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Characterization of zinc-releasing three-dimensional bioactive glass scaffolds and their effect on human adipose stem cell proliferation and osteogenic differentiation

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Abstract

While the addition of zinc ions to bioactive ceramics has been shown to enhance the proliferation and osteogenic differentiation of osteoblast-like cells, contradictory results have been found. Therefore, the effect of zinc-releasing ceramics on cell proliferation and differentiation into osteogenic lineages requires further clarification. The aim of this study was to evaluate the effects of zinc addition on the degradation profile of three-dimensional bioactive glass scaffold, and on the proliferation and osteogenesis of human adipose stem cells (hASCs) in these scaffolds. Bioactive glass scaffolds containing Na_2O, K_2O, MgO, CaO, B_2O_3, TiO_2, P_2O_5 and SiO_2 were prepared. The degradation was evaluated by weight loss measurement, scanning electron microscopy and elemental analysis. The degradation profile of bioactive glass was shown to slow down with the addition of zinc. Qualitative live/dead staining showed that zinc addition to bioactive glass inhibits cell spreading and proliferation of hASCs. However, zinc addition had no significant effect on DNA content, alkaline phosphatase activity and osteopontin concentration of hASCs when measured quantitatively. Our results suggest that the possible stimulatory effect of addition of zinc on hASC proliferation and osteogenesis was not detected because addition of zinc slowed down the degradation rate of the studied bioactive glass scaffolds.

Keywords: Bioactive glass; Zinc-release; Adipose stem cells; Bone tissue engineering

1. Introduction

Zinc is known to play an important role in bone metabolism; its stimulatory effect on bone formation and its ability to promote the expression and maintenance of osteoblastic phenotypes has been shown in vitro [1–3]. Depletion or supplementation of dietary zinc has been shown to be responsible for variations in body weight, bone length and bone biomechanical properties in growing rats. The positive effect of zinc on bone metabolism has been associated with growth hormone (GH) or insulin-like growth factor 1 (IGF-1) [4].

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Despite the numerous works reporting the stimulating effects of zinc, there are also studies that show other effects of zinc ions on bone cells. Popp et al. studied the effect of Zn^{2+}-supplemented osteogenic medium on osteoblastic proliferation and differentiation. They used concentrations of 0.20, 0.65 and 2.62 mg l^{-1} and found no significant effects of such zinc amounts on rat bone marrow stromal cells [5]. Wang et al. have shown that the effect of zinc on the osteogenic and adipogenic differentiation of mouse primary bone marrow stromal cells and on the adipogenic transdifferentiation of mouse primary osteoblasts depends on ion concentration and incubation time, with zinc even having an inhibitory effect [6].

The use of synthetic bone substitutes for delivery of zinc ions has recently gained attention in research communities. There are numerous studies regarding the development and evaluation of zinc-containing calcium phosphate ceramics both in vitro and in vivo [1,2,7–9]. There are also reports concerning the use of different bioactive glass compositions as short- or long-term zinc delivery vehicles [10–13].

Bioactive glass, due to its osteostimulative and bone-bonding properties, has been successfully used clinically in dental, craniomaxillofacial and spinal applications during the last few decades [14–19]. Its bone-bonding ability arises from the high rate of formation of hydroxyl-carbonate apatite (HCA) at the surface of the material after reaction with the surrounding biological fluids [20,21]. The bone-like, low-crystalline HCA, together with the ionic products resulting from the degradation process of the material, are also correlated to the intrinsic and characteristic bioactive glass bone regeneration potential. They have been shown to be responsible for enhancing the proliferation and differentiation of osteoprogenitor cells [22].

A number of bioactive glass compositions have been investigated as possible zinc ion delivery vehicles. It has been proposed that due to the incorporation of zinc ions in the forming HCA layer, bioactive glass allows a tailored modulation of zinc release in the biological system, hence avoiding the cytotoxicity-related problems observed with the zinc-containing calcium phosphate materials described above [23,24]. Despite the incorporation mechanism of zinc ions in HCA, the importance of the dosage as well as the possible cytotoxic effect of zinc ions have also been reported for certain bioactive glass and glass–ceramic compositions [2,10].

Many in vitro studies of the analysis of the zinc effect on cells, from rat to human, and from primary bone marrow stromal cells to osteoblasts, have been reported. However, no study has yet investigated the use of human adipose stem cells (hASCs), which have emerged as an attractive source of multipotent cells. hASCs have shown the ability to differentiate into osteogenic lineages in vitro [25,26], and have also been used to treat bone defects in clinical cases [27,28]. Additionally, it is easy to procure hACSs: their expansion in vitro is rapid and their harvest yield is approximately 40-fold higher compared to bone marrow mesenchymal stem cells [29].

Since there is much debate on the effects of zinc on cell proliferation and osteogenic differentiation, there is a clear need for more research to be carried out. Furthermore, because zinc has been shown to favour crystallization of bioactive glass at lower temperatures with respect to zinc-free glass [23,30], the effect of the ion on the manufacturing of zinc-containing bioactive glass three-dimensional (3-D) scaffolds also needs to be analyzed. This report represents the first attempt to verify the possibility of manufacturing zinc-containing 3-D porous bioactive glass scaffolds suitable for bone tissue engineering applications, and to study the effect of zinc addition on construct degradation, osteogenic differentiation and proliferation of hASCs.

2. Materials and methods

2.1. Scaffold manufacturing

Bioactive glass with a composition of 10–12 mol.% Na_2O, 10–12 mol.% K_2O, 4–6 mol.% MgO, 10–18 mol.% CaO, 1–4 mol.% P_2O_5, 1–2 mol.% B_2O_3, 0–1 mol.% TiO_2, 50–56 mol.% SiO_2, ZnO 0–5 mol.% was manufactured. The family used in this study included glasses with 0, 0.25, 0.5, 1.0, 1.5, 2.0 and 5.0 mol.% of ZnO, substituting CaO. In the cell culture experiments only 0, 0.25 and 1.0 mol.% zinc-containing bioactive glass scaffolds were studied. The manufacturing of the 3-D scaffolds was done as previously described [31]. Briefly, the glass manufacturing process consisted of melting of the reagents in a covered platinum crucible above 1360 °C, cooling down to room temperature, crushing and remelting in order to obtain better material homogenization. The glass was then used for the manufacturing of 3 mm long and 75 μm thick bioactive glass fibres by melt spinning. The collected fibres were further packed into metallic moulds and sintered to obtain 3-D scaffolds with the desired structural and mechanical characteristics. Scaffolds with dimensions of $14 \times 14 \times 5$ mm were used in the cell culture experiments.

Scaffolds characterized by a total porosity of 70% were chosen for the study based on a previous study by our group, where this porosity was shown to be suitable for hASC proliferation and osteogenic differentiation [32]. In addition, the same porosity was previously studied with respect to degradation [33], and in vivo behaviour [34].

2.2. Material and 3-D scaffold characterization

The compositional variation effect on the structure of the material was evaluated by Raman spectroscopy (S1000, Renishaw, New Mills, UK) and by Fourier transform infrared attenuated total reflectance spectroscopy (FTIR-ATR; Perkin–Elmer, Waltham, MA, USA) with respect to the crystallites that eventually formed.

The effect of zinc addition on the degradation behaviour of the material was evaluated both in deionized water and in simulated body fluid (SBF). About 110 mg of chopped fibres of the different compositions were immersed in

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