Appendix to *A Mineralogical View of Apatitic Biomaterials*

Jill Dill Pasteris, Washington University in St. Louis, St. Louis, MO 63130

American Mineralogist, 2016

Keywords: apatite, calcium phosphate, biomaterial, synthesis, bone, hydroxylapatite

Table of Contents

Physical Forms of Apatitic and Related Calcium Phosphate Biomaterials

This appendix contains descriptions of the major physical forms of Ca-P biomaterials that are made commercially and marketed for medical usage. The following subsections briefly address various uses of apatitic compounds as biomaterials or components of them.

It also includes descriptions of additional reactions and formulations to produce these materials,

as well as examples of biological reactions to different Ca-P materials and composites. More detailed discussions of clinical applications of individual biomaterials can be found in Hench (1998). The current state of knowledge about the structure and composition of high-temperature, high-pressure carbonated hydroxylapatite is documented and discussed in Fleet (2015).

Dense, porous, and particulate apatitic ceramics

This subsection focuses on applications in which apatite is the sole biomaterial, i.e., the bioceramic. In addition to the self-setting cements, coatings, and injectable forms treated in subsequent subsections below, apatitic ceramics can be formulated as dense blocks, porous blocks (scaffolds), granules, and powders.

Dense HA has less than 5 volume % porosity and a maximum pore size of ~ 1 μm. Such *microporosity* (defined as <10 μm) is created during the sintering process described in the primary article, such that pores become smaller as sintering temperature and duration increase (LeGeros and LeGeros 1993; Dorozhkin 2010).

Macroporous HA can be created in more than one way. For instance, if additional porosity is needed, some volatile compound such as hydrogen peroxide can be added to the powder and then evaporated off at about 80 °C (LeGeros and LeGeros 1993). Alternatively, a polymer foam can be added to a thermally resistant Ca-P phase and subsequently "burned off," e.g., polyurethane foam introduced into a TCP slurry, so that α-TCP coats all the surfaces of the foam. Subsequent sintering at elevated temperatures burns off the polymer foam, creating a porous, strut-like structure resembling cancellous bone and also converts the TCP to apatite or carbonated apatite (Ishikawa 2010). As described in the primary article, biomineralized materials of porous $CaCO_3$ can be replaced by apatite during an exchange reaction, while

retaining their porosity. To encourage bone regeneration in an apatitic scaffold, the optimal pore size is reported to be 300 μm (Lew et al. 2012).

Apatite powder (see Figure 1S) created via any of the synthesis methods described in the primary article can be compacted into a mold of a specific shape. Either alone or mixed with some volatile binder, the powder can be compressed in the mold under 60-80 MPa. The shaped body that results typically is sintered at the maximum desired temperature for several hours. An alternative approach is hot-pressing, in which heat and pressure are applied simultaneously. In this way, final products such as dense HA blocks or tooth forms (for replacing tooth roots after extractions) are produced commercially. The blocks can be carved into specific shapes, such as for implants into the middle ear, or milled to form HA particles of specific sizes and morphologies, such as for filler in bone defects or feedstock for plasma-spraying of apatite coatings (LeGeros and LeGeros 1993).

HA in composite biomaterials

Various types of composites (structured mixtures) are used as biomaterials for selected purposes. One group comprises polymers with incorporated ceramic particles, in which HA alone or together with other ceramic materials constitutes the particulate phase. The HA serves to stiffen the polymer, which in turns toughens the composite material. Such composites overcome two disadvantage of ceramic and metal biomaterials, i.e., a large mismatch in stiffness (i.e., elastic modulus) in the spatial transition from bioceramics such as HA into natural bone/tooth, and the low fracture resistance of ceramics. Already in 1981, Bonfield and colleagues had introduced a composite of inert high-density polyethylene (HDPE) “reinforced” with HA particles as a mechanically compatible bone substitute (Bonfield et al. 1981). They

tailored the HA:HDPE ratio to match the Young's modulus of bone in order to avoid abrupt changes in mechanical properties at the bone-implant interface. Over subsequent decades, the Bonfield group explored the optimization of particle size, particle:polymer ratio, and particle composition. For instance, their cell-culture tests in vitro showed enhanced cellular proliferation and differentiation in composites with 40 vol% HA compared to 20 vol%. Further experiments in vitro demonstrated that their composite directly bonded to bone (i.e., bioactive adhesion) instead of forming a transitional collagenous capsule of lower mechanical stability (Nath and Basu 2009).

A more recent improvement on the above is the selection of additional ceramic phases, such as Al_2O3 or ZrO_2, to incorporate into the HDPE to improve the mechanical properties of the composite without lessening the bioactive properties of the HA phase. An example is a composite of HDPE developed by Nath et al. (2009). They found that addition of 20 volume % each of bioactive HA and bioinert Al_2O_3 of 2-5 μm particle size produced a higher elastic modulus (6.2 GPa) and greater hardness (226.5 GPa) than HDPE composites with only 20 volume % of either HA or Al_2O_3 particles alone. The 3-component composite containing HA also showed favorable wear-resistance (Nath et al. 2009).

A yet more specific biomaterial demand is for apatite-polymer composites that are functional bone scaffolds, whose composition and nanostructure better replicate those of natural bone. The intended application is to the full-scale regeneration of bone tissue. Much of the work here involves the selection and additional tailoring of the polymer (not the focus of this paper), whereas the mineralogic challenge is to produce apatite crystallites that mimic the size, composition, and both intrafibrillar and extrafibrillar arrangement of the mineral in bone

(Newcomb et al. 2012).

Other types of nanocomposites with HA bring their own challenges. For instance, high-density composites of HA and carbon nanotubes (CNTs) clearly would be desirable as biomaterials. HA is bioactive, but has relatively low strength and toughness, whereas CNTs are biocompatible as well as possessing high strength and toughness. The challenge comes in trying to optimize the densification process for the composite, which typically is done through high-temperature sintering. Complications arise, however, in selecting temperature-atmosphere conditions that will not destabilize either component (White et al. 2010). To maintain the stability, structure, and stoichiometry (including hydroxyl content) of HA requires a relatively high partial pressure of water in the atmosphere (see Figure 3 in the main article). However, water at high temperature will react with the CNTs (through the water-gas reaction) to destroy them and release CO and H_2 gas. White et al. (2010) found that both phases could be stably densified during high-temperature sintering in an atmosphere produced by bubbling CO and H_2 through ice water, i.e., by increasing the concentration of the product compounds in the breakdown reaction.

Apatitic cements

"Invention of the self-setting apatite cements is held as one of the breakthroughs in reconstruction for bone defects" (Ishikawa 2010, p. 1152). The term cement may seem out of place in a biological/medical context, but cement-like filler is needed for various orthopedic (bone) and dental applications. The mechanism behind these so-called bone cements is similar to that for construction cement or plaster, i.e., creating a low-temperature aqueous environment in which mineral crystals are induced to grow and interlock. The Ca-P product in bone cement is

a physically (and, to some degree, mechanically) stable construct, either of some form of apatite (e.g., non-stoichiometric HA; poorly crystalline, nanocrystalline, carbonated apatite) or brushite. Ca-P bone cements were considered a major breakthrough when introduced in the 1980s by dental researchers (LeGeros et al. 1982; Brown and Chow 1983 and their 1985 patent) as low-temperature, moldable, often injectable materials that were self-setting once introduced into the body. They are biocompatible, bioactive, and bioresorbable, and they also can be used for controlled release of drugs or bioactive molecules (Ginebra et al. 2010; Dorozhkin 2010). As with construction cement, control over the setting time is important in apatitic cements so as to allow sufficient time for their proper placement and filling of the void.

Bone cements are based on dissolution-precipitation reactions analogous to those that pertain in the use of plaster of Paris (Ishikawa 2010). Some impetus for crystal growth is required in both materials. First-generation bone cements relied on (1) chemical reaction between acidic and basic phosphate compounds, such as monocalcium phosphate MCP and tetracalcium phosphate TCP, respectively (Hatim et al. 2015), (2) a single Ca-P phase that hydrolyzes rapidly, or (3) a single Ca-P phase that is not stable at physiological pH (7.4) but rather transforms into apatite in body fluid. In all cases, the relative solubility of the pertinent Ca-P phases is critical to the cement formulation (Ishikawa 2010).

Many second-generation apatitic cements are self-setting, injectable, intrinsically porous, and readily moldable to the desired shape. This porosity, representing both interstitial voids between original reactant grains and previously water-filled reaction regions, promotes osteo-induction, -conduction, and -integration. It has been observed in biomaterials that bone will fill in pores with diameters greater than 100 μm. Moreover, osteonal (bone-growth) structures

develop in pores exceeding 150 μm, implying that resorption of the apatitic implant could be followed by eventual replacement by new bone (Brown and Fulmer 1991). By the 1990s, there were over a dozen different formulations of Ca-P phases that produced self-setting pastes when mixed with water (Dorozhkin 2013). As seen in a graph of pH vs. Ca concentration (cf. Fig. 2 in the main article), representing the differential solubility of the Ca-P phases, the final product (i.e., the least-soluble Ca-P phase) of each and every formulation of cement is expected to be poorly crystalline HA at pH > 4.2 and brushite at pH < 4.2 (Ishikawa 2010). Results from medical use of the cements as well as from laboratory experiments suggest that brushite actually can form at pH values up to ~ 6. As seen in Figure 2 (main article), HA is the least soluble and most stable of the Ca-P solids, but intermediate metastable phases can nucleate and crystallize before hydroxylapatite (Dorozhkin 2013). Many different pairs of Ca-P phases, one more acidic and the other more basic than HA, have been used to create bone cements (Dorozhkin 2013).

When they explored the reaction kinetics of 1.5 TTCP + 3 DCP (see Table 1, main article, for abbreviations of Ca-P phases) in the formation of calcium-deficient hydroxylapatite, CDHA, $Ca_9(HPO_4)(PO_4)_5OH$, Brown and Fulmer (1991) found that the reaction initially was controlled by the surface area of the reactants, particularly that of the DCP. Later stages of reaction were slower and diffusion-controlled. The latter result, as they excellently documented photomicrographically, is due to epitaxial build-up of HA on the surfaces of DCP grains, slowing the dissolution of DCP that was needed to induce supersaturation with respect to HA. Experiments at 5 to 38 °C demonstrated that reaction rate increased with temperature and that addition of pH-neutral NaCl solution also increased the reaction rate. However, reaction typically ceased before all the reactants were consumed (Brown and Fulmer 1991). This

unsatisfactory outcome appears to be due to the negative effects of mixing two powdered reactants each of a random grain size; the size dependence of relative rates of solubility of the reactants should be considered so as to aid optimization of grain sizes and assure effective dissolution-precipitation (Ishikawa 2010).

The second mechanism of cement formation is the hydrolysis of a single Ca-P phase, e.g., ACP, TCP, DCP, OCP, or TTCP. The typical product is a material that ultimately forms CDHA during setting. TCP is the most-used reactant, probably because its initial Ca:P ratio is 1.5, just like that of the CDHA phase $Ca_9(HPO_4)(PO_4)_5OH$, which is the sole product phase (Ginebra et al. 2012).

Reactions that produce brushite-forming cements operate analogously to those producing apatitic end-products. Brushite cements have faster setting times and are resorbed more rapidly than apatitic ones. Compositional additives such as citrate compounds can slow the setting time (Dorozhkin 2013).

Although the standard reactions attributed to the formation of HA or CDHA do not include CO_3, the materials retrieved from bone defects shortly after the cement has set typically reveal the presence of carbonated apatite (Dorozhkin 2013). This observation is a reminder both that carbonation of apatite occurs readily in vivo (a boon to those who favor biomimetic pathways to bone repair) and that the total replacement of Ca-P biomaterials by natural bone (comprising carbonated apatite) over time is a reasonable goal. It also has been observed that the smaller the grain size of the reactant powder, the smaller and more needle-like (cf. platelet-like) are the apatitic precipitates (Ginebra et al. 2012). Due to their injectability and ease of shaping, cements frequently are used to fill voids in bones and to repair bones of the face and skull. The

phase composition and porosity of bone cements, however, make them inappropriate for load-bearing situations. Dorozhkin (2013) details the controls on mechanical properties of cements and lists about two dozen commercial Ca-P cements used for bone and tooth repair.

The low-temperature, more biomimetic nature of modern bone cements compared to traditional high-temperature, essentially unresorbable HA ceramics has encouraged alternative uses of the cement material. Recent applications call for different processing of the standard Ca-P cement material (see Figure 2S), e.g., to be made into porous scaffolds for ingrowth of cells, vasculature, and new bone, as porous osteogenic particles to accompany bone grafts (see Figure 3S), or to fill bone separations greater than the critical size (Ginebra et al. 2010). Bone cements also can be formulated with additives, such as water-soluble polymers, to control their setting times, compressive strength, and injectability. They can accommodate chemical additives, such as Sr, which enhance osteoinduction (Combes and Rey 2010). As detailed below, the application of self-setting mixtures of HA and calcium sulfate also is increasing. In summary, apatitic bone cements are highly valued and widely applied due to their osteoconductivity, injectability, intrinsic porosity, and low setting temperature.

Amorphous calcium phosphate

The main paper focuses on crystalline Ca-P phases, but a few remarks on amorphous calcium (ortho)phosphate (ACP) are in order. Consideration of a 3 nm X 3 nm X 3 nm crystal of apatite illustrates the difficulty in defining the boundary between nanocrystalline, as above, and amorphous (see discussion in Wopenka and Pasteris 2005). An X-ray amorphous Ca-P phase exists naturally in a number of primitive animals (Lowenstam 1981) and is recognized to exist in small amounts in a number of higher animals, especially in their teeth (Lowenstam and Weiner

1985; Beniash et al. 2009; Gordon et al. 2015). ACP can be synthesized by attainment of supersaturation through rapid cooling or rapid mixing of Ca- and P-bearing solutions. It is metastable with respect to crystalline Ca-P phases, such as HA, depending on bulk composition. Most ACP (cf. Tas 2013) transforms readily into a crystalline phase except if doped with certain elements, such as Mg, and it is much more soluble than any of the crystalline Ca-P phases (Dorozhkin 2012a). ACP is therefore a useful synthetic precursor material to the formation of HA, and it has been suggested as a possible natural precursor in the formation of bone mineral (Gower 2008; Addadi et al. 2012).

Bioactive glasses

The story behind the first bioactive glass is one that mineralogists and petrologists would appreciate (Hench 2006). The glass was explicitly developed as a strong material that would not be treated as a foreign contaminant by the body, as were metal and plastic prosthetics that rapidly became encased in scar tissue. The guiding principles in the formulation of the glass were that its composition should include the major components of natural bone mineral and that it should be easy to melt. After consulting *Phase Diagrams for Ceramists*, ceramic engineer L.L. Hench chose compositions near the eutectic of the CaO-Na_2O-SiO_2 ternary to which were added several wt.% P_2O_5. The selected compositions were melted, cast into small rectangular prisms, and implanted in the femurs of rats. Variation in the selected CaO:Na_2O:SiO_2 ratio (plus significant P_2O_5 concentration) produced biological responses that varied from bone-bonding to soft-tissue-bonding to non-bonding. Optimized compositions produced remarkably strong bonds with the rat bone after the pre-specified 6 weeks in vivo (Hench 2006).

Bioactive glasses are often considered to be a subgroup of bioceramics. They have been

used primarily as bone-graft substitutes. More recently, bioactive glasses have been employed as bone tissue scaffolds in tissue engineering and as implants that also contain either resorbable or resistant polymers (Hupa 2012). Several compositions of bioactive glass are now commercially available, but all within a relatively limited compositional range. Somewhat unexpectedly, some of the formulations do not contain phosphorus. The original Bioglass® developed by L.L. Hench and colleagues has been in clinical use since 1985. The advantages of bioactive glasses over HA and TCP are their higher resorption rate, stronger and more rapid chemical bonding with bone, and their greater strength. Although bioactive glasses have been shown to induce more bone regeneration than other ceramic biomaterials, they are not used nearly so much as crystalline HA and TCP. This is due in part to historic recognition of the composition of bone mineral (~ HA) and to limitations in some types of processing of these glasses into useful biomaterials (Shi 2006; Jones 2013).

In the presence of biofluids or their synthetic analogs, bioactive glasses undergo five stages of response: (1) ion exchange between the surface of the glass (especially its Na^+) and the surrounding aqueous solution (particularly H^+), (2) depolymerization of the Si-O-Si network of the glass followed by formation of Si-OH silanol groups and loss of soluble silica, (3) partial repolymerization of the silanols to form a siliceous gel, (4) diffusion of Ca^{2+} and PO_4^{3-} outward from the glass into the gel, forming a Ca- and P-rich layer on the glass, and (5) incorporation of CO_3^{2-} and OH^- from solution followed by solidification of the amorphous material into a poorly crystalline carbonated hydroxylapatite phase like that in bone (Hench 1991; Lockyer et al. 1995; Cerruti and Sahai 2006; Jones 2013).

The properties of these bioactive glasses, just like their petrologic counterparts, are

affected by their degree of polymerization, as explained in more detail by Cerruti and Sahai (2006). One of the most fundamental determinants of a bioglass's reactivity (bioactivity) is its solubility. The lower the degree of polymerization of the glass (i.e., smaller the proportion of bridging oxygens) the higher its solubility and reactivity, but the lower the glass-transition temperature, T_g. In addition, the lower the T_g, the lower is the hardness of the glass. When fluoride was introduced into a bioglass system while maintaining the glass's degree of polymerization, the T_g was reduced, which led to lower hardness but increased bioactivity (Farooq et al. 2012). Although their discussion is outside the scope of this paper, multiple formulations of silicate glasses do not contain P, yet are bioactive. Their chemical-structural criteria for bioactivity are explored by Cerruti and Sahai (2006).

The originally developed composition, Bioglass 45S5, not only bonds strongly with bone at their mutual interface, but it also appears to stimulate bone growth beyond that surface. Exactly how this desirable response occurs is not totally clear, but it seems that the breakdown products of the glass stimulate the differentiation of stem cells into bone-forming osteoblasts. Experiments show that proteins adsorb onto the crystalline apatite layer and that cells attach to it and produce extracellular collagenous matrix, which then mineralizes to bone. Bioglass 45S5 and competing commercial products have been used to repair bone in both orthopedic and dental (jaw) sites. These glasses also have been found to be very useful for the dental purposes of remineralizing small volumes of defective dentin to decrease tooth sensitivity and for remineralizing and polishing the surface of tooth enamel. In both dental and orthopedic applications, bioactive glass has been especially useful in the form of particles or granules up to a few hundred μm in diameter, which are very bioactive. Glass monoliths (the term used

medically) do not have the strength necessary for weight-bearing applications (Jones 2013).

Bioactive glasses can be produced either by melting the components at about 1300 °C followed by quenching or through sol-gel processes at room temperature. An advantage of the latter is that sol-gel glasses inherently possess nanopores and nanotopography, which reflect the gaps between the sol particles. In contrast, the condensed low-porosity nature of the melt-glasses does not encourage cellular responses and vascularization (Jones 2013).

Apatitic coatings on implants

Bioengineers have long recognized the biomechanical advantages of metals as substitutes for missing or degraded bone. Because metals lack osteoinductive or osteoconductive properties, however, a transitional phase is required between the metal and the pre-existing or newly forming bone. The application of HA coatings on metal implants has proved very successful for the integration of metal implants, as especially well demonstrated by titanium hip replacements.

Such HA coatings can be produced in different thicknesses (nanometers to several hundred μm). If the coating is too thick, it can crack and delaminate, but a coating that is too thin may dissolve away too quickly through resorption. The challenge is to tailor its solubility so that the apatite coating does not passively dissolve or undergo active biological remodeling before the implant is optimally stabilized. For instance, a metal prosthetic could be coated with a bioactive glass, but such material typically is resorbed so rapidly that new bone does not have time to stabilize the implant (Shi 2006; Dorozhkin 2012b; Jones 2013; Heimann and Lehmann 2015). A different challenge arises in coating the insides of pores within non-apatitic biomaterials (Oliveira and Reis 2005).

HA-based coatings can be applied at a wide range in temperature from less than 40 to

many hundreds of °C. The most common high-temperature technique is HA plasma-spraying. A feedstock of HA particles is melted and rapidly ejected in a plasma up to 20,000 °C. As the molten droplets splat onto the target implant metal, they form a stratified coating (Carayon and Lacout 2003; Shi 2006; Dorozhkin 2012b; Heimann 2013, 2016). ACP forms in the initial droplets during flight (see Figure 4S) and becomes a significant phase in the coating, which is dominated by HA, and may be accompanied by other crystalline Ca-P phases. CaO is the first phase to crystallize, followed by TTCP and TCP, the ratio of phases depending on the (variable) initial Ca:P ratio of the amorphous phase (Combes and Rey 2010).

Plasma-sprayed coatings are highly variable in their properties, which means that such properties can be tweaked, but also that all parameters must be controlled carefully to ensure appropriate and reproducible thickness, adhesion, resistance to cracking, and solubility. For instance, the high plasma temperatures can cause complete or partial dehydroxylation of the apatite (Heimann et al. 1997; Wen et al. 2000; Gross and Berndt 2002; Pasteris et al. 2004; Heimann 2013, 2016), ultimately forming oxyapatite, $Ca_{10}(PO_4)_6O$. Differences in thermal expansion coefficients between the apatite coating and the underlying biomaterial can cause cracking and delamination of the coating (Heimann 2013, 2016). The larger the proportion of an amorphous Ca-P phase (referred to as glass by some and amorphous calcium phosphate, ACP, by others), the more bioactive and abrasion-resistant the coating is. During biointegration of a metal implant with a composite coating, the latter likely undergoes gradual dissolution of the ACP component followed by new precipitation of biological apatite, while the bioinert particles remain associated with the metal and possibly act as nucleation sites for growth of new bioapatite (Nath and Basu 2009). Among Ca-P phases, stoichiometric HA has the slowest

dissolution rate, i.e.,15 - 30μm per year (Shi 2006). The degree of crystallinity of the coating, which is controllable, affects its resorption rate and cellular interaction.

In recognition of the low resorption rate of highly crystalline HA, low-temperature apatitic coating methods have been developed. For examples, sol-gel synthesis or aqueous precipitation onto the metal by long-term soaking in simulated body fluid, SBF, produces apatite of low degree of crystallinity but in very dense, homogeneous films. If the solution is saturated with CO_2, then the phase produced is (bone-like) carbonated apatite. The latter phase emulates ACP in that it is resorbed more rapidly than HA. The absence of ACP as the initial well-bonded underlying phase, however, reduces the adhesion of the coating and can lead to failure (Combes and Rey 2010).

Apatitic coatings are also applied to complexly shaped materials and the pores of bioinert porous ceramics, such as alumina, in order to increase their bioactivity (Shi 2006). Low-temperature coatings also can be produced from the introduction of particles of bioactive glass into concentrated SBF, causing precipitation of an apatitic layer onto metals, ceramics of various oxide compounds, and polymers, such as silicone and biodegradable polymers. The ongoing challenge is to create a sufficiently strong bond between the apatite layer and the underlying implant material (Oliveira and Reis 2005).

In recognition of functionally graded tissues in the body (e.g., tendon-to-bone gradient in the shoulder's rotator cuff), HA coatings are now being prepared with a graded microstructure to control their dissolution. Thus, a highly crystalline HA layer (of lowest solubility) is applied directly onto the metal implant, e.g., by ion-beam or radio-frequency sputtering, whereas much more soluble ACP forms a top layer. Such a solubility gradient is deemed beneficial for the

early stages of implant fixation (Shi 2006).

The functionality and widespread application of plasma-sprayed HA depend on its uniformity in thickness, coverage, and crystallinity (Oliveira and Reis 2005). In a recent review paper, Heimann (2016) explains the experimental controls on the above properties of HA coatings and proposes specific methodologies to help optimize the properties of plasma-sprayed coatings.

Mixtures of calcium sulfate and HA

Calcium sulfate (Ca-S), in the form of the hemihydrate $CaSO_4 \bullet 0.5H_2O$ (i.e., the mineral bassanite, Plaster of Paris), has been used for over a century to enhance bone growth in bone defects, as from tumors and unhealed fractures. More recent research has shown great promise for the use of mixtures of $CaSO_4 \bullet 0.5H_2O$ (Ca-S) and HA in cements and prefabricated, shaped blocks for bone and dental (tooth socket) repair. Ca-S is resorbed faster than TCP and much faster than HA, does not induce inflammation, and accelerates growth of new bone compared to HA alone. Whereas HA requires osteoclast activity for its resorption, Ca-S dissolves passively in body fluid, releasing calcium needed for new bone growth. Such dissolution enhances porosity in the mixed-implant material, which encourages vascular development, cell incorporation, and influx of nutrient-rich body fluid. Such porosity also encourages bulk replacement of the implant by bone ingrowth rather than by circumferential substitution inward toward the center. Its relatively rapid resorption also makes Ca-S useful for drug delivery (Thomas and Puleo 2009; Nilsson et al. 2013).

Drug delivery via Ca-P cements, ceramics, and glasses

Their porosity and the low temperature at which setting reactions occur in bone cements

make them ideal for drug delivery (Ginebra et al. 2012). In biomaterial mixtures containing Ca-S, as described above, the latter is a useful delivery medium for antibiotics due to its relatively rapid dissolution. One of the concerns in drug delivery is possible chemical reaction of the drug with the cement, which might cause undesired co-precipitation, longer setting times, or changes in the viscosity or mechanical properties of the cement. Dissolution is not the only means of releasing the drug. If the cement is not rapidly resorbed, then the mechanism of drug delivery is by diffusion through the cement. The chemistry of the specific drug is critical to the delivery process as detailed in Ginebra et al. (2012).

Nanocrystalline apatite, due to its reactive, hydrated surface, can be functionalized with multiple types of inorganic ions or biomolecules, such as bisphosphonates (for treating osteoporosis), cancer-fighting drugs, and antibacterial enzymes (Cazalbou et al. 2005, 2015). The very surgery in which biomaterials are introduced into a defective bone site can increase the need for localized antibiotics. Studies have been done to quantify the release rates and to understand the release mechanisms of antibiotics incorporated in biomimetic nanocrystalline HA. For instance, the adsorption isotherm of the broad-spectrum antibiotic tetracycline was studied as an example of the behavior of a polar molecule without terminal groups that strongly bind to apatite, such as phosphates or carboxylates (Cazalbou et al. 2015).

There is a group of ions now acknowledged as useful for specific medical applications. These so-called therapeutic inorganic ions (TII) are readily incorporated into both melt-derived and sol-gel-derived bioactive glasses, allowing the ions to be released in a controlled fashion at a selected site. Among the TII are Ce, Zn, and Ga for their antibacterial properties and for stimulating bone production. Borate-enriched glasses are resorbed more quickly than silicate

glasses without boron, and they appear to aid the healing of wounds. Strontium ions encourage differentiation of osteoblasts to produce new bone, whereas copper encourages formation of blood vessels. Iron introduction to produce ferromagnetic bioglasses enables hyperthermia techniques that treat cancer (Hoppe et al. 2013). The biological effects of inorganic ions and the breakdown products of silicate-based bioactive glasses are tabulated in Hoppe et al. (2011).

Studies continue on the dissolution kinetics and ion-release mechanisms of plausibly bioactive glasses. A further challenge is to translate those dissolution data into specific manufactured products. Fine-tuning of glass compositions and properties for medical purposes is an interdisciplinary endeavor, historically among biologists, medical researchers, and materials scientists (Hupa 2012). It would appear, however, that mineralogists, experimental petrologists, and geochemists also have much to offer to such studies.

Additional Implications for Mineralogists-Geochemists of Biomaterials Studies

Consideration of the mineral apatite via mineralogy-geochemistry compared to medicine-biomaterials reveals multiple, in some cases unexpected, contrasts between the mineralogical view and the materials-science view. Synthetic materials often are prized for their purity and structural perfection. The most crystalline and stoichiometric form of HA, however, is not the most desirable for a biomaterial. Likewise, the most stable, least soluble of the Ca-P phases (i.e., HA) is not the ideal reactant for biomaterial application. The high crystallinity and relatively low surface-area-to-volume ratio (SA:V) of typical ceramics are the exact opposite of what is required of substitutes for solid bone. Rather, low crystallinity and high SA:V are needed. These requirements are not what formulators of most ceramics are accustomed to.

Features that are representative of and necessary to bone mineral typically would be seen as defects (vacancies) and contaminants (trace and minor elements) by materials scientists. It is, however, the non-stoichiometry of bioapatite (especially the vacancies in Ca and OH sites) that accounts for its enhanced solubility and resorption rate in situ compared to stoichiometric HA.

In distinction from the commonly assessed bulk properties of a synthetic material, it is in large part the surface characteristics of an apatite phase that control the desirability of its properties as a biomaterial. It is surface reactivity that accounts for the chemical and physical maturation of non-stoichiometric apatite both in the body and during immersion in the synthesis solution. Bioapatite and its synthetic equivalent exhibit a type of core-shell morphology (Cazalbou et al. 2004; Jäger et al. 2006), in which the shell (including hydration layer) decreases in relative volume as the over-all crystal grows and matures. The importance of this hydration layer is illustrated in some of the more biomimetic synthesis techniques. For example, if there is enough water and ion mobility on the surfaces of the mineral (Cazalbou et al. 2005), the sample can be "chemically sintered" by uniaxial compression at such low temperatures (150 °C maximum) that the molecular water is not removed from the apatite (Drouet et al. 2009).

Thermodynamic energy considerations are well illustrated in the apatite group of minerals. In its ready incorporation of so many different elements, apatite has shown itself to be a material that can be changed substantially in its chemistry and properties with a relatively small input of energy. Better understanding of this relationship could lead to identification of other phases that would respond similarly. Most importantly for the biomaterials community is that HA is the most stable, least soluble of a large number of Ca-P phases. One could introduce almost any of those more soluble Ca-P phases into the body, and some composition of

hydroxylapatite eventually would be biologically precipitated to replace them. It appears in the body that, geochemically speaking, "all Ca-P roads lead to hydroxylapatite." So, there are many chemical pathways to the production of HA, and success in generating "HA" in the body does not ensure that the optimal process or pathway has been used.

"The ideal basic premise, if following the tissue engineering paradigm, is that the materials will be resorbed and replaced over time by, and in tune with, the body's own newly regenerated biological tissue" (Stevens 2008, p. 19). The good news revealed by in vitro and in vivo studies is that the body can make use of almost any Ca-P material we implant, no matter how non-biomimetically formulated or clumsily introduced. The better news is that we can improve our materials and the methods of their introduction into the body. If they are interested in this system or its analogs, geochemists and mineralogists can be among those to investigate and optimize such materials.

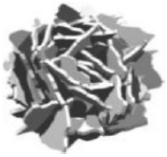
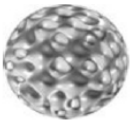
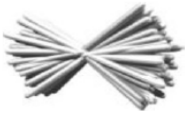
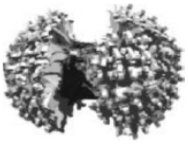
Shape	Name(s) in literature	Approx. size range
	irregular, formless, sphere	5 nm-200 μm
	sphere, microsphere, nanosphere, ball	10 nm-1000 μm
	rod, needle, tube, filament, fiber, wire, whisker, hexag. prism, worm, lath, strip	length: 10 nm-150 μm, diam.: 3 nm-50 μm, l:w = 2 - 1200
	plate, flake, sheet	length (L): 40 nm-50 μm, width (W): 20 nm-35 μm, thickness: 5 nm-3 μm
	self-assembled nanorods, bundles of nanorods, oriented raft, like enamel prisms, bundled needles	L: 200 nm-80 μm, W: 100 nm-50 μm; bundled rods 10 nm-13 μm diam., 200 nm-75μm length
	dandelion, chrysanthemum, flower, feathery structure, bundle of fibers, rosette, self-assembled nanorods	1-8 μm (organized nano-rods 80-500 nm diam., 600 nm - 5 μm length
	leaf, flake, sheet, plate	800 nm-10 μm (organized nanoplates of 20-100 nm thickness
	flower	700 nm-60 μm (organized petals of 20 nm-10 μm width, 180 nm-50μm length
	porous microsphere, mesoporous sphere	0.5-7 μm (pores of 20-150 nm)
	bowknot, self-assembled nanorods	1.5-2.5 μm (organized nanorods 100-150 nm diam., 1-2 μm length
	dumbbell	2-3 μm (organized nanoparticles of ~50 nm size)

Figure 1S. Examples of the modulated shapes that can be created by HA nanostructures. Modified and used by permission of Elsevier, from Sadat-Shojai et al. (2013), Acta Biomaterialia, vol. 9, Table 3, p. 7596.

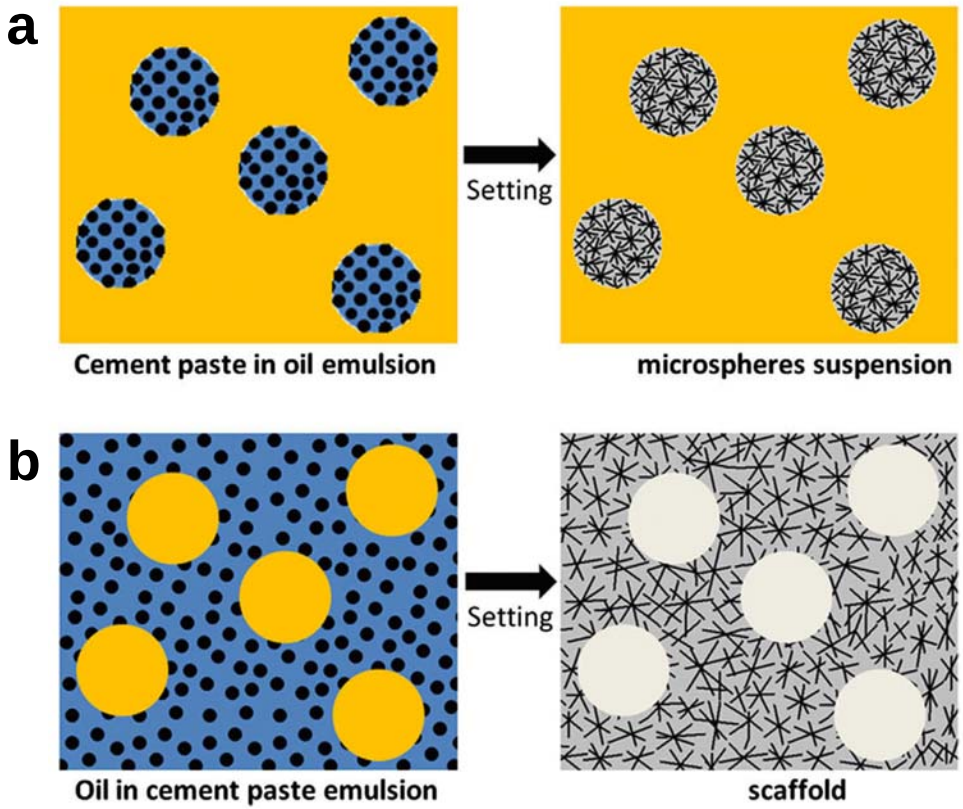


Figure 2S. Use of oil-emulsion with calcium phosphate cement to create (**a**) Ca-P microspheres and (**b**) porous Ca-P scaffolds. See Figure 3S for magnified view of such microspheres. Used by permission of Elsevier, from Ginebra et al. (2010), Acta Biomaterialia, vol. 6, Fig. 2, p. 2867.

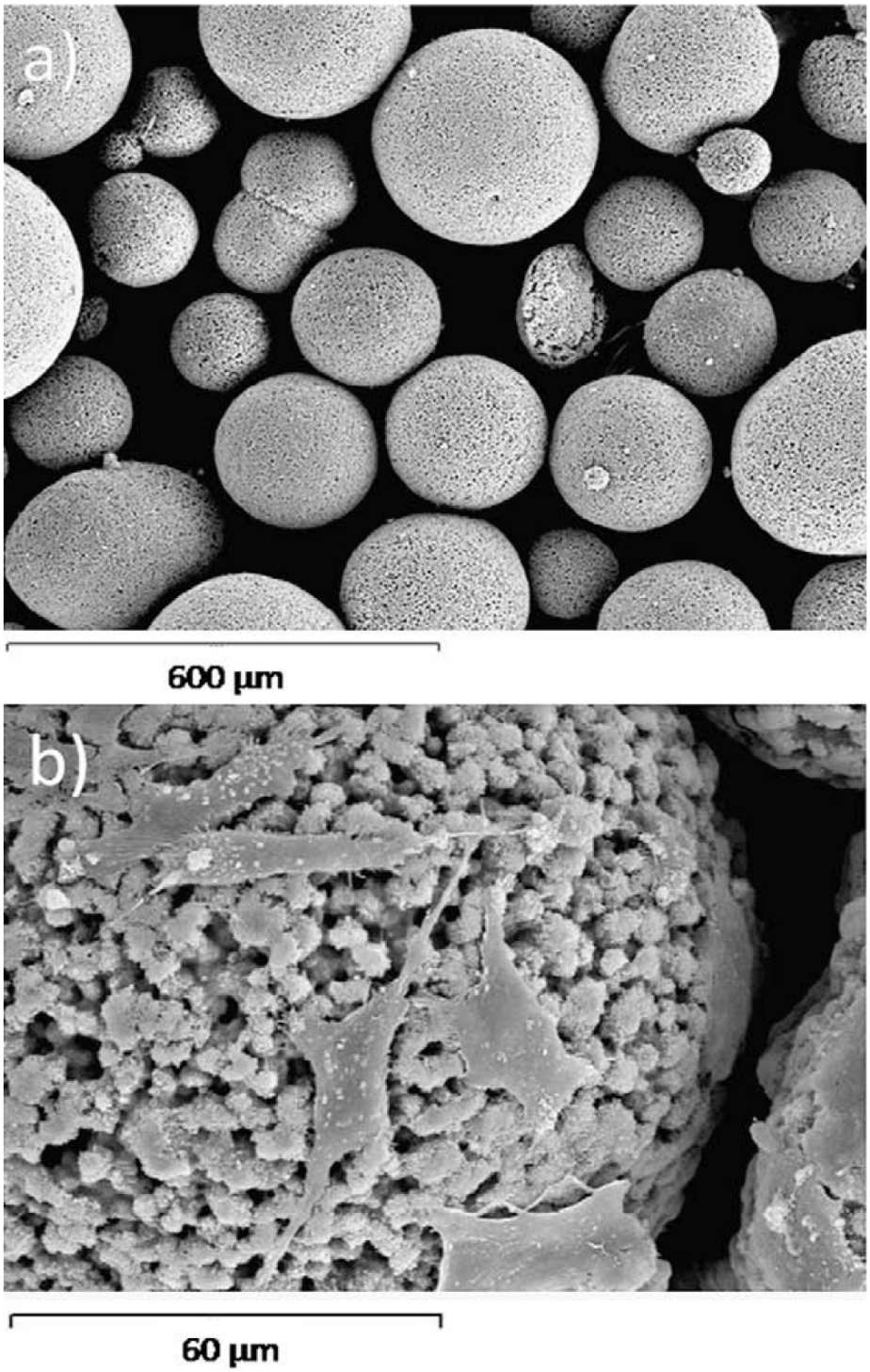


Figure 3S. SEM images of hybrid organic-inorganic microspheres formed in an emulsion as shown in Figure 2S. (**a**) Gelatine-hydroxylapatite composite. (**b**) Apparent osteoblast (bone-forming) cells growing on the microspheres. Used by permission of Elsevier, from Ginebra et al. (2010), Acta Biomaterialia, vol. 6, Fig. 3, p. 2869.

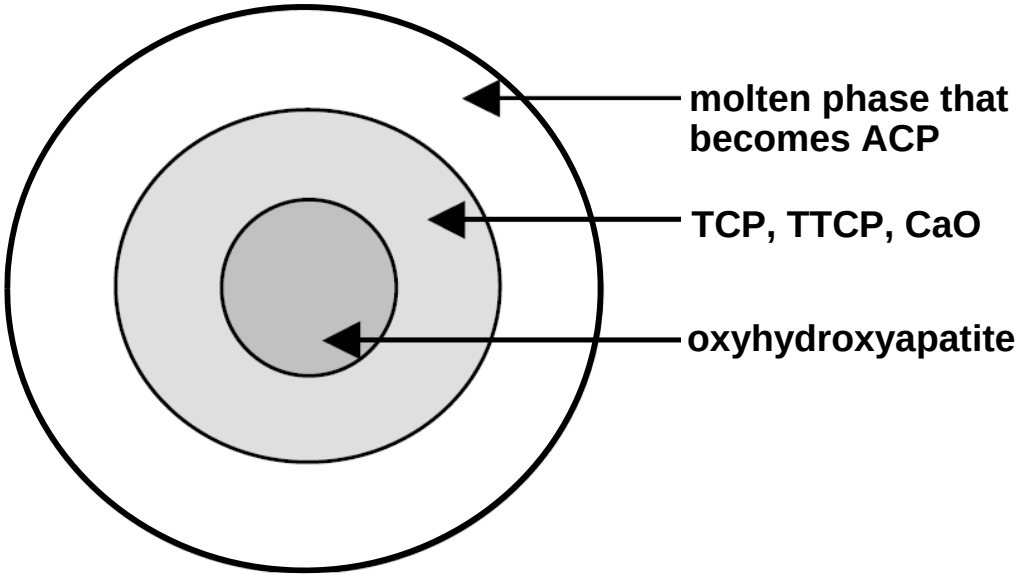


Figure 4S. Particle illustrating the effects of a temperature gradient during cooling of plasma-sprayed HA coating on a prosthetic device. CaO is the first crystalline phase to form. It is followed by calcium tetraphosphate (TTCP), calcium triphosphate (TCP), and partially dehydroxylated HA (oxyhydroxyapatite). Amorphous calcium phosphate (ACP) may recrystallize during sufficiently slow quenching (Combes and Rey, 2010). Modified and used by permission of Elsevier, from Carayon and Lacout (2003), Journal of Solid State Chemistry, vol. 172, Fig. 12, p. 347.

Reference List

Addadi, L., Vidavsky, N., and Weiner, S. (2012) Transient precursor amorphous phases in biomineralization. In the footsteps of Heinz A. Lowenstam. Zeitschrift für Kristallographie, 227, 711-714.

Beniash, E., Metzler, R.A., Lam, R.S.K., and Gilbert, P. (2009) Transient amorphous calcium phosphate in forming enamel. Journal of Structural Biology, 166, 133-143.

Bonfield, W., Grynpas, M.D., Tullly, A.E., Bowman, J., and Abram, J. (1981) Hydroxyapatite reinforced polyethylene – A mechanically compatible implant material for bone replacement. Biomaterials, 2, 185-186.

Brown, P.W. and Fulmer, M. (1991) Kinetics of hydroxyapatite formation at low temperature. Journal of the American Ceramic Society, 74, 934-940.

Brown, W.E., Chow, L.C. A new calcium phosphate setting cement. Journal of Dental Research 62, 672. 1983. Abstract.

Carayon, M.T. and Lacout, J.L. (2003) Study of the Ca/P atomic ratio of the amorphous phase in plasma-sprayed hydroxyapatite coatings. Journal of Solid State Chemistry, 172, 339-350.

Cazalbou, S., Combes, C., Eichert, D., and Rey, C. (2004) Adaptive physico-chemistry of bio-related calcium phosphates. Journal of Materials Chemistry, 14, 2148-2153.

Cazalbou, S., Eichert, D., Ranz, X., Drouet, C., Combes, C., Harmand, M.F., and Rey, C. (2005) Ion exchanges in apatites for biomedical applications. Journal of Materials Science: Materials in Medicine, 16, 405-409.

Cazalbou, S., Bertrand, G., and Drouet, C. (2015) Tetracycline-loaded biomimetic apatite: An adsorption study. Journal of Physical Chemistry B, 119, 3014-3024.

Cerruti, M. and Sahai, N. (2006) Silicate biomaterials for orthopaedic and dental implants. In N. Sahai and M.A.A. Schoonen, Eds., Medical Mineralogy and Geochemistry, 64, p. 283-313. Reviews in Mineralogy and Geochemistry, Mineralogical Society of America, Chantilly, VA.

Combes, C. and Rey, C. (2010) Amorphous calcium phosphates: Synthesis, properties and uses in biomaterials. Acta Biomateriala, 6, 3362-3378.

Dorozhkin, S.V. (2010) Bioceramics of calcium phosphate. Biomaterials, 31, 1465-1485.

Dorozhkin, S.V. (2012a) Amorphous calcium orthophosphates: Nature, chemistry and biomedical applications. International Journal of Materials and Chemistry, 2, 19-46.

Dorozhkin, S.V. (2012b) Calcium orthophosphate coatings, films, and layers. Progress in

Biomaterials, 1, 1-40.

Dorozhkin, S.V. (2013) Self-setting calcium othophosphate formulations. Journal of Functional Biomaterials, 4, 209-311.

Drouet, C., Bosc, F., Banu, M., Largeot, C., Combes, C., Dechambre, G., Estournes, C., Raimbeaux, G., and Rey, C. (2009) Nanocrystalline apatites: From powders to biomaterials. Powder Technology, 190, 118-122.

Farooq, I., Imran, Z., Farooq, U., Leghari, A., and Ali, H. (2012) Bioactive glass: A material for the future. World Journal of Dentistry, 3, 199-201.

Fleet, M. (2015) Carbonated Hydroxyapatite: Materials, Synthesis, and Applications, Pan Stanford Publishing, Singapore.

Ginebra, M.P., Espanol, M., Montufar, E.B., Perez, R.A., and Mestres, G. (2010) New processing approaches in calcium phosphate cements and their applications in regenerative medicine. Acta Biomaterialia, 6, 2863-2873.

Ginebra, M.-P., Canal, C., Espanol, M., Pastorino, D., and Montufar, E.B. (2012) Calcium phosphate cements as drug delivery materials. Advanced Drug Delivery Reviews, 64, 1090-1110.

Gordon, L.M., Cohen, M.H., MacRenaris, K.W., Pasteris, J.D., Seda, T., and Joester, D. (2015) Amorphous intergranular phases control the properties of tooth enamel. Science, 347, 746-750.

Gower, L.B. (2008) Biomimetic model systems for investigating the amorphous precursor pathway and its role in biomineralization. Chemical Reviews, 108, 4551-4627.

Gross, K.A. and Berndt, C.C. (2002) Biomedical application of apatites. In M.J. Kohn, J. Rakovan, and J.M. Hughes, Eds., Phosphates – Geochemical, Geobiological and Material Importance, 48, p. 631-672. Reviews in Mineralogy and Geochemistry, Mineralogical Society of America, Chantilly, Virginia.

Hatim, Z., Freche, M., Kheribech, A., and Lacout, J.L. (2015) The setting mechanisms of a phosphocalcium biological cement. Annales de Chimie Science des Materiaux, 23, 65-68.

Heimann, R.B. (2013) Structure, properties, and biomedical performance of osteoconductive bioceramic coatings. Surface & Coatings Technology, 233, 27-38.

Heimann, R.B. (2016) Plasma-sprayed hydroxylapatite-based coatings: Chemical, mechanical, microstructural, and biomedical properties. Journal of Thermal Spray Technology, 25, in press.

Heimann, R.B. and Lehmann, H.D. (2015) Bioceramic Coatings for Medical Implants, Wiley-VCH, Berlin.

Heimann, R.B., Vu, T.A., and Wayman, M.L. (1997) Bioceramic coatings: State-of-the-art and recent development trends. European Journal of Mineralogy, 9, 597-615.

Hench, L.L. (1991) Bioceramics: From concept to clinic. Journal of the American Ceramic Society, 74, 1487-1510.

Hench, L.L. (1998) Bioceramics. Journal of the American Ceramic Society, 81, 1705-1728.

Hench, L.L. (2006) The story of Bioglass. Journal of Materials Science: Materials in Medicine, 17, 967-978.

Hoppe, A., Guldal, N.S., and Boccaccini, A.R. (2011) A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. Biomaterials, 32, 2757-2774.

Hoppe, A., Mourino, V., and Boccaccini, A.R. (2013) Therapeutic inorganic ions in bioactive glasses to enhance bone formation and beyond. Biomaterials Science, 1, 254-256.

Hupa, L. (2012) Tailoring of bioactive glasses. In M. Ramalingam, P. Vallittu, U. Ripamonti, and W.-J. Li, Eds., Tissue Engineering and Regenerative Medicine: A Nano Approach, p. 43-58, CRC Press, Boca Raton, FL.

Ishikawa, K. (2010) Bone substitute fabrication based on dissolution-precipitation reactions. Materials, 3, 1138-1155.

Jäger, C., Welzel, T., Meyer-Zaika, W., and Epple, M. (2006) A solid-state NMR investigation of the structure of nanocrystalline hydroxyapatite. Magnetic Resonance in Chemistry, 44, 573-580.

Jones, J.R. (2013) Review of bioactive glass: From Hench to hybrids. Acta Biomaterialia, 9, 44547-4486.

LeGeros, R.Z. and LeGeros, J.P. (1993) Dense hydroxyapatite. In L.L. Hench and J. Wilson, Eds., An Introduction to Bioceramics, p. 139-180. Advanced Series in Ceramics, vol. 1, World Scientific, Singapore.

LeGeros, R.Z., Chohayeb, A., and Shulman, A. (1982) Apatitic calcium phosphates: Possible dental restorative materials. Journal of Dental Research 61, 343. Abstract.

Lew, K.-S., Othman, R., Ishikawa, K., and Yeoh, F.-Y. (2012) Macroporous bioceramics: A remarkable material for bone regeneration. Journal of Biomaterials Applications, 27, 345-358.

Lockyer, M.W.G., Holland, D., and Dupree, R. (1995) NMR investigation of the structure of some bioactive and related glasses. Journal of Non-Crystalline Solids, 188, 207-219.

Lowenstam, H.A. (1981) Minerals formed by organisms. Science, 211, 1126-1131.

Lowenstam, H.A. and Weiner, S. (1985) Transformation of amorphous calcium phosphate to crystalline dahllite in the radular teeth of chitons. Science, 227, 51-53.

Nath, S. and Basu, B. (2009) Materials for orthopedic applications. In B. Basu, D. Katti, and A. Kumar, Eds., Advanced Biomaterials: Fundamentals, Processing, and Applications, p. 53-100, John Wiley & Sons, Inc., Hoboken, NJ.

Nath, S., Bodhak, S., and Basu, B. (2009) HDPE-Al_2O_3-HAp composites for biomedical applications: Processing and characterizations. Journal of Biomedical Materials Research, Part B, 88B, 1-11.

Newcomb, C.J., Bitton, R., Velichko, Y.S., Snead, M.L., and Stupp, S.I. (2012) The role of nanoscale architecture in supramolecular templating of biomimetic hydroxyapatite mineralization. Small, 14, 2195-2202.

Nilsson, M., Zheng, M.H., and Tagil, M. (2013) The composite of hydroxyapatite and calcium sulphate: A review of preclinical evaluation and clinical applications. Expert Reviews of Medical Devices, 10, 675-684.

Oliveira, A.L. and Reis, R.L. (2005) Bonelike apatite coatings nucleated on biodegradable polymers as a way to induce bone mineralization: Current developments and future trends. In R.L. Reis and J.S. Roman, Eds., Biodegradable Systems in Tissue Engineering and Regenerative Medicine, p. 205-217, CRC Press, Boca Raton, FL.

Pasteris, J.D., Wopenka, B., Freeman, J.J., Rogers, K., Valsami-Jones, E., van der Houwen, J.A.M., and Silva, M.J. (2004) Lack of OH in nanocrystalline apatite as a function of degree of atomic order: Implications for bone and biomaterials. Biomaterials, 25, 229-238.

Sadat-Shojai, M., Khorasani, M.-T., Dinpanah-Khoshdargi, E., and Jamshidi, A. (2013) Synthesis methods for nanosized hydroxyapatite with diverse structures. Acta Biomaterialia, 9, 7591-7621.

Shi, D. (2006) Introduction to Biomaterials, World Scientific, Singapore.

Stevens, M.M. (2008) Biomaterials for bone tissue engineering. Materials Today, 11, 18-25.

Tas, A.C. (2013) X-ray amorphous calcium phosphate (ACP) synthesis in a simple biomineralization medium. Journal of Materials Chemistry B, 1, 4511-4520.

Thomas, M.V. and Puleo, D.A. (2009) Calcium sulfate: Properties and clinical applications. Journal of Biomedical Materials Research Part B, 88B, 597-610.

Wen, J., Leng, Y., Chen, J., and Zhang, C. (2000) Chemical gradient in plasma-sprayed HA

coatings. Biomaterials, 21, 1339-1343.

White, A.A., Kinloch, I.A., Windle, A.H., and Best, S.M. (2010) Optimization of the sintering atmosphere for high-density hydroxyapatite-carbon nanotube composites. Journal of the Royal Society Interface, 7, S529-S539.

Wopenka, B. and Pasteris, J.D. (2005) A mineralogical perspective on the apatite in bone. Materials Science and Engineering C, 25, 131-143.