Reinforcement of injectable calcium phosphate cement by gelatinized starches

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Abstract: Current injectable calcium phosphate bone cements (CPC) encounter the problems of low strength, high brittleness, and low cohesion in aqueous environment, which greatly hinder their clinical applications for loading-bearing bone substitution and minimally invasive orthopedic surgeries. Here, a strategy of using gelatinized starches to reinforce injectable CPC was investigated. Four types of starches, namely corn starch, crosslinked starch, cationic starch, and Ca-modified starch, were studied for their influence on CPC mechanical properties, injectability, setting times, anticollapsibility, and cytocompatibility. Gelatinized starch significantly improved compressive strength and modulus as well as strain energy density of CPC to different extents. Specifically, both corn starch and Ca-modified starch revealed sixfold and more than twofold increases in the compressive strength and modulus of CPC, respectively. The addition of gelatinized starches with proper contents increased the injectability and anticollapsibility of CPC. In addition, osteoblast proliferation tests on leaching solution of modified cements showed that gelatinized starches had no adverse effect on cell proliferation, and all cement samples resulted in better osteoblast proliferation compared to phosphate-buffered solution control. The mechanisms behind the reinforcing effect of different starches were preliminarily studied. Two possible mechanisms, reinforcement by the second phase of gelatinized starch and strong interlocking of apatite crystals, were proposed based on the results of starch zeta potential and viscosity, cement microstructure, and resultant mechanical properties. In conclusion, incorporating gelatinized starches could be an effective, facile, and bio-friendly strategy to reinforce injectable CPC and improve its mechanical stability, and thus, should be further studied and developed. © 2015 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater, 104B: 615–625, 2016.

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INTRODUCTION

Calcium phosphate cement (CPC), first developed in the 1980s, has been increasingly used for orthopedic and orthodontic applications. CPC usually refers to the mixture of one or more types of calcium phosphate which undergo chemical reactions accompanied by setting and subsequent hardening when mixing with water or other aqueous solutions. The advantages of CPC over other types of bone cement like poly(methyl methacrylate) (PMMA) include high biocompatibility, bioactivity, biodegradability, and its chemo-physical similarity to natural bone mineral.1,2 Owing to these attractive traits, CPC becomes a promising biomaterial that can be potentially used for bone substitution and regeneration.3–5 In addition, because CPC is moldable and injectable before setting or hardening, it has the capability of conforming to the bone cavity of irregular shape6 or being injected into
bone defects without open surgery. Therefore, CPC is becoming a promising candidate for minimally invasive treatment of musculoskeletal injury and therefore has received lots of attention in the past decades.

For example, percutaneous vertebroplasty surgery and its variation like kyphoplasty are minimally invasive surgical procedures that rely on the use of bone cements (or plasticizer) such as PMMA or CPC. They have been recently introduced for medical treatment of osteoporosis-induced vertebral compression fracture by stabilizing the fractured vertebral body and restoring its original height before fracture. Taking kyphoplasty as an example, the minimally invasive procedure usually contains a few steps: (1) the fracture site and the circumference of both pedicles in vertebral body are examined and marked by fluoroscopy under the lateral and anterior–posterior image guidance; (2) a working cannula in the vertebral body is created through needle puncturing and a balloon is placed into the fracture site via the cannula under fluoroscopic observation; (3) vertebral height is restored by inflating the balloon, leaving a cavity to get filled by bone cement; and (4) a setting paste of bone cement is injected into the cavity of vertebral body and the cement can stabilize and restore vertebral body after solidification. The advantages of kyphoplasty and other percutaneous vertebroplasty surgeries include minimal trauma, less bleeding, fast and sustained pain relief, all of which are desirable for recovering physical function of patients and improving their living quality.

For applications in minimally invasive surgery, however, current CPC materials have a number of drawbacks that urgently need improvements. These problems are primarily associated with mechanical properties, including insufficient strengths, high brittleness, and prone to collapse when CPC setting in blood or other body fluids. The problems seriously restrict the application of CPC in repairing load-bearing bone and narrow its applicable range to the patients of different ages. Because of this reason, many efforts have so far been made to increase CPC mechanical strengths and improve its cohesion and anticollapsibility (also known as antiwashout property). For example, CPC doped with strontium has demonstrated fewer pores and decreased pore size compared to nondoped CPC, resulting in a higher strength at the early stage of surgical treatment. Polymer or ceramic fibers, hydroxyapatite crystals, and carbon nanotubes have also been incorporated to CPC as reinforcing components. In addition, adding hydrogels of polysaccharide derivatives to CPC is also an effective strategy for toughening and strengthening CPC.

To improve anticollapsibility of CPC, a strategy of adding starch to CPC has been reported recently. Starch is an important food source and has been processed to make all kinds of drugs and medical products owing to its attractive properties such as good biocompatibility, high stability, biodegradability, and so on. Also, starch possesses unique gelatinization property, which is a function of water content and temperature, and gelatinized starch has proper viscoelastic behavior to form robust and injectable paste. Therefore, CPC mixed with different kinds of starches have been shown to possess excellent injectability and anticollapsibility in liquids. In addition, our recent study showed that the compressive strength of gelatinized starch after drying reached as high as 400 MPa (measured in this study), suggesting that gelatinized starch may be a strong reinforcing phase when added to CPC whose compressive strength is only tens of MPa.

In light of this, we proposed that gelatinized starch might be used as additional component to increase the mechanical stability and integrity of CPC. In this study, the effects of various gelatinized starch on CPC properties, including injectability, anticollapsibility, compressive strength and modulus, strain energy density and cytotoxicity, were investigated. Four types of starches, corn starch, crosslinked starch, cationic starch, and Ca-modified starch, were studied. In addition, physical properties of these starches were measured and compared. Based on the experimental results, possible mechanisms behind the reinforcing effects of gelatinized starches on CPC were also discussed and proposed.

**MATERIALS AND METHODS**

**Chemicals**

Analytical dicalcium phosphate dehydrate CaHPO4·2H2O (DCPD), calcium carbonate (CaCO3), calcium chloride (CaCl2), and calcium nitride (Ca(NO3)2) were purchased from Sigma-Aldrich. Tetracalcium phosphate (TTCP) was purchased from Ensail (Beijing, China). Sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) were purchased from Shanpu (Shanghai, China). β-Tricalcium phosphate (β-TCP) was synthesized by solid reaction of DCPD and CaCO3 (molar ratio, 2:1) at 1300°C for 6 h according to the method reported by others. After calcination, the β-TCP was cooled in air and ground into powder.

**Starches**

Food-grade corn starch and waxy starch were purchased from Yuying (China) and cationic starch was bought from Jinlingta (Wuxi, China).

Crosslinked starch was prepared from corn starch by following the previously described methods. In brief, dry corn starch, sodium sulfate (10 wt % on starch basis), and 5 wt % of a 99:1 (weight ratio) mixture of STMP and STPP were mixed in deionized (DI) water. The mixture was adjusted to pH of 11.5 by 1.0M of NaOH solution and then stirred continuously for 3 h at 45°C. After the reaction, the starch slurry was adjusted to pH of 6.5 by 1.0M of HCl and then centrifuged at 5000 rpm for 10 min. The starch was then thoroughly rinsed with DI water to ensure the removal of unreacted inorganic salts. After drying overnight in an oven at 40°C, the crosslinked starch cake was ground into powder.

Ca-modified starch paste was prepared by treating the pristine waxy starch with calcium salts such as CaCl2 and Ca(NO3)2 in water at the temperature >60°C. In this study, Ca-modified starch paste was blended with CPC powder and the detail is in the following section.

**CPC and gelatinized starch-modified CPC**

In this study, the formulation of CPC powder contained 70 wt % of β-TCP, 20 wt % of TTCP, and 10 wt % of DCPD,
receiving to the published literatures.\textsuperscript{21,22} Before use, the
mixture of TTCP, β-TCP, and DCPD was ball-milled with
ethanol (weight, CPC:ball:ethanol = 4:30:9) for 15 h at
1400 rpm using a planetary ball mill. The slurry was then
dried at 80°C and ground into powder.

For preparing CPC paste, CPC powder was mixed with
dI water at a liquid-to-solid ratio of 0.4. After setting for 3
min, the paste was then injected through a 1-mL syringe
and formed a cylindrical bar with diameter of 4.8 mm for
further testing.

For preparing gelatinized starch-modified CPC samples,
dry CPC powder was mixed with different types of starches
at starch contents of 3 or 10 wt %. The powder mixtures
with starch contents of 3 and 10 wt % were then blended
with DI water at liquid-to-solid ratios of 0.75 and 1.05,
respectively. The starch suspension was then mechanically
stirred and gelatinized at 80°C to form a uniform paste. For
preparing CPC/Ca-modified starch, CPC powder with starch
and 9 wt % calcium salts was mixed with water and heated
to 80°C until starch was gelatinized to form a paste. The
CPC/gelatinized starch pastes were cooled down to room
temperature and then injected through a 1-mL syringe with
an outlet diameter of 4.8 mm, forming cylindrical paste bars
for further testing.

Characterization

\textbf{Zeta potential.} Surface charge of particles, represented by
zeta potential, was measured using Malvern Zetasizer Nano
ZS90 zeta meter. CPC powder, corn starch, crosslinked
starch, and cationic starch (before gelatinization) were
measured at acidic (pH = 4), neutral (pH = 7), and basic
(pH = 10) conditions, respectively. For measurement, 1 g of
powder was added to 2 mL of DI water and sonicated for 2
min before pH adjustment and subsequent testing. The pH
was adjusted by diluted HCl and NaOH and the test at each
pH value was repeated three times.

\textbf{Viscosity of gelatinized starch.} Viscosity is an important
physical property of gelatinized starch. In brief, 3.8 and 8.7
wt % of starch gelatinized in DI water were tested because
these contents were corresponding to the starch contents in
the pastes of CPC modified with 3 and 10 wt % of starches,
respectively. The viscosity was measured on a rotary viscom-
eter (LVDV-1, Fangrui, Shanghai, China) at a temperature of
80°C after the starch was gelatinized in water. Corn starch
was measured using No. 1 cone spindle at the speeds of 12
and 0.6 rpm for 3.8 and 8.7 wt % samples, respectively. Cross-
linked starch was measured using No. 0 cone spindle at the
speed of 60 rpm. For cationic and Ca-modified starches, 3.8
wt % of samples were measured using No. 2 cone spindle at
the speed of 3 rpm, whereas 8.7 wt % of samples were mea-
ured using No. 4 cone spindle at the speed of 12 rpm.

\textbf{Injectability, setting times, and shrinkage.} Injectability of
the cement was qualitatively evaluated by the following cri-
eteria. The cement paste was considered to have high inject-
ability when: (1) the paste could easily pass through 1-mL
syringe with an outlet of 4.8 mm in diameter under a force
about 50 N (a force equal to that of surgeon hand during
injection\textsuperscript{23}); and (2) morphology of the injected paste is a
continuous cylindrical and nonhollow bar with a smooth
surface. The injectability was categorized as medium to low
when the paste could be injected into a bar but its morphol-
yogy showed observable pores or rough surface. The paste
was considered noninjectable when it failed to meet either
of the two criteria mentioned above.

Setting times of the starch-modified CPC were measured
by following the method stated in the ASTM C266-89 and
other well-known studies.\textsuperscript{24} Briefly, a Gilmore indenter end-
capped with light and thick needle (weight, 113 ± 0.5; diam-
er, 2.12 ± 0.05 mm) and heavy and thin needle (weight, 453.6 ± 0.5 g; diameter, 1.06 ± 0.05 mm) was used to test
the initial and final setting times of newly formed cement
sample, respectively. During the test, both needles were
dropped onto the cement surface and the indentation was
repeated at intervals of 1 min until the cement bore the
needles without leaving perceptible mark. The total times
using different needles were defined as initial and final set-
ting times, respectively. Each test was repeated four times
and mean value and standard deviation were calculated.

Shrinkage of cement was evaluated by calculating shrink
rate of the cross-section of injected paste bar before and
after drying. The initial diameter of the paste bar was
4.8 mm (same as the outlet diameter of 1-mL syringe) and
the final diameter of the bars after 3-day drying at 37°C
was measured. The shrink rate of the cross-section equals
to \((1 - \frac{\text{[final area]} - \text{[initial area]}}{100\%} \times \frac{\text{[final area]} - \text{[initial area]}}{100\%})\).

\textbf{Anticollapsibility.} For evaluating anticollapsibility, cement
paste bar was injected into DI water directly, hardened in
water, and subjected to soaking and shaking tests. Specifi-
cally, the cement bars were tested by soaking in water for 7
days and then moved to an orbital shaker for shaking test
at the speed of 50 rpm for 1 day and then at 100 rpm for
another day. The cement sample was considered to have
a good anticollapsibility if it survived both the soaking and
the shaking tests.

\textbf{Compressive strength, compressive modulus, and strain
energy density.} For compression test, the cement bar was
Cut into cylindrical samples of 4.2 mm in diameter and
8.4 mm (two times of the diameter) in length, and then
dried at 37°C in air for 1 day. Both ending surfaces of each
cylindrical sample were polished against 500-grade sandpa-
paper to get parallel and smooth surfaces before the test. Uni-
axial compression tests were performed on a mechanical
tester (HY-1080, testing range, 0–500 N with precision of
2.5 N; Hengyi, Shanghai) operating at a crosshead speed of
1 mm/min. During the compression, force and displacement
were recorded until the sample failed and engineering
stress–strain curve was generated. The maximum stress
before failure was determined as compressive strength and
the linear range in the stress curve before failure was used
to calculate compressive modulus. Strain energy density of
sample was calculated by integrating the stress over strain
to the maximum point in the stress curve (i.e., the point of

compressive strength). Compression test was repeated for at least five times.

**Microstructure.** After compression test, fractured surface of cement sample was examined for microstructural features by scanning electron microscopy (SEM, FEI Quanta 250, acceleration voltage of 5 kV under a vacuum of 1.56 × 10⁻⁴ Pa). The samples were sputtered with Au–Pd layer for 2 min. (Quorum Technologies, SC7620) before SEM examination.

**Cytotoxicity.** Cytotoxicity of starch-modified CPC was evaluated by measuring osteoblast (bone-forming cell) proliferation in cell-culture medium mixed with cement-leaching solution. The cement-leaching solution was prepared by immersing 1 g of cement sample in 3 mL of DI water at 37°C for 2 days. The leach solution was then mixed with Dulbecco’s Modified Eagle’s Medium (DMEM, Invitrogen) supplemented with 10% of fetal bovine serum (FBS, Gibico) and 1% of penicillin/streptomycin (P/S, Sigma-Aldrich) for cell proliferation test up to 3 days. Briefly, in a 96-well plate, the mixture of 20 μL of leaching solution or phosphate-buffered solution (PBS, used as control) and 180 μL of DMEM supplemented with FBS and P/S was added to each well and mouse embryo osteoblasts (MC3T3-E1) were seeded at a density of 10,000 cell/well. The cells were cultured under standard cell culture conditions (5% CO₂ humidified air at 37°C) for 1 and 3 days. The 3-day period for MC3T3-E1 osteoblast proliferation test was selected by following a well-established study by Anselme et al.25 After the prescribed time periods, the cell-culture medium was removed and each well was rinsed with PBS for three times. For measuring cell proliferation, a commercially available cell counting kit-8 (CCK8, Dojindo) was used by following the manufacturer’s instructions. Specifically, 10 μL of CCK8 solution and 100 μL PBS were added to each well and incubated under the standard cell culture conditions for 2 h. After incubation, the solution in each well was transferred to a new 96-well plate and its optical density (OD) was spectrophotometrically measured on a microplate reader (BioTek MQX200R) at a wavelength of 450 nm. The measured OD value was corrected by subtracting the background of PBS and then normalized by the OD value of control sample (i.e., cells cultured with the mixture of PBS and DMEM supplemented with FBS and P/S). The normalized OD values reflected cell viability at prescribed time periods. The experiments were performed in triplicate and repeated for three times (n = 3).

**Statistical analysis**
Statistical results were analyzed using one-way analysis of variance (ANOVA) and the data were expressed as the mean ± standard deviation.

**RESULTS**
**Physical properties of starch and gelatinized starch**

**Surface charge.** Zeta potentials of different types of starch measured at acidic (pH = 4), neutral (pH = 7), and basic (pH = 10) conditions are shown in Figure 1. Zeta potential is a measurement of the average surface charge of the particles in a colloid suspension and all the samples show decreased zeta potentials as pH value increases. In this study, CPC samples were mixed with only DI water and the components of CPC became generally basic after hydrolysis and hence the zeta-potential results above pH of 7 are more relevant to working conditions in vivo. It is worth noting that zeta potential values of CPC (tested before setting) were near zero at three pH conditions. Given that CPC is a mixture of β-TCP, TTCP, and DCPA in this study, the small zeta potential suggests that CPC particles had low electrical stability in colloid and were therefore susceptible to agglomerate when pH was changed or other particles were added into the colloidal system. For different types of starch, their zeta potentials had large variations. Cationic starch, not surprisingly, had positive zeta potentials at all three pH conditions. However, corn and crosslinked starches showed negative zeta potential at all pH conditions and their zeta potential values were both similar and very large. Zeta potential of the crosslinked starch reached as high as −83 mV. The large zeta potentials of corn and crosslinked starches suggest high stability and dispersity in water as well as in acidic and basic liquids.

**Viscosity of gelatinized starch.** Viscosity of gelatinized starch is an important physical property for starch application and Table I lists the viscosity of different starches after gelatinized in water at two different starch contents. The starch contents of 3.8 and 8.7 wt % were selected because they corresponded to the starch contents in the pastes of CPC modified with 3 and 10 wt % of starch using different liquid-to-solid ratios, respectively. It is clear that starch contents had large effect on the viscosity of gelatinized starch and the viscosity drastically increased in all types of starches except crosslinked starch, whose viscosity kept extremely low (<10 mPa·s) even at the content of 8.7 wt %.
% Ca-modified starch had the highest viscosity values at both starch contents and cationic starch was also highly viscous. In fact, the 8.7 wt% Ca-modified starch and cationic starch after gelatinization were too thick and viscous to form injectable paste when mixed with CPC, excluding them from being used for injectable bone cements (see injectability results below). Gelatinized corn starch, in contrast, was very thin and had very low viscosity at the content of 3.8 wt%, but the viscosity increased by >100 times when the starch content increased to 8.7 wt%. In the case of mixing CPC with gelatinized starch, it is believed that viscosity plays an important role in cement homogeneity, injectability, and subsequently mechanical strengths after setting and hardening. These properties and their correlation to the viscosity of gelatinized starch are discussed later.

**Injectability, setting times, and shrinkage of modified CPC**

Injectability tests revealed that all CPC samples modified with 3 wt% of gelatinized starches exhibited high injectability (Table II), indicating that all the starch-modified CPC pastes could easily be injected through syringe and formed uniform cylindrical bars with smooth outer surfaces. It is important to note that the modified CPC actually had a slightly better injectability than pure CPC (Table II), suggesting the positive effect of gelatinized starches on improving CPC injectability. However, when starch contents increased to 10 wt%, the CPC incorporated with crosslinked starch, cationic starch, and Ca-modified starch revealed deteriorated injectability and the cement pastes actually failed to form uniform and continuous bars. At this content, only the CPC modified with corn starch remained acceptable injectability although the injection resistance was clearly increased compared to the case that 3 wt% of corn starch was injected through syringe.

Initial and final setting times of CPC and CPC samples modified with 3 wt% of corn starch and cationic starch are listed in Table III. It is also important to mention that most starch-modified CPC samples revealed similar initial and final setting times. Initial setting time indicates the time during which the material should not be manipulated to avoid possible damage to the cement structure, whereas final setting time defines the time when the cement has hardened, but maximum strength may not be achieved. CPC used in this study had an initial setting time about 15 min, whereas the initial setting times of CPC modified with gelatinized starches were doubled. However, final setting times of CPC and starch-modified CPC are close, all around 50 min. The addition of gelatinized starches to CPC significantly increased the initial setting times but not the final setting times, which agrees to the previous reported cases of CPC modified with other nongelatinized starches.

Interestingly, the difference between initial and final times (i.e., \( t_i - t_f \)), which is the time period for cement hardening during surgery, was significantly decreased in starch-modified CPC compared to pure CPC (~19 vs. ~28 min, Table III). For applications in vertebroplasty and kyphoplasty, increased initial setting time allows a prolonged operation window for handling and injection, whereas shortened hardening period \( (t_f - t_i) \) reduces the delay of operation and allows the surgeon to close the wounds shortly after injecting cement.

Therefore, the setting times of CPC-modified gelatinized starches are more appropriate for minimally invasive surgery applications compared to that of pure CPC.

Shrink rates of the modified CPC are listed in Table II. Pure CPC had a shrink rate of ~10% after setting and hardening and the CPC reinforced by 3 wt% of Ca-modified starch showed similar shrinkage. However, all other starches resulted in higher shrink rates, which also increased as the starch contents increased. The highest shrink rate of 35% was observed in the CPC incorporated with 10 wt% of corn starch. High shrinkage in bone cement after setting may cause failure in repairing the defects or fractures of load-bearing bones, which is discussed later.

**Anticollapsibility of modified CPC**

Anticollapsibility of CPC modified with different gelatinized starches was tested by injecting the CPC into DI water at 37°C and the integrity of the CPC after soaking and/or shaking tests was observed. Figure 2(a,b) shows that pure CPC bars completely collapsed in water and failed to harden when injected into water. Similarly, CPC modified by crosslinked starch was highly susceptible to collapse and the injected cement bars completely disintegrated within the first hour (images not shown).

In contrast, anticollapsibility of the CPC modified by corn, cationic, and Ca-modified starches was significantly

**TABLE II. Injectability and Shrinkage of CPC Modified with Gelatinized Starches**

| Cement Sample          | Injectability | Shrink Rate (%) |
|------------------------|---------------|-----------------|
| CPC                    | Medium        | 9.4 ± 3.2       |
| 3 wt% Corn starch + CPC| High          | 16.4 ± 0.4      |
| 5 wt% Corn starch + CPC| High          | 22.1 ± 2.1      |
| 10 wt% Corn starch + CPC| Medium       | 35.0 ± 1.2      |
| 14 wt% Corn starch + CPC| Poor        | –               |
| 3 wt% Crosslinked starch + CPC| High   | 13.7 ± 1.6      |
| 10 wt% Crosslinked starch + CPC| Poor | –               |
| 3 wt% Cationic starch + CPC| High   | 19.7 ± 2.7      |
| 10 wt% Cationic starch + CPC| Poor | –               |
| 3 wt% Ca-modified starch + CPC| High | 12.9 ± 2.5      |
| 5 wt% Ca-modified starch + CPC| Poor | –               |
improved and the CPC samples remained intact in water for up to 7 days. In addition, after setting in water for 7 days, three types of starch-modified CPC were further tested on an orbital shaker at speeds of 50 rpm and then 100 rpm each for a day. The starch-modified CPC samples retained its integrity in water without collapse at both speeds, implying very high anticollapsibility in these starch-modified CPC samples [Figure 2(c,d)].

**Mechanical properties of starch-modified CPC**

**Compressive strength and modulus.** Figure 3 shows typical compressive stress–strain curves of CPC and CPC modified with gelatinized starches. From the stress–strain curve, pure CPC showed a typical mechanical behavior of brittle materials, with a small strain-at-failure (1.95%) and an immediate failure when it reached to the maximum compressive stress (1.54 MPa). Pure CPC possessed a relatively low compressive strength compared to other types of CPC in literature but it is still within the normal range of CPC compressive strength reported to date.²⁷ In contrast, CPC modified with gelatinized starches exhibited plastic behavior of ductile or nonbrittle materials with increased strain-at-failure (up to ~5%) and gradual failure process after the stress reached the maximum (i.e., compressive strength). Among the CPC modified with various starches, CPC reinforced by Ca-modified starch had the highest compressive strength than any other starch-modified CPC, although its strain-at-failure (~3%) was lower than that of other samples but still greater than that of pure CPC.

The compressive strengths and moduli of CPC reinforced by different kinds of starch (3 wt %) are significantly increased to different extents compared to pure CPC as shown in Figure 4. Specifically, the average compressive strength and modulus of CPC reinforced by Ca-modified starch were 13.16 and 567.55 MPa, respectively, which were about six and four times of strength and modulus of CPC (1.74 and 147.24 MPa, respectively). For CPC modified by cationic starch or crosslinked starch, the improvements in CPC compressive strength and modulus were clear but not as high as that of Ca-modified CPC. Interestingly, addition of 3 wt % of corn starch had no effect on the compressive strength and modulus of CPC.

| Cement Sample                      | Initial Setting Time $t_i$ (min) | Final Setting Time $t_f$ (min) | $t_f - t_i$ (min) |
|-----------------------------------|----------------------------------|-------------------------------|------------------|
| CPC                               | $15.3 \pm 1.3$                  | $43.0 \pm 2.5$                | $27.7 \pm 3.8$   |
| 3 wt % Corn starch + CPC          | $31.3 \pm 2.2$                  | $50.2 \pm 3.3$                | $18.9 \pm 5.5$   |
| 3 wt % Cationic starch + CPC      | $34.8 \pm 1.9$                  | $54.3 \pm 2.9$                | $19.5 \pm 4.8$   |

**TABLE III. Setting Times of CPC and CPC Modified with Gelatinized Corn Starch and Cationic Starch**

**FIGURE 2.** Images showing anti-collapsibility of different CPC samples. (a) Immediately after the pure CPC paste injected into water, (b) CPC collapsed in water after 1 min, (c) CPC reinforced by Ca-modified starches immediately after injection, and (d) CPC reinforced by Ca-modified starches after 7-day soaking test and subsequent 2-day shaking test.
Figure 5 shows the compressive strengths and moduli of CPC reinforced by 3, 5, and 10 wt % of corn starch, respectively. Although the addition of 3 wt % of corn starch resulted in little improvement in CPC mechanical strength (Figure 4), the compressive strength was doubled and sextupled when corn starch content increased to 5 and 10 wt %, reaching 5.39 and 12.76 MPa, respectively. The compressive modulus remained about 192 MPa for the CPC samples with 3 wt and 5 wt % corn starch, whereas it increased to 324 MPa for the CPC sample with 10 wt % of corn starch.

It is important to mention that 10 wt % is almost the upper limit for adding gelatinized starch to CPC, because the gelatinized starch was extremely viscous above 10 wt % and cement paste became too thick to inject through syringe. Even for the content of 10 wt %, the injectability of cement paste was inferior to that of CPC modified with 3 or 5 wt % of corn starch. In addition, the shrinkage of CPC with 10 wt % of corn starch was very significant and reached a value of 35% (Table II). This large shrinkage is clearly a result of high starch content in the cement paste.

**Strain energy density.** The aforementioned different mechanical behaviors of modified CPC indicated that there was a large variation in strain energy density of the starch-modified CPC as summarized in Table IV. Strain energy density, calculated up to the maximum stress here, is a measurement of both elastic and plastic energy absorbed during compression and therefore is an indicative parameter of material toughness, implying the capability to resist fracture. Strain energy density in starch-modified CPC was significantly improved compared to pure CPC, which is probably attributed to the compliant gelatinized starch in the rigid CPC matrices. The strain energy density value reached as high as 353 mJ/cm³ in the CPC modified with 10 wt % of corn starch, which is a sixfold increase compared to that of pure CPC. CPC modified with other types of gelatinized starches revealed different increases in the strain energy density, suggesting the toughness of CPC was significantly improved to different extents. These results are in agreement with the morphology observation of the failed cement samples after compression tests: the starch-modified CPC samples almost retained their cylindrical shape and only cracks were seen, whereas pure CPC bars were completely smashed.

| Sample                      | Strain Energy Density (mJ/cm³) |
|-----------------------------|--------------------------------|
| CPC                         | 14.1 ± 9.0                     |
| 3 wt % Corn starch + CPC    | 25.6 ± 12.3                    |
| 5 wt % Corn starch + CPC    | 120.8 ± 21.3                   |
| 10 wt % Corn starch + CPC   | 353.3 ± 79.7                   |
| 3 wt % Crosslinked starch + CPC | 49.2 ± 7.7                 |
| 3 wt % Cationic starch + CPC | 239.4 ± 107.5               |
| 3 wt % Ca-modified starch + CPC | 175.3 ± 78.3               |
Microstructural analyses. The microstructures of CPC reinforced by gelatinized corn- and Ca-modified starches were examined and the SEM images of cross-sectional fracture surfaces are shown in Figure 6. CPC samples reinforced by corn- and Ca-modified starches revealed rougher fracture surfaces than pure CPC with a greater number of dimples and voids. In contrast, fracture surface of pure CPC was relatively smooth and few dimples were formed. Fractographic analysis on these surfaces indicates that the failure mode of starch-modified CPC was close to ductile fracture, whereas pure CPC failed in a way close to brittle ceramic. This analysis on fracture surfaces agrees well with the previous results from stress-strain curves and strain energy density, confirming that adding gelatinized starch could alter mechanical behavior of pure CPC.

Both cements reinforced by corn- and Ca-modified starches showed homogenous microstructures on fracture surfaces. Figure 6(b,d) shows well-dispersed ceramic particles on the fracture surface and there was no apparent agglomeration of segregation between organic and inorganic components, suggesting a high homogeneity of phase distribution.

Cytocompatibility evaluation
Cytocompatibility of the starch-modified CPC samples was evaluated by measuring proliferation of osteoblasts in the mixture of cement-leaching solution and cell-culture medium. Figure 7 shows the results of osteoblast proliferation after culturing for 1 and 3 days. CPC revealed good cytocompatibility and the osteoblast proliferation in CPC-
ated osteoblast proliferations with about 50% of increases and proper setting times for application in minimally invasive orthopedic surgery. In addition, all types of CPC modified with Ca-modified starch and corn starch, as biocompatible modifiers to reinforce the injectable CPC used for minimally invasive orthopedic surgeries.

The mechanism(s) of Ca-modified and corn starch in reinforcing CPC is probably associated with the physical properties of gelatinized starches. Surface charge usually plays an important role in the stability of colloidal systems, and therefore the zeta potentials of different starch particles were examined in this study. However, there was not a clear dependence of starch zeta potential and resultant CPC mechanical strength. For example, corn starch and cross-linked starch revealed similar large negative zeta potential values at pHs of 7 and 10, but the compressive strengths of CPC modified with these starches were significantly different. The CPC modified with cationic starch had higher compressive strength than the CPC with corn and crosslinked starches. This might be attributed to the opposite zeta potentials between cationic starch and CPC particles in the pH range of 7–10. But this possibility needs more investigation and verification, because the difference in zeta potentials of cationic starch and CPC was actually too small (only 10–25 mV) to form electrostatic stabilization between two kinds of particles. Also, the zeta potentials of CPC were close to zero (i.e., 0 to −20 mV) in the pH range of 7–10, indicating that CPC particles were not stable but tended to aggregate in water, and hence the CPC might not interact with starch particles but clumped together, which would cause decrease in the mechanical strength of final composite cement. More importantly, because all types of starches were gelatinized when mixed with CPC, it is very likely that the properties of gelatinized starches, rather than that of the pristine starch, play an important role in affecting the final mechanical properties of cement.

Other evidence (e.g., sixfold improvements of compressive strength in CPC/corn starch when the starch contents increased from 3 to 10 wt %) implies that the mechanism(s) different from surface charge interaction should account for the increased compressive strengths and it probably associates with the amount of starch added to CPC. As gelatinized corn starch after drying (water content, <10 wt %) has compressive strength and modulus of more than 200 MPa and 1 GPa, respectively (measured in this study), it was speculated that gelatinized starch could serve as a reinforcing phase in CPC matrix. In this case, content of the reinforcing phase is obviously crucial to the final strength and toughness of the CPC composite.

Also, as summarized in Table I, gelatinized starches had very different rheology properties. Further study revealed that gelatinized starch with low viscosity (i.e., <30 mPa·s) did not strengthen and toughen CPC, whereas the one with extremely high viscosity (i.e., >28,000 mPa·s) was too thick to form injectable cement pastes (Table II). On the contrary, gelatinized starch with the viscosity in the range of 1750–9000 mPa·s led to significantly higher compressive strength, modulus, and strain energy density of the CPC, suggesting a...
corn starch and cationic starch led to improved compressive strengths, showed homogenous microstructures where calcium phosphate particles or converted apatite crystals were well dispersed in the composite (Figure 6).

The CPC with 10 wt% of corn starch had higher strain energy density than CPC with 3 wt% of Ca-modified starch and this can be attributed to the higher content of viscoelastic polymer phase in the composite. However, high starch content in CPC resulted in large shrinkage in the cement after setting and hardening. For example, the shrink rate of the CPC modified by 10 wt% of corn starch reached as high as 35.0 ± 1.2%, almost four times of that of pure CPC (9.4 ± 3.2%). The large shrinkage after setting and hardening is potentially a severe drawback for the clinical uses of bone cement as the cements are usually required to fully occupy and conform to bone defects and, in the application like kyphoplasty, to support mechanical load and restore the height of fractured vertebrae. In contrast, CPC with 3 wt% of Ca-modified starch shrank by 12.9 ± 2.5% after hardening, which was not statistically different from that of pure CPC. This small shrinkage makes the CPC reinforced by 3 wt% of Ca-modified starch a better candidate for orthopedic applications compared to the CPC modified by 10 wt% of corn starch.

CONCLUSIONS
Low mechanical strengths and susceptibility to collapse are two major problems that injectable CPC encounters in clinical settings. This study reported a strategy of incorporating gelatinized starch with CPC to improve its mechanical properties and anticollapsibility. All four types of gelatinized starch studied here resulted in the reinforcement of CPC to different extents, including increased compressive strengths and moduli as well as strain energy density. Specifically, adding 10 wt% of corn starch and 3 wt% of Ca-modified starch led to the highest compressive strengths and moduli of the cements compared to pure CPC, both with sixfold increases in the compressive strength and at least twofold increases in modulus. Most of gelatinized starch revealed positive effect on increasing the anticollapsibility of CPC in water and resulted in appropriate setting times of cements for minimally invasive surgery. Cytocompatibility assays revealed that adding gelatinized starches to CPC had no adverse effect on osteoblast proliferation up to 3 days and all modified CPC revealed similar cell proliferation rates as pure CPC. Viscosity of gelatinized starch appeared to strongly correlate with the mechanical properties of resultant cement composites. Mechanical properties of CPC modified with gelatinized starch also revealed a dependence on the content of starch. SEM observation on CPC reinforced by corn- and Ca-modified starches showed homogenous microstructures and well-dispersed crystals in the cement after hardening. These results suggested at least two possible reinforcing mechanisms of gelatinized starches: (1) gelatinized starch after drying acts as a reinforcing second phase in the composites and (2) apatite crystals converted from CPC particles interlock tightly with the assistance of gelatinized starch. The synergy of these two mechanisms may contribute to the significant improvement in compressive strengths and moduli as well as strain energy densities of CPC reinforced by 10 wt% of corn and 3 wt% of Ca-modified starches.

However, the addition of 10 wt% of corn starch to CPC caused shrinkage as high as 35% after setting, whereas...
addition of 3 wt % of Ca-modified starch resulted in a similar shrinkage as pure CPC (~10%). The CPC reinforced by 3 wt % of Ca-modified starch is therefore a better candidate for clinical treatments of bone defect and fractured load-bearing bone by minimally invasive procedure like kyphoplasty. Most importantly, adding gelatinized starches was shown to be an effective and facile strategy to reinforce injectable CPC and improve its anticollapsibility, and thus, should be further studied and developed.

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REFERENCES
1. Canal C, Ginebra M. Fibre-reinforced calcium phosphate cements: A review. J Mech Behav Biomed Mater 2011;4:1658–1671.
2. Barinov S, Komlev V. Calcium phosphate bone cements. Inorg Mater 2011;47:1470–1485.
3. Neira IS, Kolen’ko YV, Kommareddy KP, Manjubala I, Yoshimura M, Guitián F. Reinforcing of a calcium phosphate cement with hydroxyapatite crystals of various morphologies. ACS Appl Mater Interfaces 2010;2:3276–3284.
4. LeGeros RJ. Calcium phosphate materials in restorative dentistry: A review. Adv Dent Res 1988;2:164–180.
5. Chow LC, Brown WE. A physicochemical bench-scale caries model. J Dent Res 1984;63:868–873.
6. Li D, Li Y. Effect of substitutional Sr ion on mechanical properties of calcium phosphate bone cement. J Wuhan Univ Technol 2013;28:741–745.
7. Wardlaw D, Van Meinhaeghe J, Ranstam J, Bastian L, Boonen S. Balloon kyphoplasty in patients with osteoporotic vertebral compression fractures. Expert Rev Med Devices 2012;9:423–438.
8. Zafeiris CP, Lyritis GP, Papaioannou NA, Gratsias PE, Galanos A, Chatzioannou SN, Pneumaticos SG. Hypovitaminosis D as a risk factor of subsequent vertebral fractures after kyphoplasty. Spine J 2012;12:304–312.
9. Lewis G. Injectable bone cements for use in vertebroplasty and kyphoplasty: State-of-the-art review. J Biomed Mater Res B Appl Biomater 2006;76:456–468.
10. Chen L, Xiang H, Li XX, Ye JD, Wang XP, Li L. Improvement of anti-washout performance of calcium phosphate cement using modified starch. Key Eng Mater 2007;336:1628–1631.
11. Ishikawa K, Miyamoto Y, Kon M, Nagayama M, Asaoka K. Non-decay type fast-setting calcium phosphate cement: Composite with sodium alginate. Biomaterials 1995;16:527–532.
12. Campaña S, Charpal E, De Guise J, Rillardon L, Skalli W, Mitton D. Relationships between viscoelastic properties of lumbar intervertebral disc and degeneration grade assessed by MRI. J Mech Behav Biomed Mater 2011;4:593–599.
13. Ma L, Liu C. Preparation of chitosan microspheres by ionotropic gelation under a high voltage electrostatic field for protein delivery. Colloids Surf B Biointerfaces 2010;75:448–453.