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Evaluation of an injectable bioactive borate glass cement to heal bone defects in a rabbit femoral condyle model

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ABSTRACT

There is a need for synthetic biomaterials to heal bone defects using minimal invasive surgery. In the present study, an injectable cement composed of bioactive borate glass particles and a chitosan bonding solution was developed and evaluated for its capacity to heal bone defects in a rabbit femoral condyle model. The injectability and setting time of the cement in vitro decreased but the compressive strength increased (8 ± 2 MPa to 31 ± 2 MPa) as the ratio of glass particles to chitosan solution increased (from 1.0 g ml^{-1} to 2.5 g ml^{-1}). Upon immersing the cement in phosphate-buffered saline, the glass particles reacted and converted to hydroxyapatite, imparting bioactivity to the cement. Osteoblastic MC3T3-E1 cells showed enhanced proliferation and alkaline phosphatase activity when incubated in media containing the soluble ionic product of the cement. The bioactive glass cement showed a better capacity to stimulate bone formation in rabbit femoral condyle defects at 12 weeks postimplantation when compared to a commercial calcium sulfate cement. The injectable bioactive borate glass cement developed in this study could provide a promising biomaterial to heal bone defects by minimal invasive surgery.

1. Introduction

There is a need for synthetic biomaterials to repair large bone defects resulting from trauma, malignancy, infection and congenital diseases [1]. Although current treatments based on autogenous bone grafts (the gold standard for bone repair) and allografts are effective, autografts suffer from limitations such as inadequate supply and donor site morbidity while allografts are limited by cost and uncertain healing to bone [2]. Synthetic biomaterials should be ideal implants for bone repair provided they can replicate the properties and function of living bone and can be formed into the requisite geometry for surgical implantation [3].

The use of synthetic biomaterials with a pre-designed shape in bone repair generally requires prior knowledge of the defect shape. Reconstruction of bone defects with an irregular or complex shape can be challenging if the shape of the implant cannot be easily modified during the surgical procedure. Implantation of a scaffold with a pre-designed shape also requires an invasive surgical procedure. The addition of cells and or growth factors to the implant, often required to improve the osteogenic capacity of synthetic biomaterials, can be inefficient due to cell damage, limited cell transport through the implant or loss of bioactivity of the growth factor [4].

In comparison, injectable biomaterials can provide the benefits of a better fit to the defect shape and the attainment of better bone-biomaterial contact even for defects with a complex shape [5,6]. An injectable biomaterial with the requisite workability and setting behavior can be implanted by minimal invasive surgery and can attain a suitable strength after setting in vivo [5,6]. The use of injectable biomaterials has the potential to reduce pain to the patient, treatment cost and the extent of tissue scars resulting from the surgery. 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 more easily and homogeneously into the implant.

Injectable bone cements currently used in clinical applications, such as polymethyl methacrylate (PMMA), calcium sulfate and calcium phosphate biomaterials, are effective but they suffer from a variety of limitations [7]. Heat generated in the polymerization of methyl methacrylate to form PMMA can result in damage to cells and tissues and can limit the ability to deliver heat-sensitive biomolecules. PMMA is not

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biodegradable and it can provide a surface upon which secondary bacterial infection can occur. While calcium sulfate is resorbable in vivo, it is not bioactive and it can lose its strength rapidly due to its fast degradation rate [8]. Synthetic hydroxyapatite (HA), a widely used biomaterial in clinical applications and the main phase in several calcium phosphate cements, degrades slowly and can remain in the body for several years after implantation [9–12].

Injectable cements composed of bioactive glass composites are a new class of bone substitutes [13]. Bioactive glasses have the ability to react with the body fluid and convert to HA, which leads to the formation of a strong bond with bone and soft tissue [14–16]. Bioactive silicate glasses, such as the glasses designated 45S5 and 13–93, have been widely studied and applied in orthopedic surgery and dentistry since the 1980s, mainly for repairing osseous and periodontal defects [16]. Bioactive borate glasses are a class of more recently developed glasses that are receiving interest for biomedical applications [17]. In common with bioactive silicate glasses, bioactive borate glasses are biodegradable, osteoconductive and convert to HA in vivo, but they degrade faster and convert more completely to HA [18,19].

The objective of this study was to develop an injectable cement composed of bioactive borate glass particles and a chitosan matrix and evaluate its capacity to heal bone defects in an appropriate animal model in vivo. The in-vitro properties of the bioactive borate glass alone have been studied and reported previously [19,20]. A chitosan solution was selected as the hardening phase based on its setting characteristics and its ability to degrade into non-toxic products in vivo [21]. Cements with varying ratios of bioactive glass particles to chitosan solution were formed and evaluated in vitro. Then cements with an optimal composition were implanted in a rabbit femoral condyle model to evaluate their capacity to heal bone defects.

2. Materials and methods

2.1. Preparation of bioactive glass cement

The cement was prepared from a mixture of bioactive borate glass particles and an aqueous solution of chitosan. The borate glass (composition $6Na_2O$, $8K_2O$, 8MgO, 22CaO, $54B_2O_3$, $2P_2O_5$; mol%) was prepared by conventional melting and casting, crushed, ground and sieved to give particles of size < 40 μm, as described previously [19]. The chitosan solution was prepared by dissolving chitosan (98% deacetylated) in acetic acid and adding β-glycerophosphate (Sinopharm Chemical Reagent Co. Ltd., China). Chitosan powder was dissolved in 1.0 M acetic acid (20 g l^{-1}) 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^{-1}) was prepared. The chitosan solution was mixed with the β-glycerophosphate solution (ratio = 7:1 by volume) and the resulting solution was stored at 4 °C. The cement was formed by mixing the glass particles (solid) and chitosan solution (liquid) in varying solid to liquid (SL) ratios (weight to volume) of 1.0, 1.5, 2.0 and 2.5 g ml^{-1}.

2.2. Evaluation of cement injectability, setting time and cohesiveness

The injectability of the cement was tested in vitro using a procedure described previously [22]. Cement pastes with the SL ratios given above were formed by mixing the glass particles and chitosan solution in an agate mortar and pestle for ~1 min. Then the paste was transferred to 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^{-1} using a 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 PMMA mold containing five cylindrical holes (5 mm in diameter × 10 mm) was placed in a water bath at 37.8 °C. Then the cement paste (prepared as described earlier) was injected into the mold cavity. The setting time of the paste was determined using Gilmore needles (mass = 114 g; diameter of opening = 2.12 mm). For each SL ratio, 3 samples were tested and the setting time was determined 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 to 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 procedure described previously [23]. 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 W1 is the mass of dried cement paste before soaking in PBS and W2 is the mass of dried cement paste collected after soaking.

2.3. Mechanical testing

The strength of the bioactive glass cement after setting was measured in compression in a mechanical testing machine (CMT6104; SANS Test Machine Inc., China) before and after immersion (without stirring) in PBS for different times. Cylindrical samples (5 mm in diameter × 10 mm) were tested at a crosshead speed of 0.5 mm min^{-1}. 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 24 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 group of 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 immersion in PBS was determined by X-ray diffraction (XRD) (D/max-2500VB2 +/PC X-ray diffractometer) using monochromatic CuK_α radiation (λ = 0.15406 nm) at a scanning rate of 10° min^{-1} (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 (SEM) (S-4700; Hitachi,