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Growth and differentiation of osteoblastic cells on 13–93 bioactive glass fibers and scaffolds

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Abstract

This in vitro study was conducted to evaluate the ability of two types of constructs of bioactive, silica-based 13–93 glass fibers to support the growth and differentiation of MC3T3-E1 osteoblastic cells. The two types of constructs tested included single-layer 13–93 glass fiber rafts and three-dimensional porous scaffolds formed from sintered 13–93 fibers. Scanning electron micrographs showed a closely adhering, well-spread morphology of MC3T3-E1 cells seeded on both types of constructs. The scanning electron microscopy images also showed a continuous increase in cell densities during a 6 day incubation on 13–93 glass fiber rafts and scaffolds. Quantitative fluorescence measurements of DNA also revealed a linear increase in cell density during a 6 day incubation on both types of 13–93 constructs. Examination of scaffolds incubated in MTT containing medium showed the presence of metabolically active viable cells within the interior of the scaffold. The addition of ascorbic acid to MC3T3-E1 cells cultured on the 13–93 glass fibers triggered a threefold increase in alkaline phosphatase, a key indicator of osteoblast differentiation. The sintered scaffolds were found to have open, interconnected pores favorable for tissue ingrowth with a compressive strength similar to cancellous bone. Collectively, the results indicate that 13–93 glass fiber scaffolds are a favorable substrate for the growth and differentiation of osteoblasts and a promising material for bone tissue engineering and repair of bone defects.

Keywords: Glass fibers; Scaffolds; Osteoblasts; Proliferation; Alkaline phosphatase

1. Introduction

The shortage of skeletal allografts has created a great demand for synthetic substitutes for bone, the most commonly replaced organ of the body [1]. A promising approach to this dilemma is through the use of cell support scaffolds for both in vivo reconstruction of skeletal defects [2] and in vitro engineering of new bone tissue [3,4]. The development of novel scaffold materials for these applications has been the focus of numerous investigations over the past decade [1,5]. Most scaffold materials are designed as either a macroporous matrix or a fibrous mesh to permit tissue ingrowth and the development of vessels for nutrient delivery [6]. Poly-L-lactide and other biodegradable polymers have been commonly used to prepare support scaffolds in the form of porous foams [7,8] and fiber meshes [9,10]. Bioactive glasses and glass ceramics have also been used to form porous scaffolds fabricated via sol–gel [11,12] and sintering techniques [13].

Bioactive glasses and glass ceramics are attractive as scaffold materials due to their ability to bond to either hard or soft tissue without promoting the formation of an intervening fibrous encapsulation [14]. Bioactive glass fibers that could be woven into a mesh or sintered to form a scaffold of a desired shape would be useful in some

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applications. Currently, the only bioactive glass containing products approved by the Food and Drug Administration for clinical use are those with silica-based 45S5 glass [15]. However, 45S5 glass cannot be easily pulled into fibers due to devitrification that occurs during the fiber drawing process.

One approach for enhancing the ability to pull fibers involves increasing the silica content of the glass and adding both sodium oxide and potassium oxide to take advantage of the "mixed-alkali effect" [16]. A new silica-based glass, designated S520, is an example of a mixed-alkali, high-silica-content glass that can be pulled into continuous fibers [17]. The S520 glass was recently reported to be bioactive based on evidence that S520 glass fibers developed a hydroxyapatite surface layer during incubation in simulated body fluid and also supported the formation of mineralized nodules by primary human osteoblasts [18].

An extensive investigation of mixed-alkali, high-silica-content glasses was conducted by Brink and colleagues to identify new bioactive glasses suitable for use as coatings and formation of fibers [19,20]. The latter investigators developed 26 different glasses within the Na_2O–K_2O–MgO–CaO–B_2O_3–P_2O_5–SiO_2 system and implanted samples of each glass in rabbit tibia for 8 weeks to test bioactivity in vivo. Follow-up physical analyses of resected specimens revealed that 10 of the 26 compositions showed direct bonding to bone via a bioactive calcium phosphate layer. One of the 10 bioactive compositions within this family, a glass designated as 13–93 glass, can be easily pulled into continuous fibers [21]. Favorable mechanical properties of 13–93 glass fibers include the retention of approximately three-quarters of the initial flexural strength after 3 weeks of immersion in simulated body fluid (SBF) [22].

Based on a combination of attractive features, there is interest in 13–93 glass as a strengthening component of bioabsorbable composites and for the manufacturing of porous scaffolds. Bioabsorbable composite membranes containing pulverized 13–93 glass particles as a strengthening component were tested for repair of maxillary alveolar defects in rabbits, but were found to be devoid of osteogenic activity [23]. In a related investigation, 13–93 glass microspheres (50–125 μm) were thermally bonded to the surface of self-reinforced poly-L-lactide plates to form an osteoconductive surface that would prevent fibrous encapsulation [24]. The latter composites were found to provide limited support for the growth of human osteoblasts in vitro, although the ability of the microspheres to remain bonded to the composite when exposed to tissue fluids in vivo was not presented.

The present in vitro study was conducted to further assess the suitability of 13–93 glass fibers as a scaffold material. Two types of 13–93 glass fiber constructs were fabricated and tested for their ability to support the attachment, growth and differentiation of MC3T3-E1 osteoblastic cells. The constructs included single-layer 13–93 glass fiber rafts prepared as simple model scaffolds plus three-dimensional constructs of sintered 13–93 glass fibers formed to create reasonably strong porous scaffolds. Because 45S5 glass fibers were not available for use, tests with 45S5 glass discs and 13–93 glass discs were included in this study to compare growth and differentiation on the two glasses. In addition, samples of the 13–93 glass were soaked in SBF and analyzed for the formation of HA as a test for bioactivity.

2. Materials and methods

2.1. Preparation of glass fibers and discs

Type 13–93 glass (nominal composition listed in Table 1) was prepared at MO-SCI Corporation, Rolla, MO, by melting a homogeneous mixture of reagent-grade Na_2CO_3, K_2CO_3, MgO, $CaCO_3$, $CaHPO_4$ and SiO_2 in a platinum crucible. Some of the 13–93 glass melts were poured into metal molds to form 12 mm diameter rods. Other batches of 13–93 glass were re-melted in a Pt-resistance-type bushing from which fibers with a diameter between 25 and 40 μm were pulled. A controlled speed take-up drum was used to align and collect the fibers. In addition to the 13–93 glass, 12 mm diameter rods of 45S5 glass were prepared by melting a mixture of reagent-grade Na_2CO_3, $CaCO_3$, $CaHPO_4$ and SiO_2 mixed in the proportions listed in Table 1. A low-speed wafering saw was used to cut the annealed 13–93 and 45S5 glass rods into discs approximately 1 mm thick. The discs were ground to a final finish with 600 grit alumina and then ultrasonically cleaned for 15 min in acetone followed by 15 min in ethyl alcohol. The cleaned glass discs were blotted and dry-heat sterilized for 3 h at 250 °C.

2.2. Fabrication of glass fiber rafts and glass fiber scaffolds

Preparation of glass fiber rafts began with a strip (~3 cm × 15 cm) of unidirectionally aligned fibers positioned on a glass sheet and carefully oriented into a tightly juxtapositioned, single-thickness layer. The fibers were bonded together with a continuous bead of silicone adhesive (GE "Silicone I") applied perpendicular to the fibers at 1 cm intervals. After allowing the silicone to cure, the layer was sectioned to obtain 1 cm × 1 cm square rafts, as shown in Fig. 1. The preparation of three-dimensional glass fiber scaffolds began by slightly crushing the 13–93 fibers between two glass plates to obtain fragmented fibers with a mean length of 3 mm. The fiber fragments were weighed, placed in short tubular ceramic molds (7 mm

Table 1
Nominal composition (mol%) of test glasses

Glass	Na_2O (%)	K_2O (%)	MgO (%)	CaO (%)	P_2O_5 (%)	SiO_2 (%)
13–93	6	7.9	7.7	22.1	1.7	54.6
45S5	24.3	–	–	26.9	2.5	46.3

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