the polymer chains (O dian, 2004). In contrast to linear and branched polymers, chemically cross-linked polymers do not dissolve in solvents, but only swell in them. The extent of the polymer network swelling by a solvent is dependent on the density of cross-linkages: the higher the cross-linking density, the lower the degree of swelling. As a chemically cross-linked polymer is in fact a single giant molecule, it cannot be processed at temperatures above its melting temperature or glass transition temperature either.

Important examples of cross-linked polymers are hydrogels. These polymers are based on hydrophilic, water soluble oligomeric or polymeric chains that have been reacted with each other to form a covalent network. In one preparation route, polyethylene glycol (PEG) is functionalized at both termini with acrylic groups that in a subsequent stage are linked by radical photopolymerization reactions (Sawhney et al., 2003). To prepare a degradable and resorbable polymeric scaffold for tissue engineering, it will be necessary to have cleavable bonds in the polymer main chain or in the cross-linkages between the polymer chains.

## 6.2.2 Biodegradable Polymers: Applications and Synthesis

Significant efforts have been devoted to prepare biodegradable polymers and use them in medical applications as implants, drug delivery devices, and tissue engineering scaffolds (Ratner et al., 2013; Uhrich et al., 1999). Polymer degradation and decrease of its molecular weight occurs through scission of the main chain. Although degradation reactions can occur during processing at elevated temperatures or upon sterilization with high-energy radiation, for a polymeric material to resorb in the body it is necessary that the main chain of the macromolecule contains labile bonds. Such bonds can then be scissioned by hydrolysis or oxidation reactions (also through enzymes and cellular activity) to yield (soluble) compounds of lower molecular weight (Göpferich, 1996; Tamada and Langer, 1993). Figure 6.11 gives an overview of important cleavable bonds that can be present in the main chain of biodegradable polymers.

**FIGURE 6.11**

Overview of important cleavable labile bonds in the main chain of biodegradable polymers.

### 6.2.2.1 Biodegradable Polymers in Tissue Engineering

Of the currently employed biomaterials, synthetic polymers possess most characteristics required to fabricate scaffolds for tissue engineering applications. The most often used synthetic polymers and their areas of applications are summarized in [Table 6.2](#).

Of the synthetic polymers, linear aliphatic polyesters like PLA, PGA, and PLGA are very well known in the medical field and have therefore been broadly used in tissue engineering. In general, they elicit a minimal or mild foreign body reaction, and as such are considered to be biocompatible. By varying their molecular weight and in case of PLGA also their copolymer ratio, the biodegradation rate and the mechanical properties can be tailored. They are suited for tissue engineering applications as their degradation products (lactic and glycolic acids) obtained resulting from hydrolysis are normally present in the metabolic pathways of the human body. However, their bulk degradation behavior (see [Section 6.2.3](#)) may lead to the formation and accumulation of relatively large amounts of acidic degradation products that cannot be easily disposed of. This can result in local inflammation ([Bostman et al., 1989](#); [Fu et al., 2000](#)). Furthermore, in semicrystalline polymers, the crystalline residues may take a long time to resorb completely. Another linear aliphatic polyester commonly used in

**Table 6.2** Overview of Synthetic Polymeric Biomaterials Used in Tissue Engineering Applications

Degradable Polymer	Abbreviation	Tissue Engineering Applications
Poly(lactic acid)	PLA	Skin, cartilage, bone, ligament, tendon, vessels, nerve, bladder, liver
Poly(glycolic acid)	PGA	Skin, cartilage, bone, ligament, tendon, vessels, nerve, bladder, liver
Poly(lactic-co-glycolic acid)	PLGA	Skin, cartilage, bone, ligament, tendon, vessels, nerve, bladder, liver
Poly( $\epsilon$ -caprolactone)	PCL	Skin, cartilage, bone, ligament, tendon, vessels, nerve
Multiblock copolymers comprising poly(ethylene oxide) and butylene terephthalate units	PEOT/PBT	Skin, cartilage, bone, muscle
Polyphosphoesters	PPEs	Cartilage, bone, nerve, liver
Polyphosphazenes	PPAs	Bone, nerve
Polyanhydrides	PAs	Bone
Polyorthoesters	POEs	Bone
Poly(propylene fumarate)-diacrylate networks	PPF-DA	Bone, vessels
Poly(ethylene glycol)-diacrylate hydrogel networks	PEG-DA	Skin, vessels, nerve

tissue engineering is poly( $\epsilon$ -caprolactone) (PCL). This polymer has found many applications as a result of its good biocompatibility and favorable mechanical properties. It degrades at a much lower rate than PLA, PGA, and PLGA, making it attractive when long-term implants are desired ([Hutmacher et al., 2001](#)).

Another family of thermoplastic polymers that has been developed recently for tissue engineering and drug delivery are multiblock copolymers comprising poly(ethylene oxide) (PEO) and poly(butylene terephthalate) (PBT) (PEOT/PBT)). They are prepared by polycondensation of (dimethyl) terephthalate (T), butanediol (B), and polyethylene glycol (PEG). These poly(ether ester) copolymers exhibit good physical properties like elasticity, toughness, and strength in combination with easy processability ([Bezemer et al., 1999](#)). Their properties result mainly from a phase separated morphology in which soft, hydrophilic PEO segments are physically cross-linked by hard, semicrystalline PBT segments. In contrast to chemically cross-linked materials, these cross-links are reversible and will be disrupted at temperatures above their glass transition- or melting point, giving the polymer material its good processability.

The interest arisen in tissue engineering applications is due to the fact that by varying the molecular weight of the starting PEG segments and the weight ratio of PEOT and PBT blocks, it is possible to tailor properties, such as wettability, swelling, biodegradation rate, protein adsorption, and mechanical properties ([Bezemer et al., 1999](#); [Deschamps et al., 2002](#)).

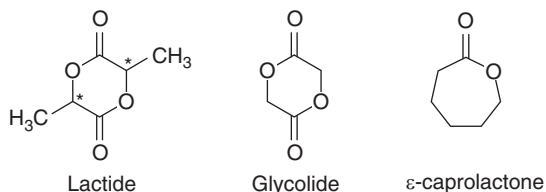


An alternative to the preparation of tissue engineering scaffolds by processing polymers from melts or solutions is making use of injectable polymers. This class of polymeric materials is very attractive as they can be used in minimally invasive surgery such as arthroscopy, which is highly beneficial to the patient. Furthermore, they can fill irregularly shaped defects (Burdick et al., 2001), and cells and bioactive agents can easily be incorporated (Elbert et al., 2001; Mann et al., 2001). In particular, photopolymerizable systems based on poly(propylene fumarate)-diacrylates (PPF-DA) and poly(ethylene glycol)-diacrylates (PEG-DA), have been much investigated. They even can be hardened transdermally by applying light (He et al., 2000). Despite the advantages in using these polymers, some issues may still rise from their low mechanical properties and possible cytotoxicity remains due to the acrylic groups.

Biodegradable polyesters synthesized by ROP in the past years, biodegradable aliphatic polyesters, such as poly(lactide) [poly(lactic acid), PLA], poly(glycolide) [poly(glycolic acid), PGA], poly PCL, and their copolymers, have attracted much attention (for reviews see Albertsson and Varma, 2002; Uhrich et al., 1999).

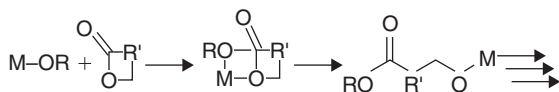
Although polyesters can be synthesized by polycondensation of hydroxyl acids such as lactic acid, it is difficult to achieve high molecular weights and control the molecular weight, molecular weight distribution, and architecture of the polymer. In most cases, biodegradable polyesters are synthesized by ROP of cyclic esters (or lactones). Structures of some of these lactones often used are shown in Figure 6.12.

Ring-opening polymerization of cyclic esters proceeds through cationic polymerization, anionic polymerization, or coordination-insertion reactions. The coordination-insertion reactions (Kowalski et al. (2000); Kricheldorf et al., 2000; Nijenhuis et al., 1992) are often initiated by metal alkoxides, which contain a covalent metal–oxygen bond and have a weak Lewis acid character. The propagation proceeds by coordination of the monomer to the metal alkoxide, followed by the insertion of the monomer into the metal–oxygen bond. A scheme of the propagation step of this mechanism is shown in Figure 6.13.



**FIGURE 6.12**

Structures of cyclic esters used in ring-opening polymerizations. Lactide has two optically active carbon atoms in the ring.

**FIGURE 6.13**

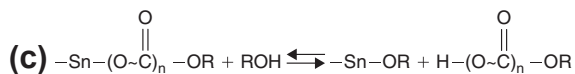
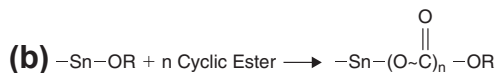
Scheme illustrating the propagation step of lactone ring-opening polymerization via a coordination-insertion mechanism.

It should be noted that the growing chains contain the metal–oxygen bond during the propagation, i.e., they remain active.

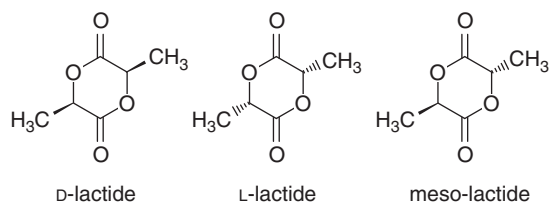
Tin(II) 2-ethylhexanoate (stannous octoate or  $\text{SnOct}_2$ ) is the most often used catalyst for the ROP of cyclic esters.  $\text{SnOct}_2$  is a highly active catalyst that is soluble in the monomer melts, allowing the ROP to be performed in bulk. Although there is some concern about the cytotoxicity of  $\text{SnOct}_2$ , the American Food and Drug Administration has approved many degradable implants in which the polymer has been prepared using small amounts of this catalyst.

The mechanism of the ROP process using  $\text{SnOct}_2$  as a catalyst has been studied in depth by [Kowalski et al. \(2000\)](#) and [Kricheldorf et al. \(2000\)](#). For the ROP of L-lactide and  $\epsilon$ -CL, Kowalski and coworkers proposed the following mechanism shown in [Figure 6.14](#): first, a tin–alkoxide bond is formed via a reversible reaction between  $\text{SnOct}_2$  and a compound containing a hydroxyl group (a). The monomer is then coordinated to and inserted into the formed tin–alkoxide bond. Subsequent coordination and insertion of a next monomer then propagates the polymerization (b). Chain transfer reactions between the growing polymer chains (in which the tin–alkoxide bonds are still present) and compounds containing hydroxyl groups can also occur, leading to an increase in the molecular weight distribution (c).

The compounds containing hydroxyl groups (shown in [Figure 6.14](#)) can have very small amounts of impurities present in the monomer, catalyst, or reaction environment. If stringent purification and reaction conditions are applied, very

**FIGURE 6.14**

Ring-opening polymerization of cyclic esters using  $\text{SnOct}_2$  as a catalyst: (a) formation of a tin–alkoxide bond by reaction of an alcohol with  $\text{SnOct}_2$ ; (b) propagation by monomer coordination and insertion; (c) chain transfer reaction.

**FIGURE 6.15**

Structures of lactide stereoisomers. D,L-lactide is an equimolar racemic mixture of LLA and DLA.

high molecular weight polyesters (such as PLA with  $M_n$  of  $310 \times 10^3$ g/mol) can be synthesized. These compounds can also be added on purpose to control molecular weight, composition, and architecture of the polymer that is to be synthesized. When, e.g., monomethoxy-PEG (mPEG) or a PEG-diol is used together with  $\text{SnOct}_2$ , the corresponding diblock or triblock copolymers of PEG and polyesters can be synthesized.

### 6.2.2.2 Lactides and Polylactides

Of the degradable polyesters used in medical applications, PLA has received special interest due to the presence of asymmetrical carbon atoms in the lactide monomers. Lactide compounds exist in three different stereoisomeric forms (Figure 6.15): D-(R-) lactide (DLA), L-(S-) lactide (LLA), and meso-lactide. It should be noted that with D,L-lactide (DLLA), an equimolar racemic mixture of LLA and DLA isomers is meant. The structures of these different compounds are shown in the following figure.

In the ROP with  $\text{SnOct}_2$ , the asymmetric carbon atoms in the lactide monomers can retain their conformation. Therefore, PLA polymers with different stereoregularities (isotactic poly(LLA) (PLLA), poly(DLA) (PDLA), and atactic poly(DLLA) (PDLLA)) can be prepared.

Poly(LLA) and poly(DLA) are semicrystalline polymers with a glass transition temperature ( $T_g$ ) of approximately  $60^\circ\text{C}$  and a peak melting temperature ( $T_m$ ) of approximately  $180^\circ\text{C}$ . PDLLA is amorphous with a  $T_g$  of approximately  $55^\circ\text{C}$ . Both semicrystalline PLLA and amorphous PDLLA polymers are rigid materials. Their modulus of elasticity and stress at break ( $\sigma_{\text{break}}$ ) values are close to, respectively, 3.5GPa and 65MPa. However, these polymers are relatively brittle with an elongation at break ( $\epsilon_{\text{break}}$ ) less than 6%.

Interestingly, mixtures of PLLA and PDLA can form the so-called stereocomplexes with even higher melting temperatures of up to  $230^\circ\text{C}$ .

Both polymers degrade by hydrolysis in which naturally occurring lactic acid is formed. Degradation of the polymers starts with water uptake, followed by random cleavage of the ester bonds in the polymer chain. The degradation takes

place throughout the bulk of the material. Upon degradation, the number of carboxylic end groups increases, which leads to a decrease in pH and autocatalytic hydrolytic degradation (Li et al., 1990). During the degradation of semicrystalline PLLA, crystallinity of the residual material increases as hydrolysis preferentially takes place in the amorphous domains. In general, the rate of degradation and erosion of amorphous PDLLA is faster than those of PLLA.

### 6.2.3 Mechanisms of Polymer Degradation and Erosion

#### 6.2.3.1 Definitions

In polymer degradation, chain scission occurs, and oligomers, monomers, and other low molecular weight species are formed (Tamada and Langer, 1993). The degradation of polymers can be induced by thermal activation, oxidation, photolysis, radiolysis, or hydrolysis processes (Gopferich, 1996). When degradation is affected by the biological environment, the term biodegradation can be used. As a consequence of degradation, *erosion* of the material can occur. Erosion specifically refers to the loss of material by monomers and oligomers leaving the polymer mass. Obviously, if polymer erosion and resorption is to occur, first soluble components need to be formed.

##### 6.2.3.1.1 Chain Scission and Polymer Degradation

For polymeric biomaterials, the most important degradation reaction is hydrolysis. There are several factors that influence the rate of this reaction: *the nature of the chemical bond, the pH, the copolymer composition, and the extend of water uptake* are the most relevant. Of these factors, it is mainly the type of bond in the polymer backbone that determines the rate of hydrolysis. Anhydrides and *ortho*-ester bonds are the most reactive ones. In water, anhydrides are more reactive than esters and amides. Such ranking must be viewed with circumspection. However, reactivity of the bonds is much dependent on catalysis and steric or electronic effects.

In organic chemistry, ester hydrolysis of organic compounds occurs by reaction of water with the ester bond. However, in the presence of acidic or basic environments, the hydrolysis reactions are catalyzed and the rates are much higher. The hydrolytic degradation of poly(CL), poly(DLLA), and related aliphatic polyesters involves the generation of carboxylic end groups that are also able to catalyze the hydrolysis reaction. Based on a first-order kinetic model, Pitt et al. (1981) related the rate of chain scission of an aliphatic polyester autocatalyzed by the generated carboxylic acid end groups to the decrease of the average number molecular weight in time:

$$\ln(\bar{M}_n) = \ln(\bar{M}_n^0) - kt$$

where  $\bar{M}_n$  is the average number molecular weight at the start of the hydrolysis,  $k$  is the rate constant, and  $t$  is the degradation time.

If the hydrolysis is not autocatalytic, chain cleavage will follow the rate law (Pitt and Gu, 1987):

As discussed earlier, the composition can have a large influence on the chemical and physical properties of a (co)polymer. The rate of degradation of a polymer is dependent on the ease of hydrolysability as well as on the accessibility of the cleavable main chain bonds to enzymes and water. Besides the nature of these labile bonds, the hydrophilicity of the material, the morphology and crystallinity of the polymer, and its molecular weight are important parameters in determining the degradability of a polymer.

In the hydrolysis of a polyester, which initially is hydrophobic and not soluble in water, the average molecular weight of the polymer will decrease in time. It will require a certain degree of main chain hydrolysis before appreciable amounts of water soluble oligomers and low molecular compounds are formed.

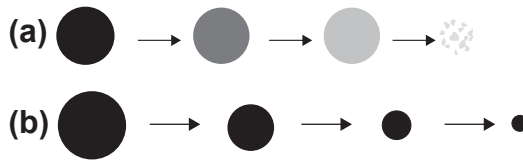
### **6.2.3.2 Bulk- and Surface-Erosion**

Polymer degradation processes can result in erosion, i.e., loss of mass of the materials, when degradation products diffuse and dissolve into the degradation environment. Polymer erosion can be a more complex process as it depends on many other processes besides degradation, such as morphological changes and characteristics of the oligomers formed (Göpferich, 1996).

In polyesters there are four main factors that determine the erosion diffusion and dissolution phenomena (Vert, 2005):

1. The hydrolysis rate constant of the ester bond.
2. The diffusion coefficient of water within the polymer matrix.
3. The diffusion coefficient of chain fragments within the polymer matrix.
4. The solubility of the degradation products (oligomers) in the surrounding medium.

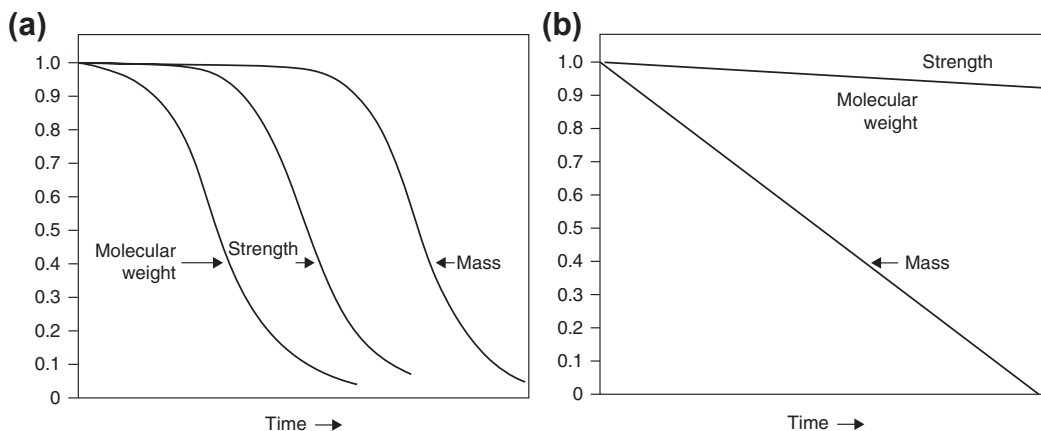
Erosion of polymers can be classified as a bulk erosion process or surface erosion process (Göpferich, 1993, 1996; Von Burkersroda et al., 2002). In bulk erosion, the polymer chain scission occurs throughout the specimen. The molecular weight and the mechanical strength of the specimens decrease in time. The decrease in molecular weight occurs essentially from the beginning of the degradation process, whereas loss of mass is much delayed. The external dimensions of the polymer specimens remain essentially unchanged, until the specimens disintegrate at a critical time point. It should be noted that the loss in mechanical properties of the material precedes the loss in mass. The process of bulk erosion is schematically described in Figure 6.16(a).

**FIGURE 6.16**

Scheme illustrating degradation of materials by bulk erosion (a) and surface erosion (b) processes.

In surface erosion, loss of material is confined to the surface of the polymer device only. Size and mass of the device decrease in time, whereas molecular weight and mechanical properties of the polymer device remain unchanged. In surface erosion, the rate of mass loss is proportional to the surface area. Surface erosion is schematically depicted in [Figure 6.16\(b\)](#).

Most biodegradable polyesters that are currently available degrade by a bulk erosion process that predominantly involves simple hydrolysis of main chain ester bonds ([Middleton and Tipton, 2000](#)). First, small amounts of water diffuse into the bulk of the material preferentially attacking the ester bonds present in the amorphous domains of the polymer. Initially, this decrease in molecular weight will not affect the mechanical properties of the device as physical cross-linkages due to entanglements and regions of crystallinity maintain the structure of the material. At a certain point, however, the reduction in molecular weight will lead to a decrease in the mechanical properties of the material. As more carboxylic acid end groups are formed, the rate of the hydrolysis reaction will increase and loss of mass will occur when the degradation products become soluble in water. [Figure 6.17\(a\)](#) shows a graphic representation of these effects.

**FIGURE 6.17**

(a) Representation of the bulk erosion process of degradable polymers; (b) representation of the surface erosion process of degradable polymers.



In a surface erosion process (Figure 6.17(b)), the rate of hydrolysis of labile bonds is relatively fast in comparison to the diffusion rate of water into the bulk, and the degradation reactions are limited to the surface of the polymer material. The molecular weight of the polymer, and therefore its mechanical properties, remain more or less unchanged with time, while the mass of the device decreases at an appreciable rate. A surface-eroding polymer could provide constant and well controlled release rates in drug delivery applications, but only few polymers show surface erosion characteristics: examples are polyanhydrides (copolymers of sebacic acid, 1,3-bis(*p*-carboxyphenoxy) propane and 1,3-bis (*p*-carboxyphenoxy)hexane poly(adipic anhydride)) and poly(orthoesters). These polymers have very hydrolytically labile bonds in their main chains, which react with water rapidly. Therefore, the degradation process occurs at the polymer surface resulting in a surface eroding material.

### 6.2.3.3 *In vivo* Degradation

In the development of a novel degradable polymer, most degradation experiments are most often conducted *in vitro* by incubating the polymeric specimen in phosphate buffered saline (PBS) at body temperature (37°C). However, *in vitro* degradation behavior can considerably differ from *in vivo* degradation. In most cases, *in vivo* degradation is faster than *in vitro* degradation (Mainil-Varlet et al., 1997). The faster *in vivo* degradation can be attributed to the tissue response (Stoll et al., 2001). Acute inflammation, due to the injury of implantation, and foreign body reaction, due to the site (Anderson, 1994; Anderson and Shive, 1997). Reactive species, like super oxide and hydrogen peroxide species the presence of the implant, induce the migration of polymorphonuclear leucocytes and macrophages to the implant, produced by these inflammatory cells can oxidize the polymer chains (Deschamps et al., 2002). Therefore, the *in vivo* decrease in molecular weight values is not only due to hydrolytic degradation caused by the extracellular fluid, but probably also by the influence of the oxygen free radicals and of the other species generated by the inflammatory cells (Ali et al., 1994). In some cases, however, such as in hydrogel-like polymer systems, a slower *in vivo* degradation has been reported compared to the *in vitro* degradation in phosphate buffer (Changez et al., 2005). The presence of less fluid near the implant site retards swelling and subsequently inhibits degradation. For copolymers that degrade only by hydrolysis in the bulk, the *in vitro* degradation will mimic the *in vivo* degradation (Deschamps et al., 2003).

Besides the increased chain scission rate, the erosion rate *in vivo* differs from the *in vitro* situation. Mass loss *in vivo* can be based on either *passive transport* of monomers and oligomers by dissolution and diffusion (similar to

in vitro) or by *active transport* by phagocytes or a combination of these processes. The solubility of the oligomers may be increased in vivo due to the presence of lipids, resulting in a faster mass loss. The dissolved degradation products will be removed from the implantation site via the lymphatic system and subsequently secreted from the body by the kidneys. The in vivo mass loss can be further increased by mechanical stresses and cellular activity. Various studies demonstrated the fragmentation of a biomaterial in time. Implants based on PEOT/PBT copolymers have been evaluated extensively in various implantation sites and extensive fragmentation of the implants was visible within 4 weeks, thereby significantly enlarging the implant surface (Lu et al., 2000). Higher fragmentation rates and smaller average particle sizes were observed at intramuscular implantation sites as compared to subcutaneous sites. This could be attributed to the higher stresses and higher extents of tissue vascularization in muscular tissue. Ultimately, the implant breaks down into particles smaller than 10  $\mu\text{m}$ , which undergo phagocytosis by macrophages (Anderson and Shive, 1997). Transmission electron microscopy (TEM), Raman spectroscopy, and imaging showed phagocytes containing intracellular polymeric particles.

Besides the site of implantation, other parameters that affect the in vivo degradation are the *size* and the *shape* of the implant. The tissue response to a biomaterial depends to a large extent on the shape of the implant (Anderson, 1994; Matlaga et al., 1976). Being a surface related process, a higher surface area (due to fragmentation or porosity) can affect the tissue response. In addition, sharp angular shapes can induce a higher response than more rounded shapes (Matlaga et al., 1976). The formation of a fibrous capsule as part of the foreign body response may also affect the rate of degradation. Furthermore, one would expect that larger implants require longer degradation times. However, this may depend upon auto or normal catalysis of the degradation process. In the case of poly(lactide-co-glycolide) (PLGA), for example, the in vivo degradation is autocatalyzed due to formation of acidic degradation products (see Classical Experiment). Various studies showed that the autocatalytic effect can be affected by the size of the implant (Grizzi et al., 1995). Comparison of PLGA implants of various sizes indicated that a critical thickness can be determined earlier, which hydrolytic degradation is accelerated due to accumulation of acidic degradation products in the implant.

In conclusion, the in vivo degradation of a biodegradable polymer is affected by many factors, which are influenced by the implant characteristics. This complex situation stresses the importance of performing relevant in vivo experiments when selecting a biodegradable polymer for a specific tissue engineering application.

### 6.3 FUTURE PERSPECTIVES FOR DEGRADABLE BIOMATERIALS IN TISSUE ENGINEERING

Current research on degradable biomaterials in tissue engineering focuses on balancing the ability to support tissue growth with the degradation rate. A degradable biomaterial should disappear without leaving inert residues behind which may lead to mechanical instability. The optimal biomaterial degradation characteristics and resorption rates are specific for each specific application. Future research on degradable biomaterials will not only focus on the supporting task of these materials but harness degradation characteristics to instruct tissue regrowth. For example, CaPs can be used not only as scaffolds to support cells but can also be designed to incorporate and release growth factors such as growth hormone, BMPs, and insulin-like growth factor to stimulate bone formation or drugs such as bisphosphonates to limit bone resorption (Chapter 11). In order to achieve a sustained release of these molecules, more research is needed to carefully tune the degradation rate while maintaining structural rigidity to support new tissue formation. Trace ions can also be integrated into the crystal structure to stimulate the differentiation of osteoblasts and inhibit bone resorption of osteoclasts. Calcium phosphate nanoparticles are even being researched to deliver cell-death-inducing genes to tumor cells for cancer therapy (Bose and Tarafder, 2012).

There is much interest in the development of responsive degradable polymeric biomaterials in tissue engineering. Such polymers can be made to degrade on demand by action of the cells that grow into or surround the implanted scaffold or supporting hydrogel structure. As cells infiltrate and degrade the temporary cell-supporting scaffold, e.g., by secretion of proteolytic enzymes, newly formed tissue will infiltrate the structure and tissue remodeling can occur (Dispinar et al., 2012; Gustafson et al., 2013; Jo et al., 2010). In addition, responsive degradable scaffolds can be designed to have thermosensitive properties as well. Polymer solutions that are liquid at room temperature can be designed to gel upon implantation at body temperature. In this manner, suspensions of cells can be injected in a minimally invasive manner and form a semisolid cell-loaded construct in situ (Galperin et al., 2010; Jeong et al., 2009).

### 6.4 SUMMARY

- Degradable biomaterials are suitable as scaffold material for tissue engineering as interference with the development and growth of new tissue and unwanted long-term reactions are prevented.

- Degradable biomaterials for use in tissue engineering should be biocompatible, and the resulting degradation products should be nontoxic. The time required to complete degradation resorption depends on the intended application and preferably matches the formation of functional tissue.
- The *in vivo* degradation of biomaterials combines physico-chemical degradation (chain scission and dissolution in wet environment), enzymatic activity, and cellular degradation (inflammation, foreign body response).
- The soluble biomaterial degradation products are transported from the implantation site via the lymphatic system to the kidneys, which excrete them from the body.
- Polymers are long-chain macromolecules formed by covalent coupling of monomers. Copolymerization, *i.e.*, the preparation of polymers from two or more types of monomers, is often employed to tune their properties.
- CaPs and BGs have unique bone-bonding properties related to their superficial phase transformation mechanisms involving dissolution and precipitation.
- The bioceramics are (partially) crystallized minerals resembling bone mineral. Their degradation profile is partly dictated by the thermodynamic solubility of the crystalline phase.

The nonresorbable HA and the resorbable  $\beta$ -TCP are the most commonly used ceramics in clinics, and they can be combined to tailor their degradation rate.

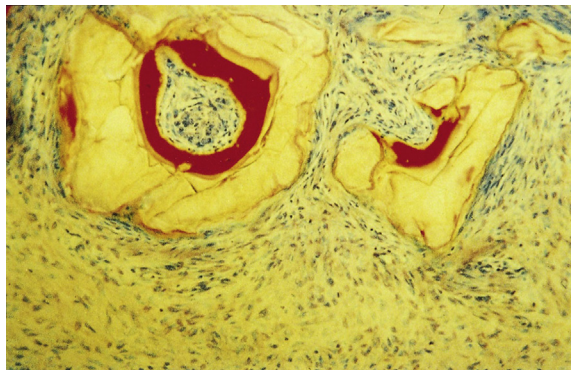
- The most important degradable polymers are polyesters. In the presence of water, chain scission occurs by hydrolysis of ester bonds, which lowers the molecular weight. The resulting oligomers dissolve in the surrounding environment or undergo further hydrolysis.
- More advanced degradable biomaterials will have an optimal balance between mechanical properties and degradation rate. In addition, incorporated functional groups will attract cells and/or growth factors required for tissue formation.

## CLASSICAL EXPERIMENT: THE DISCOVERY OF BIOACTIVE GLASSES

While attending a US Army Materials Research Conference in 1967, Larry Hench was horrified by the stories of Vietnam War battlefield casualties that were causing thousands of amputations in young soldiers. "Instead of making materials to destroy people," Hench took the challenge to "make materials to repair people." Indeed, in the 1960s, bone implants made out of metals and plastics were not suitable for bone repair, as they were rejected too often by the body. His idea was to develop a material that would be accepted by the host bone. Because bone is composed of a mineral phase made up of calcium and phosphate, he hypothesized that an implant containing calcium and phosphate in the right proportions would not be rejected by the

body. He designed three compositions of glass that contained calcium and phosphate, silicon dioxide to hold the structure together, and soda to stimulate the melting of glass in the furnace. These samples were then implanted into the thighbones of rats. Larry Hench was called by the surgeon who was taking care of the animal experiments and reports his conversation:

Six weeks later Ted called me and yelled "Larry, what is that stuff you gave me?" He was so excited, I thought for sure the glasses had killed the rats. So I quickly replied, "Calm down, Ted. They're only the first tries. There are lots of other compositions I can make." I'll never forget his answer. He said "Larry, you don't need to make any other glasses. The first ones work. Those

CLASSICAL EXPERIMENT: THE DISCOVERY OF BIOACTIVE GLASSES *Continued***FIGURE CE1**

Bone tissue formation in excavated bioactive glass particles of narrow size range. Bone tissue is stained red. Also note the channels connecting the interior of the particle with the surrounding milieu (Ducheyne and Qiu, 1999).

implants won't come out. They are bonded to the bone. I've never seen anything like it before." He was right. The glass implants did not come out ... A bond formed that was as strong as bone. In contrast, control implants of other materials slipped easily out of the bone because of the scar tissue formed at their interface (Figure CE1).

Several BGs were derived from the first Bioglass 45S5 composition by varying the percentage of  $\text{SiO}_2$ ,  $\text{CaO}$ ,  $\text{Na}_2\text{O}$ , and  $\text{P}_2\text{O}_5$ . Their ability to form bone bonding with time was evaluated in vivo. This thorough study described the relationship between glass composition,

their dissolution/precipitation properties, and their bone-bonding ability. Along with the development of new biological assays, it has also been found that the dissolution products of BGs stimulated osteogenic differentiation. Although these bioceramics are relatively "old" biomaterials, their ability to trigger bone formation are incomparable with other biomaterials. Nowadays, they benefit from new technologies allowing, on one hand, to investigate the reason behind their bone-bonding ability, and on the other hand to process them into high-performance scaffolds for bone tissue engineering.

## CLASSICAL EXPERIMENT: DEGRADATION BEHAVIOR OF POLY-DL-LACTIC ACID

Amorphous, noncrystallizable copolymers based on L-lactide, D-lactide, and glycolide show a surface to center differentiation upon hydrolysis, resulting in a hollowing out of the specimens during degradation (Grizzi et al., 1995; Li et al., 1990). This phenomenon is related to the autocatalytic hydrolysis of the ester bonds in the main chain.

Until the publications of Vert and coworkers, the extent of degradation of these polymers both in vitro

and in vivo was investigated by visual observation, viscometry, and changes in specimen mass. Polymer degradation was considered to be a homogeneous process, where water absorption throughout the polymer is followed by hydrolysis reactions. However, careful analysis of the molecular weight of the degrading polymer in 2 mm thick specimens showed a bimodal molecular weight distribution. It was found that in such poly(lactide) specimens the



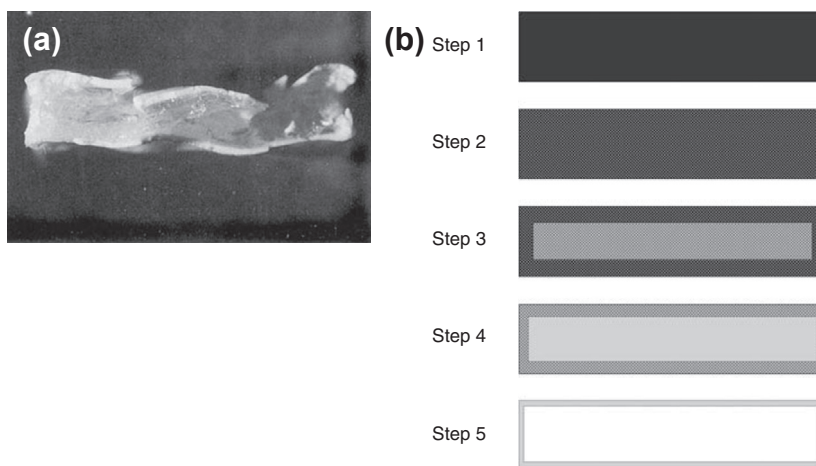
## CLASSICAL EXPERIMENT: DEGRADATION BEHAVIOR OF POLY-DL-LACTIC ACID

### Continued

inner part of the specimen degrades at a higher rate than the outside (Grizzi et al., 1995; Vert, 2005).

When the specimen is placed in the aqueous medium, water penetrates into the material. Hydrolytic cleavage of the polymer chains occurs and as carboxylic acid groups are generated, autocatalysis occurs as well. Within the specimen, degradation of the still insoluble polymer chains proceeds homogenously via an autocatalytic mechanism. As soon as the molecular weight of the

oligomers formed becomes low enough to allow solubility in the surrounding aqueous medium, these oligomers diffuse to the surface of the specimen and into the surrounding medium as they continue to degrade. This process, which combines hydrolytic degradation, diffusion, and solubilization, results in a differentiation between the rates of degradation at the surface and at the interior of the polymer specimen. As a result, hollow specimens can be formed during degradation, as shown in Figure CE2.



**FIGURE CE2**

(a) Cross-section of a PDLLA specimen degraded for 5 weeks in saline buffer. (b) Schematic representation of the different steps of the degradation of PDLLA specimens in aqueous medium. (Reproduced with permission from Li et al. (1990).) Step 1: initial specimen; Step 2: water absorption, start of ester bond cleavage, and decrease in molecular weight; Step 3: differentiation between surface and center, with dramatic decrease in molecular weight in inner part of the specimen; Step 4: diffusion of oligomers through thinning surface when molecular weight is low enough to allow solubilization in the medium; Step 5: hollow shell remaining after release of oligomers and slow degradation of the shell. (Reproduced with permission from Li et al. (1990).)

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