Periodic Macroporous Hydroxyapatite-Containing Calcium Phosphates

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Three-dimensionally ordered macroporous materials containing the bioceramic hydroxyapatite as well as other calcium phosphate and carbonate phases have been synthesized using close-packed poly(methyl methacrylate) latex spheres (256−375 nm diameter) as templates. The interstitial spaces of the colloidal crystal were penetrated by a water/alcohol cosolvent solution of calcium nitrate and phosphoric acid. Calcination in the range 475−1000 °C removed the polymer and promoted crystallization of calcium phosphate phases in the pore walls. The materials were characterized by powder X-ray diffraction, FT-IR spectroscopy, and SEM. The best combination of composition and three-dimensional order was found in a crystalline hydroxyapatite/tricalcium phosphate templated with 375-nm spheres and calcined at 700 °C. An in vitro antibiotic drug release application was tested using this product.

Introduction

Hydroxyapatite, $Ca_5(PO_4)_3OH$, is a bioceramic analogous to the mineral component of bone. Its biocompatibility and osteoconductive properties make it desirable as an implant material and drug delivery agent. Precipitation[1,2] and solid-state methods[3] have been employed to synthesize hydroxyapatite. Obtaining stoichiometric hydroxyapatite by precipitation requires careful control of solution conditions (pH, temperature, rate of reagent addition, etc.) followed by sintering at high temperatures (>1000 °C). High-temperature sintering is also necessary for solid-state synthesis, along with thorough mixing of powders. Sol−gel methods offer mixing of calcium and phosphorus precursors on a molecular scale, which is advantageous in obtaining products of homogeneous composition. These methods allow hydroxyapatite to be processed into films and coatings on ceramic substrates such as alumina and titanium.[4−12] A bioactive calcium phosphate coating can improve bonding between natural bone and a titanium or titanium alloy implant.[13−16] Precursor combinations for sol−gel syntheses include $Ca(NO_3)_2{\cdot}4H_2O$ and P_2O_5,[5] $(HOCH_2CO_2)_2Ca$ with H_3PO_4 and $P(OH)_x(OCH_2CH_3)_{3-x}$,[7] $Ca(OCH_2CH_3)_2$ and $P(OCH_2CH_3)_3$,[4,17] $Ca(OCH_2CH_3)_2$ and H_3PO_4,[18] $Ca(NO_3){\cdot}4H_2O$ and *N*-butyl acid phosphate,[6] $(CH_3CO_2)_2Ca{\cdot}xH_2O$ and $PO(OCH_2CH_3)_3$;[19,20] and $Ca(NO_3)_2{\cdot}4H_2O$ and H_3PO_4.[10,11] Other phases that have been observed in the syntheses of hydroxyapatite include tricalcium phosphates, calcium oxide, and carbonatehydroxyapatite. Although many researchers strive for highly crystalline and pure hydroxyapatite, biphasic calcium phosphate ceramics might be more effective in biomedical applications. Biphasic hydroxyapatite/tricalcium phosphate has been found to promote osteogenesis through dissolution of the tricalcium phosphate phase, resulting in local concentrations of calcium and phosphate species that contribute to the formation of new bone.[21]

Hydroxyapatite's biocompatibility makes it a good candidate for implant drug delivery. It might be used to release antitumor agents or antibiotics for the treatment of osteomyelitis, a bone infection that is often treated by excision of necrotic tissue and irrigation of the wound. A hydroxyapatite cement loaded with antibiotic has been proposed as a treatment for osteomy-

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elitis, the cement being a material that would replace excised bone.[22,23] Doxorubicin hydrochloride adsorbed onto hydroxyapatite was studied as an implantation treatment in white rabbits for hepatic cancer.[24] The researchers also performed in vitro experiments with a combination of doxorubicin hydrochloride and buthionine sulfoximine adsorbed onto the material.[25] Hydroxyapatite blocks loaded with antibiotics have been used in human clinical studies and found to be very effective in fighting osteomyelitis.[26] Hydroxyapatite has also been studied as a protein delivery agent.[27]

Porosity in a biomaterial implant allows for the possibility of ingrowth of natural bone. The increase in surface area resulting from porosity would also contribute to a delivery agent's capacity to adsorb a drug. A new class of highly ordered porous materials, 3DOM (three-dimensionally ordered macroporous) materials, is receiving much attention. Colloidal crystals consisting of close-packed polymer or silica latex spheres are used as templates.[28−30] The interstices are penetrated by precursor mixtures, and then polymer is removed by calcination or silica by HF dissolution, leaving a structure of interconnecting spherical cavities. The wall compositions of 3DOM materials have been reviewed and include various metals, metal oxides, carbons, and polymers.[31−34] 3DOM products might find applications as catalysts, supports, battery materials, and photonic crystals. A 3DOM calcium oxide/silica bioglass displayed strong bioactivity in simulated body fluid, dissolving to form hydroxycarbonate apatite at a much higher rate than nontemplated bioglass of the same composition.[35]

The synthesis of 3DOM sol−gel-derived hydroxyapatite-containing calcium phosphates, 3DOM CaPOs, is reported here. Aqueous alcoholic cosolvent solutions of $Ca(NO_3)_2{\cdot}4H_2O$ and H_3PO_4 are used to fill the interstitial spaces of close-packed poly(methyl methacrylate) (PMMA) spheres. The wall composition and pore structure are affected by the precursor concentration and calcination temperature. An antibiotic drug adsorption/release study is also reported.

Experimental Section

Chemicals. $Ca(NO_3)_2{\cdot}4H_2O$ (Fisher), H_3PO_4 (85%, EM Science), absolute methanol (Pharmco), and norfloxacin (Sigma) were used as received without further purification. 2,2′-Azobis(2-methylpropionamidine)dihydrochloride (Aldrich) was recrystallized from water. Water was deionized to 17.6 MΩ·cm.

Synthesis of 3DOM CaPOs. Poly(methyl methacrylate) latex spheres were synthesized by literature methods, without a cross-linking agent, using 2,2′-azobis(2-methylpropionamidine)dihydrochloride as the initiator.[36,37] Two batches of monodisperse PMMA spheres were used in the syntheses reported here, having diameters of 256 (±5) and 375 (±4) nm. Colloidal crystal templates were prepared from the polymer in its original synthesis mixture by centrifugation at 1000 rpm to form pellets. PMMA was annealed at 130 °C for 10 min for some syntheses. A typical synthesis involved placing 0.5−1.5-cm pieces of broken polymer pellet inside a vacuum filtration flask to which a Buchner funnel with filter paper was attached. Precursor solutions were prepared by dissolving $Ca(NO_3)_2{\cdot}4H_2O$ and H_3PO_4 in 2:1 vol % H_2O/methanol, all solutions having a Ca/P ratio of 1.67. Products designated LC-CaPO (low-concentration calcium phosphate) were made using 0.167 M $Ca(NO_3)_2$/0.100 M H_3PO_4 concentrations. For MC-CaPO (medium-concentration) products, 0.666 M $Ca(NO_3)_2$/0.400 M H_3PO_4 solutions were used, and 1.34 M $Ca(NO_3)_2$/0.800 M H_3PO_4 solutions were used to prepare HC-CaPO (high-concentration) materials. Precursor solution was poured into the filtration flask and suction was applied for 5 min, causing mild agitation of the polymer latex to provide a thorough soaking. The wetted latex was then collected by suction filtration and set out to dry in air. Polymer was removed by calcination in a muffle furnace. The temperature was raised by 1 °C/min to 300 °C, held at this temperature for 5 h under flowing air, and then raised by 10 °C/min to 475, 600, 700, 800, or 1000 °C and held for 1 h. The final calcination temperature is added to a product's designation. For example, LC-CaPO-475 was calcined at 475 °C. HC-CaPO-MeOH products were made using only methanol as a precursor solvent. Annealed latex was required for syntheses using pure methanol.

Norfloxacin Drug Charging/Release. HC-CaPO-700 (0.600 g) was stirred in a 7.83×10^{-4} M solution of norfloxacin in simulated body fluid (SBF) for 2 h. Norfloxacin is an antibiotic that decomposes upon prolonged exposure to UV−visible light.[38] SBF was prepared by the literature method and buffered to pH 7.4 with tris(hydroxymethyl)aminomethane and 1 M HCl.[39] The charged product was collected by filtration and air-dried in a dark place. Samples of charged HC-CaPO-700 (25 mg) were placed inside polypropylene bottles containing 50 mL of SBF maintained at 37 °C and covered for protection from light. After determined periods, the solids were collected by filtration, and the filtrates were analyzed for norfloxacin by UV−visible spectrophotometry. Norfloxacin has a strong absorbance band at 274 nm, and a calibration curve was obtained using solutions of known concentration.

Characterization. FT-IR spectra were obtained on a Nicolet Magna-IR 760 spectrometer using KBr pellets. Powder X-ray diffraction (XRD) experiments were performed on a Siemens D5005 X-ray diffractometer using Cu Kα radiation, $\lambda = 1.540\ 56$ Å. Scanning electron micrography (SEM) images were recorded on a Hitachi S-800 scanning electron microscope operating at 4 kV. Samples for SEM were dusted on an adhesive conductive carbon disk attached to an aluminum mount and coated with 100 Å of Pt. Ultraviolet−visible spectrophotometry was performed with a Hewlett-Packard 8452A diode array spectrophotometer using 1-cm quartz cuvettes.

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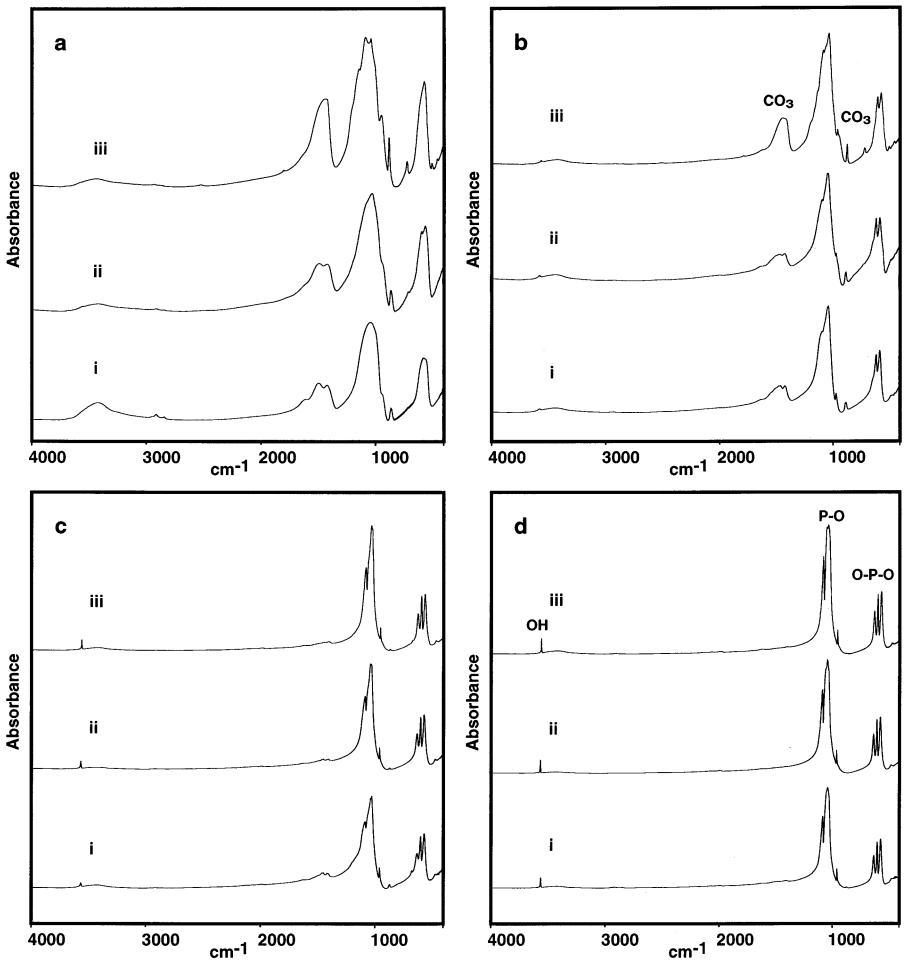

Figure 1. FT-IR spectra of CaPO products calcined at (a) 475, (b) 600, (c) 800, and (d) 1000 °C. Spectra of LC-CaPOs, MC-CaPOs, and HC-CaPOs are labeled i, ii, and iii, respectively. Absorbance bands attributed to O−H, P−O, and O−P−O vibrations are labeled in the spectrum of HC-CaPO-1000. Carbonate vibrations are labeled CO_3 in the spectrum of HC-CaPO-600.

Results and Discussion

Effects of Precursor Concentration and Temperature on Wall Composition and Pore Structure. Precursor concentrations and final calcination temperatures were varied in syntheses using PMMA spheres of 256-nm diameter centrifuged at 1000 rpm and partially annealed at 130 °C. Partial annealing of latex helps prevent disruption of its close-packed order and dispersion in hydrophobic solvents. It might also help maintain the integrity of the periodic macroporous structure, as the pore walls become more crystalline with increasing calcination temperature. Crystalline hydroxyapatite yields a distinct infrared spectrum consisting of intense P−O stretching bands at ca. 1040 and 1090 cm^{-1} (υ_3) and bands at ca. 962 (υ_1) and 499 cm^{-1} (υ_2) of lesser intensity. The υ_4 O−P−O bending mode appears in the range 570−630 cm^{-1}, and OH stretching is seen at ca. 3573 cm^{-1}. Thus, FT-IR spectroscopy was utilized to immediately evaluate the relative crystallinity of hydroxyapatite in various CaPO products. LC-CaPO and MC-CaPO products calcined at 475 °C (Figure 1a) consisted of noncrystalline calcium phosphate and a relatively large amount of carbonate (IR bands at 1430−1550, 875, and 720 cm^{-1}). The macropore walls appeared to be amorphous by powder XRD. HC-CaPO-475 produced a more crystalline XRD pattern in which a calcite ($CaCO_3$) reflection dominated over that of a calcium phosphate of indeterminate phase. All of the products displayed periodic macroporous structures when studied by SEM (Figure 2a).

Bands due to phosphate stretching were better resolved in the FT-IR spectra of CaPO products calcined at 600 °C (Figure 1b). XRD patterns of LC-CaPO-600 and MC-CaPO-600 showed poorly crystalline hydroxyapatite, calcite, and tricalcium phosphate (Figure 3). HC-CaPO-600 yielded a well-defined XRD pattern showing hydroxyapatite, calcite, β-tricalcium phosphate (β-TCP), and α-tricalcium phosphate (α-TCP). FT-IR spectra of materials calcined at 800 °C displayed good resolution of the υ-P−O and υ-O−P−O bands and relatively intense υ-OH peaks (Figure 1c). Only hydroxyapatite reflections were observed in the XRD patterns of LC-CaPO-800 and MC-CaPO-800, and the pattern of HC-CaPO-800 also displayed β-TCP (Figure 4). Carbonate bands were still seen in the FT-IR spectra at 1460 and 1550 cm^{-1} (υ_3) and 875 cm^{-1} (υ_2). Some carbonate substitution into the hydroxyapatite crystal lattice is expected when CO_3^{2-} or CO_2 is present. The locations of the bands are indicative of type A substitution in which CO_3^{2-} replaces two OH^- groups.[40,41] SEM images showed a degradation of the periodic macroporous

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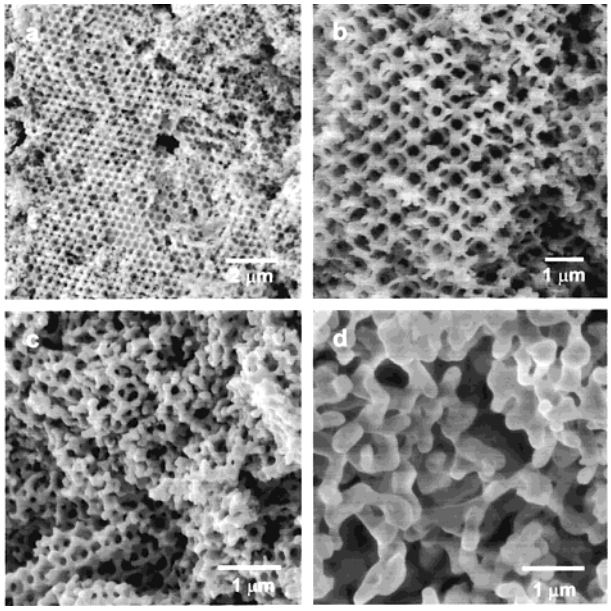


Figure 2. SEM images of HC-CaPOs calcined at (a) 475, (b) 600, (c) 800, and (d) 1000 °C. Three-dimensionally ordered macropore structures are seen for products calcined at 475 and 600 °C. The 3DOM structure is partially degraded in the material calcined at 800 °C and not present in the product calcined at 1000 °C.

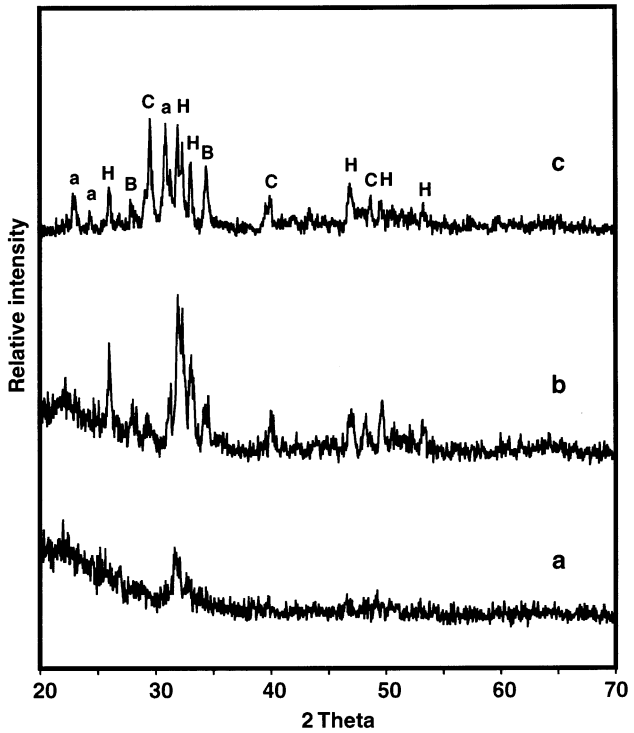


Figure 3. Powder XRD patterns of (a) LC-CaPO, (b) MC-CaPO, and (c) HC-CaPO calcined at 600 °C. Reflections corresponding to specific phases are labeled H (hydroxyapatite), B (β-tricalcium phosphate), C (calcite), and a (α-tricalcium phosphate).

structure, most notably for the HC product (Figure 2c).

The sol−gel process combined with calcination at 1000 °C produced CaPOs containing high-quality hydroxyapatite, evident by FT-IR spectroscopy (Figure 1d) and XRD (Figure 5), with virtually no carbonate. As with LC-CaPO-800, LC-CaPO-1000 yielded only hydroxyapatite reflections in its XRD pattern. Traces of

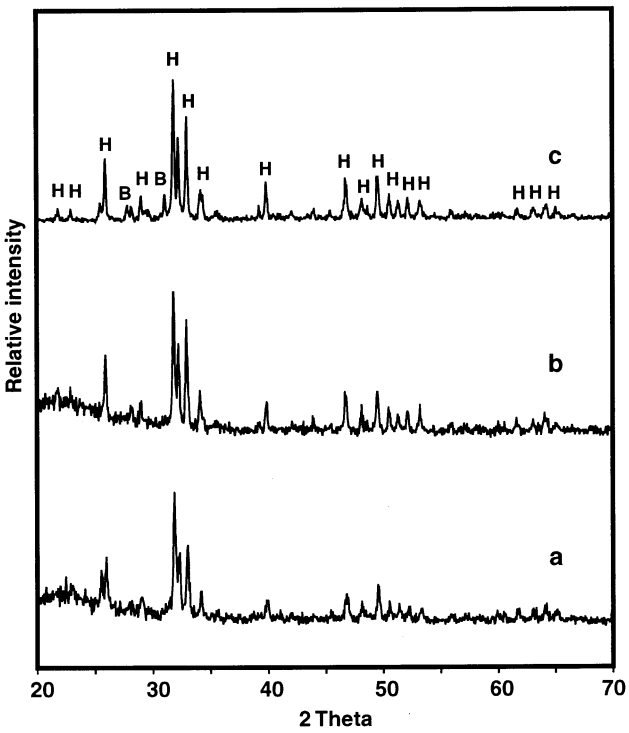


Figure 4. Powder XRD patterns of (a) LC-CaPO, (b) MC-CaPO, and (c) HC-CaPO calcined at 800 °C. Reflections corresponding to specific phases are labeled H (hydroxyapatite) and B (β-tricalcium phosphate).

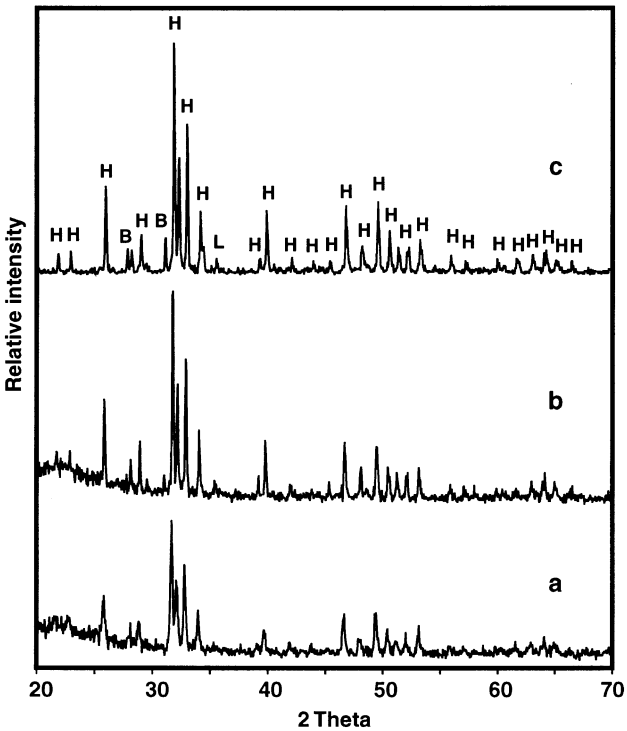


Figure 5. Powder XRD patterns of (a) LC-CaPO, (b) MC-CaPO, and (c) HC-CaPO calcined at 1000 °C. Reflections corresponding to specific phases are labeled H (hydroxyapatite), B (β-tricalcium phosphate), and L (lime).

β- and α-TCP were present in the XRD pattern of MC-CaPO-1000. HC-CaPO-1000 was found to be composed of crystalline hydroxyapatite (major phase) and β-TCP. A small amount of calcium oxide (lime) might also have been present, although this phase is difficult to distinguish because its most intense XRD reflection ($d = 2.41$

Å) overlaps with a β-TCP peak. Decomposition of hydroxyapatite to tricalcium phosphate and calcium oxide has been suggested to occur at higher temperatures as a result of minute impurities or nonstoichiometry.[42,43] The high crystallinity of the phases came at the expense of the ordered macroporosity. No 3DOM structures could be seen by SEM in products calcined at 1000 °C (Figure 2d), including LC-CaPO-1000.

Although fewer tricalcium phosphate phases were found in LC-CaPO products, HC-CaPO syntheses provided more material for study. HC-CaPOs were also synthesized from precursor solutions using only methanol as the solvent to more thoroughly wet the hydrophobic latex. HC-CaPO-MeOH products were calcined at 475, 600, and 800 °C. Undesired phases were obtained without water, with calcite dominating in the XRD patterns of products calcined at 475 and 600 °C and calcium oxide as the most crystalline phase in the material calcined at 800 °C. As with HC-CaPO materials made from aqueous alcoholic precursor solutions, 3DOM structures were obtained for products calcined at 475 and 600 °C. The periodic structure was lost upon calcination at 800 °C.

The most attractive combination of ordered macroporous structure and wall composition was found in HC-CaPO products calcined at 700 °C. Annealing the latex was not necessary, as the colloidal crystals maintained their integrity in the water/methanol cosolvent and the periodic porous structure was not threatened by crystallite growth at this calcination temperature. PMMA latex spheres 375 nm in diameter were used to synthesize the product shown in Figure 6b. An average pore size of 313 nm was measured by SEM. CaPO products calcined at 700 °C or higher were white in color, whereas those calcined at lower temperatures tended to be gray or brown as a result of incomplete decomposition of polymer. HC-CaPO-700 materials displayed some iridescence, the diffraction of light being an indicator of the quality of the 3DOM structure. XRD showed the presence of hydroxyapatite (major phase) and β-TCP (Figure 6a). One low-intensity reflection (d = 2.91 Å) was indexed to α-TCP. Some carbonate was naturally present and observable by FT-IR spectroscopy.

Conventional sol−gel or precipitation processes could not be applied successfully to this system. Adapting the method of Kordas and Trapalis,[19,20] an aqueous alcoholic cosolvent precursor solution of calcium acetate and triethyl phosphate was used to wet close-packed latex, followed by addition of more alcohol to cause gelation. Another attempt employed a solution of calcium nitrate and diammonium hydrogen phosphate as described by Jarcho et al.[1] The polymer spheres used in the above approaches were synthesized with potassium persulfate initiator. Anionic sulfate, hydroxyl, and carboxylic acid groups exist on the surfaces of spheres made with this initiator,[37,44] as shown in Scheme 1a. Acid was added to the precursor solution to prevent immediate precipitation. Aqueous ammonia or ammonia vapor was applied to the wetted latex to raise the pH and form a calcium phosphate gel. Such two-step reactions either did not produce 3DOM structures or resulted in mixtures of bulk and 3DOM products. 3DOM products are most reproducible when gelation or precipitation occurs quickly upon wetting of the latex.

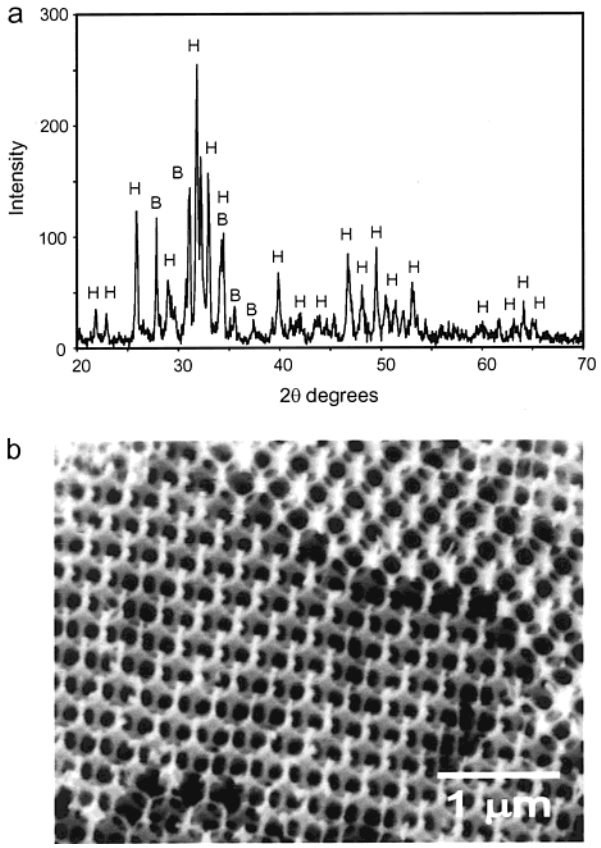
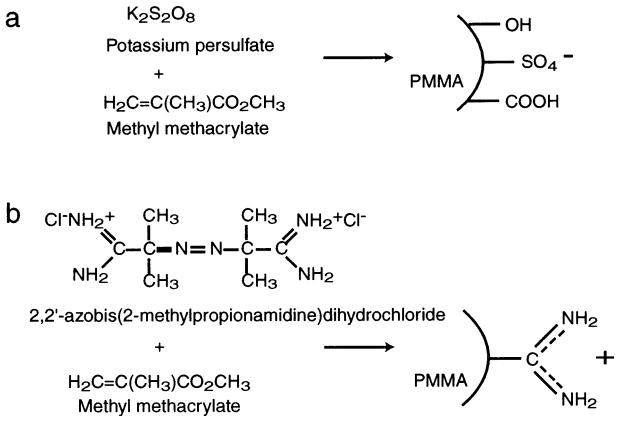


Figure 6. (a) Powder XRD pattern and (b) SEM image of HC-CaPO-700. Reflections corresponding to hydroxyapatite and β-tricalcium phosphate are labeled H and B, respectively.

Scheme 1. Radical Initiators (a) Potassium Persulfate and (b) 2,2′-Azobis(2-methylpropionamidine)dihydrochloride Used in PMMA Syntheses and the Resulting Sphere Surface Groups (Adapted from ref 37)



An azo initiator was used in the synthesis of PMMA spheres that were able to template 3DOM structures with acidic precursor solutions. This initiator provides cationic amino groups of the type shown in Scheme 1b on a sphere's surface.[37] The PMMA does not cause a noticeable change in pH when dispersed in water. However, such groups might cause a pH increase in

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their immediate environment, promoting calcium phosphate gelation, as seems to be the case here. Another possibility is that the cationic surface groups might serve as anchoring points for anionic phosphate species, contributing to the initiation of a calcium phosphate network. Chemical reactivity is the best evidence for the presence of surface groups. The IR absorbance bands of such groups were not distinguishable from those of PMMA in FT-IR spectra of the polymer.

Calcination serves a dual purpose, removing polymer to create a porous structure and crystallizing calcium phosphate in the walls. Higher temperatures resulted in hydroxyapatite phases of higher crystallinity and larger grain size, as seen by a decrease in the fwhm of the XRD reflections. The existence of tricalcium phosphate [$Ca_3(PO_4)_2$] might be unavoidable in the system studied here, as the low pH of the precursor solutions contributes to the formation of calcium phosphate of lower stoichiometry.[12] Indeed, more tricalcium phosphate is seen in products synthesized with higher-concentration precursor solutions, the phosphate source being phosphoric acid. Biphasic calcium phosphate is beneficial for some biomedical applications. The presence of soluble tricalcium phosphate increases the ability of a calcium phosphate material to be resorbed by the body while stimulating new bone growth.[3,21,45] Another difficulty encountered in this system is the loss of ordered porosity at higher calcination temperatures. The use of larger diameter spheres would naturally provide larger interstitial spaces for crystallite growth to occur without damaging the 3DOM structure. Much larger spheres would be needed to template the pore size required for osteoinduction to occur in a bone implant (100 μm). Dental implant materials only require pore sizes as low as 5−10 μm.

Norfloxacin Adsorption and Release. Norfloxacin is an antibiotic that has been used as a target drug in studies of hydroxyapatite cement as a drug release agent.[22,23] In those reports, dry drug was mixed with a calcium phosphate paste, which was then hardened. For this study, HC-CaPO-700 was charged in a saturated solution of norfloxacin in simulated body fluid. The UV−visible absorbance spectrum of the filtrate was noticeably lower in intensity compared to that of the original charging solution, suggesting an adsorption of 5.9 wt %. A distinct release curve was obtained from exposure of charged HC-CaPO-700 to SBF at 37 °C (Figure 7). The plateau of the curve indicates an actual loading of 4.4 wt %, a result of some of the drug solution bleeding out while the charged material dried. The quick release of norfloxacin within 1 h is not unexpected, as 3DOM CaPOs have open structures with pores much larger than the size of a norfloxacin molecule. Functionalization of a 3DOM CaPO will be required for controlled release.

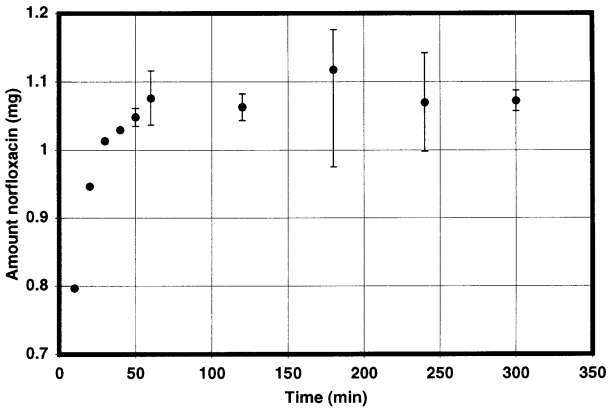

Figure 7. Release of norfloxacin from charged HC-CaPO-700 in SBF at 37 °C.

Conclusion

Colloidal crystals of PMMA spheres have been used to template periodic macroporous calcium phosphate bioceramics. Calcination at a temperature in the range of 475−1000 °C removes the polymer and crystallizes calcium phosphate in the walls. Hydroxyapatite is commonly present, along with tricalcium phosphate phases. Three-dimensional order, existent phases, and degree of crystallinity are affected by precursor solution concentration and calcination temperature. A well-ordered product consisting of hydroxyapatite and tricalcium phosphate was synthesized with an aqueous alcoholic solution of 1.34 M $Ca(NO_3)_s$/0.800 M H_3PO_4 and 375-nm PMMA spheres followed by calcination at 700 °C. The material could adsorb and release an antibiotic in vitro, although it did so too quickly for a controlled release application.

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