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Enhanced osteocalcin expression by osteoblast-like cells (MC3T3-E1) exposed to bioactive coating glass (SiO_2–CaO–P_2O_5–MgO–K_2O–Na_2O system) ions

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Abstract

This study tested the hypothesis that bioactive coating glass (SiO_2–CaO–P_2O_5–MgO–K_2O–Na_2O system), used for implant coatings, enhanced the induction of collagen type 1 synthesis and in turn enhanced the expression of downstream markers alkaline phosphatase, Runx2 and osteocalcin during osteoblast differentiation. The ions from experimental bioactive glass (6P53-b) and commercial Bioglass™ (45S5) were added to osteoblast-like MC3T3-E1 subclone 4 cultures as a supplemented ion extract (glass conditioned medium (GCM)). Ion extracts contained significantly higher concentrations of Si and Ca (Si, 47.9 ± 10.4 ppm; Ca, 69.8 ± 14.0 for 45S5; Si, 33.4 ± 3.8 ppm; Ca, 57.1 ± 2.8 ppm for 6P53-b) compared with the control extract (Si < 0.1 ppm, Ca 49.0 ppm in α-MEM) (ANOVA, $p < 0.05$). Cell proliferation rate was enhanced (1.5× control) within the first 3 days after adding 45S5 and 6P53-b GCM. MC3T3-E1 subclone 4 cultures were then studied for their response to the addition of test media (GCM and control medium along with ascorbic acid (AA; 50 ppm)). Each GCM + AA treatment enhanced collagen type 1 synthesis as observed in both gene expression results (day 1, Col1α1, 45S5 GCM + AA: 3× control + AA; 6P53-b GCM + AA: 4× control + AA; day 5, Col1α2, 45S5 GCM + AA: 3.15× control + AA; 6P53-b GCM + AA: 2.35× control + AA) and in histological studies (Picrosirius stain) throughout the time course of early differentiation. Continued addition of each GCM and AA treatment led to enhanced expression of alkaline phosphatase (1.4× control + AA after 5 days, 2× control + AA after 10 days), Runx2 (2× control + AA after 7 days) and osteocalcin gene (day 3, 45S5 GCM + AA: 14× control + AA; day 5, 6P53-b GCM + AA: 19× control + AA) and protein expression (40×–70× control + AA after 6 days). These results indicated the enhanced effect of bioactive glass ions on key osteogenic markers important for the bone healing process.

Keywords: Bioactive glass ions; Osteogenesis; Osteoblasts; Silicon; Calcium

1. Introduction

Implants are used in dental applications to replace missing teeth, or in orthopedic and craniofacial applications to replace lost bone. These implants must restore the physiological structure and function while also facilitating complete bone apposition [1]. Currently, Ti implants are used successfully for tooth replacement in mandibular or maxillary bone (86% and 76%, respectively [2]) and nearly 93% for bone replacement in the cranium [3]. However, faster osteointegration and improved implant–bone attachment are desired to improve implant success rates and longevity.

Various coating technologies to improve the implant–bone interface have been attempted. Hydroxyapatite (HA) was used for a number of years. However, delamination of HA occurred at the implant–ceramic interface [4] or

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bone–ceramic interface [5]. Commercial Bioglass [6,7] is widely known for its benefits in various bone substitutes [8] and periodontal procedures [9], however, it is difficult to use as an implant coating because it cracks at the Ti–glass interface when cyclically loaded [10]. Both HA and Bioglass cracking is primarily due to a large thermal expansion mismatch with Ti [10].

Improvement in glass coating technology led to the development of a family of bioactive glasses (50–59 wt.% SiO_2) to enhance the osteointegration potential of Ti [10–17] or as polymer–bioactive glass composite scaffolds for hard and soft tissue regeneration [18]. By doping the glass with additional constituents and partial substitution of CaO with MgO and Na_2O with K_2O (Table 1, 6P53-b vs. 45S5), bioactive coating glass had improved adhesion to Ti alloy during the coating process. In previous *in vitro* studies, these glasses facilitated direct mineralized tissue attachment (compared with mechanical attachment of mineralized tissue to Ti surfaces [16]). The bioactive glass coating formed a HA surface layer which facilitated the direct bond to bone [16,19].

In general, the surface of the glass material and its corrosion behavior in the physiological environment influence its apposition to bone. Another study [20] attempted to determine the separate impact of the initially fabricated bioactive glass surface and the initial dissolution of ions from that surface. The goal was to determine whether additional surface treatment was required to promote a favorable osteoblast response. It was found that the initial *in vitro* dissolution of the glass was rapid, which increased the pH of the *in vitro* environment to which osteoblasts were exposed. This increased pH appeared to decrease the amount of cell proliferation and alkaline phosphatase activity [20]. For these reasons, the glass materials were presoaked (or conditioned) in simulated body fluid (SBF) for a period of 10 days. This conditioning period (1) stabilized the *in vitro* pH to near physiological (7.0–7.4) [20], (2) reduced exposure of osteoblasts to contaminants from the fabrication process [21], and (3) induced the formation of a HA surface layer for mineralized tissue attachment. The enhanced marker expression observed by Foppiano et al. [22] in fact occurred when the bioactive glass was conditioned *in vitro*. This study focuses on the corrosion of ionic products (isolated from the bioactive glass surface and after this initial conditioning period) to discover whether they also influence the osteoblasts' cell cycle.

Interestingly, They have suggested that these ions may play an active role in osteoblast behavior. They have suggested that these ions may alter the expression of osteoblast differentiation markers associated with bone matrix formation [23–32], while other studies showed that individual ions may alter osteoblast function [33–37]. For example, commercial Bioglass has been shown to influence Runx2 and osteocalcin expression [38], which are expressed within the first 10 days of the differentiation compartment of the osteoblast cell cycle. Furthermore, Foppiano et al. [22] found that these dissolution products promoted the up-regulation of Runx2. Yet, no significant effort has been attempted to connect the enhanced gene expression (within 7 days) as it impacts downstream matrix protein expression (within 10 days) by osteoblasts under the influence of these bioactive coating glass corrosion products. To determine the effect of these ions on osteoblasts, a materials extract was isolated as a dissolved product from the bioactive glass surface.

Table 1
Composition (wt.%) of Bioglass™ (45S5) and experimental bioactive glass (6P53-b).

	SiO_2	Na_2O	K_2O	MgO	CaO	P_2O_5
6P53-b (LBL)	52.7	10.3	2.8	10.2	18.0	6.0
45S5 (Mo-Sci)	45.0	24.5			24.5	6.0

This study tests the hypothesis that bioactive glass ions enhance osteoblast differentiation. The aims of this study are (1) to demonstrate that specific osteoblast markers are enhanced in the presence of bioactive coating glass (6P53-b) ions; (2) to demonstrate that similar osteoblast behavior is observed for commercially available Bioglass™ (45S5) ions; (3) to determine a corresponding effect on gene, protein and extracellular matrix expression of osteogenic biomarkers.

To study the effect on osteoblasts of ionic products of bioactive glass dissolution, a well-characterized cell line, MC3T3-E1 subclone 4, was used. This cell line mimics osteoblast progenitors in that it expresses markers associated with differentiation into a mineralizing phenotype. MC3T3-E1 subclone 4 cells proliferate to confluence within 6–7 days after seeding (40,000–50,000 cell cm^{-2}). Once confluent (~2–2.5× initial seeding density within 6–7 days), these cells can be induced to differentiate by the addition of (ascorbic acid) AA and glycerol 2-phosphate. Ascorbic acid is used to induce collagen type 1 synthesis, while glycerol 2-phosphate is used to differentiate this cell line fully into a mineralizing phenotype. Since collagen type 1 forms the biological support to which mineralized tissue binds (for later bone formation) [39–42], the focus is on the effect that the ionic products of bioactive glass dissolution have on collagen type 1 synthesis (Col1α1 and Col1α2 chains) and downstream expression of other key differentiation markers, alkaline phosphatase, core binding factor a (cbfa/Runx2) and osteocalcin. Runx2 is a key transcription factor associated with early expression of the osteoblast phenotype [43]. Alkaline phosphatase is a key dephosphorylating enzyme expressed by osteoblasts to turn over expressed collagen into a form that is amenable for bone matrix formation [35,39–42]. Osteocalcin is a key non-collagenous protein which binds extracellular calcium to bone matrix [44].

2. Materials and methods

2.1. Study design

Pertaining to the goals described above, three experiments were designed to ascertain the effect of the ionic

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