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High-strength, *in situ*-setting calcium phosphate composite with protein release

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Abstract

The aim of this study was to develop a mechanically-strong calcium phosphate cement (CPC) with protein release. Chitosan was used to strengthen CPC and control protein release. Mass fraction of protein release = mass of released protein/mass of total protein incorporated into the specimen. Flexural strength (mean $\pm$ sd; $n = 6$) of CPC containing 100 ng/mL of protein increased from 8.0 ± 1.4 MPa with 0% chitosan, to 19.8 ± 1.4 MPa with 15% chitosan ($p < 0.05$). The latter exceeded the reported strengths of sintered porous hydroxyapatite implants and cancellous bone. When the chitosan mass fraction was increased from 0% to 10% and 15%, protein release varied from 0.60 ± 0.03 to 0.41 ± 0.04 , and to 0.23 ± 0.07 , respectively ($p < 0.05$). When powder:liquid ratio increased from 2:1 to 3:1 and 4:1, protein release changed from 0.89 ± 0.10 to 0.41 ± 0.04 , and to 0.23 ± 0.07 , respectively $p < 0.05$. Therefore, chitosan content and powder:liquid ratio successfully controlled the protein release. The protein release mass fraction, M , was related to CPC porosity P by: $M = 16.9 P^{4.5}$. In summary, a mechanically-strong CPC with controlled protein release was formulated. Protein release was proportional to CPC porosity. The *in situ*-hardening, nano-apatite composite may have potential for bone tissue engineering, especially when both mechanical strength and controlled release of therapeutic/bioactive agents are needed. © 2007 Wiley Periodicals, Inc. *J Biomed Mater Res* 85A: 388–396, 2008

Keywords

calcium phosphate cement; protein release; chitosan; stress-bearing; bone tissue engineering

INTRODUCTION

More than a million surgical procedures are performed annually in the U.S. to repair bone defects caused by trauma, disease, or other congenital defects.¹ Furthermore, the need for osseous treatment is increasing dramatically as the world population ages.¹ Extensive studies have been performed to develop biomaterials and scaffolds for bone repair.^{2–6} Hydroxyapatite has been used as a matrix for hard tissue repair because of its similarity to the carbonated apatite in bones.^{7–10} There are several calcium phosphate cements (CPC) that can self-harden *in*

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Certain commercial materials and equipment are identified to specify experimental procedures. In no instance does such identification imply recommendation by NIST or the ADA Foundation or that the material identified is necessarily the best available for the purpose. Unless otherwise specified, one standard deviation was used as the estimated standard uncertainty of the measurements. These values should not be compared with data obtained in other laboratories under different conditions.

situ to form hydroxyapatite, with the advantage of intimate adaptation to neighboring bone, without the machining needed by sintered hydroxyapatite implants.^{11–15}

The first CPC was developed in 1986, consisting of a mixture of tetracalcium phosphate and calcium phosphate anhydrous.¹¹ The CPC powder was mixed with water to form a paste that was sculpted during surgery to conform to the defects in hard tissues. The paste then set *in situ* to form crystalline hydroxyapatite.¹⁶ A major disadvantage of current orthopedic implant materials was that they existed in a hardened form, requiring the surgeon to fit the surgical site around the implant or to carve the graft to the desired shape.¹ This led to increases in bone loss, in trauma to the surrounding tissue, and in surgical time.¹ Therefore, CPC's moldability and *in situ* hardening ability, together with its excellent osteoconductivity, made it highly desirable for orthopedic repair. As a result, CPC was approved in 1996 by the Food and Drug Administration for repairing craniofacial defects in humans, thus becoming the first CPC available for clinical use.¹⁶

However, because of its low strength, the use of CPC was "limited to the reconstruction of non-stress-bearing bone",¹⁷ and "clinical usage was limited by ... brittleness...".¹⁶ Therefore, in more recent studies, stronger CPC was formulated to extend the use to stress-bearing repairs.^{18–20} Absorbable fibers and meshes provided excellent reinforcement to the CPC implant.^{19,20} The fibers could then dissolve away to create long, cylindrical macropores in CPC for cell infiltration and tissue ingrowth.^{19,20} The addition of a biocompatible and biodegradable polymer, chitosan lactate, also significantly increased the strength and toughness of CPC.^{21–23}

Recently, growth factors and proteins were incorporated into CPC.^{24–28} Transforming growth factor- β (TGF- β) in a cement was found to stimulate bone cell differentiation and osteoconductivity.^{24,26} In these studies, a commercial cement was used without further reinforcement, and no relationship between protein release and calcium phosphate microstructure was investigated. Another study developed a poly(DL-lactic-co-glycolic acid)-calcium phosphate composite, and demonstrated the feasibility of delivering recombinant human bone morphogenetic protein-2.²⁸ However, the strength of the protein-releasing CPC was significantly degraded compared to that without protein.²⁸ Another study found that about half of the protein was released from CPC in 140 h.²⁵ The diametral tensile strength of the protein-containing CPC was relatively low, ranging from 3.5 to 6.4 MPa.²⁵ However, no effort was made to improve the strength of protein-releasing CPC for stress-bearing applications, no effort was made to systematically vary the CPC microstructure to control the protein release, and no relationship was explored between CPC porosity and protein release.

Accordingly, the objectives of the present study were to: (1) develop mechanically-strong CPC with the capability of protein release; (2) control the protein release via tailoring the CPC microstructure; and (3) establish the relationship between CPC porosity and protein release. The chitosan content in the CPC composite and the powder:liquid ratio were systematically varied to serve a twofold purpose: to enhance the mechanical properties of the protein-containing CPC and to control the protein release.

MATERIALS AND METHODS

Cement powder and liquid

The CPC powder consisted of a mixture of tetracalcium phosphate (TTCP: $\text{Ca}_4[\text{PO}_4]_2\text{O}$) and dicalcium phosphate anhydrous (DCPA: CaHPO_4) at a TTCP:DCPA molar ratio of 1:1. As described previously,^{11,18} the TTCP powder was synthesized from a solid-state reaction between CaHPO_4 and calcium carbonate, then ground and sieved to obtain TTCP particles

with a median size of 17 μm . The DCPA powder was ground to obtain particles with a median diameter of 1 μm . The TTCP and DCPA powders were then mixed to form the CPC powder.

Chitosan and its derivatives are natural biopolymers found in arthropod exoskeletons; they are biocompatible, biodegradable, and hydrophilic.²⁹ Although chitosan is not bioactive, the bioactivity can be provided by CPC in a CPC-chitosan composite. The purpose of incorporating chitosan into CPC in the present study was to strengthen CPC and to potentially control the protein release via changing the CPC porosity. The CPC liquid used in this study consisted of chitosan lactate (Technical grade, VAN-SON, Redmond, WA; referred to as chitosan)^{21,30} mixed with a phosphate buffered saline solution (PBS) (Invitrogen, Carlsbad, CA). The PBS contained concentrations of 0.21 g/L of KH_2PO_4 , 9.00 g/L of NaCl, and 0.726 g/L of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, at a pH of 7.2. Four cement liquids were made at chitosan/(chitosan + PBS) mass fractions of 0, 5, 10, and 15%, following a previous study.²¹ Chitosan mass fractions of 20% or higher were not included because the CPC paste became relatively dry.

Specimen fabrication

Protein A, fluorescently-labeled with fluorescein isothiocyanate (FITC), was used as a model compound for protein and growth factor release. Protein A (Sigma, St. Louis, MO) is a cell surface receptor consisting of a single poly-peptide chain of a molecular weight of 42 kDa (Da = g/mol and stands for Daltons, and is a measure of molecular mass for proteins and biological molecules). It was selected because it was similar in size and structure to bone morphogenetic protein-2 and TGF- β , and because previous studies indicated that protein A was a suitable model protein for release measurements.^{25,31} The relative molecular mass of protein A was close to the 36 kDa of bone morphogenetic protein-2 and the 25 kDa of TGF- β , while protein A was much less expensive.^{32,33} In the present study, as-received FITC-labeled protein A was dissolved in PBS (pH of 7.2) at a concentration of 5 mg/mL, and was kept frozen at -80°C until needed.

Two groups of specimens were fabricated to measure the mechanical properties of protein-containing CPC. The purpose of the first group was to examine whether incorporating protein A would change the mechanical and physical properties of CPC. The purpose of the second group was to investigate the effect of chitosan content and powder:liquid ratio on the mechanical properties of the CPC-protein composite.

For the first group, protein A-FITC was added to the PBS (without chitosan) at two different concentrations: 0 ng/mL and 100 ng/mL. The CPC paste was formed by mixing the CPC powder manually with each solution at a powder:liquid mass ratio of 3:1. This mixture was then placed into a 3 mm $\times$ 4 mm $\times$ 25 mm stainless steel mold to make flexural specimens. The composite mixture was covered with a glass slide on each side, clamped and incubated in a humidor with 100% relative humidity at 37°C for 24 h.²³ The specimens were then demolded for mechanical testing.

For the second group, a 3×4 full factorial design was used with four chitosan mass fractions (0, 5, 10, and 15%) and three powder:liquid mass ratios (2:1, 3:1, and 4:1). Protein A was added to each cement liquid at a fixed concentration of 100 ng/mL. The specimens were fabricated as described earlier for mechanical testing.

Testing of mechanical and physical properties

Each specimen was fractured using a three-point flexural test with a span of 20 mm at a crosshead speed of 1 mm/min on a computer-controlled Universal Testing Machine (5500R, MTS, Cary, NC).¹⁸ The standard uncertainty is estimated to be about 3% based on specimen dimension measurement. The following properties were evaluated: flexural strength, elastic

modulus, and work-of-fracture (the energy required to fracture the specimen; obtained from the area under the load-displacement curve divided by the specimen's cross-sectional area).¹⁸

The specimen halves from the flexural test were used to measure the density and porosity. The specimens were dried in a vacuum oven at 60°C for 24 h. The density was measured by the specimen mass divided by the specimen volume.¹⁸ The volume was calculated by the specimen dimensions measured with a micrometer, with each linear dimension the average of three locations along the specimen.¹⁸ Six specimens were thus measured for each composition. The standard uncertainty is estimated to be about 3% based on specimen dimension measurement.

For CPC specimens without chitosan, the porosity of the specimen, P , can be obtained by

$$P = (d_{\text{HA}} - d) / d_{\text{HA}} \quad (1)$$

where d_{HA} is the density of fully-dense hydroxyapatite without chitosan and is equal to 3.14 g/cm³,¹⁸ and d is the measured density of the specimen. For CPC specimens with chitosan, the porosity can be similarly calculated by using the measured density of the composite specimen, the density of chitosan lactate (which is 0.55 g/cm³), together with the masses of the components used to make the specimen.³⁴

Hydroxyapatite formation in protein-containing CPC was examined with X-ray diffraction (XRD). The 002 peak intensity of hydroxyapatite was used to measure the percentage of CPC conversion to hydroxyapatite. The specimens were dried and milled into powder, and the XRD patterns were recorded with a powder X-ray diffractometer (Rigaku, Danvers, MA) with graphite-monochromatized copper K_{α} radiation ($\lambda = 0.154$ nm) generated at 40 kV and 40 mA. Data were collected in a continuous scan mode ($1^{\circ} 2\theta \text{ min}^{-1}$, step time 0.6 s, step size 0.01°). The uncertainty for this measurement was estimated to be about 1%.

Protein A-FITC release measurement

Two groups of specimens were fabricated to measure protein release. The first group was to study the effect of chitosan content. The four cement liquids at 0, 5, 10, and 15% chitosan were used. Protein A-FITC concentration was fixed at 100 ng/mL and the powder:liquid ratio was fixed at 3:1.

For the second group, preliminary studies showed that CPC with 10% chitosan had a high strength, while 5% chitosan had little effect. At powder:liquid = 4:1, 15% chitosan could not be mixed because the paste was too dry, while 10% chitosan could be readily mixed. Therefore, the 10% chitosan liquid was selected for this group. Protein A-FITC was added to the liquid at a concentration of 100 ng/mL. The CPC powder was mixed with the liquid at three different mass ratios: 2:1, 3:1, and 4:1 to examine the effect of powder:liquid ratio.

To measure protein release, the fluorescence emission intensity of FITC-labeled protein A was measured using a microplate reader (Wallac 1420 Victor,² Perkin Elmer Life Science, Gaithersburg, MD), with the excitation filter set to 485 nm and the emission filter set to 535 nm. Each specimen of approximately $3 \times 4 \times 12 \text{ mm}^3$ was placed into a 15 mL centrifuge tube, 10 mL of PBS (pH 7.2) was added and the tube was firmly capped. Centrifuge tubes were placed in a 37°C incubator for the duration of the experiment. At each time interval, 100 μL of solution was removed from each tube and added separately to the wells of a 96-well microplate. A standard curve of protein A-FITC concentration versus intensity was constructed and used to calculate the protein concentration. The uncertainty for this measurement was estimated to be about 1%.

A scanning electron microscope (SEM, JSM-5300, JEOL, Peabody, MA) was used to examine the specimens. One-way and two-way ANOVA were performed to detect the significant effects in the data, and Tukey's multiple comparison test was used at p of 0.05.

RESULTS

Mechanical properties of protein-containing CPC

Effect of protein incorporation—The effect of protein incorporation on the mechanical properties and hydroxyapatite conversion of CPC is shown in Figure 1. Each value is mean $\pm$ standard deviation (sd); $n = 6$. Adding protein to the CPC liquid (without chitosan) at a concentration of protein/CPC liquid = 100 ng/mL did not significantly change the physical properties of CPC ($p > 0.1$), except significantly increasing its work-of-fracture ($p < 0.05$).

Effect of chitosan and powder:liquid ratio on protein-containing CPC—Two-way ANOVA for the 3×4 design showed significant effects ($p < 0.05$) of powder:liquid ratio and chitosan content on the mechanical properties of protein-containing CPC (Fig. 2). There was a significant interaction between powder:liquid ratio and chitosan ($p < 0.05$). Increasing the chitosan mass fraction from 0 to 15% significantly increased the strength and work-of-fracture of CPC-protein composite ($p < 0.05$). This was especially evident for powder:liquid of 3:1, for which the strength was increased by more than 2-fold from 8 MPa at 0% chitosan, to 19.8 MPa at 15% chitosan ($p < 0.05$). Meanwhile, the work-of-fracture was increased from 39.9 J/m² at 0% chitosan, to 107 J/m² at 15% chitosan ($p < 0.05$). However, elastic modulus was not significantly increased ($p > 0.1$) except for powder:liquid of 4:1, for which the modulus at 10% chitosan was significantly higher than that at 5% chitosan ($p < 0.05$). At a powder:liquid ratio of 4:1, when the chitosan mass fraction was increased to 15%, the cement paste became dry and difficult to mix, hence no specimens were made.

Protein release

Effect of chitosan content—The mass fraction of protein released from the first group of specimens is plotted in Figure 3. The mass fraction of protein release = Mass of protein released from the specimen/Total mass of protein incorporated into the specimen. Two-way ANOVA showed significant effects of chitosan content and immersion time, with a significant interaction between the two parameters ($p < 0.05$). When no chitosan was present, the release profile showed a moderate release of protein during the first 400 h, up to a mass fraction of 0.51 ± 0.05 . After this point, there was minimal release for the remaining 600 h of the experiment, up to a mass fraction of 0.60 ± 0.03 . Specimens containing 15% chitosan did not display a significant initial release burst. Instead, it exhibited a slow increase in protein concentration up to a 0.29 ± 0.05 fraction at 1200 h.

Effect of powder:liquid ratio—The effect of powder:liquid ratio on protein release from the second group of specimens is plotted in Figure 4. Two-way ANOVA showed significant effects of powder:liquid ratio and immersion time, with a significant interaction between the two parameters ($p < 0.05$). At a 2:1 ratio, there was a strong initial burst of protein release followed by a steady increase, reaching a high mass fraction of 0.88 ± 0.09 at 800 h. When the powder:liquid ratio was increased to 4:1, there was little initial burst; rather, a slow and moderate increase in protein release was seen up to 0.23 ± 0.07 mass fraction at 1000 h.

SEM micrographs of specimen surfaces are shown in Figure 5 for CPC with 0 and 15% chitosan, respectively, at a powder:liquid ratio of 3:1. In Figure 5(A), arrows indicate pores. SEM examination showed reduced porosity with chitosan incorporation in Figure 5(B). At a much higher magnification, arrows in Figure 5(C,D) indicate the elongated hydroxyapatite crystals. The thickness of these crystals was about 50–200 nm.

DISCUSSION

The incorporation of growth factors and proteins into the bone graft is highly beneficial in tissue engineering. This is because bone regeneration can become difficult because of factors such as older age, disease, and large-sized defects, thus necessitating therapeutic means to facilitate repair and new bone formation.³⁵ Hence, it would be desirable for a bone grafting system to be comprised of a stress-bearing extracellular matrix (natural or synthetic) containing diffusible growth factors/proteins.^{3,4} For example, TGF- β has been shown to stimulate the production of cartilage-specific proteoglycans by mesenchymal stem cells and induce the proliferation of osteoblasts and osteoblast-mediated collagen deposition.³⁶ Acidic and basic fibroblast growth factors have also been shown to increase the proliferation of numerous cell populations *in vivo*, including osteoblasts, chondroblasts, fibroblasts, and endothelial cells.³⁷ For these reasons, growth factors and proteins have been incorporated into CPCs in previous studies.^{24–28}

The present study differs from these previous studies in two respects: (1) The mechanical strength and work-of-fracture of the protein-releasing CPC were substantially increased via the reinforcement of chitosan; (2) The chitosan content and cement powder:liquid ratio were systematically tailored to control the protein release from CPC. The incorporation of protein A-FITC into CPC did not compromise the mechanical properties of CPC except significantly increasing its work-of-fracture or toughness (Fig. 1). The flexural strength of CPC at a powder:liquid ratio of 3:1 was ~8 MPa, similar to previous measurements.^{22,23,30} At the same powder:liquid ratio, with the incorporation of 15% chitosan, the flexural strength of CPC containing 100 ng/mL of protein reached 19.8 MPa. This exceeded the flexural strength of 2–11 MPa for sintered porous hydroxyapatite implants⁷ and a tensile strength of about 3.5 MPa for cancellous bone.³⁸ Hence the protein-containing CPC-chitosan composite may be useful in moderate stress-bearing applications.

The incorporation of protein did not retard the CPC conversion to hydroxyapatite (Fig. 1). The nano-sized hydroxyapatite crystals in protein-containing CPC, both without and with chitosan (Fig. 5), appeared similar in size and morphology to those observed in CPC without protein in previous studies.^{30,39–41} It should also be noted that the hydroxyapatite from CPC has been shown to be bioresorbable.^{16,17} It is suggested that because the hydroxyapatite from CPC is formed in an aqueous environment at body temperature of 37°C, it is more similar to the biological apatites than sintered hydroxyapatite formed at high temperatures.⁷ In the biomimetic fabrication of biomaterials, bone is considered to be a nanocomposite of nano-sized apatite minerals and proteins.¹⁰ Tooth enamel rods consist of apatite crystallites about 100 nm in diameter.⁴² Dentin and bone have smaller apatite crystals, with dimensions of 5 nm $\times$ 30 nm $\times$ 100 nm.⁴³ The nano hydroxyapatite crystals of protein-containing CPC and CPC-chitosan composite (Fig. 5) had sizes similar to those found in natural bone and teeth.

The chitosan content and powder:liquid ratio significantly affected the protein release. Chitosan is a biopolymer and could block some of the intrinsic pores in CPC, thus reducing the porosity. Increasing the powder:liquid ratio reduced the water content in the paste which in turn reduced the porosity. At 10% chitosan, the pore volume fraction, P , was measured (mean $\pm$ sd; $n = 6$) to be $(51.2 \pm 0.4)\%$ at powder:liquid of 2:1, $(44.1 \pm 0.3)\%$ at powder:liquid of 3:1, and $(33.8 \pm 1.0)\%$ at powder:liquid of 4:1. A porosity of 51.2% and 0.512 are used interchangeably in this article. The porosity P values are plotted in Figure 6 versus the mass fraction of protein released, M . The curve in Figure 6 is a regression power-law fit to the data, resulting in the following relationship:

$$M=16.9 P^{4.5} \quad (2)$$

with a correlation coefficient $R = 0.97$. Three points should be noted here. First, this equation suggests that increasing the volume fraction of pores in a specimen leads to a significant increase in the release of protein from the construct. Therefore, porosity is a key microstructural parameter that can be tailored to control the protein release. Second, Eq. (2) was obtained from experimental data within a porosity range from 0.338 to 0.512. There likely exists a high porosity P above which M reaches the maximum value of 1, and does not further increase with increasing P . Third, care should be taken in applying this equation to other biomaterials with pore sizes and interconnections vastly different from the CPC-chitosan composite. While the general form of $M = \alpha P^\beta$ may still be valid, different biomaterials and different CPC compositions may have different values of α and β .

Cellular response is dictated not only by these soluble molecules themselves, but also their state and mobility at the healing site. For example, when only a buffer was used as a carrier for bone morphogenetic protein (BMP), results indicated that there was a reduced number of responsive stem cells and insufficient retention of BMP at the repair site to promote bone regeneration.⁴⁴ Alternately, slowly releasing BMPs from an appropriate carrier could provide a physiological concentration of BMPs in the implant area and allow cells to be attracted by chemotaxis.⁴⁵ Along with the retention of BMPs at the repair site, BMPs mixed with CPC would be beneficial because of the fact that their bioactivity could be maintained.⁴⁶ In order for a particular bone graft therapy to be clinically relevant, an appropriate carrier must be designed that will maintain therapeutic levels of diffusible growth factors/proteins at the repair site. For the CPC with 10% chitosan, after 1200 h of immersion, the released protein mass fraction was about 0.4. Hence more than half of the protein was still retained in the CPC. Sustained protein release from this reservoir could occur as the hydroxyapatite matrix was gradually resorbed while new bone was formed *in vivo*. To this end, it should be noted that the present study did not examine the cellular response or new bone formation. The present study (1) formulated a mechanically-strong CPC with protein release; (2) determined the effects of systematic variations of powder:liquid ratio and chitosan content; and (3) established a relationship between protein release and CPC scaffold porosity. Further studies are needed to investigate the effects of controlled protein release from CPC on: (1) cellular response and (2) new bone formation in animal models.

SUMMARY

A mechanically-strong CPC with the capability of controlled protein release was formulated. This study represented the first effort in controlling the protein release from CPC by systematically changing the chitosan content and the powder:liquid ratio, thereby establishing a relationship between protein release and CPC porosity. The incorporation of chitosan more than doubled the strength of protein-containing CPC over that without chitosan. The protein-releasing composite had strengths matching/exceeding the strengths of sintered porous hydroxyapatite and cancellous bone. The strong CPC with protein release may be useful for bone repair in moderate stress-bearing locations. A relationship was established for the first time between the mass fraction of protein release, M , and the CPC porosity, P : $M = 16.9 P^{4.5}$. Hence protein release from CPC could be regulated to be application-specific by altering the CPC porosity. The relatively high-strength and osteoconductive CPC-chitosan composites with various porosities may be an effective delivery vehicle for osteoinductive growth factors, antibiotics, and other molecules necessary to promote bone regeneration.

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Figure 1.

Effect of protein incorporation on properties of CPC. Each value is mean $\pm$ standard deviation (sd); $n = 6$. Error bars show one standard deviation. Horizontal line connects values that are not significantly different (Student's t ; $p > 0.1$). Adding protein to the CPC liquid (without chitosan) at a concentration of protein/CPC liquid = 100 ng/mL did not change the physical properties of CPC ($p > 0.1$), except for significantly increasing the work-of-fracture ($p < 0.05$).

Figure 2. Effect of chitosan and powder:liquid ratio on mechanical properties of protein-containing CPC. Each value is mean $\pm$ standard deviation (sd); $n = 6$. Error bars show one sd.

Figure 3.

Effect of chitosan content in CPC on protein release. The mass fraction of protein release = Mass of protein released from the specimen/Total mass of protein incorporated into the specimen. Each value is mean $\pm$ sd ($n = 6$), with the error bar showing one sd. Chitosan had a significant effect ($p < 0.05$) on protein release from CPC.

Figure 4.

Effect of powder:liquid mass ratio on protein release. The mass fraction of protein release = Mass of protein released from the specimen/Total mass of protein incorporated into the specimen. Each value is mean $\pm$ sd ($n = 6$), with the error bar showing one sd. Powder:liquid ratio had a significant effect ($p < 0.05$) on protein release. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 5. SEM micrographs of typical surfaces of CPC specimens. Both materials contained 100 ng/mL protein in the cement liquid. (A, B) Lower magnification showing pores. (C, D) High magnification showing the nano hydroxyapatite crystals in CPC. In (A), arrows indicate pores. In (B), chitosan incorporation appeared to reduce the porosity in CPC. In (C) and (D), arrows indicate nano hydroxyapatite crystals. Chitosan incorporation in (D) appeared to render the crystals slightly smaller and less elongated.

Figure 6.

Effect of CPC porosity on protein release from CPC. The amount of protein release was taken at 1000-h immersion. Error bars show one standard deviation. The curve is a regression power-law fit to the data yielding $M = 16.9 P^{4.5}$, with a correlation coefficient $R = 0.97$. Protein release mass fraction $M = \text{Mass of protein released from the specimen} / \text{Total mass of protein incorporated into the specimen}$. A pore volume fraction of 50% and 0.50 are used interchangeably.