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An injectable borate bioactive glass cement for bone repair: Preparation, bioactivity and setting mechanism

Xu Cui [a,1], Yadong Zhang [b,1], Hui Wang [a], Yifei Gu [a], Le Li [a], Jie Zhou [a], Shichang Zhao [b], Wenhai Huang [a,*], Nai Zhou [a], Deping Wang [a], Haobo Pan [c], Mohamed N. Rahaman [d,*]

[a] Institute of Bioengineering and Information Technology Materials, Tongji University, Shanghai 200092, China
[b] Shanghai 6th People's Hospital, Shanghai Jiaotong University, Shanghai 200233, China
[c] Center for Human Tissues and Organs Degeneration, Shenzhen Institute of Advanced Technology, Chinese Academy of Science, Shenzhen 518055, China
[d] Department of Materials Science and Engineering, Center for Biomedical Science and Engineering, Missouri University of Science and Technology, MO 65409-0340, USA

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ABSTRACT

There is a need for synthetic biomaterials to reconstruct bone defects using minimal invasive surgery. In this study, the preparation, bioactivity and setting mechanism of an injectable cement composed of borate bioactive glass particles and a chitosan solution was evaluated as a function of varying solid to liquid (SL) ratio. As the SL ratio increased from 1.0 g/ml to 2.5 g/ml, the injectability and initial setting time decreased from 97 ± 1% to 84 ± 10% and from 16.9 ± 0.9 min to 3.0 ± 0.5 min, respectively, while the compressive strength of the cement increased from 8 ± 2 MPa to 31 ± 2 MPa. The cement maintained its cohesiveness in a vigorously stirred aqueous medium. When immersed in phosphate-buffered saline, the glass phase reacted and converted to hydroxyapatite. The setting mechanism of the cement appeared to involve a combination of processes, such as the sol–gel transition of the chitosan solution, formation of an interlocked interface between the converted glass surface and the chitosan, and bonding between the converted glass surface and the functional groups of the chitosan. By controlling the reaction between the glass particles and the chitosan solution, injectable cements can be created with the requisite workability, degradation, strength and bioactivity for bone repair applications.

1. Introduction

Large bone defects resulting from trauma, malignancy, infection and congenital diseases are a common occurrence in orthopedic and craniofacial surgery. Clinically, those defects are reconstructed using autologous bone grafts, allografts and biocompatible materials [1]. Autografts are the gold standard for treatment but they suffer from problems such as limited availability, donor site morbidity and increased surgery time. Allografts are alternatives but they are expensive, have unreliable healing to bone and carry the risk of disease transmission and adverse host immune response. These problems have increased the need for synthetic bone graft substitutes [1].

The use of synthetic bone grafts composed of preformed scaffolds commonly requires prior knowledge of the size and shape of the defect. Reconstruction of defects with an irregular shape can be problematic when the implant cannot be readily shaped prior to surgery using the available tools. Furthermore, implantation of a preformed bone graft requires an invasive surgical procedure. Seeding synthetic implants with cells, often required to improve their osteogenic capacity, can be inefficient due to cell damage and poor cell transport through the implant [2].

In comparison, an injectable bone graft can provide a better fit to the defect, better bone–biomaterial contact even for defects with complex geometrical shapes and the ability to be implanted by minimal invasive surgery [3,4]. Consequently, the use of injectable bone grafts has the potential to reduce pain to the patient, risk of infection, treatment cost and tissue scars. As the components of the injectable implant are commonly formed into a paste from a solid phase and a solution prior to being injected, biological molecules such as osteogenic growth factors and antibacterial agents can be incorporated homogeneously into the implant.

Injectable bone cements composed of poly(methyl methacrylate) (PMMA), calcium sulfate and calcium phosphate biomaterials have been widely studied and developed for clinical applications but they suffer from limitations [5]. Polymerization of methyl methacrylate to form PMMA releases heat which can damage the surrounding tissue and can restrict the ability to deliver heat-sensitive biomolecules. PMMA is not biodegradable and it can provide a surface upon which secondary bacterial infection can occur. Calcium sulfate degrades but its lack of bioactivity limits its capacity to regenerate bone [6]. Synthetic hydroxyapatite (HA), a widely used calcium phosphate material in clinical application, is almost non-degradable and remains in the body for several years after implantation [7–10].

* Corresponding authors.
E-mail addresses: whhuang@tongji.edu.cn, huangwe@mst.edu (W. Huang), rahaman@mst.edu (M.N. Rahaman).
[1] These authors contributed equally to this work.

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Injectable cements composed of bioactive glasses (BGs) represent a new class of bone substitutes. BGs have the ability to react with the body fluid and convert to HA, which leads to the formation of a strong bond with the bone and soft tissue [11–13]. Silicate BGs, such as the glass designated 45S5, have been successfully applied in orthopedics and dentistry, mainly for repairing osseous and periodontal defects since the 1980s [13]. Borate glasses are a class of more recently developed BGs that are receiving interest for biomedical applications [14]. Certain compositions of borate glasses are bioactive, biodegradable, and osteoconductive [12]. Some borate BGs degrade faster and convert more completely to HA than silicate BGs [15,16]. Borate BGs in the form of granules, porous scaffolds or coatings are being investigated for biomedical applications [12,14]. However, an injectable bone cement composed of borate BG is not yet available for clinical application.

The objective of this study was to investigate the development of an injectable bone cement composed of borate BG particles dispersed in a chitosan solution that served as the hardening liquid. The borate BG has been used in previous studies [16–18]. A chitosan solution was used because it can undergo a sol–gel transition to form a hardening phase and later degrade into non-toxic products [19]. The properties of the cement relevant to potential clinical application such as injectability, setting time, cohesiveness, compressive strength, and bioactivity, as well as the setting mechanism of the cement were studied.

2. Experimental procedure

2.1. Preparation of borate glass cement

The cement was composed of borate BG particles in an aqueous solution of chitosan. Borate BG (composition $6Na_2O{\cdot}8K_2O{\cdot}8MgO{\cdot}22CaO{\cdot}54B_2O_3{\cdot}2P_2O_5$; mol%) was prepared by conventional melting and casting [16]. The glass was crushed, ground and sieved to give particles of size <40 μm. The liquid phase was prepared by dissolving chitosan (98% deacetylated) in acetic acid and adding β-glycerophosphate (all chemicals from Sinopharm Chemical Reagent Co. Ltd., China). Chitosan powder was dissolved in 1.0 M acetic acid (20 g/l) and the mixture was stirred for 1 h, after which it was stored at 4 °C. Then a solution composed β-glycerophosphate in deionized water (560 g/l) was prepared. Finally, the chitosan solution was mixed with the β-glycerophosphate solution (ratio = 7:1 by volume) and the solution was stored at 4 °C.

2.2. Evaluation of cement injectability, setting time and cohesiveness

The injectability of the cement was tested in vitro using a procedure described previously [20]. Cement pastes with solid to liquid (SL) ratios (weight/volume) of 1.0, 1.5, 2.0 and 2.5 g/ml were formed by mixing the glass particles and chitosan solution in an agate mortar and pestle for ~1 min. Then the paste was transferred into a 10 ml syringe (diameter of opening = 1.7 mm) and extruded by applying a force of 100 N at a crosshead speed of 10 mm/min using mechanical testing machine (CMT6104; SANS Test Machine Inc., China). The percent injectability (I) of the cement was determined using the equation:

$$I = [(M_o - M)/M_o] \times 100 \quad (1)$$

where M_o is the initial mass of the cement in the syringe, and M is the mass remaining in the syringe after the extrusion. For each SL ratio, 3 samples were tested and the results are expressed as a mean ± standard deviation (SD).

The initial setting time of the cement paste was determined according to ASTM C266. A poly(methyl methacrylate) mold containing five holes (5 mm in diameter × 10 mm) was placed in a water bath at 37.8 °C. Then the paste was prepared as described above and injected into the mold cavities. The initial setting time of the paste was determined using Gilmore needles (mass = 114 g; diameter of opening = 2.117 mm). For each SL ratio, 3 samples were tested and the setting time is expressed as a mean ± SD. The temperature increase due to the setting reaction was measured using a thermocouple (Voltcraft Data-Logger K202, Conrad Electronics, Germany) placed at the center of the paste (mass = 10 g) which was contained within a polystyrene mold in an environment at 37 °C.

The cohesiveness of the cement paste was evaluated from its resistance to disintegration in a vigorously stirred liquid. Two grams of the paste was added to 30 ml of phosphate-buffered saline (PBS) (pH = 7.2–7.4; PO_4^{3-} concentration = 0.06 M) in a beaker at 37 °C which was rotating at 180 rev/min. At selected times, the turbidity of the PBS was assessed by measuring the optical transmittance of the PBS at a wavelength of 362 nm. The disintegration resistance of the cement was further evaluated using a method described previously [21]. At each time, the amount of cement paste remaining was collected, freeze-dried and weighed. The measurement was performed in triplicate. The disintegration resistance (D) was determined using the equation:

$$D = (W_2/W_1) \times 100 \quad (2)$$

where W_1 is the mass of dried cement paste before soaking in PBS and W_2 is the mass of dried cement paste collected after soaking.

2.3. Mechanical testing

The strength of the cement after setting was measured in compression in a mechanical testing machine (CMT6104; SANS Test Machine Inc., China) on dry condition. Cylindrical samples (5 mm in diameter × 10 mm) were tested at a crosshead speed of 0.5 mm/min. Five samples of each cement were tested and the compressive strength was determined as a mean ± SD.

2.4. Evaluation of degradation and bioactivity in vitro

The degradation of the cements with different SL ratios was studied as a function of immersion time in PBS at 37 °C. Cylindrical samples of each cement (4.7 mm in diameter × 3.5 mm) were set for 4 h and immersed in 10 ml PBS in polyethylene containers. At each time point, the samples were removed, washed with deionized water, dried at 90 °C and weighed. The PBS was cooled to room temperature and its pH was measured using a pH meter. Four samples of each cement were measured at each immersion time and the results are expressed as a mean ± SD.

The presence of crystalline phases in the cements after they were immersed in PBS was determined by X-ray diffraction (XRD) (D/max-2500VB2+/PC X-ray diffractometer) using graphite monochromatized Cu K_α radiation ($\lambda = 0.15406$ nm) at a scanning rate of 10°/min (in the range 10–80° 2θ). Composition analysis of the cements was performed using FTIR (BRUKER, EQUINOXSS/HYPERION2000) in the wavenumber range 400–2000 cm^{-1} on disks prepared from a mixture of 2 mg of the cement powder and 150 mg of high-purity KBr. Each sample was scanned 32 times at a scan rate of 0.04 cm^{-1}. The morphological features of the cements before and after immersion in PBS were examined in a field emission scanning electron microscope (FESEM, Hitachi S-4700). The samples were sputter-coated with gold prior to examination.

2.5. Setting mechanism of the cement

Experiments were performed to study the setting mechanism of the cement. In one set of experiments, 2 g of glass particles (0.1–0.2 mm) was immersed in 20 ml of the solution. This low SL ratio was selected to ensure that the liquid contained enough $(PO_4)^{3-}$ ions to react

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