MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2015; 21: 1162-1172
DOI: 10.12659/MSM.893845

Received: 2015.02.10
Accepted: 2015.04.13
Published: 2015.04.23

Preparation of Calcium Phosphate Cement and Polymethyl Methacrylate for Biological Composite Bone Cements

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1,2 Jun Yang*
BCF 1 Kairui Zhang*
DF 1 Sheng Zhang*
CF 2 Jiping Fan
AF 2 Xinhui Guo
BCF 1,3 Weiqiang Dong
AF 1 Shengnan Wang
CD 1 Yirong Chen
ABCDEFG 1 Bin Yu

1 Department of Orthopaedics and Traumatology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, P.R. China
2 Department of Orthopaedics, 421 Hospital of PLA, Guangzhou, Guangdong, P.R. China
3 Department of Orthopaedics, The First Affiliated Hospital to Guangzhou Medical University, Guangzhou, Guangdong, P.R. China

* Jun Yang, Kairui Zhang, Sheng Zhang and Jiping Fan are co-first authors who contributed equally to this work

Corresponding Author: Bin Yu, e-mail: 859487053@qq.com
Source of support: Departmental sources

Background: We studied the biological safety, biomechanics, and tissue compatibility of calcium phosphate cement and Polymethyl Methacrylate composite bone cement mixed in different ratios.

Material/Methods: CPC and PMMA were mixed in different ratios (3: 1, 2: 1, 1: 1, 1: 2, 1: 5, 1: 10, 1: 15, and 1: 20). PMMA solvent is a general solvent containing a dissolved preparation of the composite bone cement specific to a given specimen to determine biological safety, biomechanics, and tissue compatibility.

Results: The CPC/PMMA (33%) group, CPC/PMMA (50%) group, CPC/PMMA (67%) group, and CPC/PMMA (75%) group were more in line with the composite bone cement without cytotoxicity requirements. The compressive strength of the CPC/PMMA (67%) group and CPC/PMMA (75%) group was 20Mpa–30Mpa, while that of the CPC/PMMA (4.8%) group, CPC/PMMA (6.25%) group, CPC/PMMA (9.1%) group, CPC/PMMA (16.7%) group, CPC/PMMA (33%) group, and CPC/PMMA (50%) group was 40Mpa–70Mpa. Curing time was longer in the CPC group (more than 11 min) and shorter in the PMMA group (less than 2 min). The results of weight loss rate showed that there were no significant differences between the CPC/PMMA group (4.8%, 6.25%, 9.1%, 16.7%, 33%) and PMMA control group ($p>0.05$). With the decrease of CPC content, the rate of weight loss gradually decreased.

Conclusions: The CPC/PMMA (50%) group, CPC/PMMA (67%) group, and CPC/PMMA (75%) group provide greater variability and selectivity for the composite bone cement in obtaining better application.

MeSH Keywords: Bone Cements • Materials Testing • Methylmethacrylate

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/893845

4174 — 10 34

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

CPC and PMMA have been widely studied respectively. The combination of CPC and PMMA is a mixture of CPC/PMMA, but it remains uncertain whether or not the new mixture would still maintain its traditional advantages.

However, through the study on reports of composite PMMA and other materials (e.g. PG [1] and PCL [2]), preparation and characterization of drug-loaded PMMA/PG composites have been confirmed in 2002 [1]. And partially biodegradable composites have been prepared with polymethyl methacrylate/poly (e-caprolactone) (PMMA/PCL) as an alternative to the drug delivery systems which can be polymerized *in vivo* and also, can provide some structural support before degradation [2].

So we believe that PMMA and CPC composite are feasible. New or modified PMMA formulations are being used in many clinical and experimental researches. Modifications to these fillers may vary from different physicians and procedures. To date, there exist no standardized formulations, biomechanical standards, or safety guidelines when preparing or modifying PMMA or any other bone void filler to be used in the spine [3]. Polymethylmethacrylate is an effective vertebral augmentation filler material, which is inert, biomechanically sound, adaptable to different techniques and cost-effective. The choice of filler depends on the ultimate development of certain material with favorable biomechanical and biological properties as well as good radiopacity and cost-effectiveness [3].

Although there lacks background of the preparation of composite bone cement, many scholars have obtained successful experience on PMMA, PG, PMMA and PCL composite materials. Then the idea of mixing these materials into new compounds, and study whether the complexes possess good biomechanical and biological characteristics occurs to us. Therefore, the experiments were conducted, and the results indicate that CPC and PMMA can be composited, and bone cement composites possess favorable biological and biomechanical characteristics.

These advantages would offset the deficiencies of PMMA, which is neither degradable nor conducive to bone or new bone ingrowth [4]. Hence, if the composite can be generated successfully, a new type of composite bone cement will be obtained with the advantages of both independent components (i.e., CPC and PMMA); in addition to the benefits of CPC listed above, PMMA will be able to enhance the mechanical support of the material to meet the needs of new bone cement as implantation material in the future.

Material and Methods

According to different mixing ratios of composite bone cement specimens, injection-type PMMA bone cement (Heraeus, Germany) was mixed with injection-type CPC bone cement (Ruibang company, China) in order to create a bone cement solid series with mass ratios of 3:1, 2:1, 1:1, 1:2, 1:5, 1:10,1:15,1:20, corresponding to PMMA to CPC and vice versa according to the biological material experimental detection and safety standards in detecting related experiments [5]. CPC and PMMA were measured in accordance with the best ratio of liquid to solid (1 ml: 2 g), and the concentrations of both CPC and PMMA were in accordance with the desired bone cement mixing ratios (3:1, 2:1, 1:1, 1:2, 1:5, 1:10, 1:15, 1:20). The bone cement mixing ratios (3:1, 2:1, 1:1, 1:2, 1:5, 1:10, 1:15, and 1:20) are in line with group75%, group 67%, group 50%, group 33%, group 16.7%, group 9.1%, group 6.25% and group 4.8%.

A pasty mixture was created and then filled in a sterile mold for 60 s of compaction at 37°C in a 100% humidity environment. Samples were removed and cured at 37°C at 100% humidity for 23 hours.

Cell toxicity test

CPC group, PMMA group, and CPC+PMMA composite bone cement group materials were arranged separately in mouse source medium (3 cm^2/ml) at 37°C for 120 h [6] in preparation for a medium extract of CPC, PMMA and composite bone cement group [7]. Then, MC3T3-E1 osteoblastic progenitor cells were inoculated in a 10 cm^2 dish and cultured at 37°C, 5%CO_2, 100% humidity for 2 days, during which the cells grew logarithmically. The culture medium was then discarded and washed by PBS solution twice. 0.25% trypsin was added into the culture dish (2 ml/10 cm^2) until the cells became round in the medium after termination of digestion. Centrifugation was given at 1000 rpm for 5 min. The supernatant was then discarded, 1ml medium was added (i.e., 10 µl), and the cells were plated at a density of 2.5×10^3–5×10^3/hole into a 96-well plate (5 holes/group). Cells were then cultured for 24 h at 37°C, 5%CO2, 100% humidity. The original culture medium was then discarded, and the bone cement soaking liquid prepared was added at 100 µl/hole. These specimens were cultured for 24 h, after which CCK8 detection reagent (Sigma, America) was added; cultivation continued for an additional 3 h-4 h (37°C, 5%CO2, 100% humidity). OD values were then measured in each group using the enzyme mark instrument volume at a wavelength of 450 nm. The relative cell growth rate was calculated respectively.

Sensitization test

Eleven bone cement samples were extracted under aseptic conditions. SD rats were reared in a quiet state following the

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

principle of animal protection. A 1-ml aliquot of each leaching liquor was drawn into a disposable syringe for later use. The injection site on the medial thigh was disinfected, and the materials were injected via local intradermal injection at 0.1 ml per injection point. The control group was injected with physiological saline. The injection sites of the SD rats were observed for erythema, edema, induration and eschar formation after 15 min, 30 min, 1 h, and 24 h from injection and compared with the control group. The scale used in these observations was based on the Magnusson and Kligman classification standard [8].

Compressive strength test and tensile strength test

The bone cements were mixed uniformly and then injected into stainless steel molds at room temperature. The resulting specimens were cylindrical with diameters of 5 mm and heights of 10 mm. The bone cement specimens were placed on an Instron universal testing machine (n=10 times) to determine their compressive strength and tensile strength.

Three-point bending test

After storing in a water bath at 37°C for 48±2 h, the flexural strength of the composite bone cement specimens was measured by a three-point bending test. Applying a universal testing machine (EZ20, Lloyd Instruments Ltd., UK), flexural strength tests were carried out with a supporting span of 50 mm with a crosshead speed of 5 mm/min until failure(n=10 times) [9].

Solidification time measurement

A mold with a diameter of 10 mm and a height of 5 mm was prepared and then filled in with different bone cement blends. These molds were placed into an environment of 37°C and 100% humidity. A vertical pressure head on the bone cement surfaces was applied for 5 seconds to measure the degree of deformation into the bone cement using a Vicat apparatus every 30 seconds until the indentation could no longer be seen. The solidification time was measured from the end of mold filling until no additional indentation was observed. The result was the average of 3 replicates.

Scanning electron microscopy morphology

The bone cement samples were immersed in ethanol to stop the hydration reaction and were allowed to dry naturally. A JSM-5600LV type low vacuum scanning electron microscope was then adopted to observe and detect structural changes in the bone cements' internal micro holes; 500 cycles were implemented for accuracy.

Phase composition analysis

The tested samples were dried naturally at room temperature. Their structure and phase were analyzed with an X 'Pertpro type X-ray from the Holland PANalytical company. X-ray diffraction with a Cu rake, a tube voltage of 40keV, a tube current of 30 mA, a continuous scan range of 20 from 10° to 90°, and a scan rate of 15.24/min was applied. The phase of the samples was determined with the X-ray software Jade 5.0.

Animal model of the bone defect

To establish an animal model of the bone cement samples after implantation in a bone defect [10,11], muscle pouch tissues were investigated. SD rats were placed supine with fixed limbs and trunks. The medial skin of their hind limbs was disinfected using iodophor and alcohol after anesthesia. After a straight incision along the medial tibial surface, a gap in the muscle was created and the periost was exposed. A hole was then drilled into the bilateral medial tibial bone using a hand drill with a borehole diameter of 5 mm, resulting in a borehole area of 19 mm^2 and a medial tibial unicortical critical bone defect. The borehole area was greater than the previously reported bone defect areas [12]. The skin was sutured immediately after the bone defect was created to establish the control group. For the experimental group, a muscle cover was sutured, the bone defect was created, and bone cement with a diameter of 5 mm and a thickness of 2 mm was implanted in bone defect; the skin was then sutured. The skin of the right hind limb was then cut, muscle tissues were separated to create a muscle cavity, in which different bone cements were implanted. The muscle and skin were then sutured, the skin was disinfected with alcohol again, and each mouse was fed feeding after being numbered. X-rays of the implanted bone cements were recorded after 4 weeks and 15 weeks. The limbs of SD rats were fixed in the animal plate with a rubber band and the metabolism of the bone cement implantation in the hind limbs of SD rats was measured and recorded.

Histological observation

SD rats were sacrificed after the composite bone cements had been implanted for 15 weeks. The bone cements in the muscle cavity were removed, and the bone cements in the bone defects and surrounding bones were fixed with 10% formalin before observation with dyeing. Specimens were embedded in paraffin, decalcified and stained to observe the internal void structure and the degree of new bone formation on the bone cement materials.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

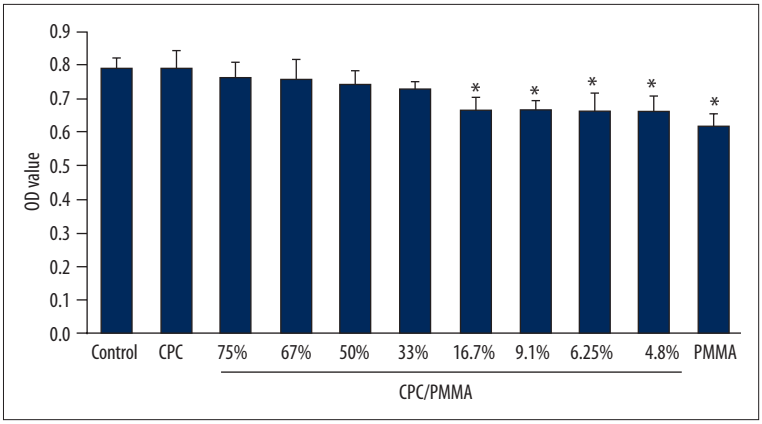


Figure 1. The subjects of the bone cement material extracts CPC/PMMA (16.7%) group, CPC/PMMA (9.10%) group, CPC/PMMA (6.25%) group, CPC/PMMA (4.80%) group, and PMMA group did experience cell toxicity. Data represents the mean ±SD (n=5,* $P<0.05$).

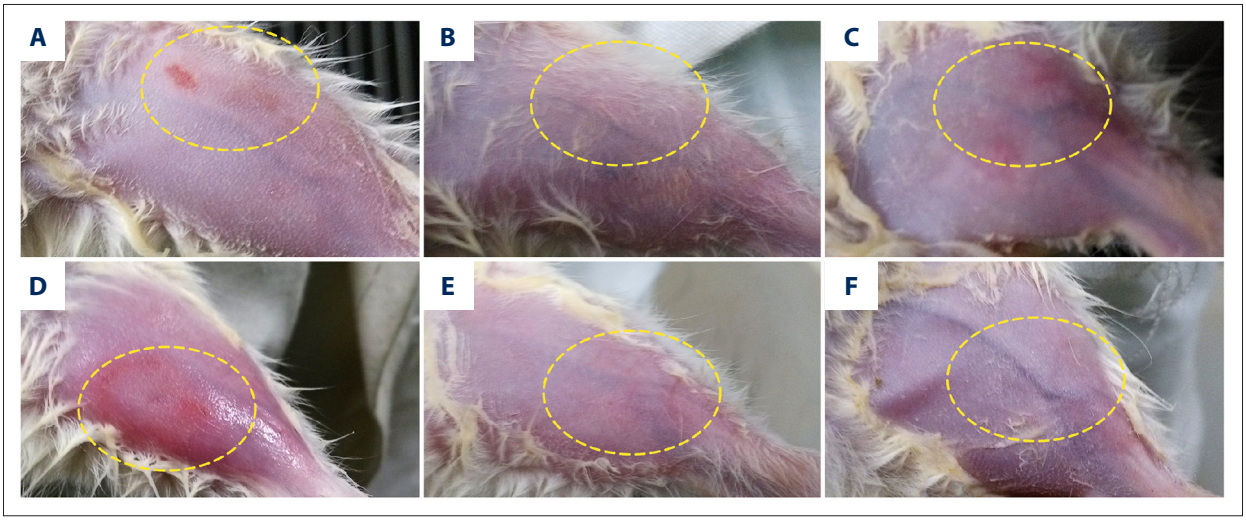


Figure 2. Sensitization test results, the PMMA monomer group showed significant punctate erythema and edema on the skin. There were no significant findings in the other groups. (**A**) Control (0.9%N.S.), (**B**) CPC extract, (**C**) PMMA extracts, (**D**) PMMA monomer, (**E**) CPC/PMMA (75%) extracts, (**F**) CPC/PMMA (4.8%) extracts

The weight loss rate calculation

Implantation of bone cement specimens weighing (W_0), W_0 represents the initial weight. Bone cement specimens were implanted into the medial tibial muscle bag of SD rats before being removed after 15 weeks. And W_1 represents the weight of the specimen *in vivo* after degradation. The weight loss rate=$(W_0–W_1)/W_0×100\%$ so as to evaluate the degradation of bone cements after implantation *in vivo*.

Statistical analysis

All data were parametric after statistical analysis using SPSS19.0. They all followed a normal distribution after data exploring analysis and were expressed as mean ±SD (n=5). The data were analyzed by one-way ANOVA. Should the data satisfied variance homogeneity, the regular F value and P value would be obtained, otherwise, Welch or Brown-Forsythe would be used to compare the population means.

Results

Cell toxicity test

Cell toxicity grading standards are divided into six levels [13]: level 0, ≥100%; level 1, 75–99%; level 2, 50–74%; level 3, 24–49%; level 4, 1–25%; level 5, 0%. Level 0 is considered non-toxic, while level 5 is highly toxic. Compared with the control group, differences were found among the groups (PMMA group, 4.8% group, 6.25% group, 9.1% group and 16.7% group ($P<0.05$). Other groups of bone cement extracts had no effect on the relative MC3T3-e1 cell growth rate, and the toxic reaction was level 1 (i.e., non-toxic). Thus, the subjects of the bone cement material extracts (33% group, 50% group, 67% group, 75% group and CPC group) did not experience cell toxicity (Figure 1).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

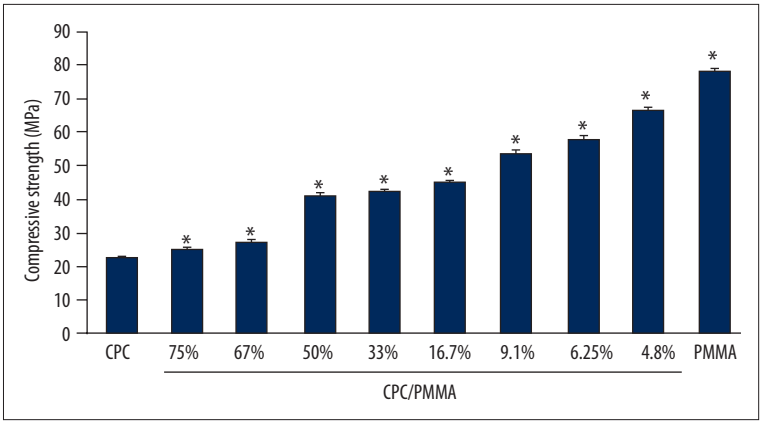


Figure 3. CPC group in control and CPC/PMMA (75%, 67%, 50%, 33%, 16.7%, 9.1%, 6.25%, 4.8%) test group, PMMA test group. There were significant differences between the compressive strength of test groups. Data represents the mean ±SD (n=10,* *P*<0.05).

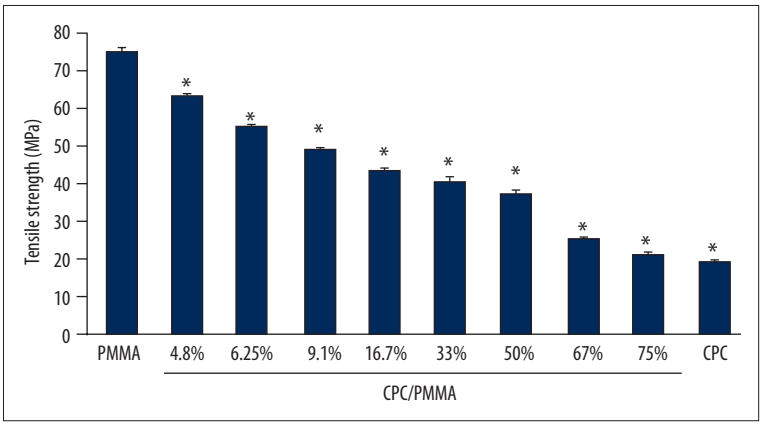


Figure 4. PMMA group in control and CPC/PMMA (75%, 67%, 50%, 33%, 16.7%, 9.1%, 6.25%, and 4.8%) test group, CPC test group. There were significant differences between the tensile strength of test group. Data represent the mean ±SD (n=10,* *P*<0.05).

Sensitization test results

The PMMA injection sites showed erythema on the skin, while the PMMA monomer group showed significant punctate erythema and edema on the skin with a Magnusson and Kligman score of 1 for prompt sensitization. There were no significant findings in other groups, as they demonstrated a Magnusson and Kligman score of 0 (Figure 2).

Testing results of compressive strength and tensile strength

Both the compressive strength and the tensile strength were found to enhance gradually with increasing PMMA concentration and decreasing CPC concentration in each test group. There were significant differences between the compressive strength of each test group ($P<0.05$) (Figures 3, 4).

Testing results of three-point bending test

The compressive strength was found to enhance gradually with increasing CPC concentration and decreasing PMMA concentration in each test group. As demonstrated, there were significant differences of the flexural strength in each test group ($P<0.05$) (Figure 5).

Results of curing time

The curing time was longer in the CPC group (more than 11 min) but was shorter in the PMMA group (less than 2 min), and that of the composite cement groups were approximately 2–6 min. There were significant differences among the CPC group, the PMMA group and the groups of composite bone cements ($P<0.05$) (Figure 6).

Scanning electron microscopy morphology

To reconcile each group of bone cements by PMMA monomer, there were no significant differences found among CPC, PMMA and the composite bone cements in terms of surfaces and structures, or in the interface between the CPC and PMMA composite bone cements; thus, good compatibility was found between these two materials. PPMA, a type of microstructure, was also distributed into the CPC with many benefits of the material's mechanical properties, composite air permeability and electrical properties. Spherulitic grains of the CPC group were found as spherulites stacked close together, which did not rule out the adhesive effects of the PMMA monomer solvent. But with increasing PMMA concentration, the crystalline region loosened marginally. Even though the result was not

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

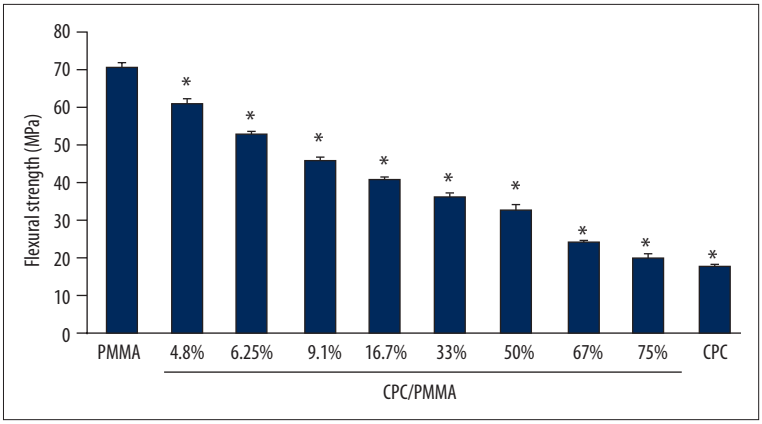


Figure 5. PMMA group in control and CPC/PMMA (75%, 67%, 50%, 33%, 16.7%, 9.1%, 6.25%, 4.8%) test group, CPC test group. There were significant differences between the flexural strength of test group. Data represent the mean ±SD (n=10,* $P<0.05$).

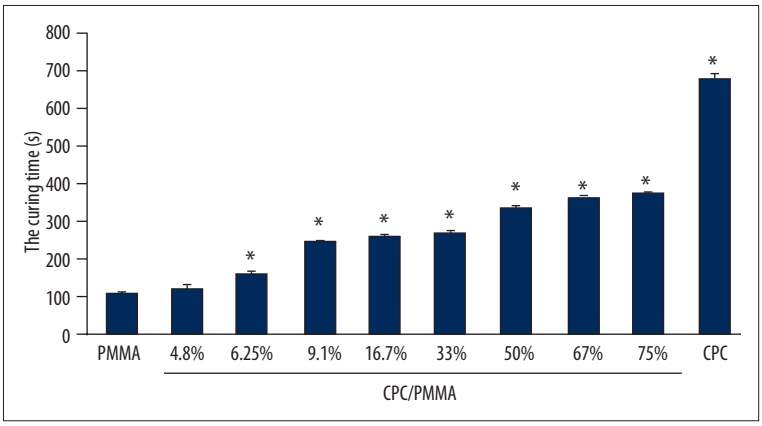


Figure 6. The curing time was longer in the CPC group (more than 11 min) but shorter in the PMMA group (less than 2 min). The curing time of the composite cement groups were approximately 2–6 min. The PMMA group in the control and CPC/PMMA (75%, 67%, 50%, 33%, 16.7%, 9.1%, 6.25%, and 4.8%) test group, CPC test group. There were significant differences between the curing times of the test groups. Data represent the mean ±SD (n=5,* $P<0.05$).

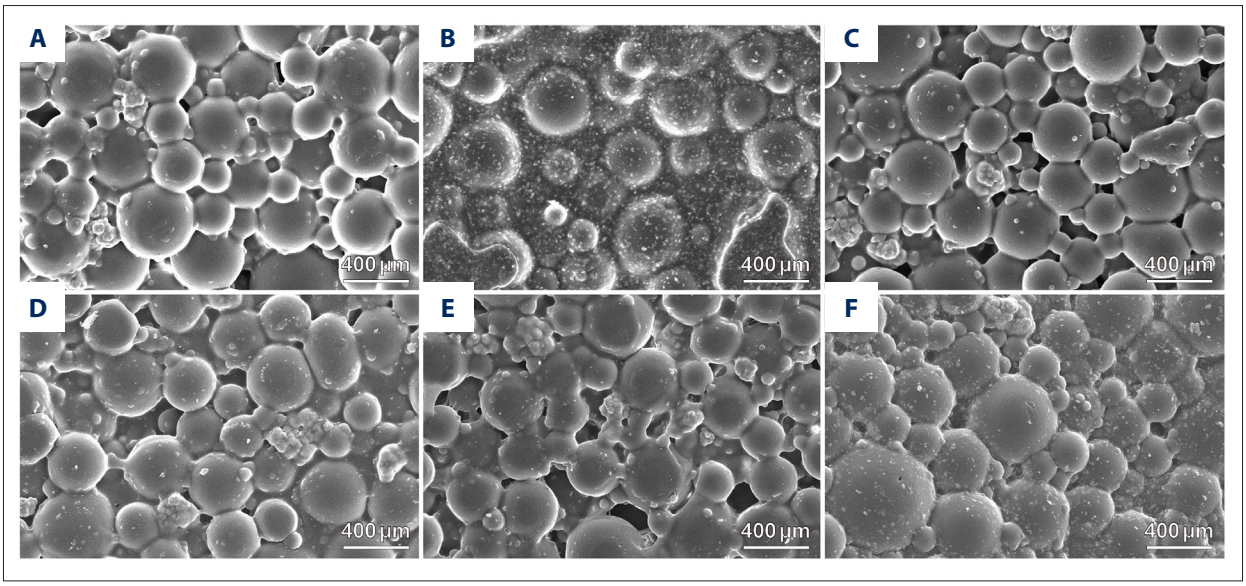
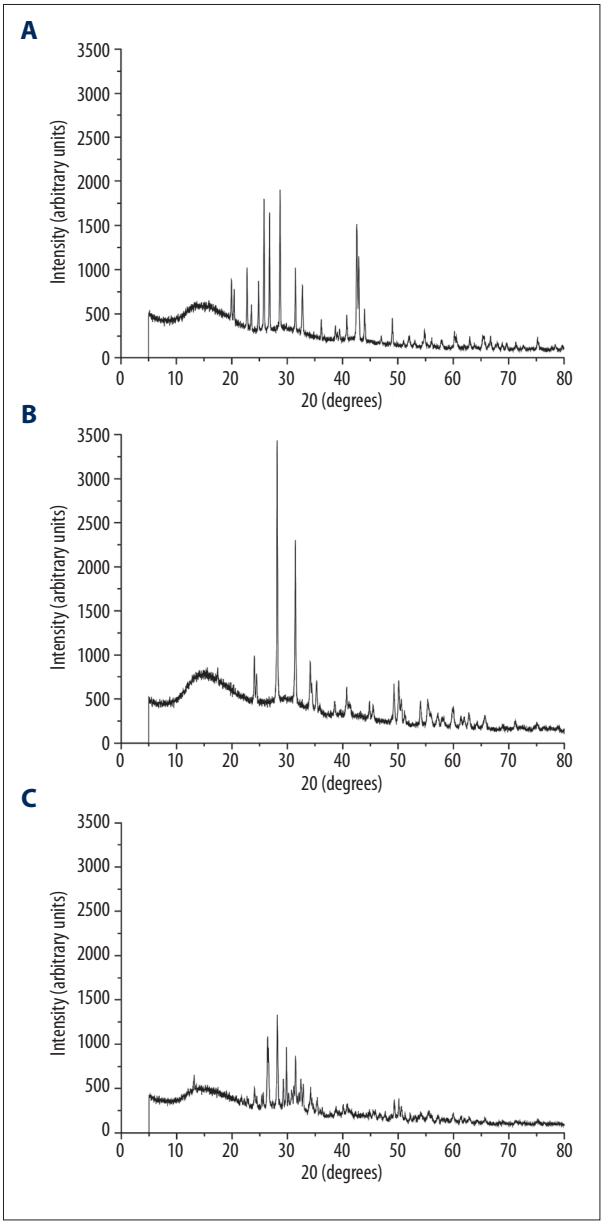


Figure 7. By scanning electron microscopy observation, bone cement particles were spherical, CPC group particles aperture are loose, between 100 um and 400 um (**B**), spherical particles of the PMMA group and CPC/PMMA (4.8%) group are arranged densely, pore aperture is between 50 um and 100 um (**A, F**), pore aperture of the CPC/PMMA (75%) group, CPC/PMMA (67%) group and CPC/PMMA (50%) group are between the group CPC and the group PMMA about 100–200 um (**C–E**). (**A**) PMMA group, (**B**) CPC group, (**C**) CPC/PMMA (75%) group, (**D**) CPC/PMMA (67%) group, (**E**) CPC/PMMA (50%) group, (**F**) CPC/PMMA (4.8%) group.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

significant, it indicated the formation of a continuous system after mixing and that the materials were compatible.

The minimum aperture that bone cells needed for growth should be 70 um. 200–400 um apertures of the general materials make the most suitable sizes of range for bone cells growth as the percentage of above 70 um pore aperture is extremely important for the bone cements pore structure [14,15]. In this experiment, above 70 um aperture would be called the effective aperture of cell growth. By scanning electron microscopy observation, bone cement particles were spherical, and particles in the group CPC were loose; spherical particles of the group PMMA were arranged at a density between 100–400 um, with the pore aperture between 50–100 um, and pore aperture of the group CPC/PMMA (75%), group CPC/PMMA (67%) and group CPC/PMMA (50%) between the group CPC and the group PMMA between 100–200 um. Therefore, the pore structure of the considered composite bone cements was suitable for bone cells growth (Figure 7).



Analysis of X-ray diffraction

The card of the control group was compared under the powder diffraction standard of the International Diffraction Data Centre. PMMA bone cements were amorphous substances with no crystalline diffraction peaks; thus, it only demonstrated the crystalline diffraction peaks of barium sulfate as a developer. The diffraction peaks shown by CPC crystalline were based on hydroxyapatite. The original crystallization properties, which included low CPC crystallinity, witnessed significant decrease with the increase of PMMA after the composite bone cements were mixed together. Sharp diffraction peaks in the XRD map were found weakened, but the crystalline region with barium sulfate and hydroxyapatite with composite bone cement material still existed. The mixture of CPC and PMMA had no significant effect on the final products of the diffraction peaks of hydroxyapatite. Thus, there was no new crystalline phase appeared in the reactant (i.e., PMMA bone cements did not participate in the curing reaction of CPC bone cements) (Figure 8).

X-ray and Histological gross observation

X-ray examinations of the bone cements were performed after 4 weeks. There were no significant degradations in the groups (Figure 9A–9C). 15 weeks later, the X-ray examinations of the bone cements were performed again. Except for the PMMA

Figure 8. Analysis of X-ray diffraction. (**A**) PMMA group, (**B**) CPC group, (**C**) CPC/PMMA (50%) group. To compare the card of the control group with the powder diffraction standard of the International Diffraction Data Centre, PMMA bone cements are amorphous substances with no crystalline diffraction peaks; thus, it only showed the crystalline diffraction peaks of barium sulfate as developer. CPC showed crystalline diffraction peaks based on hydroxyapatite. The original crystallization properties, which included low CPC crystallinity, decreased significantly with increasing PMMA after the composite bone cements were mixed together. Sharp diffraction peaks in the XRD map were found to be weakened significantly, and a large range of diffuse peaks appeared. This showed that the crystalline regions in the composites decreased significantly. However, the mixture of CPC and PMMA had no significant effect on the final products of the diffraction peaks of hydroxyapatite; thus, there was no new crystalline phase (i.e., PMMA bone cements did not participate in the curing reaction of CPC bone cements.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

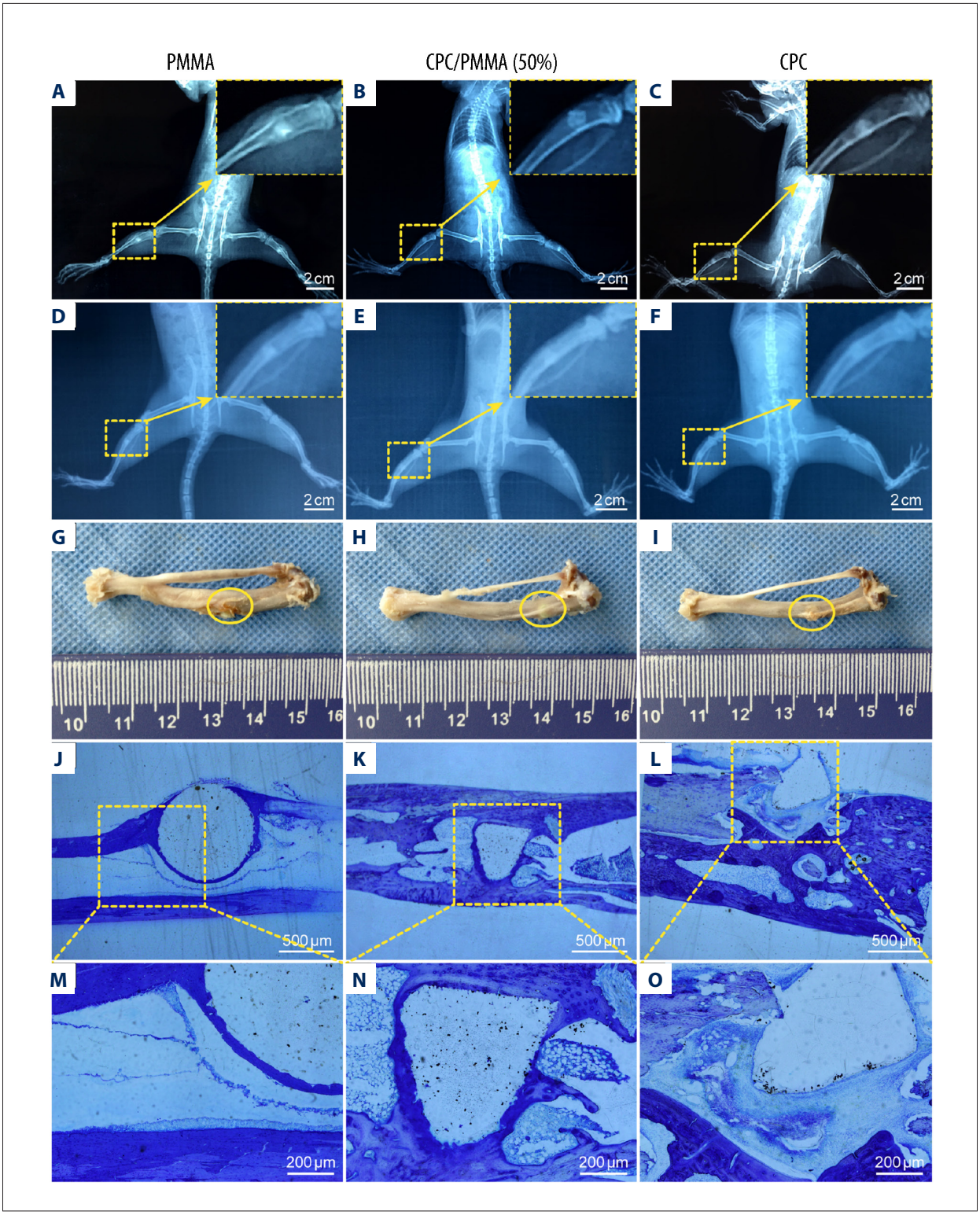


Figure 9. X-ray examinations of the bone cements were also performed after 15 weeks (**A–C**). There were significant degradations in the CPC/PMMA (75%) group, CPC/PMMA (67%) group, CPC/PMMA (50%) group, and CPC group (e.g., the CPC/PMMA (50%) group) (**D–F**). SD rat tibia with a length of approximately 4.5–5.0 cm after bone cement transplantation 15 weeks (**G–I**). Some bone cements in the CPC/PMMA (50%) group and CPC group were degraded. New bone growth was found to have closely integrated with the surrounding bone tissues (**J–L**). The PMMA group had not degraded after transplantation at 15 weeks (**M–O**).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

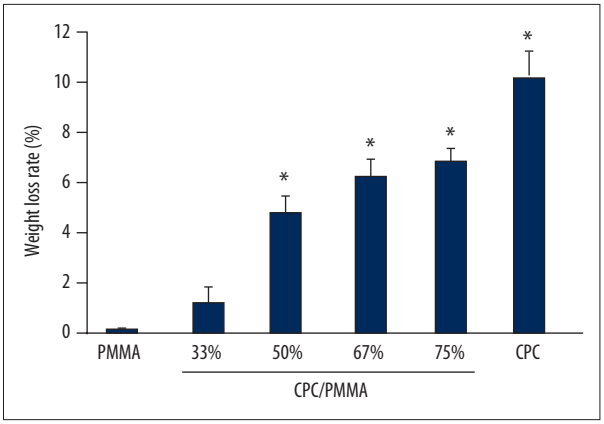


Figure 10. PMMA group weight loss rate almost no change, compared with the PMMA group, CPC/PMMA (33%) group weight loss rate without significant difference, $P>0.05$. CPC/PMMA (50%, 67%, 75%) group and CPC group were significant differences in weight loss rate, (n=5, * $P<0.05$).

group (Figure 9D–9F), significant degradations appeared in both the CPC/PMMA group (50%) and CPC group. SD rat tibia had a length of approximately 4.5–5.0 cm after composite bone cement transplantation *in vivo* after 15 weeks (Figure 9G–9I). CPC/PMMA (50%) group and CPC group bone cements were found degraded. And bone cells growth was found to have integrated with the surrounding bone tissues closely (Figure 9J–9L). PMMA group was found not degraded after transplantation *in vivo* after 15 week. A portion of the composite bone cements (group 50%) was found degraded (Figure 9M–9O).

The weight loss rate calculation

PMMA group weight loss rate witnessed almost no change. There was no significant difference in CPC/PMMA (33%) group weight loss rate, $P>0.05$. Compared with the PMMA group, CPC/PMMA (50%, 67%, 75%) group and CPC group had significant differences in weight loss rate, $P<0.05$. The most obvious change occurred in CPC/PMMA (75%) group, and the average weight loss rate was 6.78% (Figure 10).

Discussion

Although autologous bone grafts is the optimal standard for the treatment of bone defects, operation trauma, bone infection, hematoma and sensory disturbance are commonly observed. In this century, the best way to repair tissue is to use tissue engineering materials to replace self-damaged or defective tissues in the short term and to repeat repairs after body tissue cell proliferation has occurred. Despite its wide range of sources, allogeneic bone has been characterized by defects of immunogenicity and pathogenicity [16,17]; thus, it is not the most ideal material for bone tissue repair. With extensive research on artificial bone materials in recent years, biological ceramic [18–20], metal [21–23], polymer [24], cement [25,26], etc., have had clinical applications and ideal patient outcomes. To understand the advantages of each material and to achieve an accurate simulation of autogenous bone, many scholars have combined inorganic and organic materials to create substitute materials of composite bone.

The products of composite CPC [27] and PMMA can attain both material advantages concurrently while meeting the needs of clinical treatments on fractures in different parts of the human body such as bone defects, bone necrosis and bone tumors. New biomaterials will play a greater role in the field of orthopedics and will promote the development of bone tissue engineering [28]. CPC is composed of solid and liquid phases, and the solid phase consists of TTCP, TCP, DCPD, DCPA, MCPM and other calcium sulfate salts, while the liquid phase can be a phosphate solution, distilled water or serum. CPC is biocompatible, osteoconductive, and biodegradable, and has a final degradation product of hydroxyapatite. However, due to the long curing time (approximately 11 min in this study), and relatively low heat release (equal to room temperature) during the curing process, the adhesion and strength are thus relatively poor, and it is easy to disintegrate from bone; also, since no shaping of the solid-liquid mixture can occur, clinical application becomes difficult. On the other hand, PMMA is composed of solid and liquid phases as well, and the solid phase contains two zirconium oxide, polymethyl methacrylate, methyl benzoyl peroxide, and colorant141, while the liquid phase is made up of methylmethacrylate, dimethyl-p-toluidine, hydroquinone, and colorant 141. PMMA has good compressive strength (80 MPa experimentally in this study); with a solidification temperature of approximately 80°C, a curing time of about 2 min, it is easy and quick to shape during curing. However, neither does PMMA degrade as it is not biocompatible, nor induces bone activity or conduction. PMMA's clinical application is also limited to replacing joints, selecting filling and occupying bone defects with an antibiotic carrier, local bone defect filling and supporting roles, such as vertebroplasty, etc. Using the proposed fiber reinforced method, the absorbed materials and CPC are mixed into the composite materials to improve compressive and flexural strengths and the bone repairing performance of CPC [29–31].

CPC is significantly complex, and many types of materials can be combined with CPC. Although these materials can be either organic or inorganic, higher strengths are usually produced when CPC is combined with an organic compound due to improved microstructure and decreased porosity. XU [32] added 25% large diameter absorbable fibers into CPC and increased its bending resistance strength by 3 folds and its toughness by nearly 100 folds. Regarding to each group of bone cements

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

after mixing with a PMMA monomer in an electron microscope, no significant differences of CPC, PMMA, the surface of composite bone cements and the structure were found; there were also no significant interfaces between CPC and PMMA composite bone cements, thus indicating their compatibility. However, we did find that both the crystal shape of CPC and amorphous PMMA were spherical, but PMMA was found stacked with CPC. This finding did not rule out the adhesive effects of the PMMA monomer solvent, but as PMMA concentration decreased, the crystalline region loosened marginally. Although this was not significant, it indicated the formation of a continuous system after mixing and the compatibility of both materials.

To display the control group following the powder diffraction standard of the International Diffraction Data Centre, PMMA bone cements were made by amorphous substances with no crystalline diffraction peaks. As a result, it only showed the crystalline diffraction peaks of barium sulfate as developer. CPC indicated that crystalline diffraction peaks was based on hydroxyapatite, and the original crystallization properties, which included low CPC crystallinity, decreased significantly with increasing PMMA after the composite bone cements were mixed. Sharp diffraction peaks in the XRD map were also found to have been weakened significantly with a large range of diffuse peaks appeared showing that the crystalline regions in the composites decreased greatly. However, the mixture of CPC and PMMA had no significant effect on the final products of the diffraction peaks of hydroxyapatite; thus, there was no new crystalline phase (i.e., the PMMA bone cements did not participated in the curing reaction of CPC bone cements). Therefore, the combination of CPC and PMMA proved decent compatibility.

As indicated in the previous studies, the degradation rate of CPC and the new bone formation rate were related. 2 weeks after Ooms E. M [33] had implanted CPC into the bone defect of a sheep tibia cortical defect, CPC was found to be in contact with the surrounding bone with no inflammation. CPC was resorbed gradually and 24 weeks later, a new bone was formed until the CPC was absorbed completely. The study found that with the development of composite bone cement implantations in bone defects, we did not find significant metabolic phenomena of composite bone cements. Similarly, in SD rats' transplantation model of tibia bone defect on X-ray imaging after 4 weeks, bone cements were found to have degraded partially via X-rays 15 weeks after the transplantation. This phenomenon was of vital significance among the CPC, CPC/PMMA (75%), CPC/PMMA (67%) and CPC/PMMA groups (50%).

New bone was formed in the transplantation areas of the composite bone cements, and bone cells combined well. New capillaries and new bone connection belts also appeared, and the bone density was similar to the surrounding normal bone tissues. In addition, CPC supplies the basic conditions for bone conduction growth: its basic ingredients are calcium and phosphorus elements, which is similar to inorganic bone structures. CPC itself has a cellular structure, acting as a support material and is suitable for new bone ingrowth. Its bone conduction abilities include making capillary and ingrowing cells of new bone [34]. Bone conduction ability also helps to generate pluripotent cells and isolate chondrocytes and osteoblasts in non-osseous environment, and thus has osteogenic properties.

Through this set of experiments, such phenomenon was discovered and better concentration ratio range of 50% to 75% of the composite bone cement CPC/PMMA was then selected.

Cytotoxic statistical results indicated that CPC/PMMA (33%), CPC/PMMA (50%), CPC/PMMA (67%), CPC/PMMA (75%) were more in line with the composite bone cement without cytotoxicity requirements.

The compressive strength results showed that when CPC was acting as the control group, each group of pressure was significantly higher than CPC group, but since the CPC/PMMA (67%), CPC/PMMA (75%) among the groups of compressive strength were 20Mpa-30Mpa, and the CPC/PMMA (4.8%), CPC/PMMA (6.25%), CPC/PMMA (9.1%), CPC/PMMA (16.7%), CPC/PMMA (33) and CPC/PMMA (50%) groups were at 40Mpa levels above the compressive strength, it temporarily remains uncertain to determine which group has greater advantages, because the demand of compressive strength varies in different parts of the cancellous bone. We believe that the compressive strength of composite bone cement of 20Mpa-70Mpa accords with composite clinical demands.

Results of curing time was longer in the CPC group (more than 11 min) but shorter in the PMMA group (less than 2 min). There were no significant differences among the PMMA group, CPC/PMMA (4.8%) and CPC/PMMA (6.2%) group in terms of composite bone cements ($p>0.05$). The curing time of PMMA group, CPC/PMMA (4.8%) and CPC/PMMA (6.2%) group were all too short for clinical application, and therefore, we temporarily consider that CPC/PMMA (9.1%), CPC/PMMA (16.7%), CPC/PMMA (33%), CPC/PMMA (50%), CPC/PMMA (67%) and CPC/PMMA (75%) group are consistent with the requirements of composite bone cement.

The rate of weight loss results showed that there were no significant differences between CPC/PMMA (4.8%, 6.25%, 9.1%, 16.7%, 33%) group and PMMA control group. With the decrease of CPC content, the weight loss rate gradually reduced. So we hold the idea that CPC/PMMA (50%), CPC/PMMA (67%) and CPC/PMMA (75%) groups meet the composite bone cement degradation requirements.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Conclusions

The better composite bone cement concentration are selected from CPC/PMMA (50%), CPC/PMMA (67%) and CPC/PMMA (75%) group which provide greater variability and selectivity for the composite bone cement in obtaining better application.

References:

1. Fernández M1, Méndez JA, Vázquez B et al: Acrylic-phosphate glasses composites as self-curing controlled delivery systems of antibiotics. J Mater Sci Mater Med, 2002; 13(2): 1251–57
2. Méndez JA, Abraham GA, del Mar Fernández MJ et al: Self-curing acrylic formulations containing PMMA/PCL composites: properties and antibiotic release behavior. Biomed Mater Res, 2002: 61(1): 66–74
3. Lieberman IH, MD, Togawa D, Kayanja MM: Vertebroplasty and kyphoplasty: filler materials. Spine J, 2005; 5: 305S–16S
4. Brown WE, Chow LC: Dental restorative cement pastes U.S. Patent No.4518340, 1985
5. ISO10993: Biological evaluation of medical devices. Part 1: Evaluation and testing within a risk management process. Geneva: ISO, 2009
6. ISO10993: Biological evaluation of medical devices. Part 5: Tests for cytotoxicity: *in vitro* methods. Geneva: ISO, 1992
7. ISO10993: Biological evaluation of medical devices. Part 12: Sample preparation and reference materials. Geneva: ISO, 2009
8. ISO10993: Biological evaluation of medical devices. Part 10: Test for irritation and skin sensitization. Geneva: ISO, 2009
9 Xue W, Liu X, Zheng X, Ding C: *In vivo* evaluation of plasmasprayed wollastonite coating. Biomaterials, 2005; 26: 3455–60
10. Schmitz JP, Hollinger JO: The critical size defect as an experimental model for craniomandibulofacial nonunions. Clin Orthop Relat Res, 1986; (205): 299–308
11. Theyse LFH, Osterlaken-Dijksterhuis MA, Doorn J et al: Growth hormone stimulates bone healing in a critical-sized bone defect model. Clin Orthop Relat Res, 2006; 446: 259–67
12. Bernabe PF, Melo LG, Cintra LT et al: Bone healing in critical-size defects treated with either bone graft, membrane, or a combination of both materials: a histological and histometric study in rat tibiae. Clin Oral Implants Res, 2012; 23(3): 384–88
13. Zhang CX: A new method of measuring the cytotoxicity by spectrophotometer for amalgam of different cupric quantity. Zhonghua Kou Qiang Yi Xue Za Zhi, 1990; 25(4): 216–18, 252
14. Adachi T, Osako, Tanaka M et al: Framework for optimal design of porous scaffold microstructure by computational simulation of bone regeneration. Biomaterials, 2006; 27: 3964–72
15. Okuda T, loku K, Yonezawa I et al: The effect of the mierostructure of betatricalcium phosphate on the metabolism of subsequently formed bone tissue.Biomaterials, 2007; 28: 2612–21
16. Fischer J, Kolk A, Wolfart S et al: Future of local bone regeneration-Protein versus gene therapy. J Craniomaxillofac Surg, 2011; 39(1): 54–64
17. Kolk A, Haczek C, Koch C et al: A strategy to establish a gene-activated matrix on titanium using gene vectors protected in a polylactide coating. Biomaterials, 2011; 32(28): 6850–59
18. Stan GE, Pina S, Tulyaganov DU et al: Biomineralization capability of adherent bio-glass films prepared by magnetron sputtering. J Mater Sci Mater Med, 2010; 21(4): 1047–55
19. Lu J, Descamps M, Dejou J et al: The biodegradation mechanism of calcium phosphate biomaterials in bone. J Biomed Mater Res, 2002; 63(4): 408–12
20. Huang J, Best SM, Bonfield W et al: *In vitro* assessment of the biological response to nano-sized hydroxyapatite. J Mater Sci Mater Med, 2004; 15(4): 441–45
21. Nagraj N, Nagarjuna M, Desai AK et al: Double-loop technique using titanium molybdenum alloy wire for fabrication of nasal stents in nasoalveolar molding therapy for cleft lip and palate patients. Cleft Palate Craniofac J, Cleft Palate Craniofac J, 2015; 52(2): 246–49
22. Lorenzetti M, Biglino D, Novak S et al: Photoinduced properties of nanocrystalline TiO2-anatase coating on Ti-based bone implants. Mater Sci Eng C Mater Biol Appl, 2014; 37: 390–98
23. Frandsen CJ, Brammer KS, Noh K et al: Tantalum coating on TiO2 nanotubes induces superior rate of matrix mineralization and osteofunctionality in human osteoblasts. Mater Sci Eng C Mater Biol Appl, 2014; 37: 332–41
24. Joy A, Cohen DM, Luk A et al: Control of surface chemistry, substrate stiffness, and cell function in a novel terpolymer methacrylate library. Langmuir, 2011; 27(5): 1891–99
25. Horch HH, Sader R, Pautke C et al: Synthetic, pure-phase beta-tricalcium phosphate ceramic granules(Cerasorb) for bone regeneration in the reconstructive surgery of the jaws. Int J Oral Maxillofac Surg, 2006; 35(8): 708–13
26. Cui X, Zhao D, Zhang B et al: Osteogenesis mechanism of chitosan-coated calcium sulfate pellets on the restoration of segmental bone defects. J Craniofac Surg, 2009; 20(5): 1445–50
27. Horch HH, Sader R, Pautke C et al: Synthetic,pure-phase beta-tricalcium phosphate ceramic granules(Cerasorb) for bone regeneration in the reconstructive surgery of the jaws. Int J Oral Maxillofac Surg, 2006; 35(8): 707–13
28. Rezwan K, Chen QZ, Blaker JJ et al: Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials, 2006; 27(18): 3413–31
29. Xu HHK, Quinn JB: Calcium phosphate cement containing resorbable fibers for short-term reinforcement and macroporosity. Biomaterials, 2002; 26(3): 193–202
30. Xu HHK, Eichmiller FC, Giuseppetti AA: Reinforcement of a self-setting calcium phosphate cement with different fibers. J Biomed Mater Res, 2000; 52: 107–14
31. Xu HHK, Takagi S, Quinn JB et al: Fasting-setting calcium phosphate scaffolds with tailored macropore formation rates for bone regeneration. J Biomed Mater Res, 2004; 68: 725–34
32. Xu HHK, Quinn JB: Calcium phosphate cement containing resorbable fibers for short-term reinforcement and macroporosity. Biomaterials, 2002; 23(1): 193–202
33. Ooms EM, Wolke JGC, Van de Heuvel MT et al: Histological evaluation of the bone response to calcium phosphate cement implanted in cortical bone. Biomaterials, 2003; 24(6): 989–1000
34. Urist MR, Silverman BF, Buring K et al: The bone induction principle. Clin Orthop Rel Res, 1967; 53: 243–83

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]