Development, Characterization, and Validation of Porous Carbonated Hydroxyapatite Bone Cement

Pei-Fu Tang,[1] Gang Li,[2,3] Ji-Fang Wang,[1] Qiu-Jian Zheng,[4] Yan Wang[1]

[1] Department of Orthopaedic Surgery, The General Hospital of Chinese People's Liberation Army, Beijing 100853, People's Republic of China

[2] Department of Orthopaedics & Traumatology, Lika Shing Institute of Health Sciences, The Chinese University Hong Kong, Clinical Sciences Building, Prince of Wales Hospital, Shatin, Hong Kong, People's Republic of China

[3] School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast BT9 1BL, United Kingdom

[4] Department of Orthopaedics and Traumatology, The People's Hospital of Guangdong Province, Guangzhou, Guangdong Province, People's Republic of China

Received 1 October 2008; revised 27 November 2008; accepted 12 January 2009
Published online 7 April 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.31360

Abstract: Carbonated hydroxyapatite (CHA) bone cement is capable of self-setting and forming structures similar to mineralized bone. Conventional CHA leaves little room for new bone formation and delays remodeling. The purposes of this study were to develop porous CHA (PCHA) bone cement and to investigate its physicochemical properties, biocompatibility, biodegradation, and in vivo bone repair potential. Vesicants were added to modify CHA, and the solidification time, porosity, and pore size of the PCHA cements were examined. The cytotoxicity and bone repair potential of PCHA were tested in a rabbit bone defect model and assessed by x-ray, histological examination, and mechanical testing. The porosity of the modified PCHA was 36%; 90.23% of the pores were greater than 70 μm, with a calcium/phosphate ratio of 1.64 and a solidification time of 15 minutes. The PCHA did not affect bone cell growth in vitro, and the degrading time of the PCHA was two and four times faster in vitro and in vivo when compared to CHA. In the bone defect model, the amount of new bone formation in the PCHA-treated group was eight times greater than that of the CHA group; the compressive strength of the PCHA setting was relatively weak in the first weeks but increased significantly at 8 to 16 weeks compared to the CHA group. The PCHA has stable physicochemical properties and excellent biocompatibility; it degrades faster than CHA, provides more porous spaces for new bone ingrowths, and may be a new form of bone cement for the management of bone defects. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 90B: 886–893, 2009

Keywords: carbonated hydroxyapatite (CHA); porous CHA (PCHA); bone cement; biocompatibility; bone defect

INTRODUCTION

Carbonated hydroxyapatite (CHA) bone cement is a relatively new biomaterial that is being developed and used in implants for orthopedic and dental applications. CHA is capable of self setting and forming granules of well-defined geometry in variable sizes and shapes, and it can be filled into the bone defects/voids of irregular and patient-specific shape platforms.[1–2] Despite its common use, CHA has been reported to have complication rates that are as high as 32%.[2] When failed CHA implants were being removed, implant fractures had been reported as a potential cause of failure. One possible explanation is that conventional CHA has a lower volume of pores, has little room for new bone to grow into, and is hard to remodel.[3]

The lack of pores and slow resorption of CHA cement hinders its wide clinical application. Pore sizes in human cortical bones are known to range from 1 to 100 μm, whereas in trabecular bones, pores vary from 200 to 700 μm.[4] Pore size from 200 to 400 μm is most suitable for new bone ingrowths.[1,3,5,6] When the pore volume is above 30%, pores are able to form network connections;

Correspondence to: Y. Wang (e-mail: yanwang301@yahoo.com)
Contract grant sponsor: China Natural Science Foundation; contract grant number: 30672135
Contract grant sponsor: Beijing City Natural Science Foundation; contract grant number: 7072072

the biomechanical prosperities decrease when the pore volume increases, so that the pore volume should be around 30–50% to provide necessary porous connectivity and biomechanical strength[7,8] According to scanning electron microscopy (SEM) results, CHA cement forms small pores ranging from several micrometers to 30 μm, and these small pore sizes cannot meet the needs of new bone formation. The degradation of CHA cement is also a slow process, as the resorption relies mainly on surrounding tissue fluid.[9–11] Therefore, modifying CHA cement to form pore sizes greater than 70 μm is necessary to facilitate optimal bone formation and rapid material resorption. The purposes of this study were to develop porous CHA (PCHA) bone cement and to investigate its physicochemical properties, biocompatibility, biodegradation, and in vivo bone repair potential.

MATERIALS AND METHODS

Preparation of PCHA Bone Cement

CHA was prepared as previously reported.[2,12,13] CHA powders were sieved, and particles of 3 to 5 μm diameter, as measured by laser particle analyzer, were selected. The vesicant calcium biphosphate [$Ca(H_2PO_4)2\text{-}H_2O$] was added to the CHA powder at seven different concentrations of 0, 0.5, 1, 1.5, 2, 2.5, and 3% w/w. Sodium hydrogen carbonate [$NaHCO_3$],another vesicant, was added to water for mixing with the newly prepared CHA powders at a concentration of 0.6 *M*. The NaHCO3 solution and CHA powders were mixed at a ratio of 0.4 mL/g at room temperature. After self-setting, the materials were prepared for further examination.

Mercury Intrusion Porosimetry, Solidification Time, and pH of PCHA Cement

The microstructure of the newly prepared materials of various CHA powder settings, including porosity, pore size, and pore distribution, were determined by the mercury intrusion porosimetry method using a Pascal 140/240 porosimeter (CE instrument, Wigan, UK) according to the manufacturer's instructions. All the tests were done at 37°C, 100% moisture, and the mean times from five samples were calculated. To test pH stability, the sample materials were put in the middle of a 100 mm culture dish with PBS solution (pH 7.4). The pH of the solution was measured at 1, 5, 10, 20, 30, 60, 120, 600, and 1440 minutes, and a time curve with pH changes was plotted.

SEM Assay

For SEM examination, the samples were fixed by immersion into 2.5% glutaraldehyde (w/v in PBS solution) for 2 hours, washed with sodium dimethylarsenate buffer (pH 7.4), and then treated with 1% osmium tetroxide (w/v). Samples were dehydrated in ascending concentrations of ethanol (30, 50, 70, 90, 95, and 100%) for 10 minutes each and air dried overnight. The dried specimens were sputter coated with gold and examined using JSM SEM (JEOL, Tokyo, Japan).

Rabbit Bone Marrow Mesenchymal Stem Cells (MSCs) Isolation, Culture, Cytotoxicity, and Cell Proliferation Assays

Rabbit bone marrow mesenchymal stem cells (BMSCs) were harvested and cultured in vitro based on established protocols.[14–16] Two grams of PCHA and CHA powder were separately incubated in 100 mL basal media (HG-DMEM without phenol red) at 37°C, 5% CO_2 for 10 days. Then the media were cleared by centrifugation at 4°C, 4000 g for 15 minutes, filtered with a 0.75 μm filter mesh, and stored at −20°C for future use as conditioned media for a cytotoxicity assay. The effect of the PCHA and CHA on MSC proliferation was evaluated using a MTT cell proliferation assay kit (Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer's instructions.

Biodegrading Experiments of PCHA and CHA Cement

For in vitro experiments, the columnar PCHA and CHA specimens (diameter 8 mm; length 14 mm) were prepared, dried for 48 hours, and weighed. They were then incubated in 5 mL DMEM at 37°C for 12 weeks, and four samples were collected, dried, and weighed at 2, 4, 8, 12, and 16 weeks. The medium was changed twice a week. For in vivo implantation experiments, 24 NZW rabbits (8–10 weeks old, 2–2.5 kg) were randomly divided into four groups, with six rabbits in each group. One PCHA and one CHA sample were implanted into the back muscles of rabbits. Six rabbits were terminated at 2, 4, 8, 12, and 16 weeks following implantation. The specimens were collected and subjected to weight loss analysis and histological examination. Five specimens from each time point group were subjected to a compression test as described below.

Rabbit Distal Femoral Condyle Bone Defect Model

Forty NZW rabbits (8–10 weeks old, 2–2.5 kg) were randomly divided into five groups with eight rabbits in each group. With the rabbits under general anesthesia, a 2 cm cut was made on the lateral aspect of the distal femur on both legs with the lateral femoral condyle exposed; at the center of the lateral femoral condyle, a tunnel of 5.5 mm (diameter) × 12 mm (depth) was drilled using a 5.5 mm drill. The tunnel was washed with saline and then immediately filled with PCHA cement (right femur) and CHA cement (left femur). Eight animals were terminated at 2, 4, 8, 12, and 16 weeks after surgery. Calcein (30 mg/kg) was injected intravenously 2 and 5 days before termination. After termination, femurs from four animals were prepared for plastic or paraffin embedding. Femurs from four animals were collected and carefully cut open at the lateral

condyle region. The implanted PCHA or CHA materials were identified under a dissecting microscope, carefully removed as plugs (tissue blocks) of ~5.5 mm × 12 mm, and stored at −80°C until mechanical testing.

Mechanical Testing of the PCHA and CHA Plugs

The plugs of PCHA and CHA were obtained from the in vitro biodegrading tests; the in vivo implantation experiments and the femur bone defect sites from various time points were subjected to a compression test. Both ends of the specimens were carefully cleaned to be free from soft tissue attachments, and the length and diameter of each specimen was measured and recorded prior to mechanical testing. Specimens were tested to failure by compression with force applied at 5 mm/min using a mechanical tester with a 100 N load cell (Lloyd Instruments, UK). The maximal resistance to failure was calculated as MPa = Maximal Load/ Surface area of the specimen. In each instance, the same person carried out the test, and every sample was coded blind to the investigator.

Histological and Histomorphometry Examinations

For nondecalcified histological examinations, the specimens were processed and embedded in methyl-methacrylate (MMA). Slices of 300 μm were cut and grounded to thin sections of 100 μm and were subject to contact soft x-ray examination and von Kossa staining for detection of mineralization. The newly formed bone labeled by fluorochromes was revealed by epifluorescent microscopy. For calculation of the mineral appositional rate, the distance between the two fluorescent lines labeled by calcein was measured. The mineral appositional rate was calculated by the distance between two fluorochrome labeled lines per day. For decalcified histological examinations, the specimens were coded and fixed for 48 hours in 10% buffered formalin, then placed in 20% formic acid at 4°C for 3 weeks to decalcify. Decalcified samples were processed through graded alcohols, xylene and embedded in paraffin wax. Sections of 6 μm were cut and stained with haematoxylin and eosin.

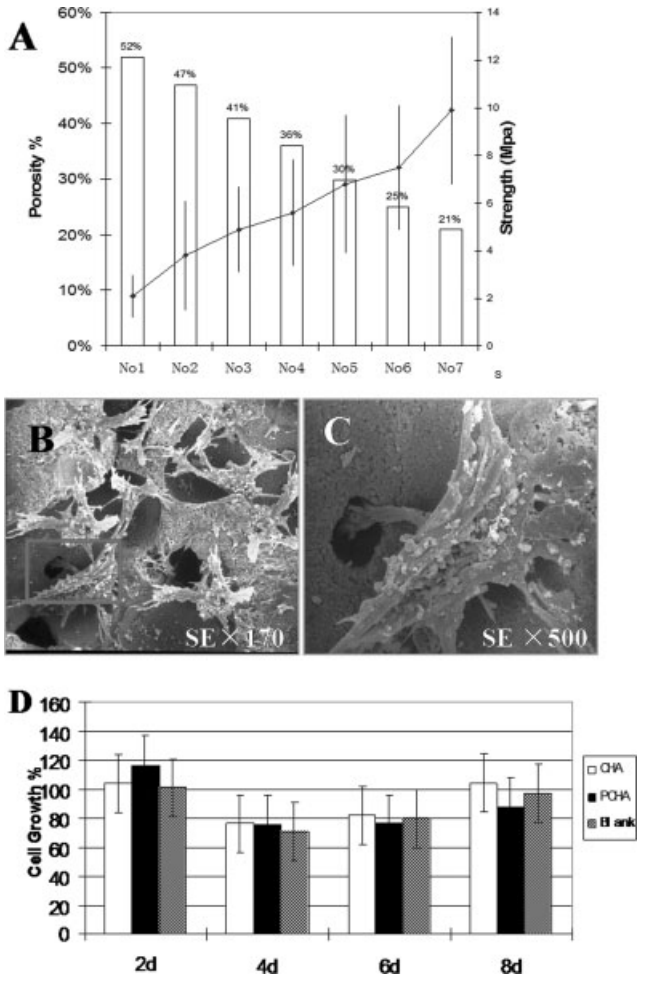

Figure 1. (A) The porosity of PCHA bone cement of various formulations and the compression strength of PCHA bone cement after consolidation. The porosity increased when the concentrations of the vesicants increased, from 21% (No. 7 preparation) to 52% (No. 1 preparation). The compression strength of the materials decreased with the increased porosity. Based on the porosity and compression strength, No. 4 preparation was selected as the ideal preparation, as it had porosity of 36% and compression strength of 6 MPa. (B) SEM showed that the MSCs attached well on the surfaces of PCHA cement, ×170. (C) The boxed area in B shows that a cell is entering the pore of the PCHA cement, ×500.

RESULTS

Physicochemical Properties of PCHA and Cytotoxicity Tests of PCHA and CHA

Porosity increased when concentrations of the vesicants increased, from 21 to 52%. The compressive strength of the materials decreased with increased porosity [Fig. 1(A)]. Based on the porosity and compressive strength, the No. 4 preparation, in which calcium biphosphate [$Ca(H_2PO_4)$ 2-H_2O] was added to the CHA powder at 1.5% w/w, was selected as the ideal preparation. The selected PCHA preparation had a porosity of 36% and a compressive strength of 6 MPa. Results of mercury intrusion porosimetry test for the No. 4 preparation confirmed that the porosity was 36% (34–39%) and the size of the pores was normally distributed, with 90.23% pores greater then 70 μm, 80% of the total pores between 158 and 394 μm, and the highest numbers of pores at the size of 259 μm. The mean pH of all the PCHA powders was 8.6. The primary solidification time was 6 minutes for PCHA and 4 minutes for CHA, and the final solidification time was 15 minutes for PCHA and 13 minutes for CHA. The ratio of calcium to phosphate was 1.64 for PCHA cement. Conditioned media from PCHA and CHA powders were added to the rabbit MSCs culture system, and there was no significant effect on cell proliferation at all the time points tested when compared to the blank (control) medium. SEM examinations showed

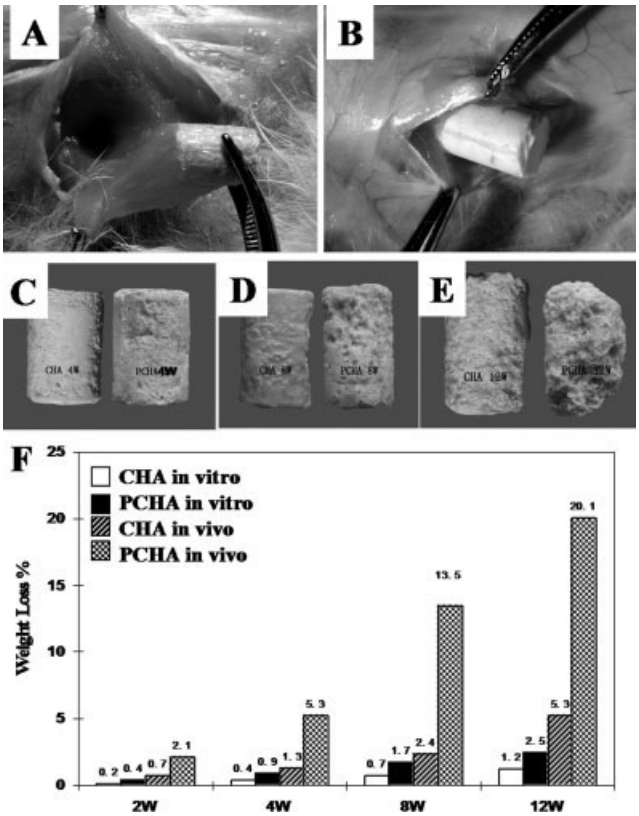

Figure 2. (A) At 12 weeks after intramuscular implantation, dense fibrous tissues grew into the PCHA cement, and the material was well-integrated with the muscle tissues. (B) In contrast, the CHA cement still had smooth surfaces, and there were few connective tissues growing into the material at 12 weeks after intramuscular implantation. (C–E) Gross samples showed the appearance of PCHA and CHA plugs following in vivo biodegrading experiments (intramuscular implantation) at 4 weeks (C), 8 weeks (D), and 12 weeks (E). At all time points (4, 8, and 12 weeks) more resorption was observed in the PCHA plugs than the CHA plugs. (F) Quantitative data showing weight loss of PCHA and CHA plugs when incubated with culture medium at 37°C in vitro or implanted intramuscularly in vivo for 4, 8, and 12 weeks. The weight loss of PCHA samples was almost two times faster in vitro and four times faster in vivo when compared to the CHA samples.

that the MSCs attached on the surfaces of the PCHA cement (No. 4 preparation) and grew into the pores of the PCHA materials [Fig. 1(B,C)].

Biodegrading of PCHA and CHA Cement In Vitro and In Vivo

Conditioned media from PCHA and CHA powders (50%) mixed with fresh culture medium were added to the rabbit MSCs culture system and cultured for 2, 4, 6, and 8 days. Cytotoxicity of the PCHA on MSCs was examined by MTT assay, and the results showed that there was no significant effect on cell proliferation at all the time points (2, 4, 6, and 8 days) tested when compared to the group with only blank (control) medium added [Fig. 1(D)].

Twelve weeks after intramuscular implantation, dense fibrous tissues grew into the PCHA cement, and the material was well integrated with muscle tissues [Fig. 2(A)]. In contrast, the CHA cement still had smooth surfaces, and there was little connective tissue growing into the material at 12 weeks after intramuscular implantation [Fig. 2(B)]. The fibrous membrane surrounding the biomaterial in the PCHA group formed at 2 weeks, and the thickness of the membrane continued to increase till 12 weeks and remained unchanged afterwards. The changes of thickness in fibrous membranes of CHA implants were similar to those of PCHA implants, but the fibrous membranes were slightly thinner and their thickness increased slowly from 8 to 12 weeks. The weight loss of the PCHA cement plug was two and four times faster in vitro and in vivo compared to the CHA cement plug [Fig. 2(C)].

Repair of Rabbit Distal Femur Condyle Bone Defect Using PCHA and CHA Cement

Once the PCHA and CHA cements were filled into the bone defect sites, the CHA cement plug did not change its radiographic appearance significantly over the 16 week period, whereas the PCHA cement plug showed signs of resorption from 8 weeks and at 16 weeks the plug was remodeled and integrated into the surrounding bone without distinguishable boundaries (Fig. 3). Macroscopically and

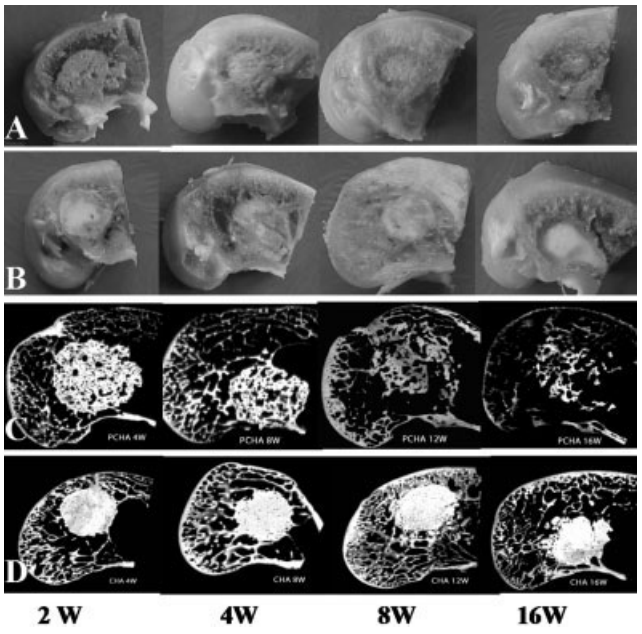

Figure 3. (A,B) Gross appearances of bone cement plugs in the femoral condyle defect at various time points as indicated. (A) The PCHA cement plug started showing signs of resorption from 4 weeks, progressed at 8 weeks, and by 16 weeks, the PCHA cement plug was barely recognizable inside the bone. (B) In contrast, the CHA cement plug did not change significantly over the 16 week period, and it was still clearly identifiable at the end of 16 weeks. (C,D) Radiographs of the femur condyle defect repaired with PCHA or CHA cement at various time points as indicated. PCHA cement in the defect site was progressively resorbed over the 16 week period (C), whereas the CHA cement in the defect remained relatively unchanged in that same period (D).

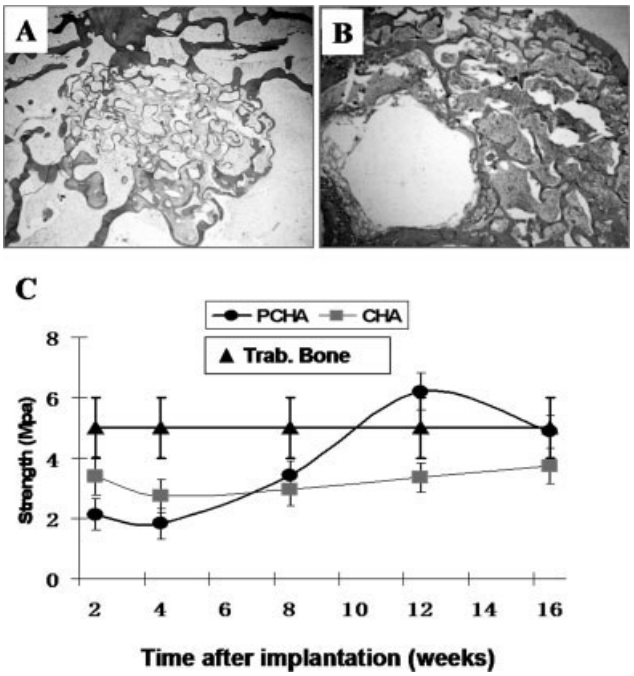

Figure 4. (A,B) Histological appearances of PCHA cement (A) and CHA cement (B) in the femoral condyle defect at 16 weeks. At all time points, the PCHA cement had more new bone ingrowths than the CHA cement, indicting a superior bone conductive ability. (C) The changes of compression strength of the PCHA and CHA cement plugs after in vivo implantation in the femoral condyle defect sites over time. At 2 and 4 weeks, the compression strength of CHA cement was higher than PCHA cement; at 8 weeks the compression strength was similar for the PCHA and CHA groups, but both were smaller than that of normal trabecular bone; at 12 weeks, the PCHA cement plug had a higher compression strength than that of the CHA cement plug and trabecular bone; at 16 weeks, the PCHA had a similar compression strength to normal trabecular bone.

radiographically, the PCHA cement plug started showing signs of resorption from 4 weeks, progressed at 8 weeks, and by 16 weeks, the PCHA cement plug was barely recognizable inside the bone. In contrast, the CHA cement plug did not change significantly over the 16 week period, and it was still clearly identifiable at the end of 16 weeks on both gross appearance and radiographs.

Histology and Histomorphometry

In the PCHA group, the fluorescence mineralized zone appeared from the material edges to the center and increased gradually with time. In the control CHA group, fluorescent zones were only seen at the intersection points between the material and the bone, indicating that there was a lack of interconnected pores and the new bone was unable to grow into the material. At all the time points, the PCHA cement had more new bone ingrowths than the CHA cement, indicating a superior bone conductive ability [Fig. 4(A,B)]. New bone was formed inside the pores of the PCHA cement at 8 weeks following implantation, and the PCHA cement was remodeled rapidly and replaced by newly formed bone at 16 weeks following implantation. Quantification of bone ingrowths showed that the amount of new bone formation in the PCHA treated group was eight times greater than that of the CHA group. Quantitative measurements of new bone formation in the bone defect areas at 16 weeks indicated that 32.20% $\pm$ 1.47% of the defect areas were filled by new bone in the PCHA group, whereas only 3.91% $\pm$ 0.65% of the defect areas were filled by new bone in the CHA group. The bone formation rate measured by tetracycline dual labeling in both PCHA and CHA groups was similar, 3.51 $\pm$ 0.30 μm/day for the PCHA group and 3.40 $\pm$ 0.27 μm/day for the CHA group.

In the PCHA group, the fluorescence mineralized zone appeared from the material edges to the center and increased gradually with time. The new bone was arranged in a disorderly manner, suggesting that new bone was growing into the material through the interconnected pores toward to the center [Fig. 5(A,B)]. The bone formation was in a multicentered manner [Fig. 5(C,D)]. The ring-like bone islands suggest the formation of Harvard systems [Fig. 5(D)]. In the control CHA group, fluorescent zones were only seen at the intersection points between the material and the bone [Fig. 5(E-H)], indicating that there was a lack of interconnected pores and the new bone was unable to grow into the material. At all the time points, the PCHA cement had more new bone ingrowths than the CHA cement, indicting a superior bone conductive ability [Fig. 5(A–C)]. New bone was formed inside the pores of the PCHA cement at eight weeks following implantation [Fig, 5(I)], and the PCHA cement was remodeled rapidly and replaced by newly formed bone at 16 weeks following implantation [Fig. 5(J)].

Mechanical Testing

The compressive strength of PCHA bone cement in vitro was 5.6 $\pm$ 2.2 MPa prior to implantation, and it reduced to 1.8 MPa after one month in vivo implantation in the bone defect site. The compressive strength of the CHA bone cement dropped from 30 MPa (in vitro, preimplantation) to 2.7 MPa after one month in vivo implantation. The reduction of the compressive strength of CHA bone cement was greater than that of the PCHA cement. The compression strength reached its lowest point at four weeks following in vivo implantation in both the PCH and CHA groups and gradually increased at 8, 12, and 16 weeks [Fig. 4(C)]. The increase in strength of the PCHA plug was significantly greater than that of the CHA plug; PCHA plug strength reached the normal trabecular bone level at eight weeks, became greater than that of trabecular bone at 12 weeks, and returned to trabecular bone level at 16 weeks [Fig. 4(C)]. Although the strength of the CHA plug continued to increase from 8 to 16 weeks, it was below normal trabecular bone level at all the time points tested.

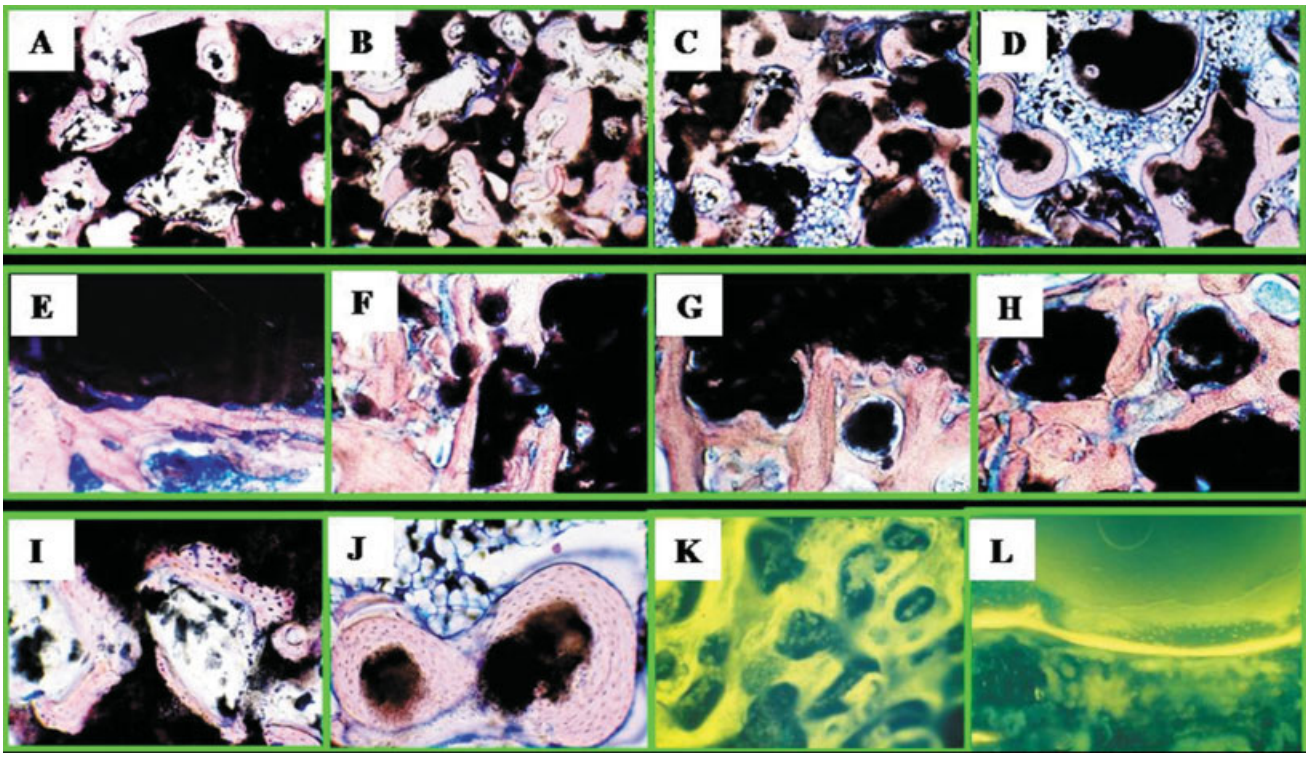

Figure 5. Histological appearances of bone cements in the femoral condyle defect at various time points from 2 to 16 weeks as indicated. (A–D) The PCHA cement at 2 weeks (A), 4 weeks (B), 8 weeks (C), and 16 weeks (D). (E–H) CHA cement at 2 weeks (E), 4 weeks (F), 8 weeks (G), and 16 weeks (H). At all the time points, the PCHA cement had more new bone ingrowths than the CHA cement, indicting a superior bone conductive ability. (A–H), von Kossa staining, original magnification ×100. (I) New bone was formed inside the pores of the PCHA cement at 8 weeks following implantation. (J) The PCHA cement was remodeled rapidly and replaced by newly formed bone at 16 weeks following implantation. I and J, von Kossa staining, ×200. (K) Fluorescent microscope showed tetracycline labeling, indicating the newly formed bone inside the PCHA cement at 12 weeks, ×100. (L) At 12 weeks, a tetracycline labeling line was only seen at the interfaces between host bone and CHA cement, ×100. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

DISCUSSION

In 1976, the hydrating characteristic of α-TCP (α-tricalcium phosphate) was first discovered, and since then, α-TCP has become the principal constituent for phosphoric acid calcium salt-based bone cement.[17,18] Until now, more than 450 formulas were tested,[19,20] among which there were more than 10 formulas that have self-solidification capability. Among them, the representative formula was the calcium phosphate bone cement, first reported by Chow and Brown in 1985[21] and the carbonated hydroxyl apatite (CHA) bone cement reported by Constanz et al. in 1996.[22,23] In particular, CHA bone cement can form carbon hydroxyl apatite after solidification, which is similar to natural bone mineral contents. In the current study, vesicants were added to the CHA powder to increase porosity and modify pore sizes. Among the seven PCHA preparation formulas, a best preparation formula was selected based on porosity, pore size, pore connectivity, and surface area. The selected formula gave 36% porosity and good connectivity between pores, and 90.23% of the pores were greater than 70 μm.

The time taken for solidification of PCHA bone cement is important for its clinical application. Khairoun et al.[12] considered the optimal time for solidification of bone cement to be 3 to 8 minutes for primary solidification and around 15 minutes for final solidification. In the current study, our PCHA has a primary solidification time of 6 minutes and final solidification time of 15 minutes, which meets the requirements for clinical application. The pH value may affect the solidification of phosphate bone cement; lower or higher pH values do not favor carbon hydroxyl apatite formation. The most suitable pH value is between 8 and 9.[7,8] In the current study, the pH value of PCHA is 8.6, in contrast to pH 10.8 for CHA, suggesting that PCHA is a more suitable material for hydroxyl apatite formation.

Mechanical strength is an important parameter for bone repair material. Many factors influence the solidification intensity of PCHA and CHA bone cement, such as the particle size of the powder, material formula, fluid/power ratio, and amount of vesicants. Among these, porosity has the biggest impact on the mechanical strength of the material. The data in this study and others have demonstrated an

inverse ratio between porosity and mechanical strength. The denser material has better mechanical strength but is difficult to resorb; porous material is good for bone formation and biodegradation but has less mechanical strength. The PCHA formula selected in this study has a similar mechanical strength as normal trabecular bone, in that the compression strength was 5.6 ± 2.2 MPa in PCHA cement and 5 MPa in trabecular bone. The porosity and mechanical strength need to be balanced for better osteoconductivity and biodegradation of the material. The clinical biomaterials have a porosity between 30 and 50%, which will provide spaces for new bone formation inside the materials and also maintain reasonable mechanical strength.[5,6] Our PCHA formula has a porosity of 36% and 90.23% pores are greater than 70 μm, which is in agreement with that previously reported for similar bone cement materials.[4–7]

According to the American standard of testing materials, all implantable biomaterials must be tested in animals with subcutaneous or intramuscular implantation to investigate tissue reactions toward the biomaterials. The assessment includes encapsulating membrane formation surrounding the biomaterials and inflammatory cell infiltration. In our study, we confirmed that there were no inflammatory responses in the surrounding tissues at the PCHA or CHA intramuscular implant sites; only some macrophages were seen around the biomaterials, which may be because of a foreign-body reaction to the biomaterial.[24–26] Fibrous membranes separate the biomaterials from host tissues; this is a protective reaction toward a foreign body.[27] The thickness of the fibrous membrane is determined by many factors, including the size, shape, surface structure, chemical composition, porosity, and site of the implant.[28,29] All the parameters used in the current experiment for in vivo implantation tests are the same; the thickness of the fibrous membranes reflects the biocompatibility of the material. The porous structure of PCHA allowed body fluids to penetrate into the material easily and therefore increased its solubility for calcium and phosphate. Thus, the stimulation of the surrounding tissues increased and resulted in thicker surrounding fibrous membranes in comparison to the CHA group, where resorption was slow because of lack of porous structures.

Animal models of bone defects are usually used to evaluate the bone induction potential of biomaterials.[15,30,31] The bone defect should have a diameter greater than 5 mm to avoid spontaneous healing.[14] In the current study, we chose rabbit femoral condyle trabecular bone region for implantation experiments, and the bone defect diameter was 5.5 mm, which is a well accepted bone defect model.

There are varied reports on the changes of mechanical properties of calcium phosphate-based bone cement when implanted in vivo. It was reported that after 1 month implantation in vivo, the compression strength of the bone cement reduced from 40 MPa to 2.8 MPa, less than that of trabecular bone.[32–34] The compression strength of the bone cement in vitro is at least two times higher than of that in vivo; this may be due to the infiltration of blood and tissue fluid during the solidification process of the bone cement in vivo.[33,34] In the current study, the compressive strength of the PCHA setting was relatively weak in the first 4 weeks but increased significantly at 8 to 16 weeks compared to the CHA group. At 12 weeks, the PCHA cement plug had a higher compression strength than that of CHA cement, and by 16 weeks, the PCHA had a similar compression strength to that of normal trabecular bone. This can be explained, in that PCHA encouraged bone formation from 8–12 weeks and increased the compression strength; at 16 weeks, the new bone was remodeled at the same time that the PCHA biodegradation resulted in a reduction in strength back to normal trabecular bone level. In the CHA group the new bone formation was slow because of the lack of porosity; hence, strength increased slowly.

In summary, the newly defined PCHA cement has stable physicochemical properties comparable to CHA bone cement. The PCHA has good biocompatibility, degrades faster than CHA, and provides more porous spaces for new bone ingrowths. Despite the PCHA having relatively weaker compressive strength at the beginning, it promotes bone formation and increases compressive strength with time when compared to CHA bone cement. The PCHA may be a new form of bone cement that can be used clinically for the management of bone defects.

The authors acknowledge the assistance from Professor Da-Wei He and his team at the Institute of Chemistry, Beijing, Chinese Academy of Sciences, for modifying the PCHA bone cement.

REFERENCES

1. Damien CJ, Parsons JR. Bone graft and bone graft substitutes: A review of current technology and applications. J Appl Biomater 1991;2:187–208.
2. Baker SB, Weinzweig J, Kirschner RE, Bartlett SP. Applications of a new carbonated calcium phosphate bone cement: Early experience in pediatric and adult craniofacial reconstruction. Plast Reconstr Surg 2002;109:1789–1796.
3. LeGeros RZ. Properties of osteoconductive biomaterials: Calcium phosphates. Clin Orthop 2002;395:81–98.
4. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005;26:5474–5491.
5. Adachi T, Osako Y, Tanaka M, Hojo M, Hollister SJ. Framework for optimal design of porous scaffold microstructure by computational simulation of bone regeneration. Biomaterials 2006;27:3964–3972.
6. Okuda T, Ioku K, Yonezawa I, Minagi H, Kawachi G, Gonda Y, Murayama H, Shibata Y, Minami S, Kamihira S, Kurosawa H, Ikeda T. The effect of the microstructure of beta-tricalcium phosphate on the metabolism of subsequently formed bone tissue. Biomaterials 2007;28:2612–2621.
7. Habibovic P, Yuan H, van den Doel M, Sees TM, van Blitterswijk CA, de Groot K. Relevance of osteoinductive biomaterials in critical-sized orthotopic defect. J Orthop Res 2006; 24:867–876.
8. Habibovic P, de Groot K. Osteoinductive biomaterials - properties and relevance in bone repair. J Tissue Eng Regen Med 2007;1:25–32.

9. Habibovic P, Yuan H, van der Valk CM, Meijer G, van Blitterswijk CA, de Groot K. 3D microenvironment as essential element for osteoinduction by biomaterials. Biomaterials 2005; 26:3565–3575.
10. Gosain AK, Riordan PA, Song L, Amarante MT, Kalantarian B, Nagy PG, Wilson CR, Toth JM, McIntyre BL. A 1-year study of osteoinduction in hydroxyapatite-derived biomaterials in an adult sheep model: Part II. Bioengineering implants to optimize bone replacement in reconstruction of cranial defects. Plast Reconstr Surg 2004;114:1155–1163.
11. Gosain AK, Song L, Riordan P, Amarante MT, Nagy PG, Wilson CR, Toth JM, Ricci JL. A 1-year study of osteoinduction in hydroxyapatite-derived biomaterials in an adult sheep model: Part I. Plast Reconstr Surg 2002;109:619–630.
12. Khairoun I, Boltong MG, Driessens FC, Planell JA. Effect of calcium carbonate on clinical compliance of apatitic calcium phosphate bone cement. J Biomed Mater Res 1997;41:560–567.
13. Ning JH, Liu HC, Mao KY, Pan HP, Wang HX. A pilot study of repair of periodontal bone defects with carbonated phosphate bone cement modified with synthesized peptides in dogs. Zhonghua Kou Qiang Yi Xue Za Zhi 2003;38:464–466.
14. Shao XX, Hutmacher DW, Ho ST, Goh JC, Lee EH. Evaluation of a hybrid scaffold/cell construct in repair of high-load-bearing osteochondral defects in rabbits. Biomaterials 2006; 27:1071–1080.
15. Wan C, He Q, McCaige M, Marsh D, Li G. Non-adherent cell population of human marrow culture is a complementary source of mesenchymal stem cells (MSCs). J Orthop Res 2006;24:21–28.
16. Chen X, Xu H, Wan C, McCaigue M, Li G. Bioreactor expansion of human adult bone marrow-derived mesenchymal stem cells (MSCs). Stem Cells 2006;24:2052–2059.
17. Durucan C, Brown PW. Alpha-Tricalcium phosphate hydrolysis to hydroxyapatite at and near physiological temperature. J Mater Sci Mater Med 2000;11:365–71.
18. Fernández E, Ginebra MP, Boltong MG, Driessens FC, Ginebra J, De Maeyer EA, Verbeeck RM, Planell JA. Kinetic study of the setting reaction of a calcium phosphate bone cement. J Biomed Mater Res 1996;32:367–374.
19. Tas AC. Preparation of porous apatite granules from calcium phosphate cement. J Mater Sci Mater Med 2008;19:2231–2239.
20. Habibovic P, Sees TM, van den Doel MA, van Blitterswijk CA, de Groot K. Osteoinduction by biomaterials–physicochemical and structural influences. J Biomed Mater Res A 2006;77:747–762.
21. Brow WE, Chow LC. US Patent 4,518,430, 1985.
22. Constantz BR, Ison IC, Fulmer MT, Poser RD, Smith ST, VanWagoner M, Ross J, Goldstein SA, Jupiter JB, Rosenthal DI. Skeletal repair by in situ formation of the mineral phase of bone. Science 1995;267:1796–1799.
23. Constanz BR. Carbonated hydroxyapatite compositions and uses. International Patent: PCT/US9309086, 1994.
24. Frayssinet P, Gineste L, Conte P, Fages J, Rouquet N. Short-term implantation effect of a DCPD-based calcium phosphate cement. Biomaterials 1998;19:971–977.
25. Ohura K, Bohner M, Hardouin P, Lemaître J, Pasquier G, Flautre B. Resorption of,and bone formation from,new β-tricalcium phosphate-monocalcium phosphate cements: An in vivo study. J Biomed Mater Res 1996;30:193–200.
26. Hong Y, Wang JT, Hong CY. The periapical tissue reactions to a calcium phosphate cement in the teeth of monkeys. J Biomed Mater Res 1991;25:484–498.
27. Jacob-LaBarre JT, Assouline M, Byrd T, Mcdonald M. Synthetic scleral reinforcement materials. I. Development and in vivo tissue biocompatibally response. J Biomed Mater Res 1994;28:699–712.
28. Behling CA, Spector M. Quantitative characterization of cells at the interface of long-term implants of selected polymers. J Biomed Mater Res 1986;20:653–666.
29. Clark AE, Hench LL, Paschall HA. The influence of surface chemistry on implant interface histology: A theoretical basis for implant materials selection. J Biomed Mater Res 1976;10: 161–174.
30. Kruyt MC, Dhert WJ, Oner FC, van Blitterswijk CA, Verbout AJ, de Bruijn JD. Analysis of ectopic and orthotopic bone formation in cell-based tissue-engineered constructs in goats. Biomaterials 2007;28:1798–1805.
31. Hanawa T, Kamiura Y, Yamamoto S, Kohgo T, Amemiya A, Ukai H, Murakami K, Asaoka K. Early bone formation around calcium-ion-implanted titanium inserted into rat tibia. J Biomed Mater Res 1997;36:131–136.
32. Hamanishi C, Kitamoto K, Ohura K, Tanaka S, Doi Y. Self-setting, bioactive, and biodegradable TTCP-DCPD apatite cement. J Bio Mater Res 1996;32:383–389.
33. Yamamoto H, Niwa S, Hori M, Hattori T, Sawai K, Aoki S, Hirano M, Takeuchi H. Mechanical strength of calcium phosphate cement in vivo and in vitro. Biomaterials 1998;19: 1587–1591.
34. 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.
35. Joschek S, Nies B, Krotz R, Goferich A. Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone. Biomaterials 2000;21:1645–1658.
36. Schopper C, Moser D, Goriwoda W, Ziya-Ghazvini F, Spassova E, Lagogiannis G, Auterith A, Ewers R. The effect of three different calcium phosphate implant coatings on bone deposition and coating resorption: A long-term histological study in sheep. Clin Oral Implants Res 2005;16:357–368.