An injectable cement: Synthesis, physical properties and scaffold for bone repair

Weitao Y, Kangmei K, Anmin J*

ABSTRACT

Micro-invasive bone grafting is to deliver bone graft materials to the desired site through local puncturation and injection. It has many advantages such as little injury, simple procedures and high efficiency of osteogenesis. Limited sources of graft materials and complicated procedures are the main factors affecting the development of the technique. Therefore, to prepare a stable, economical, efficient and easy-to-use liquid graft material is crucial for the development of the technique. Purpose: The potential efficacy of an injectable calcium phosphate cement’s handling properties and scaffold for bone repair performance was evaluated in a rabbit femoral condyles model. Study Design: A comparative study was conducted between a new cement and a commercially available calcium phosphate cement. Materials and Methods: The new cement and commercially available calcium phosphate cements were compared in terms of the setting time, injection pressure, particle size and compressive strength. Then the cements were delivered to rabbit femoral condyles through local injection and then degradation of cements and bone formation were observed regularly after operation. Results: The new injectable cement was superior to currently used cements in terms of permitted manipulation time, injection pressure, particle size, postoperative degradation and efficacy of scaffold for bone repair; nevertheless, the new cement was slightly inferior to currently used cements in compressive strength and the final setting time. Conclusions: The new injectable cement is more suitable for the clinical study of micro-invasive bone grafting. It allows a brand new bone grafting procedure and provides a new graft material and thus merits further development and wider application.

KEY WORDS: Comparative study, injectable calcium-phosphate cement, micro-invasive bone grafting

Minimally invasive technique is a newly emerging surgical technique, which includes micro-invasive bone grafting. Micro-invasive bone grafting means that the bone is taken out and bone graft materials are delivered to the desired site through a small incision or even local percutaneous puncture and injection. So the grafts used in this procedure may be liquid or fine particulate materials. Departing from other bone graft methods, this technique can avoid bone harvesting, and immunological reactions to graft materials. At the same time, the condition for bone formation is favorable; spaces, particularly irregular ones, can be filled up and influences on sites other than the graft site are minimized, thus accelerating degradation and osteogenesis.

Micro-invasive bone grafting places high demands on bone graft materials, for instance, they must be injectable, have certain biomechanical strength and in vivo osteogenic efficacy compatible with that of autogenous ilium and have no toxic or side-effects. Although new calcium phosphate cements, calcium sulfate cements and bioactive glasses developed over recent years exhibit sound degradability and osteogenic activity, their use is limited in percutaneous bone grafting because of short setting times, high viscosity and large differences in the degradation rate in vivo.

Hence, to prepare a novel liquid graft material is crucial for the development of micro-invasive bone grafting. In this study a new injectable cement with sound degradability and osteoconductive effect in vivo was prepared through improving the composition of traditional calcium phosphate cements. A comparative study was conducted between the new cement and a commercially available calcium phosphate cement to observe the physicochemical properties and osteoconductive activity of the new cement, so as to lay the foundations for further studies on micro-invasive bone grafting.

Materials and Methods

Composition of the cement and preparation of materials
The new cement consisted of a solid phase and a liquid phase. The solid phase was composed of beta-tricalcium phosphates
(beta-TCP), calcium phosphate dibasic anhydrous (DCPA) and nanocrystalline hydroxyapatite (nHA) in certain proportions. The liquid phase consisted of 0.8% sodium alginate and 0.25M Na₃HPO₄/NaH₂PO₄ buffer (pH = 7.4). The comparative cement, purchased from Shanghai Rebone Biomaterials Co., Ltd., was composed of tetracalcium phosphate (TTCP) and calcium phosphate dibasic anhydrous.

**Determination of the physicochemical properties of calcium phosphate cements**

**Determination of setting time**
The prepared cement and the purchased cement were infused respectively into a mould 2 cm in diameter and 2 cm in height and then the mould was put into a thermostat oven at 37°C and 100% humidity. The initial and final setting times (Ti and Tf) were determined by Gillmore needles method.\(^7\)

**Determination of injection pressure**
The two cements were respectively infused into a 5 ml-syringe connected with a puncture needle 2 mm in diameter and then the syringe was fixed to a compressor. The cement was pushed by a globular head 1 cm in diameter at 0.05 mm/s using MTS-858 Bionixtest system (MTS, Minneapolis, MN) till it was completely discharged from the syringe. The maximal propulsion was defined as the injection pressure.

**Measurement of cement particle size and pore diameter**
The final products of the cements were ground, fixed, coated with gold and observed by scanning electron microscopy to measure particle size and pore diameter. The micrographs were analyzed by using Image J software (Authors: Rasband, W.S. Download: http://rsb.info.nih.gov/ij/).\(^8\)

**Determination of compressive strength of final cement products**
The two cements were respectively prepared to cylinders of 2 cm in diameter and 2 cm in height in the aforementioned thermostat oven and were kept for 48 h. Then the cylinders were compressed till they broke under pressure using MTS-858 Bionixtest system (MTS, Minneapolis, MN). The maximal condensing force and rigidity were calculated according to the force and displacement curve. Compressive strength was calculated as follows: \(C = 4N/(\pi \times d^2)\), (C: compressive strength of the sample, P: maximal endurable load (N), D: diameter of the sample).

**Preparation of animal models**
Twelve New Zealand rabbits were randomly divided into four groups. After peritoneal anesthetization with pentobarbital sodium (40 mg/kg), the rabbit was placed in the prone position; bilateral lower limbs were depilated, scrubbed with soapsuds, sterilized with iodine tincture and alcohol. Then, a posterolateral incision was made in the distal part of the thigh. Lateral vastus muscles were separated and the distal part of the femur was exposed. A 6-mm hole vertical to the longitudinal axis of the femur was drilled in the condyles of the femur and the cortical bone on the opposite side was reserved so as to prevent lower limb fractures and cement leakage. The sterilized cement was prepared with a powder to liquid ratio of 1:0.6 (g/ml) and then the mixture was poured into a 5 ml-syringe using a spatula. A 16-gauge needle for abdominal puncturation was inserted into the left hole and approximately 1ml of the investigational cement was infused into the spongy bone of the condyles of the femur in a retrograde manner. With the same method, 1ml of the purchased cement was infused into the right hole to serve as control. After careful hemostasis (obvious bleeding was stopped by sealing the orifice of the hole with bone wax), the incision was sutured. The operated rabbits were housed at the experimental animal facility of Zhujiang Hospital. All the procedures employed in this study were in accordance with the standards of the guidelines for the care and use of laboratory animals of NIH.

**Sample collection and parameter determination**

**Sample collection**
Three rabbits were anesthetized peritoneally and sacrificed by injecting air into ear edge veins at Weeks 4, 8, 12 and 16 after surgery, respectively. Bilateral condyles of femur were resected and fixed in 4% paraformaldehyde.

**HE staining and observation**
The fixed samples were subjected to decalcification with nitric acid (10%), dehydration, lucidification and embedding with paraffin. Then femoral condyles were sliced with a thickness of 10 μm parallel to the longitudinal axis of the femur. All sections were oven-dried and subjected to deparaffinage and routine HE staining. Finally, the sections were observed under a light microscope for the reactions of adjacent tissues, metabolism of cement and bone formation. Area of new formed bone was measured by Image J software.

**Scanning electron microscopic (SEM) observation**

After fixation and decalcification as aforementioned, femoral condyles were split longitudinally, immersed in stepwise phosphate buffers, dehydrated with alcohol with concentrations ranging from 30% to 100%, dried at the critical point and coated with gold in vacuum. Scanning electron microscopic (Hitachi X-506, Sun Yat-sen University) observation was performed for the degradation and ultrastructure of the bone-cement interface.

**Results**

1. Initial and final setting time of cements [Table 1]
2. Injection pressure and compressive strength of cements [Table 3]
3. Area of new formed bone of two cements [Table 4]
4. Degradation of cements at week 12 after operation [Figure 1]
5. Degradation and osteogenesis [Figure 2]

These results demonstrated that the injection pressure (27.80±6.41N), particle size (2.35±0.81 μm) and compressive strength (3.28±0.85N) of the investigational cement were significantly lower than those of the control cement, while the initial setting time (23.00±3.52 minutes), final setting time (7.90±1.08 hours), poriness (46.58%±8.66%) and area of new formed bone at 12 weeks (22.12%±2.08%) and 16 weeks
Table 1: Setting time of cements in the experimental and control groups (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Ti (minute)</th>
<th>Tf (hour)</th>
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<tbody>
<tr>
<td>Experimental</td>
<td>23.00±3.52</td>
<td>7.90±1.08</td>
</tr>
<tr>
<td>Control</td>
<td>11.17±2.14</td>
<td>4.94±0.41</td>
</tr>
<tr>
<td>( P ) value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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Note: Mann-Whitney U test using SPSS 11.0

Table 2: Particle size and poriness of cements in the experimental and control groups (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Particle size (µm)</th>
<th>Poriness (%)</th>
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<tbody>
<tr>
<td>Experimental</td>
<td>2.35±0.81</td>
<td>46.58±8.66</td>
</tr>
<tr>
<td>Control</td>
<td>4.57±0.62</td>
<td>34.77±5.68</td>
</tr>
<tr>
<td>( P ) value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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Note: Mann-Whitney U test using SPSS 11.0

Table 3: Injection pressure and compressive strength of cements in the experimental and control groups (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Injection pressure (N)</th>
<th>Compressive strength (mPa)</th>
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<tbody>
<tr>
<td>Experimental</td>
<td>27.80±6.41</td>
<td>3.28±0.85</td>
</tr>
<tr>
<td>Control</td>
<td>81.50±7.40</td>
<td>5.85±0.90</td>
</tr>
<tr>
<td>( P ) value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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Note: Mann-Whitney U test using SPSS 11.0

Table 4: New bone formed area in the experimental and control groups (Mean±SD)

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<tr>
<th></th>
<th>12 weeks (%)</th>
<th>16 weeks (%)</th>
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<tbody>
<tr>
<td>Experimental</td>
<td>22.12±2.08</td>
<td>43.91±4.29</td>
</tr>
<tr>
<td>Control</td>
<td>12.55±2.04</td>
<td>31.11±4.11</td>
</tr>
<tr>
<td>( P ) value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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Note: Mann-Whitney U test using SPSS 11.0

(43.91%±4.29%) in the investigational cement were significantly higher than those of the control cement. These results indicate that the new cement is easier to inject, permits a longer time duration for the clinical procedure and is also easier to degrade in vivo. The new bone formed area after cement dissolution was superior in the experimental group than in the control group. But with the increase in poriness, its compressive strength decreases significantly.

It is one of the most distinct characteristics of new cement degrading in vivo compared with the control cement which can be seen from Figure 2. Whether in the experiment group or control group, the injected cement was biocompatible and bioactive and no adverse foreign body reaction was observed. In the experiment group, new bone formation from preexisting cancellous bone around the edge or under the surface of the cement was seen at four weeks after operation, conspicuously degraded region was formed and no gap existed between the cement and host bone. New bone grew with time and many more new bone “islands” formed at eight weeks after implantation; around them there were many osteoblasts and osteoid tissues. At the periphery of the “islands” there was new fibrous tissue composed of newly formed vessels and fibroblasts as observed under high magnification [Figure 2A]. In the control group, however, no bone or osteoid tissue was observed even at eight weeks after implantation [Figure 2B]. At 12 weeks after operation, the cement was parted and its entire surface was completely covered with newly formed bone which even penetrated into the cement. At 16 weeks of implantation, the cement was degraded completely and there were mainly new bone trabecula and plastic osteoid as well as marrow cavity.
like structures around the bone trabecula in the visual field. There was complete bridging of the defect with the restoration of the haversian canals at the level of the bone defect [Figure 2C]. At all times, no intervening fibrous tissue layer was present at the interface between bone and cement. In the control group, however, there was no bone formed at eight weeks after implantation [Figure 2B] and there was some remnant cement at Week 16 and new bone in the degraded region [Figure 2D]. There are some similarities between the two calcium phosphate cements in terms of degradation and osteoconduction: the cement was degraded from the periphery to the centre; new vessel ingrowth and involvement of osteoblasts accompanied cement degradation; degradation may initiate local bone formation.

**Discussion**

To permit easy injection of the cement in the experiment and keep its cohesion, we added sodium alginate in the liquid phase and calcium carbonate, nanocrystalline hydroxyapatite in the powder phase as components. Sodium alginate can bind Ca$_2^+$ of the cement and form a gel of calcium alginate which makes this new cement an excellent cohesive in bone tissue and reduces the viscosity of cement. At the same time, Tajima S$^{[9]}$ found that addition of sodium alginate was very useful for Biopex(one of Ca-P cements) to acquire the anti-washout property and increase the mechanical strength. Calcium carbonate was added to this cement just because it can reduce the particles of the cement and it’s one of the inorganic ingredients in bone. LeGeros$^{[10]}$ observed that the cement had smaller particles and easier salvation than others when the calcium carbonate was added in the Ca-P cement. Partial substitution of CO$_3^{2-}$ for PO$_4^{3-}$ can also give the cement a higher carbonate content, which made it uniquely similar to the mineral phase of bone-dahlite. This kind of cement is called B-type carbonate apatite$^{[11]}$ and has shown good biocompatibility and improved the mechanical function. The results showed that this new cement had a low viscosity and good cohesion which can be easily passed through the needle (27.80±6.41N). The ultimate production which used sodium alginate had smaller particles (diameter 2.35±0.81 μm, [Figures 1A and B]) and larger caves rate (46.58%±8.66%) than the control group.

To get an excellent combination effect of degrading and promote bone formation, we attempted to use beta-TCP and DCPA as the main components that composed into a brushite CPC, which in comparison beta-TCP to alpha-TCP in most calcium phosphate cement. Beta-TCP and alpha-TCP have the same macrostructure and microstructure, but different phase composition. And the solubility of alpha-TCP is much higher than that of beta-TCP. Therefore, the dissolution of porous alpha-TCP progresses at a higher rate than bone repair in vivo.$^{[12]}$ As a candidate material for bone graft, beta-TCP has excellent merit in bone formation.$^{[13]}$ Beta-TCP and DCPA mixed together for bone substitution have been used for many years. Ohura$^{[14]}$ developed a biphasic dehydrate matrix filled with beta-TCP granules and observed that the solubility of DCPA is approximately eight times higher than beta-TCP and approximately 15 times higher than HA at physiologic pH in vitro. The same orders of magnitude applied for the resorption of the materials in vivo; new bone forms at the space left by the resorption of the DCPD matrix, with beta-TCP granules acting as the guiding structure.$^{[15]}$ The more slowly resorbing granules were surrounded by newly grown bone, thus providing an inverse scaffold for bone regeneration.$^{[16]}$ So with the adding of beta-TCP granules, the overall resorption rate of the cement can be tailored to specific needs and to control the bone formation rate. In our study, the cement contains beta-TCP and DCPA, which has a complete degrades in 16 weeks in vivo.

In this study, cement degraded and new bone formed much earlier in the experiment group than in the control group and the rate of cement dissolution in the experimental group was actually near to that after fractures. The causes underlying better degradation and osteoconduction in the experiment group may be: (a) The particle size of the cement is small and the porosity is high. (b) Calcium carbonate can increase biomedical cement resorption rates just for its high solubility in vivo.$^{[17]}$ (c) Beta-TCP has good osteogenic performance. The results demonstrate that the new cement, serving as an injectable material used in micro-invasive bone grafting, is better than traditional ones in the treatment of bone defects and nonunion.

Nevertheless, there are still some problems with the new cement: (a) With increasing use of sodium alginate, the injection pressure of cement lowers, the liquid component increases greatly, the setting time prolongs and the compressive strength of the final products of cement reduces substantially. These may be associated with calcium alginate gel in the final product of the cement, because the gel disrupts interconnections between cement reactants and delay reactions. Increased distance between particles in the final products of the cement, on the one hand, accelerates degradation and resorption of cement and, decreases the compressive strength of cement. Hence, to use proper amounts of sodium alginate is one of the key factors in the preparation of cement. (b) How to reduce the final setting time? The final setting time was much longer than that of other calcium phosphate cements, possibly because of calcium alginate gel. The addition of proper setting promoters is one of the issues to be investigated. (c) The compressive strength of the final products was not high enough. This is the main weakness of currently used calcium phosphate cements, which may be attributed to factors such as the ratio of solid to liquid reactants, particle size of final products and porosity. This investigational cement also exhibited such a weakness.

**References**


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