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Guest editorial

Strontium-substituted bioactive glasses in vitro osteogenic and antibacterial effects

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ABSTRACT

Objectives. Bioactive glass forms a bone mineral apatite interface and can be engineered to promote optimal bone regeneration. Strontium (Sr^{2+}) stimulates osteoblast and inhibits osteoclast activities *in vitro*, and is used clinically as a treatment for osteoporosis. Dental bone defect repair requires rapid bone formation for early osseointegration but, can be subject to infection. The aim of this study was to investigate the osteogenic and antibacterial effects of strontium-substituted bioactive glasses *in vitro*.

Methods. Strontium-substituted bioactive glasses were designed and produced. Then the osteogenic potential and antibacterial effects of bioactive glass particulates were explored.

Results. Alkaline phosphatase activity, cell number, Type I collagen and mineral nodule formation of MC3T3-E1 cells were significantly promoted by the 5% strontium-substituted glass (5Sr). Furthermore, after incubation with 0.001 g and 0.01 g glass particulates, the growth of sub-gingival bacteria, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* was significantly inhibited; the antibacterial activity being dependent on the percentage of strontium in the glasses.

Significance. These results show that strontium-substituted bioactive glasses significantly promote osteogenic responses of MC3T3-E1 osteoblast-like cells and inhibit the growth of *A. actinomycetemcomitans* and *P. gingivalis*.

1. Introduction

When exposed to body fluids, bioactive glass grafts undergo surface reactions to from a strong bond with the living bone with the generation of an hydroxycarbonate apatite (HCA) layer, and therefore they are widely utilized in dental and orthopaedic applications [1].

Strontium ranelate (SrR), marketed as Protelos®, is an approved drug for treatment and prevention of osteoporosis, in which, strontium (Sr^{2+}) is reported to be the active component [2,3]. Numerous studies have demonstrated that Sr^{2+}

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in vitro promotes osteoblastic differentiation and bone nodule formation [4–6], stimulates the type I collagen protein levels [7], increases the expression of dental matrix protein I, and elevates the osteoprotegerin (OPG) secretion [8]. Sr^{2+} also inhibits the differentiation and activity of osteoclasts [9]. Therefore, the incorporation of $Sr^{2\pm}$ into bioactive glass and used as a bone graft substitute could provide a range of advantages especially if the locally-released $Sr^{2\pm}$ is osteogenic.

Strontium has a similar charge-to-size ratio to calcium and because of the properties detailed above, substituting SrO for CaO in bioactive glass has been widely investigated [10–16]. In a study using simulated body fluid, Fredholm et al. demonstrated that full Sr substitution provides the combined benefits of significantly enhanced early glass dissolution and apatite formation [16]. The mixed Ca-Sr-hydroxyapatite ($Sr_5Ca_5(PO_4)_6(OH)_2$) is formed more rapidly [10]. Yet, since Sr is heavier and slightly larger than Ca, expanding the network should increase the ion dissolution rates and therefore Sr has an earlier effect on bone cells compared with Ca [17,18].

A bacterial infection can hinder the process of bone healing and sometimes can lead to surgical failure [19]. In recent years, some studies have reported that the incorporation of Sr^{2+} into dental and orthopaedic biomaterials will inhibit the bacterial growth, but the antimicrobial mechanism is unclear [20–23]. Whilst phosphate is essential in biological mineralization, some studies have reported that phosphate ions can also be either bacteriostatic or bactericidal [24–26].

We have created a series of bioactive glasses with a fixed 4 mol% P_2O_5 content, and a range of SrO (substituted for CaO). The osteogenic effects on mouse osteoblast-like cells and antibacterial effects on subgingival bacteria species *in vitro* were investigated.

2. Materials and methods

2.1. Glass synthesis

Glasses in the system SiO_2-P_2O_5-CaO-Na_2O-SrO (Table 1) were prepared by the melt-quench route. Briefly, mixtures of analytical grade SiO_2 (Prince Minerals Ltd., Stoke-on-Trent, UK), P_2O_5, Na_2CO_3, $CaCO_3$ and $SrCO_3$ (Sigma–Aldrich Company Ltd., Gillingham, UK) were weighed in the appropriate amounts to give a batch size of 200 g. The batch was mixed thoroughly and placed in a platinum/rhodium crucible, heated and maintained at a temperature of 1460 °C for 90 min in an electrically heated furnace (Lenton EHF 17/3, Hope Valley, UK). After melting, the glass was quenched rapidly into deionized water and the resulting frit was washed with ethanol then dried in a drying cabinet at 37 °C overnight. 100 g of each glass was ground in a Gyro mill (Glen Creston, London, UK) for two periods of 7 min and sieved by a mesh analytical sieve (Endecotts Ltd., London, UK) to obtain fine powder (38 μm diameter). The amorphous structure of the glasses was tested using powder X-ray diffraction (PANalytical, Eindhoven, The Netherlands).

Table 1 – Compositions in mol% with Sr substituted Ca.

Glass	SiO_2	P_2O_5	CaO	SrO	Na_2O
0Sr	42.00	4.00	39.00	0.00	15.00
5Sr	42.00	4.00	37.05	1.95	15.00
50Sr	42.00	4.00	19.50	19.50	15.00
100Sr	42.00	4.00	0.00	39.00	15.00

2.2. Glass powder conditioned culture medium and ion release

Glass particles from each group were immersed in α-Minimum Essential medium (α-MEM, Lonza, London, UK) (1.5 g/L) with 1% antibiotic (penicillin and streptomycin, Invitrogen, London, UK) addition and shaken (60 rpm) at room temperature for 2, 8, 24 and 72 h. At each time point, the samples were centrifuged (800 rpm, 5 min) and filtered with 0.2 μm pore size filters (VWR, Lutterworth, UK) for sterilization.

The filtrate was diluted 1:10 with deionized water and 1% nitric acid [27] and then analyzed in an inductively coupled plasma-optical emission spectroscopy (ICP-OES; Varian Vista-PRO, Varian Ltd., Oxford, UK) to detect silicon, calcium, strontium, and phosphorus concentrations.

2.3. Cell culture and cytotoxicity of glass conditioned medium

MC3T3-E1, a mouse osteoblast-like cell line was obtained from the Culture Collections (Public Health England, Porton Down, Salisbury, UK), and maintained in α-MEM under standard conditions (37 °C, 5% CO_2/95% air, 100% humidity) with 5% foetal bovine serum (FBS, Lonza, London, UK), 1% antibiotic and 1% L-glutamine.

The glass conditioned medium (glass particles immersed for 2, 8, 24 and 72 h) was further supplemented with sterile 1% L-glutamine and 5% FBS, and used to treat MC3T3-E1 for 1 d, 3 d and 5 d. Cytotoxicity of glass conditioned medium on cell growth was visualized by MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay [28]. Briefly, medium was removed and cells were washed twice with PBS, then 30 μL 5 mg/mL tetrazolium salt MTT (Sigma–Aldrich Company Ltd., Gillingham, UK) was added to each well and incubated in 37 °C for 4 h. Formazan crystals generated by mitochondrial enzyme activity were dissolved by dimethyl sulfoxide (DMSO, Sigma–Aldrich Company Ltd., Gillingham, UK) and the intensity of purple coloured reaction product quantified by measuring the absorbance spectra at 570 nm.

2.4. Total quantification of cells cultured in glass conditioned medium

According to the cytotoxicity results, 72 h glass conditioned medium was chosen to further explore the effect on cell proliferation. MC3T3-E1 cells were treated for 7 d, 14 d and 21 d. Cell number was ascertained by quantifying the DNA in cultures using the fluorochrome, bisbenzimidazole (Hoechst 33258, Sigma–Aldrich Company Ltd., Gillingham, UK) [29,30], in which fluorescence intensity is linearly related to DNA concentration. Briefly, after treatment for the indicated time points, cells were lysed through a freezing and thawing cycle with 100 μL deionized water in each well to rupture cells and release DNA. The resulting lysate was then incubated with