Performance of 13-93 Glass Fiber Scaffolds with Osteogenic Cells

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Introduction: The numerous shortcomings associated with autografts and allografts have focused attention on a need for synthetic replacements for bone. One approach to resolving this problem involves the use of cell support scaffolds for *in vivo* repair of skeletal defects and *in vitro* engineering of new bone tissue. We recently reported the development of porous scaffolds fabricated from sintered 13-93 silicate glass fibers [1]. Here we report results of a follow-up *in vitro* study of the suitability of 13-93 glass fiber scaffolds for potential use in bone tissue engineering applications. Murine MLO-A5 cells, an established line of postosteoblast/preosteocyte cells [2], were seeded on the glass fiber scaffolds and assayed for growth and function as a model of newly developing bone tissue.

Methods: Glass with the 13-93 composition (mol %) ($54.6SiO_2$, $6Na_2O$, $7.9K_2O$, 7.7MgO, 22.1CaO, $1.7P_2O_5$) was melted and pulled to 100 to 300 μm diameter fibers. The fibers were crushed to a mean length of 3mm, placed in ceramic molds, and sintered at 720°C to form scaffolds about 2mm thick x 6mm diameter [1]. The scaffolds were seeded with 50,000 MLO-A5 cells, placed in αMEM medium containing 5% fetal calf serum/5% calf serum, and cultured 2 to 12 days at 37ºC. Metabolically active cells on and within the scaffolds were visualized by labeling with MTT tetrazolium salt during the last 4 hr of incubation. Scanning electron microscopy (SEM) was used to examine the morphology of cells on the scaffolds. Alkaline phosphatase (ALP) activity in freeze-thaw lysates recovered from cell-seeded scaffolds was measured spectrophotometrically as p-nitrophenol (pNP) released from pNPP substrate. Mineralization was induced by incubating the cell-seeded scaffolds in medium supplemented with β-glycerolphosphate and ascorbate [2] and the product formed was measured by uptake of alizarin red as described elsewhere [3]. Group means (n=3) were compared by Student's *t*-test for differences significant at $p<0.05$.

Results and Discussion: SEM images show a well-adhered morphology of MLO-A5 cells on the fiber scaffolds with a low density at day 2 (Fig. 1A) and much higher density at day 4 (Fig. 1B). Additional SEM images (not shown) revealed complete coverage of the scaffolds by day 6. The increasing intensity of purple formazan visible on the scaffolds (Fig. 2A) is evidence of vigorous growth of metabolically-active cells during the 6-day incubation. The MTT labeling also reveal metabolically-active cells within the interior of the scaffolds (Fig. 2B). Separate measurements (not included here) showed a linear, 6-fold increase in total protein in lysates recovered from cell-seeded samples during the 6-day incubation. Collectively, these findings indicate the glass fiber scaffolds are capable of supporting robust cell growth. ALP activity, an indicator of osteogenic function, increased dramatically in MLO-A5 cultured on the test scaffolds (Table 1). Incubation of cell-seeded scaffolds in medium containing mineralization inducing agents caused a 20-fold rise in recovery of alizarin red-positive material (Table 2). This dramatic response, a definitive indicator of osteogenic function, is further evidence the 13-93 glass fiber scaffolds are capable of supporting differentiation of osteoprogenitor cells to functional bone tissue.

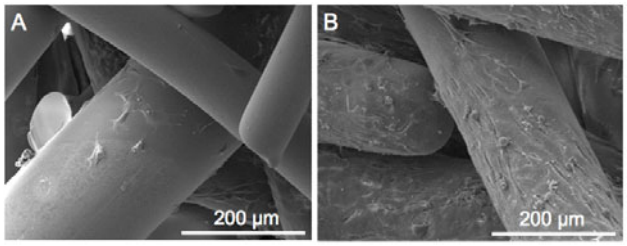

Fig. 1. SEM images of cell-seeded 13-93 glass fiber scaffolds cultured for: (A) 2 days; and (B) 4 days.

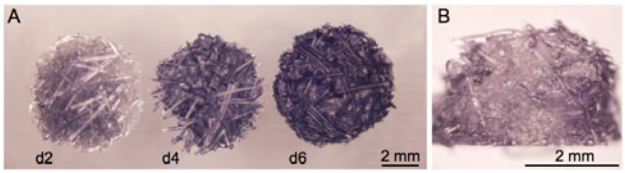

Fig. 2. (A) Cell-seeded scaffolds labeled with MTT after incubations of 2, 4, & 6 days; (B) fracture face of day 6 sample showing MTT labeled cells in interior of scaffold.

Table 1. ALP activity on cell-seeded scaffolds. Values are nanomoles pNP released/min/scaffold. *$p<0.05$.

Day 2		Day 4		Day 6
9.9 ± 1.7	*	44.5 ± 5.0	*	134.2 ± 46.2

Table 2. Mineralized nodules formed by MLO-A5 cells after 12 days on 13-93 scaffolds. Values are nanomoles alizarin red staining of nodules/scaffold. *$p<0.05$.

Control medium		βGP/ascorbate medium
10.4 ± 1.1	*	226 ± 20.8

Conclusions: Results show the 13-93 fiber scaffolds do support the growth and function of MLO-A5 cells, findings that suggest these porous glass constructs may be useful for bone repair and tissue engineering applications.

References:

1. Brown RF *et al.* Acta Biomaterialia. 2008;4:387-96.
2. Kato Y, *et al*. J Bone Miner Res. 2001;16:1622-33.
3. Gough, *et al*. Biomaterials. 2004;25:2039-46.

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