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Bone-Like Mineral and Organically Modified Bone-Like Mineral Coatings

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Introduction: Cell–Matrix Interactions

Cells interact with their environment, namely, the extracellular matrix (ECM), continuously at each stage of cellular life from embryonic development until death. The ECM is an interconnected network consisting primarily of different types of collagens, proteoglycans, and matricellular proteins, such as fibronectin, laminin, and vitronectin, and in some tissues inorganic mineral (Plopper 2007). This complex matrix is secreted by the cells and serves as a specialized niche microenvironment, providing the specific cues necessary to control the function of each tissue type. The cells respond to these cues by altering their biological activity and, in turn, remodeling their surrounding ECM to reflect this altered activity, resulting in a well-orchestrated feedback system. For instance, the cells in bone (osteoblasts) produce a mineralized matrix during development and form the mature bone

tissue, which is then remodeled by osteoclast-mediated resorption and new matrix formation by osteoblasts in response to changes in diet, exercise, and age (Baron 2003).

One of the most important functions of the ECM is to mediate cellular adhesion and consequent differentiation (Ruoslahti, Hayman, and Pierschbacher 1985). Most cell types need to be attached to a matrix in order to survive, grow, and differentiate. Cell adhesion occurs via heterodimeric receptors found in the plasma membrane, known as integrins, which recognize and bind to specific domains found in adhesive ECM proteins. The specific type of integrin receptor involved depends on the cell type and the composition of the ECM. A single cell usually expresses several types of integrin receptors during its lifetime, depending on the type of signals it receives from its environment. These integrins form a part of focal adhesion complexes, linking the ECM molecules to the cell cytoskeleton thus controlling cell adhesion. Furthermore, integrins are also capable of triggering specific cell signaling pathways and thus effectively modulating a variety of cellular functions including cell growth and migration, and differentiation and suppression of apoptosis (Plopper 2007; Ruoslahti, Hayman, and Pierschbacher 1985; Lebaron and Athanasiou 2000; Ruoslahti 1996). Once adhered, cells proliferate and differentiate into a specific lineage with the aid of growth factors and other signaling molecules that are sequestered by the ECM and released during the various stages of cellular activity. Hence, the cell–matrix system is dynamic and complex in nature.

Disruption of cell–cell and/or cell–matrix connections causing either lack of communication or the wrong type of communication to occur between the cells and their surroundings results in abnormal cell activity, manifested in the form of diseased tissues/organs. The fields of tissue engineering and biomimetics employ concepts from biological sciences and engineering to regenerate healthy tissues to replace diseased or damaged ones. Biomimetic materials derive inspiration from naturally occurring systems by imitating aspects of their structural and functional complexity. The main goals of these mimetic biomaterials are to facilitate cellular adhesion and production of ECM by replicating normally occurring cell–matrix interactions in order to control tissue formation.

Biomaterials to be used in bone tissue engineering applications should meet both physical requirements such as mechanical support, surface and bulk material properties and architecture, and biological requirements such as supporting cellular differentiation into osteoblasts. Biomimetic precipitation of calcium phosphate mineral onto biomaterial surfaces facilitates integration of the surface into host bone as well as allows for the incorporation of bioactive moieties under physiological conditions. This chapter focuses on the use of biomimetic apatite coatings to bond to native bone and recreate cell–matrix interactions in vitro and in vivo. The cellular and ECM components present in bone are briefly presented first, followed by a summary of the important material requirements needed to recreate cellular microenvironments in prosthetic and tissue engineering systems. The concept of biomimetic apatite formation and the use of these coatings on metals, ceramics, and polymers are then explored. Finally, a discussion of the use of biomineralization techniques to synthesize organic/inorganic hybrid (bone-like mineral (BLM) integrated with biologically active molecules) coatings that allow for mimicry of cell–matrix interactions is presented.

Engineering Cellular Microenvironments

Before designing any material system to be placed in vivo, it is important to understand the biology of the targeted tissue. Knowledge of the type of cells present, their surrounding

matrix, and the type of interactions that occur at the cell–matrix interface is required to design biomaterials that simulate the natural environment in which these materials will be implanted.

Cellular and Matrix Components of Bone

Osteoblasts are derived from mesencyhmal stem cells under the influence of growth factors such as bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs) on preosteoblastic cells (Baron 2003). Osteoblasts are mainly responsible for secreting the collagen and ground substance matrix (osteoid), which then undergoes calcification to form bone. Osteoblast–matrix interactions occur largely through β1 integrins, which mediate binding to collagens and other noncollagenous proteins found in the secreted matrix and cause activation of the mitogen-activated protein kinase (MAPK) cell signaling pathway, resulting in osteoblastic differentiation and osteogenesis (Lian, Stein, and Aubin 2003). After producing the osteoid matrix that calcifies, osteoblasts get trapped in the calcified bone tissue where they then function as osteocytes. Osteocytes are found in lacunae in the bone and interact with osteoblasts and other osteocytes, as well as the ECM via gap junctions found at the end of long cytoplasmic processes. Loss of these interactions leads to osteocyte cell death and consequent loss of bone (Baron 2003).

Osteoclasts are derived from the mononuclear/phagocytic cell lineage and are involved in bone resorption and turnover, and indirectly in the maintenance of plasma calcium and phosphate levels. Bone remodeling occurs during development and growth (determines shape and size of bones) as well as in adult bones, where the bone structure is maintained locally by replacement of old bone by new bone (Baron 2003; Martin 1989). The main events that occur during remodeling are (1) osteoclast activation and bone resorption, (2) osteoclast apoptosis, (3) preosteoblast chemotaxis, proliferation, and differentiation, and (4) formation of new bone and cessation of osteoblastic activity (Mundy, Chin, and Oyajobi 2003). The exact mechanisms involved in the coupling of osteoclastic resorption to osteoblastic bone formation are not completely understood, and several theories have been suggested to explain this phenomenon. It is thought that coupling is regulated by local and systemic chemical factors such as parathyroid hormone, 1,25-dihydroxyvitamin D, RANK ligand and its receptors, transforming growth factor (TGFβ), BMPs, and FGFs. Another theory is that once osteoclastic resorption is completed, osteoblasts present normally in the bone repopulate and reline the resorbed area without the action of any humoral factors, probably by detection of the resorption site via cell surface molecules (Mundy, Chin, and Oyajobi 2003). Imbalances in this coupling, where resorption is not followed by an equivalent amount of formation, leads to bone loss, seen in diseases such as osteoporosis. New bone formation can also occur in surfaces that have not been resorbed, such as in cases of prolonged fluoride therapy and in osteoblastic metastases (Baron 2003; Lian, Stein, and Aubin 2003; Mundy, Chin, and Oyajobi 2003; Martin 1989).

The ECM in bone is comprised of 50% to 70% inorganic mineral matrix, 20% to 40% organic matrix, 5% to 10% water, and less than 3% lipids. The inorganic matrix is composed of a hydroxyapatite mineral [$Ca_{10}(PO_4)_6(OH)_2$]. The mineral component contributes to structural support of the skeletal system. Bone mineral is a nonstoichiometric, semicrystalline, calcium, and hydroxide deficient analog of hydroxyapatite. Table 1.1 shows a variety of hydroxyapatite analogs found in bone. Most calcium phosphate precipitates containing calcium/phosphorous ratio between 1.33 to 2.0 result in a diffraction pattern resembling that of an apatite crystal. The apatite crystal size in bone is much smaller (~200 Å in the smallest dimension) than its geologic analog (Robey and Boskey 2003). This size disparity

TABLE 1.1

Calcium-Phosphate Phases with Corresponding Ca/P Ratios

Name	Formula	Ca/P Ratio
Hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Fluorapatite	$Ca_{10}(PO_4)_6F_2$	1.67
Chlorapatite	$Ca_{10}(PO_4)_6Cl_2$	1.67
A-type carbonated apatite (unhydroxylated)	$Ca_{10}(PO_4)_6CO_3$	1.67
B-type carbonated hydroxyapatite (dahllite)	$Ca_{10-x}[(PO_4)_{6-2x}(CO_3)_{2x}](OH)_2$	≥1.67
Mixed A- and B-type carbonated apatites	$Ca_{10-x}[(PO_4)_{6-2x}(CO_3)_{2x}]CO_3$	≥1.67
HPO_4 containing apatite	$Ca_{10-x}[(PO_4)_{6-x}(HPO_4)_x](OH)_{2-x}$	≤1.67
Monohydrate calcium phosphate (MCPH)	$Ca(H_2PO_4)_2H_2O$	0.50
Monocalcium phosphate (MCP	$Ca(H_2PO_4)_2$	0.50
Dicalcium phosphate dihydrate (DCPD)	$Ca(HPO_4)2H_2O$	1.00
Tricalcium phosphate (TCP)	α- and β-$Ca_3(PO_4)_2$	1.50
Octacalcium phosphate (OCP)	$Ca_8H(PO_4)_65H_2O$	1.33

Source: Segvich et al., in *Biomaterials and Biomedical Engineering*, Ahmed et al. (eds.), TTP, Switzerland, pp. 327–373, 2008. With permission.

can arise from lattice substitutions of calcium, phosphate, and hydroxide groups with magnesium and carbonate ions. These substitutions can also give rise to altered solubility of the mineral phase. Since bone mineral contains calcium and alkali reserves, this enhanced solubility can buffer systemic changes in Ca^{2+}, H_3PO_4, and CO_2 (Neuman and Neuman 1957). For instance, during acidosis, the mineral can give up a carbonate ion for a hydronium ion to supplement blood buffers. The crystalline phase contains carbonate lattice substitutions that account for 2 to 7 wt.% of biological apatite (Segvich, Luong, and Kohn 2008). The consensus is that carbonate substitutes directly into the lattice through a type B (Table 1.1) substitution that is most commonly found in biological apatite (LeGeros 2002). The organic matrix is predominantly comprised of collagen, of which type I collagen is the major component, with trace amounts of other collagen isoforms present during certain developmental stages. Noncollagenous proteins comprise the remaining 10% to 15% of total bone protein content and include proteoglycans, glycosylated proteins, and γ-carboxylated proteins. These noncollagenous proteins are involved in directing organic matrix assembly, maintaining structural integrity of the tissue, sequestering and interacting with growth factors, and regulating bone metabolism and mineralization.

Bone ECM also functions as a reservoir of growth factors that are secreted by the cells (Biondi et al. 2008). Growth factors are a major class of hormones that mediate growth, division, and proliferation, and can be involved in endocrine, autocrine, and paracrine signaling (Silverthorn 2003). Cell stimulation by growth factors is influenced by concentration gradients and stage of development at which the active molecules are present. For instance, the role of TGF-β1 in osteogenesis and bone remodeling varies with concentration. TGF-β1 is present in high concentrations during early fracture repair process, but levels off in later stages (Allori, Sailon, and Warren 2008). In the early stages of repair, TGF-β1 promotes division of fibroblasts, osteoblast recruitment, and differentiation. TGF-β1 also inhibits osteoclast proliferation and differentiation. In later stages of wound healing, TGF-β1 promotes osteoclastogenesis. Similarly, BMP2 promotes chemotaxis and cell proliferation at low concentrations and cell differentiation and bone formation at high concentrations (Allori, Sailon, and Warren 2008).

Engineering Biomaterial Surfaces

A range of materials used for both prosthetic and regenerative therapies attempts to emulate some of the compositional, structural, and/or functional characteristics of the native bone microenvironment. Whether biomaterials are designed to function in vivo in a transient or permanent manner, they should integrate with host tissue and not lead to fibrous encapsulation. Clinical success rates of prostheses correlate with implant integration with surrounding tissue. Successful implant integration with host bone is characterized by a bone-like interface that integrates the implant surface with surrounding bone. This interface contains mineral, collagen, and cellular components and functions as a site for bone formation and resorption. This nanometer-thick interface is observed on implant surfaces that are conducive to osteogenic cell attachment, proliferation, and differentiation.

Implant and scaffold materials are designed to promote osteoconduction and/or osteoinduction, thereby improving osseointegration (Albrektsson and Johansson 2001). Osteoconduction refers to the propensity of a surface to allow bone growth. An osteoconductive material implanted at the defect site allows osteogenic precursor migration, adhesion, proliferation, and differentiation (Alsberg, Hill, and Mooney 2001). Conductive materials support adhesion of cells migrating from surrounding host tissue or may be used as a carrier to transplant osteogenic precursors. Therefore, the conductive properties of a substrate surface do not guarantee osseointegration but simply allow it to take place. Integration into host tissue is governed by additional factors that direct cells and organic components to mineralize and form new bone. Therefore, osteoconduction is necessary, but not sufficient for osseointegration.

Osteoinduction refers to the process by which osteogenesis is induced. More specifically, this is the process by which osteogenic precursor cells are actively guided to develop into differentiated osteogenic cells (Albrektsson and Johansson 2001). These differentiated cells partake in the restructuring of the extracellular matrix and the subsequent formation of new bone. Osteoinduction is typically achieved via the incorporation of growth factors, peptides, and/or DNA that interact with cell surface receptors and trigger signal transduction pathways to recruit and direct cell infiltration into the defect site from the surrounding tissue or transplanted donor cells. Osteoinductive materials, functionalized with biomolecules, actively engage in cell recruitment and direction to enhance the quality, amount, and rate of bone formation compared to osteoconductive materials alone (Hirano and Mooney 2004). An osteoinductive material is implicitly osteoconductive since a biofunctionalized nonconductive material surface would negate the inductive effects that would have lead to bone formation. Both osteoinductive and osteoconductive properties of the material play an integral role in osseointegration.

Although bulk properties of a material provide structural stability for both prosthetic and regenerative therapies, surface characteristics play an equally important role in regulating conduction and integration (Mitragotri and Lahann 2009; Murphy et al. 2000b; Liu, de Groot, and Hunziker 2005; Liu, de Groot, and Hunziker 2004). Surface chemistry, surface roughness, and elasticity can affect biological responses to implanted materials (Temenoff and Mikos 2008).

Surface chemistry. Atoms at the surface of a material are not bound on all sides like they are in the bulk. Unbound surface atoms have unfilled valence electrons resulting in surface-free energy also referred to as surface tension. When implanted into the host, proteins migrate toward the implant surface to reduce this surface free energy. Two other factors that regulate protein adsorption or foreign body response are surface charge and surface hydrophilicity (Temenoff and Mikos 2008). Hydrophilic surfaces demonstrate enhanced

surface wettability by water. Wettability is the relative adhesion of a fluid to a solid surface. In the case of biomaterials and immiscible fluids, wettability refers to the ability of water to spread or adhere on an implant surface. A wettable surface has surface free energy 10 dyn/cm greater than the surface tension of the liquid. Hydrophilic surfaces that exhibit enhanced wettability can improve osteoconductivity by providing energetically favorable binding sites for integrins (Kilpadi and Lemons 1994; Rupp et al. 2006). Hydrophilic surfaces that exhibit enhanced wettability can improve osteoconductivity by providing energetically favorable binding sites for integrins (Kilpadi and Lemons 1994; Rupp et al. 2006).

An implant's surface charge resulting from dissociating ions can also have an effect on biointegration. With more dissociating surface ionic groups, oppositely charged biomolecules become electrostatically attracted to the surface. For instance, the spontaneously formed TiO_2 film on titanium implants reacts with water to form acidic and basic hydroxyl groups at the surface that enhance surface charge and protein adsorption (Kilpadi and Lemons 1994). Increasing the hydrophilicity improves osteoconduction by increasing osteoblastic cluster formation compared to unmodified titanium surfaces (Rupp et al. 2006).

Surface topography. Cells interact with the ECM through transmembrane focal adhesion kinases that allows them to transduce external cues through the cytoskeleton into the nucleus to induce transcription. Transduction of external mechanical cues elicits specific biochemical signals controlling cell cycle, proliferation, migration, and differentiation. Surface topography and elasticity are key factors that can control and direct this cellular response (Ingber 2006, 1997).

Implant surface topography has been extensively studied to identify correlations between surface structures and fixation to bone. Despite the heterogeneity in experimental methods, there is a positive relationship between surface roughness and bone to implant contact (Shalabi et al. 2006). Several approaches to modify surface roughness at the micron level have been utilized, among which sandblasting, acid etching, and sodium hydroxide treatments are the most widely used (Bollen, Lambrechts, and Quirynen 1997). For instance, osteoblasts cultured on sandblasted implants exhibit enhanced mineralization compared to osteoblasts grown on smooth surfaces (Marchisio et al. 2005). However, there is an upper limit to roughness for improving tissue integration or inducing an enhanced cellular response on the order of R_a = 4 μm (Rønold, Lyngstadaas, and Ellingsen 2003). Roughness is therefore an important design parameter to consider when altering surface characteristics to enhance osteointegration.

Surface chemistry and surface topography are also co-optimized to enhance osseointegration. For instance, sandblasted acid etched implants are contaminated by hydrocarbons minutes after exposure to air, making their surface chemistry hydrophobic. These implants are processed with nitrogen gas and stored in NaCl solution to decrease contamination and increase hydrophilicity. This surface modification procedure results in improved osseointegration (Rupp et al. 2006).

Micro- and nanostructuring techniques are also used to control molecular-level interactions between cells and the environment: soft-lithography, photolithography, sputtering, self-assembling nanostructures, and physical and chemical vapor deposition modify surface topography at the micro- and nanoscales and control cell behavior (Tan and Saltzman 2004; Martinez et al. 2009; Dalby et al. 2007, Dalby et al. 2004; Xia and Whitesides 1998). Surface micro- and nanotopography regulate cell orientation, morphology, and cytoskeletal rearrangement and promote cell adhesion, proliferation, and differentiation (Martinez et al. 2009). For instance, to identify the role of surface topography in directing osteogenic differentiation, human mesenchymal stem cells were grown on 120-nm grooves created

by electron beam lithography on polymethylmethacrylate (PMMA). Cells grown on these nanostructures engaged in osteogenic differentiation and bone mineral formation without the addition of osteogenic factors to the culture media (Dalby et al. 2007; Dalby et al. 2004).

Substrate elasticity. Substrate elasticity also plays an important role in cell adhesion, proliferation, and differentiation, thereby enhancing osteoconduction and osseointegration. Advances in materials engineering offer a variety of polymer substrates with elasticities that may be tuned to match the stiffness of specific tissues (Thompson et al. 2005; Kloxin, Benton, and Anseth 2010; Lo et al. 2000; Discher, Mooney, and Zandstra 2009). These tunable polymers are used to observe effects of substrate compliance on adhesion and proliferation independent of surface chemistry and topographical effects.

A variety of cells, including fibroblasts, epithelial cells, myocytes, and osteoblasts, show increased adhesion and proliferation on stiffer substrates (Mitragotri and Lahann 2009; Griffin et al. 2004). For example, kidney epithelial cells grown on polyelectrolyte multilayers show increased adhesion with increasing modulus between 50 and 500 kPa (Kocgozlu et al. 2010). Mesenchymal cells show markers for neurogenic, myogenic, and osteogenic differentiation when cultured on polyacrylamide gels with stiffness analogous to native brain, muscle, and osteoid, respectively (Engler et al. 2006). Therefore, material stiffness can be a useful parameter to direct osteoconduction and osseointegration. However, it is important to consider the integrated roles of surface chemistry, surface topography, and elasticity to amplify osteoconductive effects of a biomaterial surface.

One way to incorporate the desired surface properties into an implant or scaffolding material is to use coating techniques wherein the chemistry of the coatings can be controlled to provide the required roughness, elasticity, and crystallinity. Hydroxyapatite coatings are the most commonly used inorganic coatings on bone implants and in regenerative therapies, and can be used to increase modify the microtopography of substrate surfaces. Hydroxyapatite coatings with surface roughness (R_a) values in the range of 0.7–4.8 show significantly increased human bone marrow stromal cell adhesion and proliferation with increasing R_a (Deligianni et al. 2001).

HA coatings with low crystallinity show increased dissolution compared to highly crystalline coatings (Lee et al. 2009). In addition to varying crystallinity, crystallite size can also be varied by altering processing temperatures. Coatings with larger crystallite size exhibit lower dissolution and improved stability of the crystallographic lattice (Zhang et al. 2003).

In addition to affording control over dissolution and surface roughness, crystallinity is reported to improve osseointegration. For example, fibroblasts cultured on 98% crystalline HA coatings exhibit enhanced adhesion and proliferation compared to 65% crystalline, 25% crystalline, and uncoated titanium surfaces after 14 days of culture (Chou, Marek, and Wagner 1999). In vivo, canine femoral implants having 98% crystalline HA coatings showed greater integration with surrounding bone 3 months postimplantation compared to implants with 50% crystalline HA coatings (Xue et al. 2004).

However, other studies show no significant increase in osseointegration with changes in crystallinity. For example, no difference in bone formation was observed between 50%, 70%, and 90% crystalline coatings at 4, 12, and 24 weeks (Lacefield 1999). Similar studies using 100% and 40% crystalline HA coatings also resulted in no discernable difference in osseointegration (Frayssinet et al. 1994). Further research is required to more thoroughly elucidate the role of crystallinity in osseointegration. Although crystallinity maintains the osteoconductive properties of a material while providing control over coating delamination (Lacefield 1999), an osteoconductive material is not always osseointegrative. Therefore,

although osteogenic precursors are still able to adhere and grow on implants with higher crystallinity, this does not translate to an osseointegrative response.

Apatite coatings create surface chemistries more analogous to native bone. Different calcium-phosphate phases present in native bone, such as octacalcium phosphate and carbonated apatite, enhance cell adhesion and osteoconduction (Le Guehennec et al. 2007; Müller et al. 2007; Wang et al. 2004). Altering the phases of the apatite coating can have significant effects on the degree of crystallinity, surface roughness, and solubility of the coating (Barrere et al. 2003a). Apatite coatings therefore afford increased control over surface chemistry and/or topography while maintaining the elastic characteristics of the biomaterial surface. Gaining further control over surface chemistry and topography will enhance the ability to reconstruct cellular microenvironments, thereby improving osseointegration. Apatite coatings also increase the stiffness of soft substrates, providing control over cytoskeletal organization (Murphy et al. 2000b; Leonova et al. 2006). There are several approaches to depositing apatite coatings with controlled composition, topography, and/or stiffness for enhancing conduction. The subsequent sections discuss processing and applications of a biomimetically applied BLM coating precipitated from a supersaturated salt solution and how controlling biomimetic processing, composition, and structure can control biological responses in vitro and in vivo.

Biomimetic Precipitation of Mineral

Implants that do not integrate into host tissue become isolated from the surrounding tissue, limiting the efficiency of load transfer (Jacobs, Gilbert, and Urban 1998). Bioactive materials such as Bioglass 45S5 and A-W glass ceramics form a layer of apatite on the surface when placed in vivo, which is vital for implant/tissue integration (Ducheyne 1985; Nakamura et al. 1985). It is possible to simulate this apatite coating in vitro and thus provide bioactivity to non-bioactive materials using coating techniques such as plasma spraying, electrophoretic deposition, sol–gel deposition, hot isostatic pressing, frit enameling, ion-assisted deposition, pulsed laser deposition, electrochemical deposition, and sputter coating (Liu and Hunziker 2009). Each one of these methods has its own advantages and disadvantages (Table 1.2), and not all techniques can be used with all classes of materials (Table 1.3). Of these methods, the most widely used technique commercially for metals is plasma spraying. Plasma spraying, however, is not ideal with small implants and complex shapes. It requires a coating thickness of 40 to 50 μm to achieve uniform deposition, and is clinically challenged by delamination issues due to variations in the phases that constitute the coating (Le Guehennec et al. 2007). Other methods such as dynamic mixing and hot isostatic pressing are limited by the uniformity of coating that they generate as well (Wie, Hero, and Solheim 1998; Yoshinari, Ohtsuka, and Dérand 1994). However, there are a number of coating methods that deposit mineral uniformly: sputter coating, pulsed laser deposition, sol–gel deposition, and electrophoretic deposition are better suited for uniform coating on complex structures (Wolke et al. 1994; Zeng and Lacefield 2000; Li, De Groot, and Kokubo 1996). However, the use of high processing temperatures in some of these methods results in the formation of apatite that differs from the composition of natural bone apatite, and is also not amenable to soft materials such as polymers (Abe, Kokubo, and Yamamuro 1990). Among these methods, sol–gel deposition is the only other method that can achieve uniform mineral coatings at low processing temperatures, but

TABLE 1.2

Hydroxyapatite Coating Produced Using Various Deposition Technologies

Technique	Thickness	Advantages	Disadvantages	References
Thermal spraying	30–200 µm	High deposition rates; low cost	Line-of-sight technique; high temperatures induce decomposition; rapid cooling produces amorphous coatings	Gross and Berndt 1998; Gross, Berndt, and Herman 1998; Li, Khor, and Cheang 2002; Yang and Ong 2003; Zyman et al. 1993; Tao, Heng, and Chuanxian 2000; Weng et al. 1993; Chen, Wolke, and de Groot 1994; Zyman et al. 1994; Roome and Adam 1995
Sputter coating	0.5–3.0 µm	Uniform coating thickness on flat substrates; dense coating	Line of sight technique; expensive; time-consuming; produces amorphous coating	Ding 2003; Ding, Ju, and Lin 1999; Wolke et al. 2003; Massaro et al. 2001; Ong and Lucas 1994; Ong et al. 1994; Wolke et al. 1994; van Dijk et al. 1996; van Dijk et al. 1995
Pulsed laser deposition	0.05–5.0 µm	Coating with crystalline and amorphous; coating with dense and porous	Line-of-sight technique	Clèries et al. 2000; Fernández-Pradas et al. 2001; Zeng and Lacefield 2000
Dynamic mixing method	0.05–1.30 µm	High adhesive strength	Line-of-sight technique; expensive; produces amorphous coating	Yoshinari, Ohtsuka, and Dérand 1994
Dip coating	0.05–5.0 mm	Inexpensive; coating applied quickly; can coat complex substrates	Requires high sintering techniques; thermal expansion mismatch	Wenjian and Baptista 1998; Choi et al. 2003; Shi, Jiang, and Bauer 2002; Jiang and Shi 1998; Campbell et al. 2000

(continued)

TABLE 1.2 CONTINUED

Hydroxyapatite Coating Produced Using Various Deposition Technologies

Technique	Thickness	Advantages	Disadvantages	References
Sol–gel	<1 μm	Can coat complex shapes; low processing temperatures; relatively inexpensive as coating is very thin	Some processes require controlled atmosphere processing; expensive raw materials	Li, De Groot, and Kokubo 1996; Manso et al. 2002; Liu, Yang, and Troczynski 2002; Chai, Gross, and Ben-Nissan 1998
Electrophoretic deposition	0.1–2.0 mm	Uniform coating thickness; rapid deposition rates; can coat complex substrates	Difficult to produce crack-free coatings; requires high sintering temperatures	Ducheyne et al. 1986; Han et al. 1999; Han et al. 2001; Zhu, Kim, and Jeong 2001; Agata De Sena et al. 2002; Ma, Wan, and Peng 2003; Nie et al. 2001; Manso et al. 2000
Biomimetic coating	<30 μm	Low processing temperatures; can form bone-like apatite; can coat complex shapes; can incorporate bone growth stimulating factors	Time-consuming; requires replenishment and constant pH of simulated body fluid	Habibovic et al. 2002; Oliveira et al. 1999; Li et al. 1992
Hot isostatic pressing	0.2–2.0 mm	Produces dense coatings	Cannot coat complex substrates; high temperature required; thermal expansion mismatch; elastic property differences; expensive; removal/interaction of encapsulation material	Wie, Hero, and Solheim 1998

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TABLE 1.3

Classes of Materials That Can Be Coated with Different Calcium-Phosphate Coating Techniques

Coating Technique	Metals	Ceramics	Polymers	Examples
Plasma spraying	×			de Groot et al. 1987; Klein et al. 1991
Sputter coating	×		×	Ong and Lucas 1994; Feddes et al. 2004
Pulsed laser deposition	×	×	×	Baeri et al. 1992; Antonov et al. 1998; Honstu et al. 1997
Electrophoretic deposition	×	×		Ducheyne et al. 1990; Yamashita et al. 1998
Sol–gel	×		×	Kaneko et al. 2009; Montenero et al. 2000
Hot isostatic pressing	×	×		Herø et al. 1994; Li, Liao, and Hermansson 1996
Dynamic mixing method	×			Yoshinari, Ohtsuka, and Dérand 1994
Biomimetic coating	×	×	×	Tanahashi et al. 1994; Habibovic et al. 2002; Abe, Kokubo, and Yamamuro 1990

this method is expensive. A promising alternate technique is biomimetic precipitation of apatite onto implant surfaces using supersaturated salt solutions known as simulated body fluids (SBFs) (Abe, Kokubo, and Yamamuro 1990). These SBFs have similar ionic constitutions to blood plasma and form carbonated apatites at physiologic temperatures, similar to those found in bone (Table 1.4). Commonly used SBF coating regimens for different material applications are listed in Table 1.5.

The mechanism of formation of BLM can be generally outlined as (1) functionalization of the substrate to obtain a negatively charged surface, (2) nucleation by chelation of precursor Ca^{2+} ions to these negatively charged groups, and (3) growth of the BLM layer (Murphy, Kohn, and Mooney 2000a). Surface functionalization can be achieved by several methods, such as grafting functional groups, alkaline treatments, aqueous hydrolysis, heat treatments, and glow discharge treatment in oxygen (Murphy, Kohn, and Mooney 2000a; Segvich et al. 2008; Kokubo 1996; Tanahashi et al. 1995; Luong et al. 2006; Tanahashi and Matsuda 1997). The heterogeneous precipitation of biomineral from solution occurs when the precursor–substrate interfacial energy is lower than the precursor–solution energy,

TABLE 1.4

Chemical Composition (in mM) of Different Types of SBFs

Type of SBF	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO^{3-}	HPO_4^{2-}	SO_4^{2-}	Reference
1× SBF	142.0	5.0	1.5	2.5	148.8	4.2	1.0	0.5	Abe, Kokubo, and Yamamuro 1990
Revised SBF (r-SBF)	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5	Oyane et al. 2003
Newly improved SBF (n-SBF)	142.0	5.0	1.5	2.5	103.0	4.2	1.0	0.5	Takadama et al. 2004
1× mSBF	145.2	6.0	1.5	5.0	157.0	4.2	2.0	0.5	Luong et al. 2006
2× SBF	282.0	10.0	3.0	5.0	304.0	8.4	2.0	1.0	Shin, Jayasuriya, and Kohn 2007
5× SBF	710.0	25.0	12.7	7.7	739.7	21.0	5.0	2.5	Chen et al. 2008
Human plasma	142.0	5.0	2.5	1.5	103.0	27.0	1.0	0.5	Abe, Kokubo, and Yamamuro 1990

TABLE 1.5

Commonly Used SBF Treatment Applications

Application	SBF Treatment	Reference
Metals	1. 5× SBF for 24 h at 37°C 2. 5× mSBF for 24 h at 50°C	Barrere et al. 2003
	1. 5× SBF for 24 h at 37°C 2. 5× mSBF for 48 h at 50°C	Habibovic et al. 2005
Ceramics	1. SBF 4 days at 37°C	Liu, Ding, and Chu 2004
PLGA films	1. 2× mSBF at 37°C	Luong et al. 2006
PLGA scaffolds	1. 4× mSBF for 12 h at 37°C 2. SBF change every 6 h 3. 2× mSBF for 108 h at 37°C 4. SBF change every 8 h	Segvich, Luong, and Kohn 2008; Segvich et al. 2008

Source: Ong et al., in Thin Calcium Phosphate Coatings for Medical Implants, Leon and Jansen (eds.), Springer, pp. 175–198, 2009. With permission.

resulting in the formation of an apatite coating on the substrate. In the absence of this thermodynamically favorable situation, homogeneous precipitation can occur (Bunker et al. 1994). The thickness, morphology, and composition of the BLM formed are dependent on several factors, such as the incubation time and temperature, ionic concentration, composition, and pH of SBF and method of surface functionalization. For example, apatite formed from SBFs with low ionic products is more crystalline compared to apatite formed from SBFs with higher ionic products. Also, the Ca/P ratios of BLM vary inversely with SBF solution ionic activity—higher ionic products lead to lower Ca/P ratios (Shin, Jayasuriya, and Kohn 2007).

Characterization of biomimetic apatite to quantify and understand its properties can be achieved using several methods (Figure 1.1). Fourier transform infrared spectroscopy (FTIR) is useful in determining chemical composition by measuring carbonate and phosphate band intensities and ratios, and presence of any organic constituents. X-ray diffraction (XRD) and transmission electron diffraction (TED) allow the measurement of the lattice parameters of the crystallographic peaks and thereby assess the degree of crystallinity. Scanning electron microscopy (SEM) can be used to observe surface morphology and, when used along with energy dispersive x-ray spectrometry (EDX), can provide a qualitative measure of elemental composition. N-SEM, which is another form of SEM, can be used to characterize surface morphology with high natural contrast but at the cost of resolution. Microcomputed tomography (μCT) generates images of 3-D mineralized scaffolds from which total mineral content, volumetric mineral density, and distribution of mineral can be quantified (Segvich, Luong, and Kohn 2008).

The use of SBF to prepare apatite coatings on prosthetic metal and ceramic implants has been extended to soft resorbable materials used in bone tissue engineering to create a new class of osteoconductive biomaterials. Since biomimetic apatite precipitation occurs at ambient temperatures, it is possible to coat temperature-sensitive scaffold materials such as polymers with a layer of uniform mineral. The use of processing techniques based on biomineralization also allows the incorporation of biologically active molecules such as growth factors, proteins, and nucleic acids into the mineral layer without any loss of bioactivity due to high temperatures (Segvich et al. 2008; Luong et al. 2006; Luong, McFalls, and Kohn 2009). Moreover, since porosity is important for tissue infiltration and vascularization to induce biological fixation, polymer scaffolds can be coated with a spatially uniform

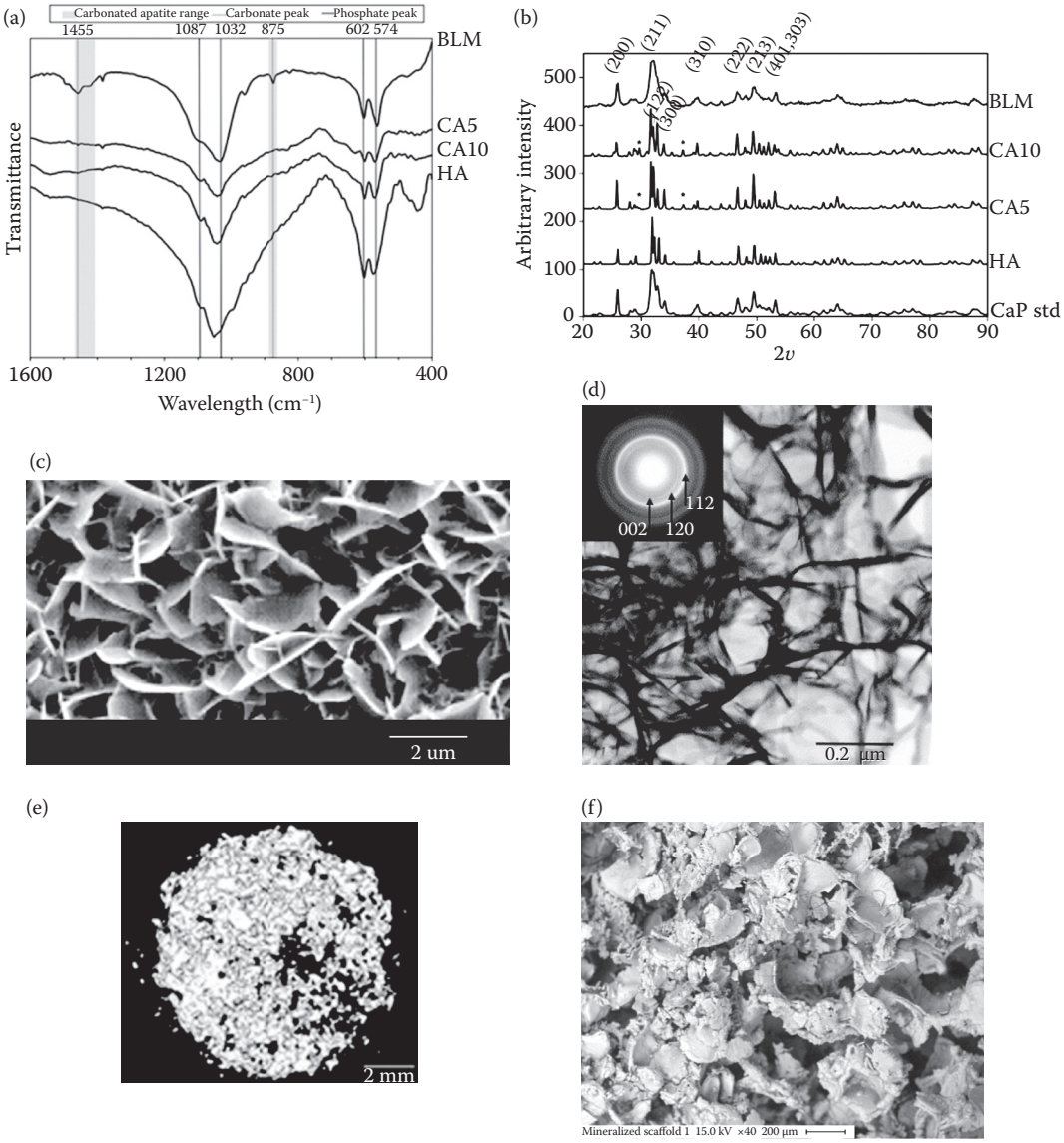

FIGURE 1.1
Different methods of characterization of bone-like mineral (BLM). (a) FTIR spectra for BLM, sintered disks from 5.6% (CA5), and 10.5% (CA10) carbonated apatite powder, sintered disks from hydroxyapatite powder (HA); (b) XRD patterns for BLM, CA5, CA10, HA, and a calcium phosphate standard (CaP std); (c) SEM image of BLM coating on PLGA film (magnification 10,000×); (d) TEM image and diffraction pattern of BLM crystals on PLGA film; (e) micro-CT image of top cross section of BLM coated on PLGA scaffold; (f) N-SEM image of top cross section of BLM coating on PLGA scaffold. (Reprinted from: (a, b) Segvich et al., *Biomaterials*, 30, 1287–1298, 2009, with permission from Elsevier; (c, d) Luong et al., *Biomaterials*, 27(7), 1175–1186, 2006, with permission from Elsevier; (e) Segvich et al., *Journal of Biomedical Materials Research B*, 84B(2), 340–349, 2008, with permission from Wiley.)

and continuous layer of biomimetic apatite throughout the thickness, without compromising porosity (Segvich et al. 2008).

Metals

Metal implants are most commonly used for their load-bearing and tribological properties. The most prevalent metal implant materials include titanium and its alloys, cobalt–chromium alloys, and stainless steel. Metal implants biomimetically coated with BLM enhance osteoconduction and osseointegration. Heat treating titanium with NaOH results in the formation of a sodium titanate layer at the surface of the implant. When this pretreated titanium is immersed in SBF, sodium ions at the titanate surface are rapidly exchanged with hydronium ions in the fluid. This exchange functionalizes the surface with Ti–OH groups. Titanium's isoelectric point is at pH 5.8; therefore, a negative charge on the surface arises when immersed in SBF that has a pH of 7.4. As sodium ion exchange continues, a thin calcium titanate layer forms on top of the sodium titanate layer in about 30 min as a result of electrostatic interaction between negatively charged units of repolymerized TiOH groups and calcium in the SBF. Amorphous calcium phosphate precipitates on the surface within 36 h due to electrostatic interaction between the increasingly positive charge of the calcium titanate layer and negatively charged phosphate ions in SBF. This interaction leads to crystalline apatite formation within 48 h after immersion (Takadama et al. 2001).

BLM coatings on metal implants enhance osteointegrative properties in vitro and in vivo (Stigter et al. 2004; Stigter, de Groot, and Layrolle 2002; Schliephake et al. 2006; Bernhardt et al. 2005; Fujibayashi et al. 2004). BLM coatings deposited on implants from solution at ambient temperature and pressure are more favorable for osteoconduction than coatings deposited by high-temperature processing methods since the calcium/phosphate ratio and crystal structure of the mineral coating are more conducive to osteoconduction (Wang et al. 2004). For instance, in a goat orthotopic model, BLM-coated Ti_6Al_4V femoral implants exhibited significantly greater implant/bone contact compared to noncoated implants (Barrere et al. 2003a, 2003b; Habibovic et al. 2005).

Ceramics

Ceramics are another class of biomaterials that have been successfully used in a variety of orthopedic and dental implants, such as acetabular cups, extracochlear implants, ilial crest replacement, alveolar ridge maintenance, dental crowns, inlays, onlays, and veneers (Hench 1991). Although ceramics do not exhibit the same mechanical strength as metals, they are ideal for some bone microenvironments since they are more susceptible to incorporation into the surrounding tissue while their degradative byproducts are biologically tolerable.

Bioactive glasses improve integration and fixation with host bone tissue. When immersed in SBF, dissolution of calcium and silicate from these bioactive glasses plays an important role in mineralization. Ceramic surfaces, such as metal surfaces, exchange hydronium ions for cations, thereby functionalizing the surface to present nucleation sites for mineralization (Abe, Kokubo, and Yamamuro 1990; Takadama et al. 2001). These functionalized Si–OH surface groups drive the deposition of calcium and phosphate ions, resulting in BLM formation and a subsequent depletion of phosphate ions from the media. Bioglass 45S5, Ceravital-type glass ceramic, glass ceramic A-W, sintered HA, apatite/β-tricalcium phosphate, and calcium sulfate exchange Ca^+ ions with the solution resulting in BLM formation at the surface (Kokubo and Takadama 2006). Nonbioactive glass ceramics do not

dissolve these initial calcium and silicate ions nor do they form a BLM layer unless their surfaces are functionalized before immersion (Kokubo 1991). As with metals, the osseointegration of BLM coated ceramics occurs more rapidly than on noncoated surfaces due to the enhanced conductive properties of the mineral layer (Liu, Ding, and Chu 2004).

Polymers

Biomineralized metals, glasses, and glass ceramics bond well to bone and serve as good implant materials. However, these materials possess high elastic moduli compared to bone, which can cause resorption of the host bone tissue (Kokubo 1996; Nagano et al. 1996). Furthermore, metals and many ceramics can only be used as prosthetic implants since they are not bulk biodegradable and thus do not allow replacement of the material by new tissue over time. Polymers are good tissue engineering substitutes and possess advantages such as biodegradability, improved flexibility in controlling structure, composition, and properties, as well as the ability to be molded into different shapes to fit the wound or defect site.

Natural polymers are readily available, inexpensive, and nontoxic. Raw silk, fibrinogen, and collagen have been incubated in SBF solutions to obtain mineralized biopolymers that can be used as biomimetic bone analogs (Takeuchi et al. 2003; Wei et al. 2008; Girija, Yokogawa, and Nagata 2002). However, since these polymers are protein-based, they may elicit undesired immunological responses when placed in vivo. Alternative natural materials are polysaccharide-based systems, several of which have been developed and used as biomineralization templates. Chitosan microparticles functionalized with Si–OH groups are made bioactive by soaking in SBF and can be used as injectable biomaterials and protein/drug delivery systems (Leonor et al. 2008). Another example is a cornstarch–ethylene vinyl alcohol (SEVA-C) polymer blend that is made bioactive by its ability to induce bone-like apatite formation in SBF after the introduction of carboxylic acid functional groups on the surface (Leonor et al. 2007).

While synthetic polymers possess most of the same advantages as biopolymers, they also offer more control in tailoring their synthesis and degradation properties to suit specific applications. Biodegradable synthetic polymers such as poly-L-lactic acid (PLLA), poly-glycolic acid (PGA) and poly-lactic-*co*-glycolic acid (PLGA) break down into nontoxic natural acid metabolites over time (Murphy, Kohn, and Mooney 2000a). Apatite coatings or polymer/apatite composite materials can compensate for this acidic release by the dissolution of basic calcium phosphate and maintain pH within physiological ranges (Linhart et al. 2001).

BLM coatings have been produced on a variety of polymers including polyvinyl chloride, poly(tetrafluoroethylene), nylon 6, poly(ethylene terephthalate), alkanethiols, and polyhydroxyalkanoates (Tanahashi and Matsuda 1997; Tanahashi et al. 1994; Misra et al. 2006). Biomimetic mineral layers formed on electrospun nanofiber poly(ε-caprolactone) meshes support proliferation of Saos-2 osteogenic sarcoma cells up to 2 weeks in culture, demonstrating potential to regenerate bone ECM (Araujo et al. 2008). Continuous and uniform BLM layers can be produced throughout the porous structure of 3-D PLGA scaffolds (Murphy, Kohn, and Mooney 2000a; Segvich et al. 2008). These mineralized scaffolds exhibit superior mechanical properties, as seen by a 5-fold increase in compressive modulus (Murphy, Kohn, and Mooney 2000a). They also support higher bone marrow stromal cell adhesion through well-distributed fibrillar contacts, and when used to transplant bone marrow stromal cells, form a higher bone volume fraction in comparison to nonmineralized polymer scaffolds (Leonova et al. 2006; Kohn et al. 2005). Other in vivo studies using

polyethersulfone (PES) coated with bone-like apatite also show bonding of the implant to native bone, accompanied by remodeling and complete resorption of the apatite layer after 30 weeks (Nagano et al. 1996). Thus, a variety of biomimetically mineralized polymer scaffolds have utility in bone tissue engineering.

While these BLM coatings mimic the mineral component of bone, they can be altered to include biomolecules such as proteins, growth factors, enzymes, and nucleic acids within the biomineral layer, resulting in organic/inorganic hybrid biomaterials that have the potential to dictate cellular events in an even more controlled manner.

Organic/Inorganic Hybrids

Native bone is a composite material consisting of both inorganic and organic phases. The organic components play important roles in the nucleation and formation of mineral, while influencing osteogenic growth and differentiation. Incorporation of organic molecules such as proteins, growth factors, and DNA into BLM coatings gives rise to a new class of organic/inorganic hybrid materials. These composites are capable of influencing mineral growth and are also able to enhance cell attachment and direct these adhering cells toward osteogenic differentiation. Using different combinations of biomolecules and mineralization regimens, it is possible to mimic physiologic spatial and temporal gradients of bioactive molecules to modulate cellular function and bone formation during development, repair, and regeneration. Other applications of organic/inorganic hybrids include

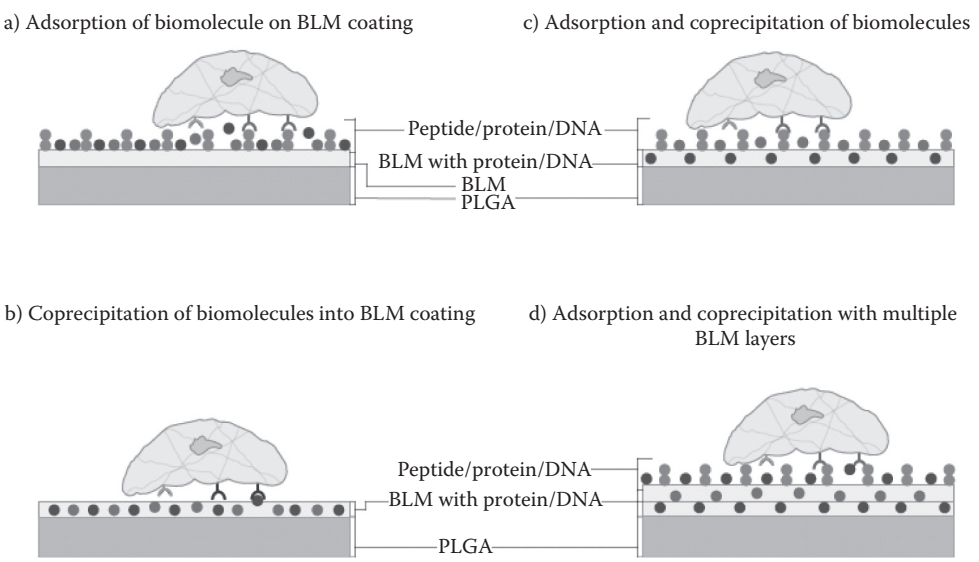

FIGURE 1.2
Schematic representation of cell substrate interactions with several variations of organic/inorganic hybrid constructs. (a, c) Cells binding to biomolecules that have been adsorbed, (b, d) demonstrate cellular interactions with adsorbed and/or coprecipitated moieties. Peptides in panels a, c, d aim to anchor the cells to the substrate surfaces while exposing basal surface for uptake of growth factors and DNA.

creating bone mimics that attempt to recreate the structural hierarchy of natural bone and can be also be used to elucidate the roles of organic molecules in biomineralization.

There are several approaches to create calcium phosphate–organic hybrids. The two main methods to produce organic/BLM hybrids with bioactive molecules discussed in this section are adsorption and coprecipitation. For materials intended as protein delivery systems, each of these approaches has advantages depending on the release profile desired. Adsorption can be used when a quick transient release of the biomolecule is desired, or to recruit cells to the material surface initially. Coprecipitation is more useful in creating gradual release profiles, where the desired molecule is needed over a longer period (Figure 1.2).

Adsorption of Proteins to BLM Surfaces

Adsorption of proteins to mineral surfaces is the simplest method of forming organic/inorganic hybrids containing biologically active molecules and involves incubating the mineralized substrate in a protein solution, allowing the protein to associate itself with the mineral. The mechanism of adsorption is thought to be via electrostatic interactions between the protein and the apatite, and hence the protein–mineral bond created by adsorption is not strong compared to the covalent attachment of proteins to apatite created by other techniques. Adsorption of protein onto biomimetic apatite does not cause any change in the morphology of the mineral (Luong et al. 2006; Liu et al. 2001), since adsorption is a surface phenomenon and does not result in protein integration into the mineral structure (Figure 1.3).

Coating substrates with BLM enhances specificity of protein adsorption. For example, apatite formed on titanium from saturated Ca–P solutions selectively adsorbs proteins from serum and in higher amounts compared to plasma-sprayed hydroxyapatite coatings (Wen, Hippensteel, and Li 2005). Similarly, bioactive glass coated with BLM specifically adsorbs high molecular weight proteins such as fibronectin when incubated in serum (El-Ghannam, Ducheyne, and Shapiro 1999). This enhanced specific adsorptive capability has been attributed to the highly nanoporous structure and high surface area and surface roughness of BLM coatings (Wen, Hippensteel, and Li 2005; Murphy et al. 2005). Increased surface adsorption of proteins onto BLM is especially advantageous for in vivo applications since the adsorbed serum protein layer that forms on implants is vital for cell migration and attachment.

Generally, the release kinetics of an adsorbed protein from a material surface involve a burst profile typified by a fast initial spike in release followed by a more gradual release over time. The amount of protein adsorbed and subsequently released is dependent on the characteristics of the protein (most importantly charge and conformation) and the mineral (surface area, charge). Depending on charge, size, and electrostatic interaction with the BLM surface, different proteins exhibit different affinities to, and therefore different release kinetics from BLM coatings. While TGF-β, Nell-1 and osteocalcin are released gradually over time, BSA exhibits a more characteristic burst release profile (Lee et al. 2009; Wen, Hippensteel, and Li 2005; Krout et al. 2005). BLM coatings may reduce the extent of burst release that occurs with some adsorbed proteins, making BLM coatings more useful therapeutic agent carriers. However, it is important to fully characterize the protein being used to understand the influence of its properties on adsorption and release.

Coprecipitation of proteins along with the mineral is another way to control burst release as the protein is physically incorporated into the mineral and distributed spatially throughout the mineral layer, as compared to surface localization seen with adsorption.

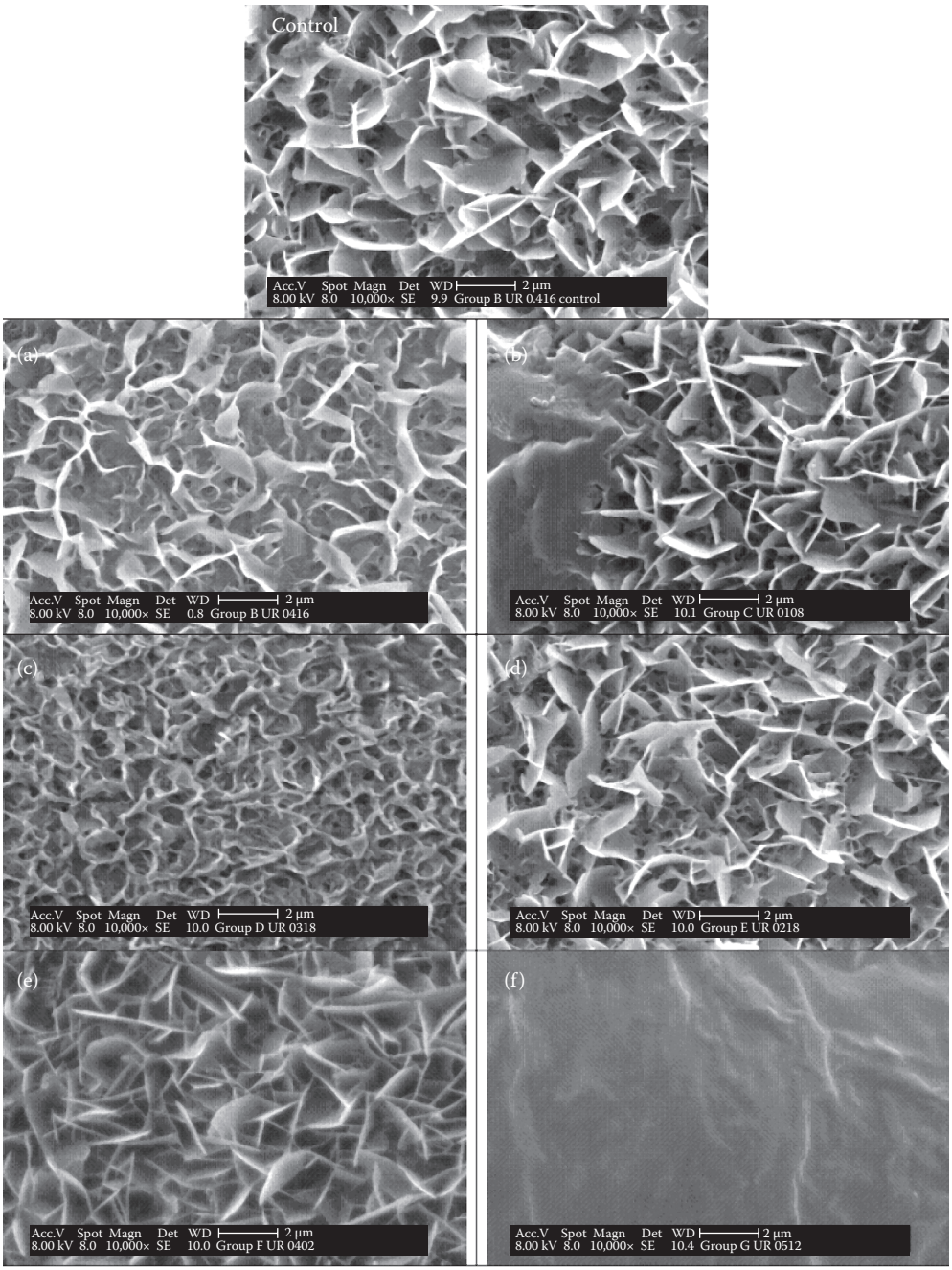

FIGURE 1.3
SEM images of representative samples examined from each of the following groups (magnification 10,000×): (Control) 6-day mineralization, (a) 6-day coprecipitation, (b) 3-day mineralization, 3-day adsorption, (c) 3-day mineralization, 3-day coprecipitation, (d) 3-day mineralization, 2-day adsorption, 1-day mineralization, (e) 3-day mineralization, 2-day coprecipitation, 1-day mineralization, (f) 3-day mineralization, 3-day acid etched adsorption. BSA incorporation via coprecipitation leads to changes in the platelike mineral structure that is observed in the control, whereas BSA adsorption does not change the mineral morphology. (Reprinted from Luong et al., *Biomaterials*, 27, 7, 1175–1186, 2006, with permission from Elsevier.)

For instance, BMP-2 adsorbed on a titanium implant coated with BLM shows a transient release up to 1 week and a sporadic osteogenic response over a 5-week period (Liu, de Groot, and Hunziker 2005).

Surface Adsorption of Peptides to BLM to Enhance Cellular Attachment

Immediately after placing an implant in the body, a protein layer is adsorbed from the surrounding body fluids onto the implant surface, and this protein layer is largely responsible for recruiting cell populations to its surface and mediating all subsequent cellular events (Horbett 2004). An approach that utilizes this adsorbed protein coating to dictate cellular events at the implant surface involves adsorption of adhesive proteins found in the natural bone ECM onto biomaterials, thus attempting to mimic the cell–matrix interactions occurring in vivo. Recognition of specific domains/motifs found within these proteins causes the cells to activate signaling pathways, ultimately resulting in cell proliferation, differentiation, and new bone formation. However, the use of recombinant human and animal proteins in vivo has several disadvantages, including adverse immune responses, enzymatic degradation, and changes in conformation arising from protein–material interactions. Also, proteins usually contain several domains that may be involved in mediating attachment of different types of cells, leading to the possibility of nonspecific cell binding.

Synthetically designed peptides that mimic specific portions of these proteins are able to overcome many of these drawbacks. Table 1.6 provides a list of peptides used in bone tissue engineering. Apart from being cheaper to produce and easier to characterize, peptides are smaller in length (typically 12–30 amino acids) and are less likely to form secondary and tertiary structures, and hence are not affected by changes in conformation. Also, peptides can be engineered to contain domains that are specific to the desired type of cell surface

TABLE 1.6

Examples of Peptides Used in Bone Tissue Engineering Applications

Peptide Sequence	Source/Derived from	Function	Reference
EEEEEEEPRGDT	Bone sialoprotein	Enhances osteoblast adhesion and differentiation	Fujisawa et al. 1997; Itoh et al. 2002
VTKHLNQISQSY	Phage display	Specific affinity toward bone-like mineral (BLM)	Segvich, Smith, and Kohn 2009
KIPKASSVPTELSAISTLYL	BMP2, osteocalcin	Promotes osteogenic differentiation of human mesenchymal stem cells	Lee, Lee, and Murphy 2009
RGDG13PHSRN	Fibronectin	Enhances osteoblast adhesion and differentiation	Benoit and Anseth 2005
KRSR	Heparin	Enhances osteoblast adhesion	Dee, Andersen, and Bizios 1998
DVDVPDGRGDSLAYG	Osteopontin	Enhances osteoblast attachment and differentiation	Shin et al. 2004a, 2004b
SVSVGMKPSPRP	Phage display	Specific affinity toward hydroxyapatite	Roy et al. 2008
(DSS)n	Dentin phosphoprotein	Specific affinity toward calcium phosphate	Yarbrough et al. 2010

Source: Segvich and Kohn 2009, with kind permission from Springer Science + Business Media.

receptors, increasing the likelihood of cell-specific responses (Lebaron and Athanasiou 2000; Segvich, Smith, and Kohn 2009; Hersel, Dahmen, and Kessler 2003).

Noncollagenous proteins found in bone, such as osteopontin, osteonectin, and bone sialoprotein, bind strongly to hydroxyapatite and are thought to be involved in nucleation and growth of crystals during mineralization in vivo (Hunter, Kyle, and Goldberg 1994; Hunter and Goldberg 1994; Fujisawa et al. 1997). These proteins are made up of several regions of acidic amino acids, which are believed to interact with hydroxyapatite crystals. Peptides designed to adhere to hydroxyapatite-based materials have been inspired by these acidic amino acid regions. Peptides consisting of consecutive glutamic acid residues derived from osteonectin bind strongly to hydroxyapatite. The type of acidic amino acid present in these peptides affects mineralization in vitro. For example, polyglutamic acid residues (Glu6) enhanced mineralization, whereas polyaspartic acid residues (Asp6) had an inhibitory effect (Fujisawa et al. 1996). Hence, during the peptide designing process, it is important to understand the contribution of each amino acid to the entire peptide's properties.

ECM proteins are usually multifunctional, containing several domains, allowing them to influence attachment to both materials and cells. Peptidomimetics capable of mediating binding to both hydroxyapatite and cells have been designed using bone sialoprotein as a model. This peptide (EEEEEEEPRGDT) contains a glutamic acid-rich (E7) sequence and the ubiquitous cell binding Arg-Gly-Asp (RGD) domain and binds well to hydroxyapatite, as well as mediates osteoblast attachment and osteogenesis in vitro (Fujisawa et al. 1997; Itoh et al. 2002).

Peptide sequences capable of binding specifically to BLM have recently been identified using a unique combination of phage display and computational modeling techniques (Segvich, Smith, and Kohn 2009) (Figure 1.4). Phage display is a powerful tool as it allows for a wide range of peptides (~10^9) to be panned against any substrate, including natural

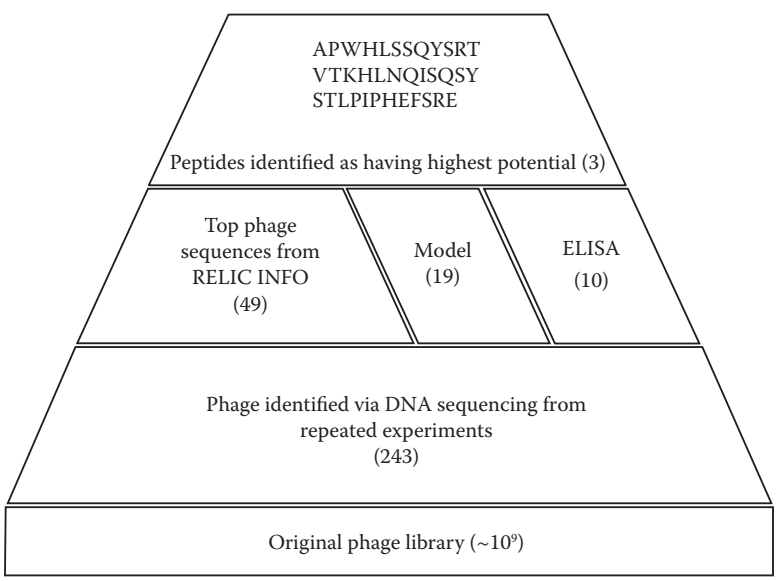

FIGURE 1.4
Schematic displaying the trifold analysis approach used to identify peptides that preferentially bind to BLM and HA. (Reprinted from Segvich, H.J., Smith, H.C., and Kohn, D.H., *Biomaterials,* Vol. 30, pp. 1287–1298, 2009, with permission from Elsevier.)

and synthetic materials, cells, proteins, and viruses, leading to the identification of a few highly specific amino acid sequences. This technique along with ELISA, peptide adsorption assays, and computer modeling were used to discover the peptide VTKHLNQISQSY (VTK), which binds with specific affinity to BLM and HA. An interesting fact is that the VTK peptide has no acidic amino acids, contrary to previously designed HA-binding peptides and therefore is unlikely to have been identified without phage panning.

The same techniques can also be applied to identify cell-specific sequences, allowing for the development of dual functioning peptides that are capable of modulating both mineral-binding and cell adhesion. Multifunctional peptides, whose sequences are inspired by known ECM proteins and growth factors, have also been designed. "Modular" peptides consisting of hydroxyapatite-binding domains derived from osteocalcin and biologically active BMP-2 derived domains bind well to BLM coatings and are capable of directing human mesencyhmal stem cells down osteoblast lineages (Lee, Lee, and Murphy 2009).

The use of protein engineering allows for the development of a new class of biomaterials that are capable of tailoring cellular attachment and function on BLM coatings. The addition of peptides with different charges and conformations into SBF solutions can be used to study nucleation and growth of mineral to gain insight into mineralization processes. Also, peptides can be modified to incorporate important posttranslational modifications such as phosphorylation and glycosylation of certain amino acid residues and can be studied for their effects on mineral formation and cellular differentiation.

Adsorption of DNA to Mineral

Gene therapy may overcome the drawbacks of bioavailability, systemic toxicity, in vivo clearance rate, and manufacturing costs associated with protein delivery (Park, Jeong, and Kim 2006). Design considerations for gene therapy include choosing an effective gene and an efficient and safe delivery system that protects from gene degradation while facilitating transfer to target cells. There are two general methods of gene administration: viral and nonviral.

Viral techniques utilize an adenoviral, retroviral, or lentiviral construct to deliver the gene of interest to the target cell. Disadvantages of using viral gene therapy include mutation risk, induction of immunogenic response, size limitation of DNA construct, and toxicity at high dosages to several tissues (Stigter, de Groot, and Layrolle 2002).

Nonviral delivery methods include naked DNA adsorption, adsorption of conjugated DNA, and coprecipitation of conjugated DNA. Nonviral plasmid DNA conjugation with cationic polymers and liposomal vectors reduces degradation and allows targeting specific cell types; however, surface aggregation and interaction with the biomaterial can reduce transfection efficiency (Luong, McFalls, and Kohn 2009; Shen, Tan, and Saltzman 2004; Park et al. 2003; Jang and Shea 2003; Kofron and Laurencin 2004). Many of the same methods that were used with growth factors can be used to incorporate DNA onto the surface of the mineral coating. Similar to growth factor delivery, adsorption of both naked DNA and DNA lipoplexes results in a burst release of nucleic acid.

Coprecipitation of Proteins and Mineral

An important requirement of a delivery system for sustained release of bioactive molecules is the ability to control the spatial and temporal localization of the molecules across the thickness of the substrate. Such control, along with the degradation and diffusion properties of the carrier will enable the release kinetics to be tailored to achieve a desired

biological response. Adsorption, being largely a surface phenomenon and characterized by weak binding forces and burst release, is not useful in producing spatial distributions and/or gradients of biomolecules. Coprecipitation involves adding the protein into the saturated calcium–phosphate solution, resulting in a heterogeneous matrix consisting of both mineral and protein being simultaneously precipitated onto the substrate. It is possible to distribute single or multiple molecules over sections or the entire thickness of the coating by varying the time periods of mineralization and coprecipitation, thus producing gradients of bioactive molecules within the biomaterial (Luong et al. 2006) (Figure 1.5). Further, since the precipitation occurs at ambient temperatures, loss in biological activity of the protein can be minimized.

Effect of Protein Addition on BLM Formation

Bovine serum albumin (BSA) is often used as a model protein to understand the influence of proteins on mineral nucleation and growth. Addition of BSA to SBF causes a delay in the mineral nucleation and growth, indicating that BSA inhibits these processes (Luong

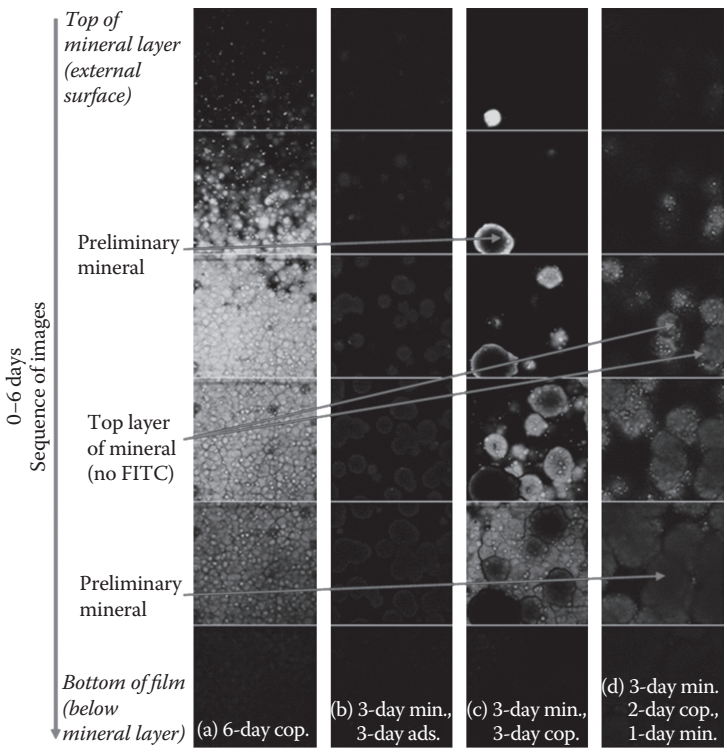

FIGURE 1.5
Images through the thickness of the mineral layer containing FITC-labeled BSA taken using confocal microscopy. Spatial distribution of the protein through the thickness of the mineral layer is exhibited for the following incorporation techniques: (a) 6-day coprecipitation, (b) 3-day mineralization, 3-day adsorption, (c) 3-day mineralization, 3-day coprecipitation, (d) 3-day mineralization, 2-day coprecipitation, 1-day mineralization. Fluorescence can be seen where coprecipitation or adsorption has occurred. Control over the spatial distribution of the protein is shown by the presence of fluorescence through the thickness of the mineral for the different coprecipitation groups. (Reprinted from Luong et al., *Biomaterials*, 27(7), 1175–1186, 2006, with permission from Elsevier.)

et al. 2006). These inhibitory effects are stronger when the BSA is in solution as compared to being preadsorbed onto the substrate (Areva et al. 2002). Also, the stage at which the protein is included affects the extent of inhibition in a concentration-dependent manner. Increasing BSA concentration causes an increase in induction periods for apatite nucleation. Addition of low concentrations of BSA (<10 g/l) during the growth phase causes an increase in the growth rate of the crystals, whereas higher concentrations (>10 g/l) inhibit the growth rate (Combes, Rey, and Freche 1999). Although the exact mechanism by which coprecipitation occurs is not completely understood and may differ from protein to protein, these results suggest that proteins may modulate apatite formation by adsorbing to the initially formed nuclei and stabilizing them by causing a decrease in interfacial energy between the crystal and solution. At low concentrations, there is no sufficient protein to coat the entire mineral surface, allowing the nuclei to grow quickly. At higher concentrations due to coverage of the mineral surface by the protein molecules, growth is prevented (Combes and Rey 2002).

Mineral crystals nucleated in the presence of BSA are smaller in size and less crystalline compared to mineral formed in the absence of BSA (Liu et al. 2001; Combes and Rey 2002). The morphology of the mineral is also affected; protein-free SBF forms sharp platelike mineral crystals that are rounded in the presence of BSA (Luong et al. 2006; Liu et al. 2001) (Figure 1.2). Coprecipitating BSA onto a premineralized surface causes higher quantities of BSA to be loaded, which has been attributed to ability of the negatively charged BSA to interact with the positively charged Ca^{2+} ions. The BSA is attracted to the Ca^{2+} ions in the preliminary mineral layer, causing it to be incorporated into the mineral, which then attracts the Ca^{2+} ions from solution, resulting in a cyclical growth process (Luong et al. 2006; Liu et al. 2001). This interaction of BSA with Ca^{2+} ions is confirmed by slower release of Ca^{2+} ions from coatings formed by coprecipitation with BSA as compared to that in the absence of BSA (Liu et al. 2003).

The above findings show that coprecipitation can be used to integrate proteins into the mineral layer, with their subsequent release being dependent primarily on the rate of dissolution of the mineral. The interaction of each protein with different types of mineral is dependent on several factors including the size, concentration, and charge of the protein, and its influence on the mineral characteristics such as size and crystallinity.

Applications of Protein Coprecipitation in Bone Tissue Engineering

Coprecipitation has been used to incorporate ECM proteins, enzymes, and drugs into biomimetic calcium phosphate coatings. Bone analogs have been produced by coprecipitation of collagen I and mineral onto PLLA substrates using a highly concentrated SBF solution such as 5XSBF. These coatings are capable of enhancing proliferation and differentiation of Saos-2 cells (human osteosarcoma cell line) in vitro (Chen et al. 2008). Coprecipitation has also been used to incorporate enzymes such as amylase and lysozyme into BLM coatings on starch-based polymers (Leonor et al. 2003). These materials can be potentially used as stimulus-responsive scaffolds, undergoing gradual degradation by the incorporated enzyme over time (Martins et al. 2009). Antibiotics such as tobramycin have also been integrated into biomimetic Ca–P coatings on titanium implants and hinder bacterial growth. Biomimetically coated implants that are coprecipitated with antibiotics not only possess the osteoconductive properties of BLM coatings, but are also capable of preventing postoperative infections (Stigter, de Groot, and Layrolle 2002).

Cell–matrix interactions in the natural bone environment orchestrate complex growth factor release profiles that help control bone resorption and formation. TGF-β1 is present

in high concentrations during early fracture repair processes, but levels off in later stages, eliciting specific responses resulting from these concentration changes. For instance, in the early stages of repair, TGF-β1 promotes division of fibroblasts, osteoblast recruitment, and differentiation, whereas in later stages, it promotes osteoclastogenesis. Therefore, a temporally graded administration of TGF-β1 would enhance the repair and regeneration process. Similarly, BMP2 promotes chemotaxis and cell proliferation at low concentrations and cell differentiation and bone formation at high concentrations (Allori, Sailon, and Warren 2008). Likewise, a pulsatile delivery of BMP2 may be optimal for tissue regeneration strategies.

Coprecipitation can tailor the administration of growth factors and bioactive molecules to control their release kinetics and the rate of tissue regeneration. For example, rat bone marrow cells cultured on titanium alloys coated with biomimetically precipitated BLM and BMP-2 showed a significant increase in bone formation compared to adsorption (Hunter and Goldberg 1994). This method of growth factor incorporation into the mineral matrix allowed for a sustained release over a period of 5 weeks as compared to the 1-week burst release of adsorbed growth factor that only produced a sporadic osteogenic response. Although the same amount of BMP-2 was used for both methods, BMP incorporated via coprecipitation showed more of a sustained osteogenic response (Liu, de Groot, and Hunziker 2004). Similarly, insulin-like growth factor-1 (IGF-1) coprecipitated with BLM on PLGA scaffolds showed a sustained and linearly increasing release profile over a 30-day period (Jayasuriya and Shah 2008).

A complex biological response such as tissue regeneration is a result of the coordinated cellular events involving the sequential secretion of multiple growth factors. Coprecipitation and surface immobilization could be used to customize and mimic these coordinated events. For example, TGF-β1 also regulates gene expression of other growth factors such as VEGF. In a rat mandibular orthotopic model, TGF-β and VEGF mRNA transcription increased 2.5-fold only 3 h after surgery and TGF-β and VEGF expression increased 3-fold compared to baseline levels for 4 weeks after wound healing commenced (Allori, Sailon, and Warren 2008). Incorporation of multiple growth factors into a single construct has the potential to enhance bioactivity. For example, the delivery of TGF-β and BMP-2 delivery from alginate gels resulted in greater bone tissue formation after 6 weeks, but no significant changes were observed even 22 weeks after implantation when they were administered alone (Simmons et al. 2004).

Coprecipitation provides the flexibility to tailor release profiles since the concentrations of different growth factors could be graded separately through the thickness of the coating. A similar coordinated response can be achieved by coupling coprecipitation of proteins with surface immobilization methods; the adsorbed molecule could be released in a burst profile and the coprecipitated molecule could be delivered in a sustained fashion.

Coprecipitation of DNA and Mineral

DNA coprecipitation allows the incorporation of nucleic acids into the biomimetically precipitated mineral layer at physiological conditions (Figure 1.6). Similar to growth factors, coprecipitation with mineral can be used to control the spatial distribution of DNA through the thickness of the coating. As the mineral layer degrades in physiological conditions, DNA will be released in a spatially and temporally controlled manner. Coprecipitation of mineral also improves the stiffness of soft substrate surfaces, which improves cellular uptake of DNA (Kong et al. 2005).

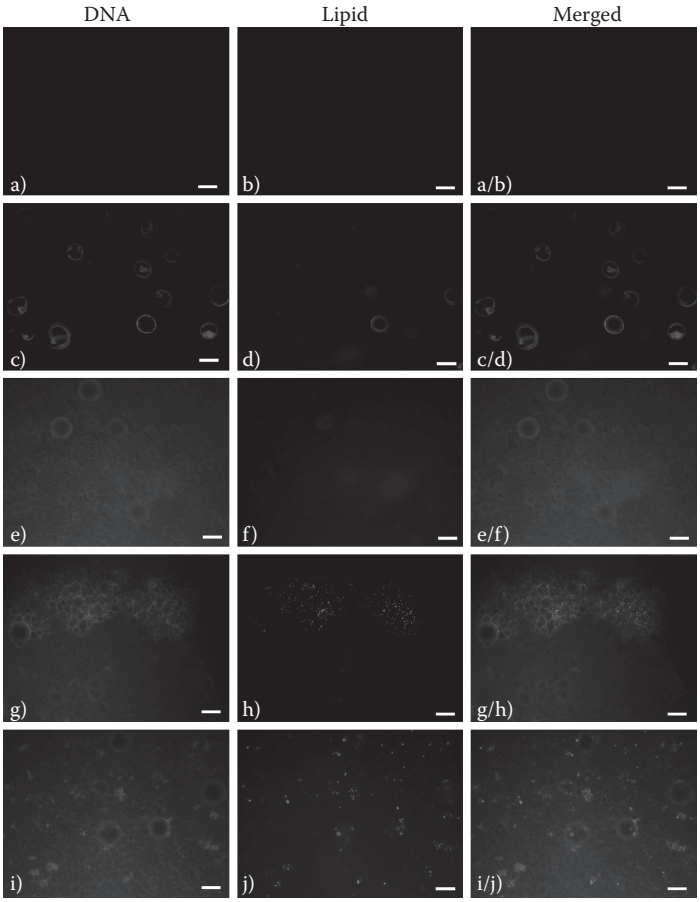

FIGURE 1.6
Fluorescence images of DNA and lipid agent components from representative samples from each of the following groups. (a–b) Mineralized controls, (c–d) plasmid DNA incorporated into PLGA, (e–f) plasmid DNA coprecipitated with mineral, (g–h) plasmid DNA-lipoplex adsorbed to mineralized films, (i–j) plasmid DNA-lipoplex coprecipitated with mineral. Distribution of both the plasmid DNA and the lipid transfection agent on the bone-like mineral was demonstrated by the colocalization of the fluorescent staining in the adsorption and coprecipitation groups and the absence of staining in the mineralized controls. Scale bars represent 100 um. (Reprinted from Luong et al., *Biomaterials,* 30(36), 6996–7004, 2009, with permission from Elsevier.)

The method of DNA application affects transfection efficiency. For example, adsorbed lipoplexes and coprecipitated lipoplexes show significantly different transfection efficiencies. Encapsulation of DNA in a calcium phosphate precipitate improves cellular uptake and produces an enhanced cellular response compared to lipoplexing techniques (Jordan 1996). DNA-lipoplexes coprecipitated with BLM show higher transfection efficiency compared to adsorbed lipoplexes, and coprecipitated naked DNA. This improved transfection efficiency arises from enhanced cellular uptake and protection from degradation as a result of cationic lipid complexation, along with the higher availability of apatite at the surface controlling the rate of release (Luong, McFalls, and Kohn 2009).

Transfection efficiency can also be improved by altering the ionic concentrations of SBF. For instance, removing Mg ions from the solution improves DNA incorporation and

facilitates efficient endocytosis (Shen, Tan, and Saltzman 2004). Surface morphology and DNA retention at the mineral surface play an important role in improving transfection efficiency. SBF concentrations and coprecipitation time can be altered to control the dissolution rate of the mineral layer and subsequent release of DNA to improve transfection efficiency (Luong, McFalls, and Kohn 2009).

Drawbacks of Using BLM

Although there is merit to using BLM coatings, there are also difficulties that may be encountered that need to be acknowledged. Challenges that arise when using SBF to coat implants, especially porous and porous-coated implants and scaffolds, include controlling coating thickness, preserving substrate stability during functionalization, and translation to industrial-scale processes. As with all apatite coating techniques, the thickness of the BLM layer on 3-D scaffolds needs to be controlled to maintain sufficient porosity for mass transport and angiogenesis. An implant coating that is too thick can occlude pores, which would interfere with tissue perfusion and vascular infiltration in vivo. Excessively thick mineral layers can result in delamination of the coating from the underlying substrate.

Substrate surface functionalization is carried out before incubation in SBF to obtain a negatively charged surface for calcium nucleation. However, prolonged treatment for functionalization can damage the underlying substrate. For instance, PLGA/PLLA materials etched with sodium hydroxide can undergo considerable hydrolysis resulting in loss of structure, thereby compromising mechanical stability. The necessity for functionalization creates some limitations over the types of substrates that can be used for biomimetic precipitation (Table 1.2) as well as the types of methods that could be used to functionalize the surface.

Achieving industrial-scale batch processing could be a problem when working with SBF. The SBF solution needs to be replenished periodically to maintain pH and ion concentrations near saturation, which would be complex in an industrial setting. Batch processing implants in large volumes of liquid under sterile conditions to prevent contamination can also prove to be difficult.

One main disadvantage of using coprecipitation to create organic/inorganic hybrid materials is the low efficiency of biomolecule incorporation. Although biomolecule retention on BLM is higher with coprecipitation than with adsorption, only about 10% loading can be achieved with coprecipitation. Therefore coprecipitation requires large concentrations of biomolecules to elicit a desired response, which becomes expensive for growth factor administration.

Conclusions

Bone is a complex and dynamic composite tissue that consists of both inorganic and organic phases, supporting cellular adhesion, proliferation, and differentiation. The technique of biomimetic calcium phosphate precipitation attempts to simulate aspects of this complexity by forming a BLM coating on the surface of natural and synthetic substrates. This mineral layer makes a biomaterial more osteoconductive, as well as enhances mechanical strength

and stiffness, which are important requirements for load-bearing implants. Inductivity can be integrated into this conductive approach by the incorporation of biomolecules such as proteins, peptides, and DNA to generate inorganic/organic hybrids that are capable of facilitating and enhancing cell–matrix interactions.

These hybrids can be synthesized using adsorption or coprecipitation techniques, or a combination of both depending on the type of response desired. Protein engineering can be utilized to recruit bone cell populations initially to implant surfaces, by designing peptides that bind specifically with strong affinity to both BLM materials and cells. These peptides mimic naturally found bone ECM adhesive proteins, such as osteopontin and bone sialoprotein, and mediate cell adhesion to apatite. Coprecipitating mineral and biomolecules can provide the signaling cues required for cell proliferation and differentiation, leading to new bone formation. Coprecipitation also provides control over spatial and temporal release of the biomolecules, allowing for multiple growth factor delivery during different stages of cellular differentiation, a concept similar to growth factor sequestration by the ECM in vivo. A blend of both adsorption and coprecipitation, in conjunction with biomimetically precipitated apatite, can be utilized to develop bone analogs that mimic the natural environment with greater precision, thereby ensuring controlled and uniform tissue regeneration.

References

Abe, Y., Kokubo, T., and Yamamuro, T. 1990, Apatite Coating on Ceramics, Metals and Polymers Utilizing a Biological Process, *Journal of Materials Science: Materials in Medicine*, Vol. 1, pp. 233–238.

Agata De Sena, L., Calixto De Andrade, M., Malta Rossi, A., and de Almeida Soares, G. 2002, Hydroxyapatite Deposition by Electrophoresis on Titanium Sheets with Different Surface Finishing, *Journal of Biomedical Materials Research,* Vol. 60, No. 1, pp. 1–7.

Albrektsson, T., and Johansson, C. 2001, Osteoinduction, Osteoconduction and Osseointegration, *European Spine Journal: Official Publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, Vol. 10, Suppl. 2, pp. S96–S101.

Allori, A.C., Sailon, A.M., and Warren, S.M. 2008, Biological Basis of Bone Formation, Remodeling, and Repair—Part I: Biochemical Signaling Molecules, *Tissue Engineering Part B, Reviews,* Vol. 14, No. 3, pp. 259–273.

Alsberg, E., Hill, E.E., and Mooney, D.J. 2001, Craniofacial Tissue Engineering, *Critical Reviews in Oral Biology & Medicine,* Vol. 12, No. 1, pp. 64–75.

Antonov, E.N., Bagratashvilli, V.N., Popov, V.K., Sobol, E.N., Howdle, S.M., Joiner, C., Parker, K.G., Parker, T.L., Doktorov, A.A., Likhanov, V.B., Volozhin, A.I., Alimpiev, S.S., and Nikiforov, S.S. 1998, Biocompatibility of Laser-Deposited Hydroxylapatite Coatings on Titanium and Polymer Implant Materials, *Journal of Biomedical Optics,* Vol. 3, No. 4, pp. 423–428.

Araujo, J.V., Martins, A., Leonor, I.B., Pinho, E.D., Reis, R.L., and Neves, N.M. 2008, Surface controlled Biomimetic Coating of Polycaprolactone Nanofiber Meshes to Be Used as Bone Extracellular Matrix Analogues, *Journal of Biomaterials Science—Polymer Edition,* Vol. 19, No. 10, pp. 1261–1278.

Areva, S., Peltola, T., Sailynoja, E., Laajalehto, K., Linden, M., and Rosenholm, J.B. 2002, Effect of Albumin and Fibrinogen on Calcium Phosphate Formation on Sol–Gel Derived Titania Coatings in Vitro, *Chemistry of Materials,* Vol. 14, pp. 1614–1621.

Baeri, P., Torrisi, L., Marino, N., and Foti, G. 1992, Ablation of Hydroxyapatite by Pulsed Laser Irradiation, *Applied Surface Science,* Vol. 54, pp. 210–214.

Baron, R. 2003, General Principles of Bone Biology, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,* American Society for Bone and Mineral Research, Washington D.C., pp. 1–8.

Barrere, F., van der Valk, C.M., Dalmeijer, R.A., van Blitterswijk, C.A., de Groot, K., and Layrolle, P. 2003a, In Vitro and in Vivo Degradation of Biomimetic Octacalcium Phosphate and Carbonate Apatite Coatings on Titanium Implants, *Journal of Biomedical Materials Research. Part A,* Vol. 64, No. 2, pp. 378–387.

Barrere, F., van der Valk, C.M., Meijer, G., Dalmeijer, R.A., de Groot, K., and Layrolle, P. 2003b, Osteointegration of Biomimetic Apatite Coating Applied onto Dense and Porous Metal Implants in Femurs of Goats, *Journal of Biomedical Materials Research. Part B, Applied Biomaterials,* Vol. 67, No. 1, pp. 655–665.

Benoit, D.S.W., and Anseth, K.S. 2005, The Effect on Osteoblast Function of Colocalized RGD and PHSRN Epitopes on PEG Surfaces, *Biomaterials,* Vol. 26, pp. 5209–5220.

Bernhardt, R., van den Dolder, J., Bierbaum, S., Beutner, R., Scharnweber, D., Jansen, J., Beckmann, F., and Worch, H. 2005, Osteoconductive Modifications of Ti-Implants in a Goat Defect Model: Characterization of Bone Growth with SR μCT and Histology, *Biomaterials,* Vol. 26, No. 16, pp. 3009–3019.

Biondi, M., Ungaro, F., Quaglia, F., and Netti, P.A. 2008, Controlled Drug Delivery in Tissue Engineering, *Advanced Drug Delivery Reviews,* Vol. 60, No. 2, pp. 229–242.

Bollen, C.M., Lambrechts, P., and Quirynen, M. 1997, Comparison of Surface Roughness of Oral Hard Materials to the Threshold Surface Roughness for Bacterial Plaque Retention: A Review of the Literature, *Dental Materials: Official Publication of the Academy of Dental Materials,* Vol. 13, No. 4, pp. 258–269.

Bunker, B.C., Rieke, P.C., Tarasevich, B.J., Campbell, A.A., Fryxell, G.E., Graff, G.L., Song, L., Liu, J., Virden, J.W., and McVay, G.L. 1994, Ceramic Thin-Film Formation on Functionalized Interfaces Through Biomimetic Processing, *Science,* Vol. 264, pp. 48–55.

Campbell, A.A., Song, L., Li, X.S., Nelson, B.J., Bottoni, C., Brooks, D.E., and DeJong, E.S. 2000, Development, Characterization, and Anti-Microbial Efficacy of Hydroxyapatite-Chlorhexidine Coatings Produced by Surface-Induced Mineralization, *Journal of Biomedical Materials Research,* Vol. 53, No. 4, pp. 400–407.

Chai, C.S., Gross, K.A., and Ben-Nissan, B. 1998, Critical Ageing of Hydroxyapatite Sol–Gel Solutions, *Biomaterials,* Vol. 19, No. 24, pp. 2291–2296.

Chen, J., Wolke, J.G.C., and de Groot, K. 1994, Microstructure and Crystallinity in Hydroxyapatite Coatings, *Biomaterials,* Vol. 15, No. 5, pp. 396–399.

Chen, Y., Mak, A.F.T., Wang, M., Li, J.S., and Wong, M.S. 2008, In Vitro Behavior of Osteoblast-Like Cells on PLLA Films with a Biomimetic Apatite or Apatite/Collagen Composite Coating, *Journal of Materials Science: Materials in Medicine,* Vol. 19, pp. 2261–2268.

Choi, J., Bogdanski, D., Köller, M., Esenwein, S.A., Müller, D., Muhr, G., and Epple, M. 2003, Calcium Phosphate Coating of Nickel–Titanium Shape-Memory Alloys. Coating Procedure and Adherence of Leukocytes and Platelets, *Biomaterials,* Vol. 24, No. 21, pp. 3689–3696.

Chou, L., Marek, B., and Wagner, W.R. 1999, Effects of Hydroxylapatite Coating Crystallinity on Biosolubility, Cell Attachment Efficiency and Proliferation In Vitro, *Biomaterials,* Vol. 20, No. 10, pp. 977–985.

Clèries, L., Martínez, E., Fernández-Pradas, J.M., Sardin, G., Esteve, J., and Morenza, J.L. 2000, Mechanical Properties of Calcium Phosphate Coatings Deposited by Laser Ablation, *Biomaterials,* Vol. 21, No. 9, pp. 967–971.

Combes, C., and Rey, C. 2002, Adsorption of Proteins and Calcium Phosphate Materials Bioactivity, *Biomaterials,* Vol. 23, pp. 2817–2823.

Combes, C., Rey, C., and Freche, M. 1999, *In Vitro* Crystallization of Octacalcium Phosphate on Type I Collagen: Influence of Serum Albumin, *Journal of Materials Science: Materials in Medicine,* Vol. 10, pp. 153–160.

Dalby, M.J., Gadegaard, N., Tare, R., Andar, A., Riehle, M.O., Herzyk, P., Wilkinson, C.D., and Oreffo, R.O. 2007, The Control of Human Mesenchymal Cell Differentiation Using Nanoscale Symmetry and Disorder, *Nature Materials,* Vol. 6, pp. 997–1003.

Dalby, M.J., Riehle, M.O., Sutherland, D.S., Agheli, H., and Curtis, A.S.G. 2004, Use of Nanotopography to Study Mechanotransduction in Fibroblasts—Methods and Perspectives, *European Journal of Cell Biology,* Vol. 83, No. 4, pp. 159–169.

De Groot, K., Geesink, R., Klein, C.P.A.T., and Serekian, P. 1987, Plasma Sprayed Coatings of Hydroxylapatite, *Journal of Biomedical Materials Research,* Vol. 21, pp. 1375–1381.

Dee, K.C., Andersen, T.T., and Bizios, R. 1998, Design and Function of Novel Osteoblast-Adhesive Peptides for Chemical Modification of Biomaterials, *Journal of Biomedical Materials Research,* Vol. 40, pp. 371–377.

Deligianni, D.D., Katsala, N.D., Koutsoukos, P.G., and Missirlis, Y.F. 2001, Effect of Surface Roughness of Hydroxyapatite on Human Bone Marrow Cell Adhesion, Proliferation, Differentiation and Detachment Strength, *Biomaterials,* Vol. 22, No. 1, pp. 87–96.

Ding, S.J., Ju, C.P., and Lin, J.H. 1999, Characterization of Hydroxyapatite and Titanium Coatings Sputtered on Ti-6Al-4V Substrate, *Journal of Biomedical Materials Research,* Vol. 44, No. 3, pp. 266–279.

Ding, S. 2003, Properties and Immersion Behavior of Magnetron-Sputtered Multi-Layered Hydroxyapatite/Titanium Composite Coatings, *Biomaterials,* Vol. 24, No. 23, pp. 4233–4238.

Discher, D.E., Mooney, D.J., and Zandstra, P.W. 2009, Growth Factors, Matrices, and Forces Combine and Control Stem Cells, *Science,* Vol. 324, No. 5935, pp. 1673–1677.

Ducheyne, P., Radin, S., Heughebaert, M., and Heughebaert, J.C. 1990, Calcium phosphate Ceramic Coatings on Porous Titanium: Effect of Structure and Composition on Electrophoretic Deposition, Vacuum Sintering and *In Vitro* Dissolution, *Biomaterials,* Vol. 11, pp. 244–254.

Ducheyne, P. 1985, Bioglass Coatings and Bioglass Composites as Implant Materials, *Journal of Biomedical Materials Research,* Vol. 19, pp. 273–291.

Ducheyne, P., Van Raemdonck, W., Heughebaert, J.C., and Heughebaert, M. 1986, Structural Analysis of Hydroxyapatite Coatings on Titanium, *Biomaterials,* Vol. 7, No. 2, pp. 97–103.

El-Ghannam, A., Ducheyne, P., and Shapiro, I.M. 1999, Effect of Serum Proteins on Osteoblast Adhesion to Surface-Modified Bioactive Glass and Hydroxyapatite, *Journal of Orthopaedic Research,* Vol. 17, pp. 340–345.

Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. 2006, Matrix Elasticity Directs Stem Cell Lineage Specification, *Cell,* Vol. 126, No. 4, pp. 677–689.

Feddes, B., Vredenburg, A.M., Wolke, J.G.C., and Jansen, J.A. 2004, Bulk Composition of RF Magnetron Sputter Deposited Calcium Phosphate Coatings on Different Substrates (Polyethylene, Polytetrafluoroethylene, Silicon), *Surface Coatings and Technology,* Vol. 185, pp. 346–355.

Fernández-Pradas, J.M., Clèries, L., Martínez, E., Sardin, G., Esteve, J., and Morenza, J.L. 2001, Influence of Thickness on the Properties of Hydroxyapatite Coatings Deposited by KrF LASER Ablation, *Biomaterials,* Vol. 22, No. 15, pp. 2171–2175.

Frayssinet, P., Tourenne, F., Rouquet, N., Conte, P., Delga, C., and Bonel, G. 1994, Comparative Biological Properties of HA Plasma-Sprayed Coatings Having Different Crystallinities, *Journal of Materials Science: Materials in Medicine,* Vol. 5, No. 1, pp. 11.

Fujibayashi, S., Neo, M., Kim, H., Kokubo, T., and Nakamura, T. 2004, Osteoinduction of Porous Bioactive Titanium Metal, *Biomaterials,* Vol. 25, No. 3, pp. 443–450.

Fujisawa, R., Mizuno, M., Nodasaka, Y., and Kuboki, Y. 1997, Attachment of Osteoblastic Cells to Hydroxyapatite by a Synthetic Peptide (Glu7-Pro-Arg-Gly-Asp-Thr) Containing Two Functional Sequences of Bone Sialoprotein, *Matrix Biology,* Vol. 16, pp. 21–28.

Fujisawa, R., Wada, Y., Nodasak, Y., and Kuboki, Y. 1996, Acidic Amino Acid-Rich Sequences as Binding Sites of Osteonectin to Hydroxyapatite Crystals, *Biochimica et Biophysica Acta,* Vol. 1292, pp. 53–60.

Girija, E.K., Yokogawa, Y., and Nagata, F. 2002, Bone-Like Apatite Formation on Collagen Fibrils by Biomimetic Method, *Chemical Letters*, pp. 702–703.

Griffin, M.A., Sen, S., Sweeney, H.L., and Discher, D.E. 2004, Adhesion-Contractile Balance in Myocyte Differentiation, *Journal of Cell Science,* Vol. 117, No. 24, pp. 5855–5863.

Gross, K.A., and Berndt, C.C. 1998, Thermal Processing of Hydroxyapatite for Coating Production, *Journal of Biomedical Materials Research,* Vol. 39, No. 4, pp. 580–587.

Gross, K.A., Berndt, C.C., and Herman, H. 1998, Amorphous Phase Formation in Plasma-Sprayed Hydroxyapatite Coatings, *Journal of Biomedical Materials Research,* Vol. 39, No. 3, pp. 407–414.

Habibovic, P., Barrère, F., Blitterswijk, C.A., Groot, K., and Layrolle, P. 2002, Biomimetic Hydroxyapatite Coating on Metal Implants, *Journal of the American Ceramic Society,* Vol. 85, No. 3, pp. 517–522.

Habibovic, P., Li, J., van der Valk, C.M., Meijer, G., Layrolle, P., van Blitterswijk, C.A., and de Groot, K. 2005, Biological Performance of Uncoated and Octacalcium Phosphate-Coated Ti_6Al_4V, *Biomaterials,* Vol. 26, No. 1, pp. 23–36.

Han, Y., Fu, T., Lu, J., and Xu, K. 2001, Characterization and Stability of Hydroxyapatite Coatings Prepared by an Electrodeposition and Alkaline-Treatment Process, *Journal of Biomedical Materials Research,* Vol. 54, No. 1, pp. 96–101.

Han, Y., Xu, K., Lu, J., and Wu, Z. 1999, The Structural Characteristics and Mechanical Behaviors of Nonstoichiometric Apatite Coatings Sintered in Air Atmosphere, *Journal of Biomedical Materials Research,* Vol. 45, No. 3, pp. 198–203.

Hench, L.L. 1991, Bioceramics: From Concept to Clinic, *Journal of the American Ceramic Society,* Vol. 74, No. 7, pp. 1487–1510.

Herø, H., Wie, H., Jørgensen, R.B., and Ruyter, I.E. 1994, Hydroxyapatite Coatings on Ti Produced by Hot Isostatic Pressing, *Journal of Biomedical Materials Research,* Vol. 28, No. 3, pp. 343–348.

Hersel, U., Dahmen, C., and Kessler, H. 2003, RGD Modified Polymers: Biomaterials for Stimulated Cell Adhesion and Beyond, *Biomaterials,* Vol. 24, pp. 4385–4415.

Hirano, Y., and Mooney, D. 2004, Peptide and Protein Presenting Materials for Tissue Engineering, *Advanced Materials,* Vol. 16, No. 1, pp. 17–25.

Honstu, S., Matsumotu, T., Ishii, J., Nakamori, M., Tabata, H., and Kawai, T. 1997, Electrical Properties of Hydroxyapatite Thin Films Grown by Pulsed Laser Deposition, *Thin Solid Films,* Vol. 295, pp. 214–217.

Horbett, T.A. 2004, The Role of Adsorbed Proteins in Tissue Response to Biomaterials, in *Biomaterials Science: An Introduction to Materials in Medicine*, B.D. Ratner, A.S. Hoffman, F.J. Schoen, and J.E. Lemons (eds.), 2nd Edition, Elsevier, San Diego, pp. 237–246.

Hunter, G.K., Kyle, L.C., and Goldberg, H.A. 1994, Modulation of Crystal Formation by Bone Phosphoproteins: Structural Specificity of the Osteopontin-Mediated Inhibition of Hydroxyapatite Formation, *Biochemical Journal,* Vol. 300, pp. 723–728.

Hunter, G.K., and Goldberg, H.A. 1994, Modulation of Crystal Formation by Bone Phosphoproteins: Role of Glutamic Acid-Rich Sequences in the Nucleation of Hydroxyapatite by Bone Sialoprotein, *Biochemical Journal,* Vol. 302, pp. 175–179.

Ingber, D.E. 2006, Cellular Mechanotransduction: Putting All the Pieces Together Again, *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology,* Vol. 20, No. 7, pp. 811–827.

Ingber, D.E. 1997, Tensegrity: The Architectural Basis of Cellular Mechanotransduction, *Annual Review of Physiology,* Vol. 59, No. 1, pp. 575–599.

Itoh, D., Yoneda, S., Kuroda, S., Kondo, H., Umezawa, A., Ohya, K., Ohyama, T., and Kasugai, S. 2002, Enhancement of Osteogenesis on Hydroxyapatite Surface Coated with Synthetic Peptide (EEEEEEEPRGDT), *In Vitro*, *Journal of Biomedical Materials Research,* Vol. 62, pp. 292–298.

Jacobs, J.J., Gilbert, J.L., and Urban, R.M. 1998, Corrosion of Metal Orthopaedic Implants, *The Journal of Bone and Joint Surgery. American Volume,* Vol. 80, No. 2, pp. 268–282.

Jang, J.H., and Shea, L.D. 2003, Controllable Delivery of Non-Viral DNA from Porous Scaffolds, *Journal of Controlled Release: Official Journal of the Controlled Release Society,* Vol. 86, No. 1, pp. 157–168.

Jayasuriya, A.C., and Shah, C. 2008, Controlled Release of Insulin-Like Growth Factor-1 and Bone Marrow Stromal Cell Function of Bone-Like Mineral Layer-Coated Poly(Lactic-*co*-Glycolic Acid) Scaffolds, *Journal of Tissue Engineering and Regenerative Medicine,* Vol. 2, No. 1, pp. 43–49.

Jiang, G., and Shi, D. 1998, Coating of Hydroxyapatite on Highly Porous Al_2O_3 Substrate for Bone Substitutes, *Journal of Biomedical Materials Research,* Vol. 43, No. 1, pp. 77–81.

Jordan, M. 1996, Transfecting Mammalian Cells: Optimization of Critical Parameters Affecting Calcium–Phosphate Precipitate Formation, *Nucleic Acids Research,* Vol. 24, No. 4, pp. 596–601.

Kaneko, A., Hirai, S., Tamada, Y., and Kuzuya, T. 2009, Evaluation of Calcium Phosphate-Coated Silk Fabric Produced by Sol–Gel Processing as a Wound Cover Material, *Sen'i Gakkaishi,* Vol. 65, No. 3, pp. 97–102.

Kilpadi, D.V., and Lemons, J.E. 1994, Surface Energy Characterization of Unalloyed Titanium Implants, *Journal of Biomedical Materials Research,* Vol. 28, No. 12, pp. 1419–1425.

Klein, Christel P. A. T., Patka, P., van der Lubbe, H. B. M., Wolke, J.G.C., and de Groot, K. 1991, Plasma-Sprayed Coatings of Tetracalcium Phosphate, Hydroxyl-Apatite and Alpha-TCP on Titanium Alloy: An Interface Study, *Journal of Biomedical Materials Research,* Vol. 25, No. 53, p. 65.

Kloxin, A.M., Benton, J.A., and Anseth, K.S. 2010, In Situ Elasticity Modulation with Dynamic Substrates to Direct Cell Phenotype, *Biomaterials,* Vol. 31, No. 1, pp. 1–8.

Kocgozlu, L., Lavalle, P., Koenig, G., Senger, B., Haikel, Y., Schaaf, P., Voegel, J.C., Tenenbaum, H., and Vautier, D. 2010, Selective and Uncoupled Role of Substrate Elasticity in the Regulation of Replication and Transcription in Epithelial Cells, *Journal of Cell Science,* Vol. 123, No. Pt 1, pp. 29–39.

Kofron, M.D., and Laurencin, C.T. 2004, Development of a Calcium Phosphate Co-Precipitate/Poly(Lactide-*co*-Glycolide) DNA Delivery System: Release Kinetics and Cellular Transfection Studies, *Biomaterials,* Vol. 25, No. 13, pp. 2637–2643.

Kohn, D.H., Shin, K., Hong, S., Jayasuriya, C.A., Leonova, E.V., Rossello, R.A., and Krebsbach, P.H. 2005, Self-Assembled Mineral Scaffolds as Model Systems for Biomineralization and Tissue Engineering, W.J. Landis, and J. Sodek (eds.), University of Toronto Press, Toronto.

Kokubo, T. 1991, Bioactive Glass Ceramics: Properties and Applications, *Biomaterials,* Vol. 12, No. 2, pp. 155–163.

Kokubo, T. 1996, Formation of Biologically Active Bone-Like Apatite on Metals and Polymers by a Biomimetic Process, *Thermochimica Acta,* Vol. 280/281, pp. 479–490.

Kokubo, T., and Takadama, H. 2006, How Useful Is SBF in Predicting In Vivo Bone Bioactivity?, *Biomaterials,* Vol. 27, No. 15, pp. 2907–2915.

Kong, H.J., Liu, J., Riddle, K., Matsumoto, T., Leach, K. and Mooney, D.J. 2005, Non-Viral Gene Delivery Regulated by Stiffness of Cell Adhesion Substrates, *Nature Materials*, Vol. 4, No. 6, pp. 460–464.

Krout, A., Wen, H.B., Hippensteel, E., and Li, P. 2005, A Hybrid Coating of Biomimetic Apatite and Osteocalcin, *Journal of Biomedical Materials Research A,* Vol. 73A, pp. 377–387.

Lacefield, W.R. 1999, Biomechanical and Morphometric Analysis of Hydroxyapatite-Coated Implants with Varying Crystallinity, *Journal of Oral and Maxillofacial Surgery,* Vol. 57, No. 9, pp. 1108–1109.

Le Guehennec, L., Soueidan, A., Layrolle, P., and Amouriq, Y. 2007, Surface Treatments of Titanium Dental Implants for Rapid Osseointegration, *Dental Materials: Official Publication of the Academy of Dental Materials,* Vol. 23, No. 7, pp. 844–854.

Lebaron, R.G., and Athanasiou, K.A. 2000, Extracellular Matrix Cell Adhesion Peptides: Functional Applications in Orthopedic Materials, *Tissue Engineering,* Vol. 6, No. 2, pp. 85–103.

Lee, J.S., Lee, J.S., and Murphy, W.L. 2009, Modular Peptides Promote Human Mesenchymal Stem Cell Differentiation on Biomaterial Surfaces, *Acta Biomaterialia*, Vol. 6, No. 1, pp. 21–28.

Lee, M., Li, W., Siu, R.K., Whang, J., Zhang, X., Soo, C., Ting, K., and Wu, B.M. 2009, Biomimetic Apatite-Coated Alginate/Chitosan Microspheres as Osteogenic Protein Carriers, *Biomaterials,* Vol. 30, pp. 6094–6101.

LeGeros, R.Z. 2002, Properties of Osteoconductive Biomaterials: Calcium Phosphates, *Clinical Orthopaedics and Related Research,* Vol. 395, pp. 81–98.

Leonor, I.B., Azevedo, H.S., Alves, C.M., and Reis, R.L. 2003, Effects of the Incorporation of Proteins and Active Enzymes on Biomimetic Calcium–Phosphate Coatings, *Key Engineering Materials,* Vol. 240–242, pp. 97–100.

Leonor, I.B., Baran, E.T., Kawashita, M., Reis, R.L., Kokubo, T., and Nakamura, T. 2008, Growth of a Bonelike Apatite on Chitosan Microparticles after a Calcium Silicate Treatment, *Acta Biomaterialia,* Vol. 4, No. 5, pp. 1349–1359.

Leonor, I.B., Kim, H.M., Balas, F., Kawashita, M., Reis, R.L., Kokubo, T., and Nakamura, T. 2007, Alkaline Treatments to Render Starch-Based Biodegradable Polymers Self-Mineralizable, *Journal of Tissue Engineering and Regenerative Medicine,* Vol. 1, No. 425, p. 435.

Leonova, E.V., Pennington, K.E., Krebsbach, P.H., and Kohn, D.H. 2006, Substrate Mineralization Stimulates Focal Adhesion Contact Redistribution and Cell Motility of Bone Marrow Stromal Cells, *Journal of Biomedical Materials Research A,* Vol. 79A, pp. 263–270.

Li, P., De Groot, K., and Kokubo, T. 1996, Bioactive $Ca_{10}(PO_4)_6(OH)_2$–TiO_2 Composite Coating Prepared by Sol–Gel Process, *Journal of Sol–Gel Science and Technology,* Vol. 7, No. 1, pp. 27–34.

Li, H., Khor, K.A., and Cheang, P. 2002, Titanium Dioxide Reinforced Hydroxyapatite Coatings Deposited by High Velocity Oxy-Fuel (HVOF) Spray, *Biomaterials,* Vol. 23, No. 1, pp. 85–91.

Li, J., Liao, H., and Hermansson, L. 1996, Sintering of Partially-Stabilized Zirconia and Partially-Stabilized Zirconia—Hydroxyapatite Composites by Hot Isostatic Pressing and Pressureless Sintering, *Biomaterials,* Vol. 17, No. 18, pp. 1787–1790.

Li, P., Ohtsuki, C., Kokubo, T., Nakanishi, K., Soga, N., Nakamura, T., and Yamamuro, T. 1992, Apatite Formation Induced by Silica Gel in a Simulated Body Fluid, *Journal of the American Ceramic Society,* Vol. 75, No. 8, pp. 2094–2097.

Lian, J.B., Stein, G.S., and Aubin, J.E. 2003, Bone Formation: Maturation and Functional Activities of Osteoblast Lineage Cells, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,* American Society for Bone and Mineral Research, pp. 13–28.

Linhart, W., Peters, F., Lehmann, W., Schwarz, K., Schilling, F.A., Amling, M., Rueger, J.M., and Epple, M. 2001, Biologically and Chemically Optimized Composites of Carbonated Apatite and Polyglycolide as Bone Substitution Materials, *Journal of Biomedical Materials Research,* Vol. 54, No. 2, pp. 166–171.

Liu, Y., and Hunziker, E.B. 2009, Biomimetic Coating and their Biological Functionalization, in *Thin Calcium Phosphate Coatings for Medical Implants*, eds. B. Leon and J. Jansen, 1st Edition, Springer, New York, NY, pp. 301–314.

Liu, D., Yang, Q., and Troczynski, T. 2002, Sol–Gel Hydroxyapatite Coatings on Stainless Steel Substrates, *Biomaterials,* Vol. 23, No. 3, pp. 691–698.

Liu, X., Ding, C., and Chu, P.K. 2004, Mechanism of Apatite Formation on Wollastonite Coatings in Simulated Body Fluids, *Biomaterials,* Vol. 25, No. 10, pp. 1755–1761.

Liu, Y., de Groot, K., and Hunziker, E.B. 2005, BMP-2 Liberated from Biomimetic Implant Coatings Induces and Sustains Direct Ossification in an Ectopic Rat Model, *Bone,* Vol. 36, No. 5, pp. 745–757.

Liu, Y., de Groot, K., and Hunziker, E.B. 2004, Osteoinductive Implants: The Mise-en-Scene for Drug-Bearing Biomimetic Coatings, *Annals of Biomedical Engineering,* Vol. 32, No. 3, pp. 398–406.

Liu, Y., Hunziker, E.B., Randall, N.X., de Groot, K., and Layrolle, P. 2003, Proteins Incorporated into Biomimetically Prepared Calcium Phosphate Coatings Modulate Their Mechanical Strength and Dissolution Rates, *Biomaterials,* Vol. 24, pp. 65–70.

Liu, Y., Layrolle, P., de Bruijn, J., van Blitterswijk, C., and de Groot, K. 2001, Biomimetic Coprecipitation of Calcium Phosphate and Bovine Serum Albumin on Titanium Alloy, *Journal of Biomedical Materials Research,* Vol. 57, pp. 327–335.

Lo, C., Wang, H., Dembo, M., and Wang, Y. 2000, Cell Movement Is Guided by the Rigidity of the Substrate, *Biophysical Journal,* Vol. 79, No. 1, pp. 144–152.

Luong, L.N., Hong, S.I., Patel, R.J., Outslay, M.E., and Kohn, D.H. 2006, Spatial Control of Protein within Biomimetically Nucleated Mineral, *Biomaterials,* Vol. 27, No. 7, pp. 1175–1186.

Luong, L.N., McFalls, K.M., and Kohn, D.H. 2009, Gene Delivery via DNA Incorporation within a Biomimetic Apatite Coating, *Biomaterials,* Vol. 30, No. 36, pp. 6996–7004.

Ma, J., Wang, C., and Peng, K.W. 2003, Electrophoretic Deposition of Porous Hydroxyapatite Scaffold, *Biomaterials,* Vol. 24, No. 20, pp. 3505–3510.

Manso, M., Langlet, M., Jiménez, C., and Martínez-Duart, J.M. 2002, Microstructural Study of Aerosol–Gel Derived Hydroxyapatite Coatings, *Biomolecular Engineering,* Vol. 19, No. 2–6, pp. 63–66.

Manso, M., Jiménez, C., Morant, C., Herrero, P., and Martínez-Duart, J. 2000, Electrodeposition of Hydroxyapatite Coatings in Basic Conditions, *Biomaterials,* Vol. 21, No. 17, pp. 1755–1761.

Marchisio, M., Di Carmine, M., Pagone, R., Piattelli, A., and Miscia, S. 2005, Implant Surface Roughness Influences Osteoclast Proliferation and Differentiation, *Journal of Biomedical Materials Research. Part B, Applied Biomaterials,* Vol. 75, No. 2, pp. 251–256.

Martin, T.J. 1989, Bone Cell Physiology, *Endocrinology and Metabolism Clinics of North America,* Vol. 18, No. 4, pp. 833–858.

Martinez, E., Engel, E., Planell, J.A., and Samitier, J. 2009, Effects of Artificial Micro- and Nano-Structured Surfaces on Cell Behaviour, *Annals of Anatomy—Anatomischer Anzeiger: Official Organ of the Anatomische Gesellschaft,* Vol. 191, No. 1, pp. 126–135.

Martins, A.M., Pham, Q.P., Malafaya, P.B., Raphael, R.M., Kasper, K.F., Reis, R.L., and Mikos, A.G. 2009, Natural Stimulus Responsive Scaffolds/Cells for Bone Tissue Engineering: Influence of Lysozyme upon Scaffold Degradation and Osteogenic Differentiation of Cultured Marrow Stromal Cells Induced by CaP Coatings, *Tissue Engineering: Part A,* Vol. 15, No. 00, pp. 1–11.

Massaro, C., Baker, M.A., Cosentino, F., Ramires, P.A., Klose, S., and Milella, E. 2001, Surface and Biological Evaluation of Hydroxyapatite-Based Coatings on Titanium Deposited by Different Techniques, *Journal of Biomedical Materials Research,* Vol. 58, No. 6, pp. 651–657.

Misra, S.K., Valappil, S.P., Roy, I., and Boccaccini, A.R. 2006, Polyhydroxyalkanoate (PHA)/Inorganic Phase Composites for Tissue Engineering Applications, *Biomacromolecules,* Vol. 7, No. 8, pp. 2249–2258.

Mitragotri, S., and Lahann, J. 2009, Physical Approaches to Biomaterial Design, *Nature Materials,* Vol. 8, No. 1, pp. 15–23.

Montenero, A., Gnappi, G., Ferrari, F., Cesari, M., Salvioli, E., Mattogno, L., Kaciulis, S., and Fini, M. 2000, Sol–Gel Derived Hydroxyapatite Coatings on Titanium Substrate, *Journal of Materials Science,* Vol. 35, No. 11, pp. 2791–2797.

Müller, L., Conforto, E., Caillard, D., and Müller, F.A. 2007, Biomimetic Apatite Coatings—Carbonate Substitution and Preferred Growth Orientation, *Biomolecular Engineering,* Vol. 24, No. 5, pp. 462–466.

Mundy, G.R., Chin, D., and Oyajobi, B. 2003, Bone Remodeling, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,* American Society for Bone and Mineral Research, Washington D.C., pp. 46–58.

Murphy, W.L., Hsiong, S., Richardson, T.P., Simmons, C.A., and Mooney, D.J. 2005, Effects of a Bone-Like Mineral Film on Phenotype of Adult Human Mesenchymal Stem Cells In Vitro, *Biomaterials,* Vol. 26, No. 3, pp. 303–310.

Murphy, W.L., Kohn, D.H., and Mooney, D.J. 2000a, Growth of Continuous Bonelike Mineral within Porous Poly(Lactide-*co*-Glycolide) Scaffolds *In Vitro*, *Journal of Biomedical Materials Research,* Vol. 50, pp. 50–58.

Murphy, W.L., Peters, M.C., Kohn, D.H., and Mooney, D.J. 2000b, Sustained Release of Vascular Endothelial Growth Factor from Mineralized Poly(Lactide-*co*-Glycolide) Scaffolds for Tissue Engineering, *Biomaterials,* Vol. 21, No. 24, pp. 2521–2527.

Nagano, M., Kitsugi, T., Nakamura, T., Kokubo, T., and Tanahashi, M. 1996, Bone Bonding Ability of an Apatite-Coated Polymer Produced Using a Biomimetic Method: A Mechanical and Histological Study, *In Vivo*, *Journal of Biomedical Materials Research,* Vol. 31, pp. 487–291.

Nakamura, T., Yamamuro, T., Higashi, S., Kokubo, T., and Itoo, S. 1985, A New Glass-Ceramic for Bone Replacement: Evaluation of Its Bonding to Bone Tissue, *Journal of Biomedical Materials Research,* Vol. 19, pp. 685–698.

Neuman, W.F., and Neuman, M.W. 1957, Emerging Concepts of the Structure and Metabolic Functions of Bone, *The American Journal of Medicine,* Vol. 22, No. 1, pp. 123–131.

Nie, X., Leyland, A., Matthews, A., Jiang, J.C., and Meletis, E.I. 2001, Effects of Solution pH and Electrical Parameters on Hydroxyapatite Coatings Deposited by a Plasma-Assisted Electrophoresis Technique, *Journal of Biomedical Materials Research,* Vol. 57, No. 4, pp. 612–618.

Oliveira, A.L., Elvira, C., Reis, R.L., Vâzquez, B., and San Român, J. 1999, Surface Modification Tailors the Characteristics of Biomimetic Coatings Nucleated on Starch-Based Polymers, *Journal of Materials Science: Materials in Medicine,* Vol. 10, No. 12, pp. 827–835.

Ong, J.L., and Lucas, L.C. 1994, Post-Deposition Heat Treatments for Ion Beam Sputter Deposited Calcium Phosphate Coatings, *Biomaterials,* Vol. 15, No. 5, pp. 337–341.

Ong, J.L., Lucas, L.C., Raikar, G.N., Weimer, J.J., and Gregory, J.C. 1994, Surface Characterization of Ion-Beam Sputter-Deposited Ca–P Coatings after In Vitro Immersion, *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* Vol. 87, No. 2, pp. 151–162.

Ong, J.L., Yang, Y., Oh, S., Appleford, M., Chen, W., Liu, Y., Kim, K., Park, S., Bumgardner, J., Haggard, W., Agrawal, C.M., Carner, D.L., and Oh, N. 2009, Calcium Phosphate Coating Produced by a Sputter Deposition Process, in *Thin Calcium Phosphate Coatings for Medical Implants*, B. León, and J.A. Jansen (eds.), 1st edn, Springer Science, New York, pp. 175–198.

Oyane, A., Kim, H., Furuya, T., Kokubo, T., Miyazaki, T., and Nakamura, T. 2003, Preparation and Assessment of Revised Simulated Body Fluids, *Journal of Biomedical Materials Research A,* Vol. 65A, No. 2, pp. 188–195.

Park, J., Ries, J., Gelse, K., Kloss, F., von der Mark, K., Wiltfang, J., Neukam, F.W., and Schneider, H. 2003, Bone Regeneration in Critical Size Defects by Cell-Mediated BMP-2 Gene Transfer: A Comparison of Adenoviral Vectors and Liposomes, *Gene Therapy,* Vol. 10, No. 13, pp. 1089–1098.

Park, T.G., Jeong, J.H., and Kim, S.W. 2006, Current Status of Polymeric Gene Delivery Systems, *Advanced Drug Delivery Reviews,* Vol. 58, No. 4, pp. 467–486.

Plopper, G. 2007, The Extracellular Matrix and Cell Adhesion, in *Cells*, B. Lewin, V.R. Lingappa, and G. Plopper (eds.), 1st edn, Jones and Bartlett, Ontario, pp. 645–702.

Robey, G.P., and Boskey, A.L. 2003, Extracellular Matrix and Biomineralization of Bone, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,* American Society for Bone and Mineral Research, Washington D.C., pp. 38–46.

Rønold, H.J., Lyngstadaas, S.P., and Ellingsen, J.E. 2003, Analysing the Optimal Value for Titanium Implant Roughness in Bone Attachment Using a Tensile Test, *Biomaterials,* Vol. 24, No. 25, pp. 4559–4564.

Roome, C.M., and Adam, C.D. 1995, Crystallite Orientation and Anisotropic Strains in Thermally Sprayed Hydroxyapatite Coatings, *Biomaterials,* Vol. 16, No. 9, pp. 691–696.

Roy, M.D., Stanley, S.K., Amis, E.J., and Becker, M.L. 2008, Identification of a Highly Specific Hydroxyapatite-binding Peptide using Phage Display, *Advanced Materials,* Vol. 20, No. 10, pp. 1830–1836.

Ruoslahti, E. 1996, RGD and Other Recognition Sequences for Integrins, *Annual Review of Cell and Developmental Biology,* Vol. 12, No. 697, p. 715.

Ruoslahti, E., Hayman, E.G., and Pierschbacher, M.D. 1985, Extracellular Matrices and Cell Adhesion, *Arteriosclerosis,* Vol. 5, No. 6, pp. 581–594.

Rupp, F., Scheideler, L., Olshanska, N., de Wild, M., Wieland, M., and Geis-Gerstorfer, J. 2006, Enhancing Surface Free Energy and Hydrophilicity Through Chemical Modification of Microstructured Titanium Implant Surfaces, *Journal of Biomedical Materials Research Part A,* Vol. 76A, No. 2, pp. 323–334.

Schliephake, H., Scharnweber, D., Roesseler, S., Dard, M., Sewing, A., and Aref, A. 2006, Biomimetic Calcium Phosphate Composite Coating of Dental Implants, *The International Journal of Oral and Maxillofacial Implants,* Vol. 21, No. 5, pp. 738–746.

Segvich, S.J., Luong, L.N., and Kohn, D.H. 2008, Biomimetic Approaches to Synthesize Mineral and Mineral/Organic Biomaterials, in *Biomaterials and Biomedical Engineering*, W. Ahmed, N. Ali, and A. Ochsner (eds.), TTP, Switzerland, pp. 327–373.

Segvich, S., Smith, H.C., Luong, L.N., and Kohn, D.H. 2008, Uniform Deposition of Protein Incorporated Mineral Layer on Three-Dimensional Porous Polymer Scaffolds, *Journal of Biomedical Materials Research B,* Vol. 84B, No. 2, pp. 340–349.

Segvich, S.J., Smith, H.C., and Kohn, D.H. 2009, The Adsorption of Preferential Binding Peptides to Apatite-Based Materials, *Biomaterials,* Vol. 30, pp. 1287–1298.

Shalabi, M.M., Gortemaker, A., Hof, M.A.V., Jansen, J.A., and Creugers, N.H.J. 2006, Implant Surface Roughness and Bone Healing: A Systematic Review, *Journal of Dental Research,* Vol. 85, No. 6, pp. 496–500.

Shen, H., Tan, J., and Saltzman, W.M. 2004, Surface-Mediated Gene Transfer from Nanocomposites of Controlled Texture, *Nature Materials,* Vol. 3, No. 8, pp. 569–574.

Shi, D., Jiang, G., and Bauer, J. 2002, The Effect of Structural Characteristics on the In Vitro Bioactivity of Hydroxyapatite, *Journal of Biomedical Materials Research,* Vol. 63, No. 1, pp. 71–78.

Shin, H., Zygourakis, K., Farach-Carson, M.C., Yaszemski, M.J., and Mikos, A.G. 2004a, Attachment, Proliferation, and Migration of Marrow Stromal Osteoblasts Cultured on Biomimetic Hydrogels Modified with an Osteopontin-Derived Peptide, *Biomaterials,* Vol. 25, pp. 895–906.

Shin, H., Zygourakis, K., Farach-Carson, M.C., Yaszemski, M.J., and Mikos, A.G. 2004b, Modulation of Differentiation and Mineralization of Marrow Stromal Cells Cultured on Biomimetic

Hydrogels Modified with Arg-Gly-Asp Containing Peptides, *Journal of Biomedical Materials Research A,* Vol. 69, pp. 535–543.

Shin, K., Jayasuriya, A., and Kohn, D.H. 2007, Effect of Ionic Activity Products on the Structure and Composition of Mineral Self Assembled on Three-Dimensional Poly(Lactide-*co*-Glycolide) Scaffolds, *Journal of Biomedical Materials Research A,* Vol. 83, No. 4, pp. 1076–1086.

Silverthorn, D.U. 2003, *Human Physiology: An Integrated Approach with Interactive Physiology*, Third Edition, Benjamin Cummings, San Francisco.

Simmons, C.A., Alsberg, E., Hsiong, S., Kim, W.J., and Mooney, D.J. 2004, Dual Growth Factor Delivery and Controlled Scaffold Degradation Enhance In Vivo Bone Formation by Transplanted Bone Marrow Stromal Cells, *Bone,* Vol. 35, No. 2, pp. 562–569.

Stigter, M., Bezemer, J., de Groot, K., and Layrolle, P. 2004, Incorporation of Different Antibiotics into Carbonated Hydroxyapatite Coatings on Titanium Implants, Release and Antibiotic Efficacy, *Journal of Controlled Release: Official Journal of the Controlled Release Society,* Vol. 99, No. 1, pp. 127–137.

Stigter, M., de Groot, K., and Layrolle, P. 2002, Incorporation of Tobramycin into Biomimetic Hydroxyapatite Coating on Titanium, *Biomaterials,* Vol. 23, pp. 4143–4153.

Takadama, H., Hashimoto, M., Mizuno, M., and Kokubo, T. 2004, Round-Robin Test of SBF for In Vitro Apatite-Forming Ability of Synthetic Materials, *Phosphorus Research Bulletin,* Vol. 17, pp. 119–125.

Takadama, H., Kim, H.M., Kokubo, T., and Nakamura, T. 2001, TEM-EDX Study of Mechanism of Bonelike Apatite Formation on Bioactive Titanium Metal in Simulated Body Fluid, *Journal of Biomedical Materials Research,* Vol. 57, No. 3, pp. 441–448.

Takeuchi, A., Ohtsuki, C., Miyazaki, T., Tanaka, H., Yamazaki, M., and Tanihara, M. 2003, Deposition of Bone-Like Apatite on Silk Fiber in a Solution That Mimics Extracellular Fluid, *Journal of Biomedical Materials Research A,* Vol. 65A, pp. 283–289.

Tan, J., and Saltzman, W.M. 2004, Biomaterials with Hierarchically Defined Micro- and Nanoscale Structure, *Biomaterials,* Vol. 25, No. 17, pp. 3593–3601.

Tanahashi, M., and Matsuda, T. 1997, Surface Functional Group Dependence on Apatite Formation on Self-Assembled Monolayers in a Simulated Body Fluid, *Journal of Biomedical Materials Research,* Vol. 34, pp. 305–315.

Tanahashi, M., Yao, T., Kokubo, T., Minoda, M., Miyamoto, T., Nakamura, T., and Yamamuro, T. 1994, Apatite Coating on Organic Polymers by a Biomimetic Process, *Journal of the American Ceramic Society,* Vol. 77, No. 11, pp. 2805–2808.

Tanahashi, M., Yao, T., Kokubo, T., Minoda, M., Miyamoto, T., Nakamuro, T., and Yamamuro, T. 1995, Apatite Coated on Organic Polymers by Biomimetic Process: Improvement in Its Adhesion to Substrate by Glow-Discharge Treatment, *Journal of Biomedical Materials Research,* Vol. 29, pp. 349–357.

Tao, S., Heng, J., and Chuanxian, D. 2000, Effect of Vapor-Flame Treatment on Plasma Sprayed Hydroxyapatite Coatings, *Journal of Biomedical Materials Research,* Vol. 52, No. 3, pp. 572–575.

Temenoff, J.S., and Mikos, A.G. 2008, *Biomaterials: The Intersection of Biology and Materials Science*, 1st Edition, Prentice Hall, New Jersey.

Thompson, M.T., Berg, M.C., Tobias, I.S., Rubner, M.F., and Van Vliet, K.J. 2005, Tuning Compliance of Nanoscale Polyelectrolyte Multilayers to Modulate Cell Adhesion, *Biomaterials,* Vol. 26, No. 34, pp. 6836–6845.

van Dijk, K., Schaeken, H.G., Wolke, J.C., Maree, C.H., Habraken, F.H., Verhoeven, J., and Jansen, J.A. 1995, Influence of Discharge Power Level on the Properties of Hydroxyapatite Films Deposited on Ti_6Al_4V with RF Magnetron Sputtering, *Journal of Biomedical Materials Research,* Vol. 29, No. 2, pp. 269–276.

van Dijk, K., Schaeken, H.G., Wolke, J.G.C., and Jansen, J.A. 1996, Influence of Annealing Temperature on RF Magnetron Sputtered Calcium Phosphate Coatings, *Biomaterials,* Vol. 17, No. 4, pp. 405–410.

Wang, J., Layrolle, P., Stigter, M., and de Groot, K. 2004, Biomimetic and Electrolytic Calcium Phosphate Coatings on Titanium Alloy: Physicochemical Characteristics and Cell Attachment, *Biomaterials,* Vol. 25, No. 4, pp. 583–592.

Wei, G., Reichert, J., Bossert, J., and Jandt, K.D. 2008, Novel Biopolymeric Template for the Nucleation and Growth of Hydroxyapatite Crystals Based on Self-Assembled Fibrinogen Fibrils, *Biomacromolecules,* Vol. 9, No. 3258, p. 3267.

Wen, H.B., Hippensteel, E.A., and Li, P. 2005, Protein Adsorption Property of a Biomimetic Apatite Coating, *Key Engineering Materials,* Vol. 284–286, pp. 403–406.

Weng, J., Liu, X., Zhang, X., Ma, Z., Ji, X., and Zyman, Z. 1993, Further Studies on the Plasma-Sprayed Amorphous Phase in Hydroxyapatite Coatings and Its Deamorphization, *Biomaterials,* Vol. 14, No. 8, pp. 578–582.

Wenjian, W., and Baptista, J.L. 1998, Alkoxide route for Preparing Hydroxyapatite and Its Coatings, *Biomaterials,* Vol. 19, No. 1–3, pp. 125–131.

Wie, H., Hero, H., and Solheim, T. 1998, Hot Isostatic Pressing-Processed Hydroxyapatite-Coated Titanium Implants: Light Microscopic and Scanning Electron Microscopy Investigations, *The International Journal of Oral and Maxillofacial Implants,* Vol. 13, No. 6, pp. 837–844.

Wolke, J.G., van Dijk, K., Schaeken, H.G., de Groot, K., and Jansen, J.A. 1994, Study of the Surface Characteristics of Magnetron-Sputter Calcium Phosphate Coatings, *Journal of Biomedical Materials Research,* Vol. 28, No. 12, pp. 1477–1484.

Wolke, J.G.C., van der Waerden, J.P.C.M., Schaeken, H.G., and Jansen, J.A. 2003, In Vivo Dissolution Behavior of Various RF Magnetron-Sputtered Ca–P Coatings on Roughened Titanium Implants, *Biomaterials,* Vol. 24, No. 15, pp. 2623–2629.

Xia, Y., and Whitesides, G.M. 1998, Soft Lithography, *Annual Review of Materials Science,* Vol. 28, No. 1, pp. 153–184.

Xue, W., Tao, S., Liu, X., Zheng, X., and Ding, C. 2004, In Vivo Evaluation of Plasma Sprayed Hydroxyapatite Coatings Having Different Crystallinity, *Biomaterials,* Vol. 25, No. 3, pp. 415–421.

Yamashita, K., Yonehara, E., Ding, X., Nagai, M., Umegaki, T., and Matsuda, M. 1998, Electrophoretic Coating of Multilayered Apatite Composite on Alumina Ceramics, *Journal of Biomedical Materials Research (Applied Biomaterials),* Vol. 43, pp. 46–53.

Yang, Y., and Ong, J.L. 2003, Bond Strength, Compositional, and Structural Properties of Hydroxyapatite Coating on Ti, ZrO_2-Coated Ti, and TPS-Coated Ti Substrate, *Journal of Biomedical Materials Research. Part A,* Vol. 64, No. 3, pp. 509–516.

Yarbrough, D.K., Hagerman, E., Eckert, R., He, J., Choi, H., Cao, N., Le, K., Hedger, J., Qi, F., Anderson, M., Rutherford, B., Wu, B., Tetradis, S., and Shi, W. 2010, Specific Binding and Mineralization of Calcified Surfaces by Small Peptides, *Calcified Tissue International,* Vol. 86, pp. 58–66.

Yoshinari, M., Ohtsuka, Y., and Dérand, T. 1994, Thin Hydroxyapatite Coating Produced by the Ion Beam Dynamic Mixing Method, *Biomaterials,* Vol. 15, No. 7, pp. 529–535.

Zeng, H., and Lacefield, W.R. 2000, The Study of Surface Transformation of Pulsed Laser Deposited Hydroxyapatite Coatings, *Journal of Biomedical Materials Research,* Vol. 50, No. 2, pp. 239–247.

Zhang, Q., Chen, J., Feng, J., Cao, Y., Deng, C., and Zhang, X. 2003, Dissolution and Mineralization Behaviors of HA Coatings, *Biomaterials,* Vol. 24, No. 26, pp. 4741–4748.

Zhu, X., Kim, K., and Jeong, Y. 2001, Anodic Oxide Films Containing Ca and P of Titanium Biomaterial, *Biomaterials,* Vol. 22, No. 16, pp. 2199–2206.

Zyman, Z., Weng, J., Liu, X., Li, X., and Zhang, X. 1994, Phase and Structural Changes in Hydroxyapatite Coatings under Heat Treatment, *Biomaterials,* Vol. 15, No. 2, pp. 151–155.

Zyman, Z., Weng, J., Liu, X., Zhang, X., and Ma, Z. 1993, Amorphous Phase and Morphological Structure of Hydroxyapatite Plasma Coatings, *Biomaterials,* Vol. 14, No. 3, pp. 225–228.