Pilot study of a calcium phosphate cement based composite as implant coating and socket grafting material

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Research Article

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Abstract

Materials that can be used in load-bearing implants are desirable. They have to display a wide range of properties in order to work well in the human body. Calcium phosphate cement (CPC) is a promising candidate for this role because of their ability to directly bond to bone, serving as a template for newly forming bone and meanwhile a load-bearing support for implant. In this study, a commercialized CPC product were blended with hemostatic gelatin sponges and minocycline, then smashed to form a self-setting composite material with porous structures. Firstly, the CPC composite was tested as a coating material for titanium implant in experimental rabbit models. The results showed the coating materials could guide bone-to-implant contact in 12 weeks, displaying a good osteoconductive ability. Then a clinical trial was carried out to evaluate the CPC composite's performance as bone substitute in 8 patients, who experienced tooth extractions and accepted the composite material grafting for ridge preservation. After 3-6 months' healing, the majority of the CPC composite was replaced by new alveolar bone, allowing successful placement of dental implant and denture restoration. This pilot study indicated that porous CPC composite could be used in implant dentistry as biomedical material for implant coating or alveolar ridge preservation.

Background

Bone grafting has been considered necessary to correct alveolar bone defects after tooth extraction, and raise the operational reliability of immediate or early implant placement.[1–3] Current commonly used bone grafts involve autogenous cancellous/ block bone, and bone substitute materials. The advantages of artificial bone graft substitutes include unlimited use of material, and no morbidity of the donor-site. Numerous bone substitutes have been developed to helped reduce the necessity of autogenous bone grafts.[4] From the clinical standpoint, an ideal bone substitutes should be moldable when packing in irregular defects, then display a self-hardening potential, and form a stable but still porous scaffold in the defect. These properties would ease the clinical application, reduce the need for barrier membranes to retain graft materials and exclude nonosteogenic tissues from influencing the bone healing process.[5, 6] Calcium phosphate cement (CPC) have been considered as an attractive bone substitutes that can be used in implant dentistry.[7] CPC consist of two phases, a powder phase of calcium phosphate salts and a liquid phase of aqueous solution, which, when mixed, form a viscous paste that is able to progressively set and harden at room/body temperature. In the paste form, CPC can be easily manipulated and shaped into a defect area, providing intimate adaptation to the surrounding bone even for irregularly shaped cavities. The cement setting reaction mainly involves the release of calcium and phosphate ions from starting powders, generation of a supersaturation in the solution, then nucleation of a new phase of hydroxyapatite crystal and entanglement of the structure.[8] The final composition of hardened CPC is more similar to the calcium phosphates found in the bone tissues than sintered bioceramics, representing a high osteoconductivity of the material.[9, 10] CPC has been successfully commercialized for clinical applications, and due to their unique mechanical properties and outstanding bone-repair abilities, the application of CPC- based biomaterials for implant dentistry has been of great interest.
However, despite a wealth of knowledge gained, clinical efforts that use CPC as bone substitutes for dental and intraoral therapies remain substantially lacking. In seeking a break-through in this area, we designed a preclinical study and a clinical trial to test a novel porous CPC composite fabricated by incorporating CPC with gelatin sponges and minocycline. The results were inspiring and gave primary evidence for the use of CPC in implant dentistry as biomedical material for implant coating or alveolar bone defect filling.

**Materials And Methods**

**CPC composite preparation and in vitro testing**

A commercial product of CPC, 3.8 gram in each ampoule, was purchased from Shanghai Rebone Biomaterials (Shanghai, China). Absorbable gelatin sponge, each size 6cm×2cm×0.5cm, was product of Jinling pharmaceutical co.(Nanjing, China). Minocycline Hydrochloride Ointment, 10mg in each syringe, was product of Sunstar INC(Japan). All the products have been approved for clinical application by the National Medical Products Administration of China. When preparing the CPC composite for use, the CPC powder and liquid were mixed firstly following the manufacturer's instruction, then three pieces of gelatin sponges and a syringe of minocycline ointment were blended into the CPC paste and smashed by using a sterilized tissue homogenizer for 20 seconds. The final composite material was filled in 0.3cm×0.4cm×2.5cm molds, setting in 100% relative humidity incubator for 24 hours. Mechanical properties of the material were evaluated by three-point bending tests on a multifunctional material-testing machine (SHIMADZU, Japan). The test span of the supporting point was 1.0cm, and the loading speed was 0.1cm/min. Material flexural strength is defined as \( S = \frac{3F_{\text{max}}L}{2bh^2} \), where \( F \) is the maximum load of the load-displacement curve, \( L \) is the span, \( b \) is the width of the specimen, and \( h \) is its thickness. Elastic modulus is calculated as \( E = \frac{F}{d} \left( \frac{L^3}{4bh^3} \right) \). Work-of-fracture (toughness) is the area under the F-d curve divided by the cross-sectional area of the specimen. The porous structure of the hardened CPC composite was observed by using a high-resolution micro-CT device (Inveon Micro-CT, Siemens, Germany). The scanner was set with the nominal resolution of 15 lm/pixel to obtain an accurate 3D reconstruction of each sample. The ultra-structure of the material was also inspected by scanning electron microscopy (SEM, ZEISS EVO18, Germany).

**Preclinical testing of CPC composite as implant coating material**

Eighteen healthy, male, adult (6–8 months old and 3.5-4 kg) New Zealand White rabbits were involved in this study. All animals were treated in accordance with the guidelines of the Laboratory Animal Care & Welfare Committee, School of Stomatology, the Fourth Military Medical University. A rabbit model for research in implant dentistry was used according to the published literature.[11] All surgical procedures were performed under general anesthesia induced by an intravenous injection of 3% pentobarbital sodium solution. The surgical site was shaved, sterilized, and given local infiltration anesthesia with lidocaine (0.5% lidocaine with 1:200,000 epinephrine). The lateral flat bone surface of the left distal femur
was exposed. An bone cavity of 8 mm in diameter and 6 mm in depth was prepared using a cylinder-shaped burr. Experimental titanium implants (ZHONGBANG Titanium Biological Materials Co., Ltd) of 4 mm in diameter and 6 mm in root length were coated with 2 mm thick CPC composite preoperatively, and placed in the artificial bone cavities. After surgical wound closing, intramuscular injection of penicillin (4 WU/kg) was applied for five days to prevent infection. The rabbits were observed daily for proper activities until they were sacrificed at 12 weeks. The femur specimens containing implants were harvested and placed in 10% neutral buffered formalin for 48 h, rinsed thoroughly with phosphate-buffered saline (PBS), and then placed in 70% (v/v) ethanol solution. Micro-CT was used for radiographic evaluation of each sample. A standard implant-centered cylindrical (8 mm in diameter, 6 mm in depth) volume of interest (VOI) was created, material degradation and new bone regeneration around the implant were examined. The osseo-implant interface was further observed by hard tissue slicing. After dehydration, femur specimens was infiltrated with a methyl-methacrylate resin from a starting solution of 50% ethanol/resin to a subsequent resin of 100%, with each step lasting 24 hours. After polymerization, blocks were sectioned and then ground down to approximately 40µm. Van Gieson staining was used to reveal the bone healing situation around the implant.

Clinical application of CPC composite as socket grafting material

The clinical trial was approved by Ethics Committee and Institutional Review Board of School of Stomatology at the Fourth Military Medical University (ethics committee protocol number: IRB-RJ-2020007; date of registration: 2021/3/12), and carried out in accordance with the Declaration of Helsinki. Verbal and written information describing the nature of the study was given to all enrolled participants and the signed informed consent forms were obtained prior to enrollment of the subjects. Eight patients, 26–56 years of age, intended for extraction and subsequent implant placement, were enrolled. The preoperative radiography revealed a substantial bone wall defect around the tooth. Exclusion criteria included patients with uncontrolled systemic diseases, history of head/neck irradiation, immune system severe deficiencies, and patients treated with oral/intravenous bisphosphonates. The tooth extraction was performed without flap reflection, and the alveolar socket was cleaned thoroughly. CPC composite was prepared and implanted into the socket by using a head-removed 5ml syringe. A sterile gauze was used to compact materials and shape its surface within the confines of the ridge. Gradually hardening of CPC composite could be observed in several minutes, then the extraction wound was sutured with silk thread. Subjects were instructed to take Ibuprofen for pain as needed and use chlorhexidine (0.12%) mouth rinse daily. Amoxicillin was provided 500 mg TID for 5 days. At 1-week post-surgery, sutures were removed. At 3 months and 6 months, clinical revision and CBCT inspection were done to evaluate the bone healing of extraction site, based on what dental implant was placed.

Statistical methods

The results were analyzed by SPSS17.0 statistical software. The mechanical test results were analyzed using an independent t test.
Results

Micro-CT inspection revealed that the smashed gelatin sponges distributed within the CPC block evenly and formed abundant porosity. Scanning electronic microscopy (SEM) demonstrated that the gelatin sponge's pieces created macropores (approximately 200–900µm) within CPC upon its intrinsic spotty micro-porosity. The elastic modulus of composite material obtained from elastic bending test was 1.30 ± 0.21GPa. (Fig. 1)

In the preclinical study, all rabbits tolerated the surgical procedure well. No gross signs of morbidity were observed. Twelve weeks after the operation, the Micro-CT inspection revealed that the coating CPC composite was replaced by new bones with legible trabecular structures, and bone-to-implant contact was satisfied. Hard tissue slicing further proved peri-implant bone healing and implant osseointegration. (Fig. 2)

In the clinical trial, ten teeth of 8 patients (6 males and 2 females) were included in the study. Because the CPC composite had fallen off the extraction socket within the first postoperative week, seven teeth were assessed in this study. The loss of graft materials all occurred in upper molar regions with excessive bone defects, two of which were connected adjacent sockets. The CPC composite hardening in these sockets had larger size and more gravity but less area bonding to bone.

No infection occurred in extraction sockets after the CPC composite grafting. No obvious discomfort or pain were reported by the patients. At 3 months, satisfied gingival healing by secondary intention was observed. There might be unabsorbed small piece of CPC composite existing on the surface of ridge in some cases. But CBCT analysis revealed the majority of the graft materials were replaced by newly formed bone in four regions of 4 patients, allowing early placement of dental implant and following denture restoration. The other three regions of 2 patients experienced a delayed implant placement at 6 months, owing to the larger volume of graft materials and longer time of osteoconduction processes. (Fig. 3–4)

Discussion

When a functional tooth has to be extracted, the obstacles that all clinicians have dealt with is how to manage extraction sites to prepare better site for the following dental implant placement or to minimize ridge shrinkage for a fixed or removable prosthesis delivery.[12] It is well documented that bone graft/substitute can contribute to reducing of the ridge dimensional changes or supporting implant placement after a tooth is extracted. Ideal bone graft/substitute materials should be able to fill irregular sockets, attach to the alveolar bone, provide a load-bearing property, and maintain biological support during new bone regeneration.[13] The superior handling characteristics of CPC have make it a promising candidate materials for application in implant dentistry. However, it is also widely accepted that there are still some crucial issues that need to be solved to satisfy real clinical requirements.[8]
Hardened CPC are intrinsically microporous with pore size in the range of submicro/micrometers, being conducive to tissue fluid impregnation into CPC, and help resorption and replacement of CPCs by bone. However, it is also desirable to create macropores of at least tens of micrometers in CPCs to favor bone colonization, accelerating the process of replacement of CPCs by bone. [14, 15] In our previous animal study, we proved collagen could be a bionic component to be integrated with CPC to improve its biocompatibility, meanwhile create macro-porosity within CPC. The CPC-collagen composite had expanded inner surface area that could facilitate bone ingrowth.[10] In this study, we chose clinically available gelatin sponges and CPC to fabricate a uniform composite for both preclinical testing and clinical trial. By using a tissue homogenizer, the gelatin sponges were smashed and mixed with CPC paste forming a fusion product. After hardening reaction, the final CPC composite showed abundant macroporosity, which was architecturally similar to cancellous bones.

In this study, the novel CPC composite also presented other beneficial properties. Due to the evenly distributed gelatin sponges, we found the modified CPC paste had improved viscosity. Addition of cohesion promoters to CPC had been considered important because CPC pastes tend to disintegrate upon early contact with blood or other aqueous fluids, which inhibits the use of these materials for clinical use as for bone repair, reconstruction and augmentation.[8, 16] The improvement of paste viscosity could help maintaining the material’s early stability in fresh alveolar socket. Another main challenge facing CPC composites was their mechanical properties, including strength, toughness, brittleness and reliability.[17, 18] In this study, the novel CPC composite was a homogeneous material having a strength comparable to cancellous bone.[20] The overall mechanical properties of CPC composite made it a favourable packing material for dental implant in non-to-moderate load-bearing environment.

The fresh alveolar socket was a more complicated open environment, comparing with the closed bone cavity in rabbit’ femur.[21] Various socket bone wall defect and dynamic bone remodeling/resorption following extraction would influence the stability of packing materials. The chewing activity would create unexpected load-bearing during the healing process. Furthermore, without extensive flapping, the wound closure after tooth extraction would generally be secondary closure, also known as healing by secondary intention. This type of healing required more time.[22, 23] Based on the concerns above, alveolar ridge preservation without dental implant placement was designed to testing CPC composite in clinical case series. However, loss of materials from upper molar sockets occurred in two patients. Different from the traditional particle materials, the CPC composite set in situ as integrated material, whose stability largely depended on a tight contact with surrounding bone of the extraction socket. We suggested that additional fixation, instead of simple suturing of the extraction wound, should be necessary for CPC composite in upper large sockets with excessive bone defects. Further studies will be carried out.

The limitations of this study include a small cohort and time-limited observation. But the evidences provided by the preclinical study and clinical trial primarily confirmed the high osteoconductivity, appropriate mechanical properties and good surface chemistry of newly fabricated CPC composite, which make it a unique candidate for dental applications. The commercial product of CPC, gelatin and
minocycline ointment are reasonably cheap. There is no need for barrier membrane guidance. Further researches will be carried out to find appropriate approach for severely damaged socket restoration by using CPC composite material allied with immediate dental implant placement.

Declarations

- Ethical Approval and Consent to participate

The study was approved by the Ethics Committee at the School of Stomatology, the Fourth Military Medical University. All participants gave informed consent. This retrospective study involved no more than minimal risk to subjects, did not adversely affect the rights and welfare of subjects, contributed to greater public good, and could not practically be carried out otherwise.

- Consent for publication

Informed consent for publication was obtained from all participants.

- Availability of supporting data

The data sets supporting the results of this article are included within the article.

- Competing interests

There are no actual or potential conflict of interest needs to be disclosed, including any financial, personal or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, their work.

- Funding

Not applicable

- Authors' contributions

Hongzhi Zhou, performed the clinical surgery and wrote the manuscript;

Yang Xue, contributed significantly to analysis and manuscript preparation;

Ping Liu, Feng He, contributed significantly to the entire study;

Xuenni Zheng, contributed to data collection and analysis.

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References


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Figures
Figure 1

The figures show the Micro-CT (a) and SEM (b) images of CPC composite, and its mechanical properties comparing with the pure CPC material as control (c).
Figure 2

The figures show the preclinical study of CPC composite: the bone cavity prepared in distal femur of rabbit (a); the placement of experimental titanium implant coating with CPC composite (b); the Micro-CT image of implant healing at 12 weeks postoperatively (c); the bone to implant contact in hard tissue slicing (d).

Figure 3

The figures show the clinical application of CPC composite: the radiography before the surgery (a); the extraction socket with graft CPC composite (b); the radiography at 3 months (c); the second healing of the extraction site (d).
Figure 4

The figures show the implant placement at healed extraction site: the surgery of dental implant placement (a); the radiography of the implant 3 months after the surgery (b); the denture restoration of the implant (c).