Unidirectional bioactive glass (13-93) scaffolds with controllable pore size for repair of load-bearing bones

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Introduction: The repair of large defects in load-bearing bones remains a clinical challenge. Scaffolds prepared by current methods from biodegradable polymers, bioactive glasses, or bioactive ceramics often lack the combination of high strength and high porosity for skeletal substitution of load-bearing bones. Scaffolds with oriented pore architectures, prepared by unidirectional freezing of aqueous suspensions, have far higher strengths than conventionally-prepared scaffolds, but their pore sizes have been limited to <50–100 μm.[1,2] In this study, unidirectional freezing of camphene suspensions was investigated for the preparation of oriented bioactive glass (13-93) scaffolds with controllable pore diameters >100 μm. The mechanical and *in vitro* cell culture performance of the scaffolds was also investigated.

Methods: Bioactive glass (13-93) particles (1 ± 0.5 μm) were dispersed in camphene containing 2 wt% stearic acid (dispersant), and ball-milled for 24 h at 55°C to form a slurry (10 vol% particles). Unidirectional freezing was performed by pouring the slurry into rubber molds (11 mm in diameter × 20 mm) placed on Cu plates at 0°C. The frozen samples (sealed in PVC tubes) were annealed at 34°C for up to 72 h, to allow coarsening of the camphene. After sublimation of the camphene, the samples were sintered in air for 1 h at 690°C (heating rate = 5°C/min) to densify the glass network. Scanning electron microscopy (SEM), Image J software, and the Archimedes method were used to characterize the scaffolds. The mechanical response of the scaffolds in compression was evaluated at a deformation rate of 0.5 mm/min. Dry heat sterilized scaffolds were seeded with 50,000 MT3T3-E1 cells, placed in 2 ml of a-MEM medium with 10% FBS plus 100 U/ml penicillin and 100 μg/ml streptomycin sulfate, and incubated for 2 days at 37°C. After incubation, cell-seeded constructs were placed in serum-free medium containing MTT salt to permit visual examination of metabolically active cells.

Results and Discussion:

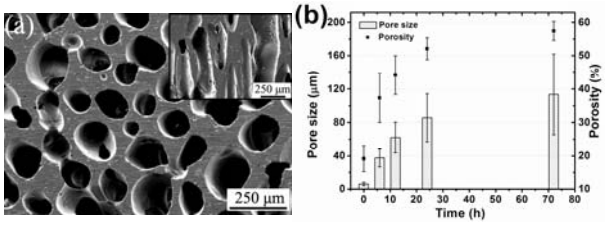

Fig. 1. (a) SEM image of scaffold prepared by unidirectional freezing of camphene-based slurries: annealing time = 72 h. (b) Porosity and pore size of sintered scaffolds vs. annealing time of frozen slurry.

SEM of the cross sections of the sintered scaffolds (Fig. 1a) showed nearly circular pores oriented in the direction of freezing. The average pore size and porosity of the scaffolds increased with the annealing time of the frozen construct (Fig. 1b). Scaffolds with porosity of 50–55% and pores of size 60–120 μm, approximately the minimum values reported for scaffolds capable of supporting cell proliferation and function, were obtained after 24 h annealing. Scaffolds with controllable porosity and pore size can be obtained using this method.

The compressive stress vs. deformation of the scaffolds (Fig. 2) was different from the response of brittle solids. After an initial linear response (region 1), the deformation increased at almost constant stress (region 2), followed by an almost linear response before failure (region 3). The strength of the scaffolds (highest stress), was dependent on the annealing time (or microstructure), decreasing from ~150 MPa (porosity = 20%) to ~30 MPa (porosity 55–60%). The strengths of cortical and trabecular bone are 125–175 MPa and 2–12 MPa, respectively.

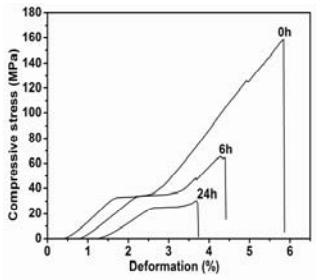

Fig. 2 Compressive stress vs. deformation

Incubation for 2 d showed that the scaffolds provided a favorable surface for proliferation of MC3T3-E1 cells (Fig. 3, inset). After the 2 d incubation, scaffolds with the larger pore sizes (annealing time ≥12h) supported the proliferation of cells down into the pores, as observed by the metabolically active cells on the lower surface of the scaffolds (Fig. 3).

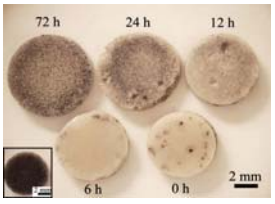

Fig. 3 Lower surface of cell-seeded scaffolds treated with MTT, for scaffolds formed from frozen slurries and annealed for the times shown. (Inset: top surface of cell-seeded scaffolds treated with

Conclusions: Unidirectional bioactive glass (13-93) scaffolds, with controllable porosity (20–60%) and pore size (10–160 μm), prepared by freezing of camphene suspensions, showed a unique mechanical response, compressive strengths as high as 150 MPa (20% porosity), and supported cell proliferation on the surface and down into the pores (>60–80 μm). These scaffolds could potentially be used in the repair of load-bearing bones.

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References

[1]Deville et al., Biomaterials 2006;27:5480-9.
[2]Fu et al., J Biomed Mater Res B 2008;86B:125-35.