SELF-GELLING, INJECTABLE HYDROGEL-BIOACTIVE GLASS COMPOSITES

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Introduction
When applying hydrogels as biomaterials for bone tissue regeneration, the introduction of an inorganic phase is considered desirable [1]. Particles of bioactive glass can be added during hydrogel formation. Bioactive glasses are not only known to promote bioactivity, but they can also induce the gelation of solutions of anionic, calcium-binding polymers and impart antibacterial activity [2]. Bioactive glasses can be doped with metal ions such as magnesium (Mg), zinc (Zn) and strontium (Sr) [3].
In this study, four different bioactive glass preparations were added to a solution of pectin, a calcium-binding polysaccharide which gels in the presence of calcium. The resulting composites where characterized physicochemically (gelation kinetics), microbiologically and cell biologically with MG63 osteoblast-like cells.

Materials and Methods
Bioactive glasses, doped with Zn, Mg and Sr and undoped, hereafter denoted P5 (undoped), P5-Zn, P5-VS-Mg and P5-VS-Sr were produced as described previously [3]. Glass particle sizes were studied by laser diffraction: average (d(0,5)) particle sizes were 31, 24, 91 and 92 µm, respectively. Glasses and pectin solution (amidated apple pectin, 0.8% (w/v)) were sterilized by autoclaving at 134ºC. Hydrogel-glass composites were prepared by vigorous mixing of glass particles with pectin solution to yield composites with glass concentration of 32% (w/v). Visually, the distribution of glass particles appeared to be more homogeneous in composites containing P5 and P5-Zn. Gelation kinetics were studied by rheometry. To assess the cytocompatibility of hydrogel-glass composites, 50 µl freshly prepared composite was dispensed into wells of a 96 well plate. Following gelation, MG63 osteosarcoma cells were seeded on top of the thin composite layer at 10,000 ($1X10^4$) cells per well in complete growth medium (Dulbeccos Modified Essential Medium, 10% Foetal Bovine Serum, 1% PenStrep). Cells were incubated at 37°C, with 5% CO_2 until required. After 1, 3 and 7 days, cell viability was assessed using the alamarBlue® assay (Thermo Fisher; DAL1025). AlamarBlue metabolic activity readings were normalised to non-seeded gels in complete growth medium to account for any effect this may have had on the resazurin reduction. Cells seeded on tissue culture plastic were used as a control to assess proliferation potentials. MG63 cells were also encapsulated into composites containing P5 and P5-Zn (10,000 cells/50 µl composite), as were the most homogeneous and easiest to handle. Antibacterial activity was tested using methicillin-resistant *Staphylococcus aureus* (MRSA) as described previously [4] and *Staphylococcus aureus (S.aureus).*

Results and Discussion
Gelation of all composites occurred within 5 minutes. Composites did not exhibit any appreciable antibacterial activity against MRSA, but composites containing P5-Zn inhibited *S.aureus* growth. MG63 cells retained viability and proliferated over 7 days on composites containing P5 and P5-VS-Sr (FIG. 1). Proliferation was markedly lower on composites containing P5-VS-Mg and P5-Zn at all time points. This is surprising, considering that Mg, as a component of ceramic materials, is able to stimulate cell proliferation [5]. MG63 cells also retained viability when encapsulated in composites containing P5 and P5-Zn (FIG. 2). After encapsulation, proliferation was markedly higher in composites containing P5-Zn than in those containing P5 after 3 and 7 days, although the proliferation values on composites containing P5-Zn were markedly lower (FIG. 1). The reasons remain unclear.

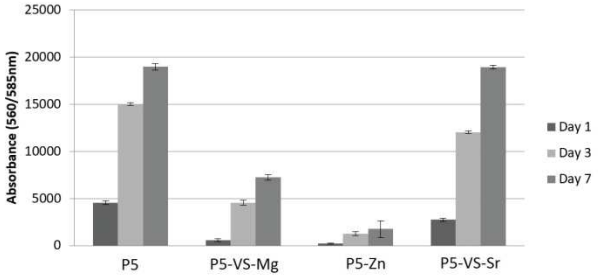

FIG. 1. MG63 cell proliferation on the surface of composites after 1, 3 and 7 d.

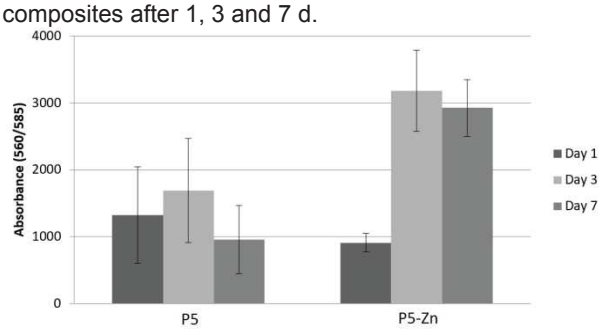

FIG. 2. Proliferation of MG63 cells encapsulated in composites after 1, 3 and 7 d.

Conclusions and Outlook
All four bioactive glass preparations induced gelation of pectin solution to form injectable hydrogel-bioactive glass composites within 5 minutes. Osteoblast-like MG63 cells were able to maintain viability and proliferate both on the surface of composites and after encapsulation. Glass distribution homogeneity and cell number depended on type of glass used. These results pave the way for further investigation of mineralizability and glass distribution.

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