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Oriented bioactive glass (13-93) scaffolds with controllable pore size by unidirectional freezing of camphene-based suspensions: microstructure and mechanical response

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Abstract

Scaffolds of 13-93 bioactive glass (composition 6Na₂O, 8K₂O, 8MgO, 22CaO, 2P₂O₅, 54SiO₂; mol %), containing oriented pores with controllable diameter, were prepared by unidirectional freezing of camphene-based suspensions (10 vol% particles) on a cold substrate (−196°C or 3°C). By varying the annealing time (0–72 h) to coarsen the camphene phase, constructs with the same porosity (86 ± 1%) but with controllable pore diameters (15–160 μm) were obtained after sublimation of the camphene. The pore diameters had a self-similar distribution that could be fitted by a diffusion-controlled coalescence model. Sintering (1 h at 690°C) was accompanied by a decrease in the porosity and pore diameter, the magnitude of which depended on the pore size of the green constructs, giving scaffolds with a porosity of 20–60% and average pore diameter of 6–120 μm. The compressive stress vs. deformation response of the sintered scaffolds in the orientation direction was linear, followed by failure. The compressive strength and elastic modulus in the orientation direction varied from 180 MPa and 25 GPa, respectively, (porosity = 20%) to 16 MPa and 4 GPa, respectively, (porosity = 60%), which were 2–3 times larger than the values in the direction perpendicular to the orientation. The potential use of these 13-93 bioactive glass scaffolds for the repair of large defects in load-bearing bones, such as segmental defects in long bones, is discussed.

Keywords

Bioactive glass; scaffolds; bone repair; unidirectional freeze casting; camphene

1. Introduction

There is a need to develop new scaffolds to repair segmental defects in long bones, using a method that is biocompatible and durable during the patient's lifetime. At present, bone allografts and custom metal augments are used to address segmental skeletal deficiency, but treatments are limited by concerns related to high costs, limited availability, unpredictable long-term durability, uncertain healing to host bone, and other variables. Biocompatible scaffolds that replicate the structure and function of bone would be ideal bone substitutes, provided they have the requisite mechanical properties for reliable long-term cyclical loading during weight-bearing. Ideally, the scaffold should have mechanical properties (e.g., elastic

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modulus and compressive strength) comparable to those of the tissue to be replaced. The scaffolds should also have a microstructure suitable for tissue growth into the porous scaffolds, to allow strong bonding and facile integration with apposing host bone and surrounding soft tissues. A porosity of ~50% and an interconnected pore size (diameter or width of the opening between adjoining pores) of 100 μm are considered to be the minimum requirements to permit tissue ingrowth and function [1].

The use of biodegradable polymers for replacing large defects in load-bearing bones (such as segmental defects in long bones) is challenging because of their very low mechanical strength and elastic modulus [2,3]. Bioactive glasses and bioactive ceramics are attractive materials for filling bone defects because of their widely recognized ability to support the growth of bone cells [4,5], and to bond strongly with hard and soft tissue [6,7]. However, porous three-dimensional scaffolds of these bioactive materials prepared by conventional methods often lack the requisite mechanical properties for repairing segmental defects in bone.

Unidirectional freezing of suspensions has been shown to provide a method for preparing oriented scaffolds with far higher mechanical properties (in the orientation direction) when compared to scaffolds prepared by more conventional methods [8]. Hydroxyapatite (HA) scaffolds prepared by unidirectional freezing of aqueous suspensions were found to have a compressive strength of 65 MPa (porosity = 56%) [9]. However, the lamellar pores obtained after sublimation of the frozen liquid had pore widths of only 10–40 μm , far smaller than the minimum pore size known to favor tissue ingrowth. Fu et al. [10,11] found that the use of binary mixtures of solvents, consisting of water and dioxane (60 wt% dioxane), produced a marked change from the lamellar microstructure, giving a columnar microstructure with far larger pore widths (pore diameter = 90–110 μm). Columnar scaffolds of silicate bioactive glass (13-93) had a compressive strength of 25 ± 3 MPa (porosity = 55–60%) in the orientation direction, and provided a more favorable substrate than the lamellar scaffolds for supporting cell proliferation and function, and cell infiltration into the interior pores of the scaffolds [12]. When implanted into subcutaneous pockets in the dorsum of rats, columnar scaffolds of 13-93 bioactive glass also showed the ability to support tissue infiltration into the interior pores of the scaffold [13].

The use of aqueous-based suspensions is limited by concerns about the degradation of the bioactive glass during processing, particularly for the more recently developed bioactive glass compositions, such as borate and borosilicate bioactive glass, which react faster than silicate bioactive glass [14,15]. Furthermore, our previous work showed that only a limited range of pore diameters (10–100 μm) were obtained in HA and bioactive glass scaffolds prepared by unidirectional freezing of aqueous suspensions [10,12]. For the intended application of bioactive glass scaffolds in bone repair, it would be useful to achieve a wider range of controllable pore diameters.

Camphene ($\text{C}_{10}\text{H}_{16}$) has been shown to have favorable characteristics for use as a sublimable vehicle in the preparation of constructs by freezing of suspensions [16]. Camphene-based suspensions can be frozen at room temperature, higher than the temperatures used for freezing aqueous suspensions [16–18]. Examples of previous work (summarized in Table I) showed that constructs prepared by freeze casting of camphene-based suspensions had pore sizes in the range 10–300 μm , far larger than the pore size range for scaffolds prepared from aqueous suspensions.

Recent work has shown that oriented constructs of Al_2O_3 with pore sizes >100 μm can be prepared using a two-step process consisting of unidirectional freezing of camphene-based suspensions, followed by annealing the frozen constructs for 24 hours at 35°C [19]. It was suggested that the larger pore sizes achieved using this two-step process resulted from growth

of the camphene crystals during the annealing step. However, a clear understanding of the kinetics and mechanisms of the camphene growth process during this annealing step is lacking.

The objective of the present work was to investigate the use of a two-step process, involving unidirectional freezing and thermal annealing of camphene-based suspensions, for creating oriented bioactive glass scaffolds with controllable pore sizes. The present work has two major differences when compared to our previous work on the preparation of oriented scaffolds by unidirectional freezing of aqueous suspensions [10–12]. First, organic (camphene)-based suspensions are used in this work, which are advantageous for limiting the degradation of the bioactive glass, particularly for future work in which more reactive glass compositions will be used. Second, a two-step process (freezing and thermal annealing) is investigated in this work for the creation of scaffolds with a wider range of pore diameters than those obtained from aqueous suspensions. In contrast to previous work [19], the kinetics and mechanism of the camphene phase coarsening during the annealing step were studied to provide a clearer understanding of the microstructure development. Silicate 13-93 glass was used as the scaffold material in this work because of its attractive bioactive properties and our extensive previous experience with its preparation and evaluation. This glass is based on the well-researched 45S5 composition, but it has a higher SiO₂ content, plus additional network modifiers, such as K₂O and MgO [7]. Products of 13-93 glass are also approved for *in vivo* use by the U.S. Food and Drug Administration (FDA). Relationships among the processing conditions, microstructure, and mechanical properties of the fabricated scaffolds were investigated.

2. Materials and Methods

2.1 Preparation of oriented 13-93 bioactive glass scaffolds

Bioactive glass (13-93) scaffolds with oriented pores were prepared in a set of sequential steps: preparation of camphene-based suspensions, unidirectional freezing of the suspensions, thermal annealing of the frozen constructs at a temperature near the solidification temperature of the suspension, sublimation of the camphene crystals, and heat treatment (sintering) to densify the glass network.

Glass with the 13-93 (composition: 6Na₂O, 8K₂O, 8MgO, 22CaO, 2P₂O₅, 54SiO₂, mol %; 6Na₂O, 12K₂O, 5MgO, 20CaO, 4P₂O₅, 53SiO₂, wt%), was prepared by melting a mixture of analytical grade Na₂CO₃, K₂CO₃, MgCO₃, CaCO₃, SiO₂ and NaH₂PO₄·2H₂O (Sigma-Aldrich, St. Louis, MO) in a platinum crucible for 1 h at 1300°C and quenching between cold stainless steel plates. The glass (density = 2.50 g/cm³) was crushed, ground in a hardened steel shatterbox (8500 Shatterbox[®], Spex SamplePrep LLC., Metuchen, NJ, USA), and ball-milled for 24 h in water with ZrO₂ grinding media. The size of the resulting glass particles was 1.0 ± 0.5 μm, as measured by a laser diffraction particle size analyzer (Model LS 13 320, Beckman Coulter Inc., CA). Camphene (C₁₀H₁₆; CAS 5794-04-7; Alfa Aesar, Ward Hill, MA, USA), with a melting temperature = 35°C and a solid density = 0.85 g/cm³ (according to the manufacturer's specifications) was used as the dispersion medium. Isostearic acid (C₁₈H₃₆O₂; MP Biomedicals LLC, Solon, OH, USA) was selected as the dispersant because of its use in previous work [27].

The optimum concentration of isostearic acid required for stabilizing the glass particles in liquid camphene at 55°C was determined by measuring the viscosity of the suspension (10 vol % particles) as a function of dispersant concentration using a rotating cylinder viscometer (VT500; Haake Inc., Paramus NJ). Suspensions containing 5–40 vol% glass particles and the optimum concentration of isostearic acid (2 wt% based on the dry mass of the glass particles) were prepared by ball milling for 24 h at 55°C in sealed polyethylene bottles. The viscosity of each suspension was measured as a function of shear rate at 55°C using a rotating cylinder

viscometer (Haake VT500). The data were used to determine the effect of particle concentration on the viscosity of the suspension.

Suspensions for unidirectional freezing, consisting of 10 vol% glass particles, 2 wt% isostearic acid, and camphene, were prepared by ball milling the mixture for 24 h at 55°C in a sealed polypropylene bottle. Suspensions containing 5 and 15 vol% particles were also used but they resulted in weak constructs (5 vol% particles) or low porosity constructs (15 vol% particles). The solidification of the slurry was monitored using differential scanning calorimetry, DSC (Model 2010; TA Instruments, City, State, USA) at a heating rate of 2°C/min. Unidirectional freezing was performed by pouring the slurry into cylindrical rubber molds (11 mm in diameter × 20 mm, or 18 mm in diameter × 15 mm) placed on a copper plate kept at 3°C using an ice–water mixture or at –196°C using liquid nitrogen. In order to optimize the unidirectional solidification, the rubber molds were warmed at 55°C prior to placing them on the cold copper plate and, after the slurry was poured, the molds were covered with rubber caps previously warmed to 55°C. For comparison, more randomly solidified samples were prepared by pouring the suspension into a cylindrical copper mold, sealing the mold, and immersing it in an ice–water mixture.

After solidification, the samples were transferred individually into polyvinyl chloride (PVC) tubes, and sealed with PVC caps to avoid camphene loss. The samples were then annealed at 34°C for up to 72 h in an incubator (Model CCC 0.5d; Boekel Industries Inc., Feasterville, PA, USA). This annealing temperature was chosen because the DSC experiments described above showed a peak in the solidification profile of the slurry at 36°C. After the annealing step, the samples were cooled to room temperature, and the camphene was removed by sublimation (24 h at room temperature). The porous constructs were sintered in air for 1 h at 690°C (heating and cooling rate = 5°C/min) to densify the glass phase. The use of this sintering temperature was based on previous work.

2.2 Microstructural characterization of porous constructs

After sublimation of the camphene (and before the sintering step), the porous constructs were too weak to be manipulated and characterized by microscopy. In order to develop adequate strength without significantly altering the pore characteristics, the porous constructs were lightly pre-sintered for 5 h at 600°C, well below the sintering temperature. The porosity of these pre-sintered constructs was determined from their mass and external dimensions. The pre-sintered samples were mounted in epoxy resin, sectioned in planes parallel and perpendicular to the freezing direction, polished, coated with Au/Pd, and examined using scanning electron microscopy, SEM (S-4700; Hitachi, Tokyo, Japan) at an accelerating voltage of 15 kV and a working distance of 18 mm. The sintered constructs were prepared and examined in the SEM using a similar method. In order to better characterize the pore network in three dimensions, the sintered scaffolds were examined using synchrotron X-ray tomography. Scanning was carried out at the Advanced Light Source (ALS, Lawrence Berkeley National Lab., Berkeley, CA) using 22 keV monochromatic X-rays and a resolution size of 1.7 µm voxel.

The porosity and pore diameters (or widths) in the cross sections were analyzed using imaging software (Image J). At least 150 pores per cross section were analyzed for each sample. Polished cross sections of the constructs in the directions perpendicular and parallel to the freezing direction were observed by optical microscopy (Optiphot-POL, Nikon Corp., Tokyo, Japan) to confirm the microstructural homogeneity along the length of the samples. The porosity of the sintered constructs was measured by the Archimedes method and the values were compared with those obtained by image analysis.

2.3 Evaluation of pore connectivity of oriented scaffolds

The connectivity of the oriented pores in the sintered scaffolds was evaluated by a capillary suction test using a blood-like solution which had a viscosity equal to that of human blood. The blood-like solution consisted of 34 wt% glycerol, 65 wt% deionized water and 1 wt% Alizarin red. The composition of the solution was based on the viscosity data of water–glycerol mixtures [28] which had a viscosity equal to that of human blood [29]. The flat surface and the circumferential surface of the porous constructs were dipped slightly (to a depth of < 1mm) into the surface of the solution. After the liquid was drawn up into the pores by capillary pressure, the constructs were allowed to dry. Both the external surface and the cross section of the constructs were visually examined to assess the infiltration of the liquid.

2.4 Mechanical response of sintered scaffolds

The mechanical response of the sintered constructs in the directions parallel and perpendicular to the freezing (orientation) direction was measured in compression using an Instron testing machine (Model 5881; Norwood, MA, USA). Cylindrical constructs (7 mm in diameter × 7 mm) and cubic constructs (7 mm × 7 mm × 7 mm) were deformed in the directions parallel and perpendicular to the orientation direction, respectively, at a rate of 0.5 mm/min. The contact surfaces of the constructs were machined flat, to provide parallel surfaces for the tests. At least 8 samples were tested for each group, and the compressive strength and elastic modulus were determined as an average ± standard deviation. For comparison, cylindrical constructs (7 mm in diameter × 7 mm) with a more random microstructure, prepared as described earlier, were tested under the same conditions.

3. Results

3.1 Viscosity of camphene-based suspensions

The viscosity of the camphene-based suspensions (10 vol% glass particles) as a function of the dispersant (isostearic acid) concentration at a constant shear rate (100 s⁻¹) (Fig. 1), showed that 1–2 wt% isostearic acid gave the lowest viscosity of the suspension, presumably corresponding to the most stable suspension. These viscosity data confirmed qualitative sedimentation tests which showed that suspensions containing ~2 wt% isostearic acid had the slowest particle sedimentation rate. Henceforth, suspensions used in the experiments were stabilized with 2 wt% isostearic acid.

For particle concentrations of 5–40 vol% used in these experiments, the viscosity of the suspension initially decreased with increasing shear rate, but became almost independent of shear rate above 50–100 s⁻¹. The results are omitted for the sake of brevity. Figure 2 shows results for the relative viscosity of the suspension (shear rate = 100 s⁻¹) as a function of the glass particle concentration. The data can be fitted by a modified Krieger-Dougherty equation [30,31]:

$$\eta_r = \left(1 - \frac{\phi}{\phi_m}\right)^{-n} \quad (1)$$

where η_r , the relative viscosity, is defined as the viscosity of the suspension, η , divided by the viscosity of the solvent (camphene) η_L , ϕ is the volume fraction of particles, ϕ_m is the volume fraction of particles at which the viscosity becomes practically infinite, and n is a fitting parameter. The maximum solids loading predicted by this model is $\phi_m = 43$ vol%, with $n = 3.2$.

3.2 Microstructure of frozen constructs

After sublimation of the camphene, pre-sintering of the constructs resulted in a linear shrinkage of <5% in the directions perpendicular and parallel to the freezing direction, regardless of the freezing temperature and annealing time used in the freeze casting process. Therefore, the microstructure of the pre-sintered constructs could be taken as a good approximation to the microstructure of the constructs after sublimation of the camphene. Furthermore, the morphology of the pores resulting from the sublimation of the camphene was well replicated by the epoxy resin used for mounting the samples for microscopy.

SEM images of the cross sections in the directions perpendicular and parallel to the freezing direction are shown in Fig. 3 for the constructs prepared by freezing on copper substrates at 3°C and -196°C (annealing time = 0 h). Apart from a size difference, the overall pore morphology of the two groups of constructs frozen at the two different temperatures was similar. The pores appeared to show an interconnected cellular morphology in the cross-section perpendicular to the freezing direction (Figs. 3a, 3c). The average diameter (or width) of the pores increased from ~15 µm for the constructs frozen at -196°C to ~40 µm for the constructs frozen at 3°C. The sections parallel to the freezing direction (Figs. 3b, 3d) showed that while the pores were oriented, they did not have a regular cylindrical shape. Instead, the pores appeared to have a dendritic-like morphology.

3.3 Effect of thermal annealing on microstructure of frozen constructs

Figure 4 shows SEM images of the cross sections of constructs frozen at 3°C and annealed at 34°C for 6, 12, and 72 h. The annealing step resulted in a change of the pore cross section from a cellular morphology to a nearly circular shape within 6 h (Fig. 4a). With increasing annealing time, the pore cross section remained circular and the average pore diameter increased (Figs. 4c, 4e). SEM images of the cross section parallel to the freezing direction (Figs. 4b, 4d, 4f) showed that after an annealing time of 6 h, the pores still had a somewhat dendritic shape. However, the pores developed a nearly cylindrical shape with smoother pore walls within an annealing time of 12 h. Furthermore, the pores remained oriented during the annealing process. Despite the wide difference in the average pore width of the constructs frozen at 3°C (40 µm) and at -196°C (15 µm), the temperature of freezing had little effect on the average pore diameter after an annealing time of 6 h. The microstructures of the annealed constructs for a freezing temperature of -196°C are therefore omitted for brevity.

The average pore diameter of the constructs frozen at 3°C or -196°C and annealed for 0–72 h at 34°C increased rapidly within the first 12 h of annealing, but then increased more slowly thereafter (Fig. 5). Starting from a pore width of 40 µm (freezing temperature = 3°C) or 15 µm (freezing temperature = -196°C), the average pore diameter increased to ~80 µm and ~125 µm after annealing times of 6 h and 12 h respectively. Annealing for 72 h resulted in a further increase in the average pore diameter to ~160 µm.

Analysis of SEM images of the cross sections of the constructs showed that the fractional area of the porous region was $67 \pm 4\%$ in the planes parallel and perpendicular to the freezing direction, and that this value was independent of the annealing time. Taken together with the results described above, this means that with increasing annealing time, the number of pores decreased, the average pore diameter increased, the thickness of the pore walls increased, but the overall pore volume remained almost constant during the annealing process.

Based on the mass and dimensions of the frozen or annealed constructs, the porosity of the constructs was found to be $86 \pm 1\%$, which is in good agreement with the expected value of 90% for constructs formed from suspensions with 10 vol% particles. On the other hand, as indicated above, the porosity determined from image analysis of SEM micrographs was $67 \pm$

4% in the planes perpendicular or parallel to the freezing direction. This difference in porosity determined by the two methods is due to the presence of fine pores between the glass particles in the walls of the construct. Furthermore, the annealing process caused a coarsening of the camphene phase (and the large pores resulting from the sublimation of the camphene), but apparently had little effect on the packing of the glass particles in the walls of the construct.

3.4 Microstructure of sintered constructs

Optical images of the cross sections of the sintered constructs (formed by freezing at 3°C and annealing for 72 h at 34°C) showed no marked difference in microstructure along the length of the sample (20 mm long) (data not included). Neglecting a thin surface layer, the microstructure of the constructs can be taken as homogeneous and independent of distance from the top and bottom of the construct. Figure 6 shows SEM images of the cross sections (perpendicular and parallel to the freezing direction) of the sintered constructs which were formed by freezing at 3°C and annealing at 34°C for the times indicated. The sections perpendicular to the freezing direction (Figs. 6a, 6c, 6e) showed a microstructure consisting of a dense glass matrix containing pores with a nearly circular cross section. The open porosity and average pore width (or diameter) of the sintered constructs increased markedly with the duration of the annealing step. When compared to the microstructure of the constructs after the freezing and annealing steps, the fine pores between the glass particles in the walls of the construct were essentially eliminated by viscous flow sintering, giving a dense glass network. The larger oriented pores resulting from the camphene sublimation also shrank (i.e., became smaller in diameter) and, if below a certain diameter, were completely eliminated.

Since shorter annealing times resulted in pores with smaller diameters, the porosity of the sintered constructs, in turn, decreased with decreasing annealing time. Images of the sections parallel to the freezing direction showed that for the frozen construct without the annealing treatment (Fig. 6b), the elongated pores resulting from the camphene sublimation shrank during sintering, leading to fine, presumably isolated pores. On the other hand, while the oriented pores in the annealed constructs became smaller in diameter, they apparently remained continuous (Figs. 6d, 6f). Constructs annealed for longer times, as outlined above, had larger pore diameters, so the corresponding sintered constructs also had larger pore diameters.

X-ray tomography images of constructs prepared by annealing for 24 h and sintering showed an oriented, columnar microstructure (Fig. 7). Although the pores are not perfectly aligned along the direction of freezing, a high degree of orientation is evident. The images also showed that the oriented pores were not discontinuous in the direction perpendicular to the freezing direction. Neighboring pores appeared to be connected at several positions along their length. In addition to showing microstructural features that were compatible with those observed by SEM, the X-ray tomography images provided a clearer view of the pore orientation.

The average pore diameter (or width) and porosity determined by image analysis are shown in Fig. 8 for the sintered constructs formed by freezing at 3°C followed by annealing at 34 °C for 0–72 h. Both the pore diameter and the porosity increased with annealing time. Without annealing, the sintered construct had a porosity of $19 \pm 4\%$ and a pore width of $6 \pm 2 \mu\text{m}$. The sintering shrinkage was approximately isotropic, equal to 46–48% in the axial direction (parallel to the freezing direction) and in the radial direction (perpendicular to the freezing direction). For an annealing time of 24 h, the porosity and pore diameter of the sintered constructs were $52 \pm 3\%$ and $90 \pm 30 \mu\text{m}$, respectively, while annealing for 72 h resulted in a porosity of $59 \pm 3\%$ and a pore diameter of $110 \pm 50 \mu\text{m}$. The sintering shrinkage decreased with annealing time became more anisotropic; for an annealing time of 72 h, the shrinkage was 31% and 38% in the axial and radial direction, respectively.

As outlined earlier, to test the interconnectivity of the pores, the planar or circumferential surface of the sintered constructs was dipped slightly (to a distance <1 mm) into a blood-like solution. All the constructs annealed for 6 h or longer were rapidly filled with the blood-like solution (within 5 s) as a result of capillary pressure of the pores, regardless of whether the planar or circumferential surface was dipped into the solution. Figure 9 shows the cross sections of the sintered constructs, prepared by freezing at 3°C and annealing for 6 h or 72 h at 34°C, after the constructs were dipped in the solution and dried. The infiltration of the solution completely throughout the constructs indicated that pores were interconnected in both the axial and radial directions.

3.5 Mechanical response of sintered constructs

The stress vs. deformation response of the sintered constructs in compression, in the freezing (orientation) direction, is shown in Fig. 10. The data are shown for the sintered constructs formed by freezing at 3°C and annealing for varying times in the range 0–72 h. The stress shown is the engineering stress, equal to the load divided by the initial cross sectional area of the construct, while the deformation is the change in length divided by the initial length. The stress increased linearly with deformation, followed by a sharp decrease due to failure. The constructs failed in a brittle manner, fracturing into several pieces. However, the constructs annealed for 72 h (with the highest porosity) showed some degree of compaction following failure, resulting in a series of small decreases in stress with higher deformation. The compressive strength of the sintered constructs, taken as the highest stress in the stress vs. deformation response, varied from 180 ± 70 MPa (0 h annealing; porosity = 20%) to 16 ± 2 MPa (72 h annealing; porosity = 60%) (Table II). The elastic modulus, determined from the slope, varied from 25 ± 5 GPa (0 h annealing) to 4 ± 0.1 GPa (72 h).

When tested in the direction perpendicular to the orientation direction, the constructs showed a response which was qualitatively similar to that in the orientation direction, but they were markedly weaker (Fig. 11). For the constructs prepared by annealing for 24 h and sintering, the compressive strength and elastic modulus in the perpendicular direction (13 ± 3 MPa and 2 ± 1 GPa, respectively), were 2–3 times smaller than those in the orientation direction (27 ± 8 MPa and 7 ± 3 GPa, respectively). For comparison, constructs with a random microstructure showed a mechanical response that was somewhat intermediate between those for the oriented scaffolds in the directions parallel and perpendicular to the freezing (orientation) direction (Fig. 11).

4. Discussion

4.1 Colloidal stability and viscosity of camphene-based suspensions

The viscosity of the suspension is an important processing parameter in the freeze casting process. For a given particle size, it determines the sedimentation velocity of the particles in the suspension [32], as well as the critical solidification front velocity for particle engulfment in the growing camphene crystal front. In the freezing process, the particles are rejected from the growing camphene crystals, and the interaction of the particles with the growing camphene crystal front determines the engulfment or rejection of the particles by the solidification front. For a given particle size, the critical solidification front velocity has been shown to vary inversely as the viscosity of the suspension [33,34].

The colloid stability of the suspension is also important in that it determines the flocculation rate of the suspension as well as the viscosity of the suspension. The use of 2 wt% isostearic acid as a dispersant resulted in the formation of suspensions with approximately the lowest viscosity (Fig. 1). For this dispersant concentration, the maximum particle loading ϕ_m in the suspension, predicted on the basis of the Krieger–Dougherty equation (Fig. 2) was only 43%.

In comparison, aqueous suspensions of 13-93 glass, with a similar particle size, stabilized with 0.5 wt% EasySpense, were found to have a ϕ_m of 55% [35]. The effectiveness of other dispersants for stabilizing bioactive glass particles in liquid camphene will be investigated in future work.

4.2 Solidification of camphene-based suspensions

In comparison with the unidirectional freezing of aqueous suspensions where the strongly anisotropic growth of hexagonal ice crystals leads to a lamellar structure [36], the tendency of camphene to form dendrites when solidified under appropriate temperature conditions resulted in a more dendritic structure upon freezing camphene-based suspensions [37]. While camphene has been reported to have a cubic crystal structure, some studies indicated that the structure was tetragonal [37]. An anisotropy of ~3% was found in the $\langle 001 \rangle$ or *c*-direction, while the *a*- and *b*-directions are equivalent in a tetragonal unit cell. Camphene dendrites therefore grow preferentially in the *c*-direction, with side-branching or secondary dendritic growth in the orthogonal *a*- and *b*-directions.

Unidirectional freezing of camphene-based suspensions on a cold substrate starts with nucleation of camphene on the surface of the substrate. The imposed temperature gradient favors the growth of camphene crystals whose $\langle 001 \rangle$ or *c*-direction coincides with the temperature gradient, leading to growth of primary dendrites down the temperature gradient. As illustrated in Fig. 12, as the primary dendrites grow, the particles are expelled. This, coupled with side-branching or secondary dendritic growth, leads to the accumulation of the particles between the secondary dendrites emanating from the primary dendrites. Therefore, after sublimation of the camphene from the frozen constructs, the oriented pores should have a rough surface, due to the camphene removed from the side-branches or secondary dendrites, as observed in Fig. 3.

Camphene can be solidified at room temperature, and the solidification of camphene-based suspensions at room temperature resulted in the formation of larger camphene dendrites (larger pores upon sublimation of the camphene) because of low dendrite velocity. However, the tendency for unidirectional growth of the primary dendrites was reduced. Therefore, in the present work, freezing on a cold substrate at 3°C or -196°C was used to provide a steep vertical temperature gradient, which favored crystal growth preferentially down the temperature gradient. The size (width) of the primary camphene dendrites (or the size of the pores resulting from their sublimation) can be controlled by manipulating the freezing rate. In the case of unidirectional freezing of aqueous suspensions of hydroxyapatite, an increase in the freezing rate from 2°C/min to 10°C/min resulted in a decrease in the lamellar pore width from ~40 μm to ~10 μm [9]. In the present system, the cooling rate cannot be accurately measured. However, a decrease in the cold substrate temperature from 3°C to -196°C (the boiling point of liquid nitrogen), which is equivalent to an increase in the cooling rate, resulted in a decrease in the pore widths from ~40 μm to ~15 μm. However, these pore widths obtained by manipulating the freezing rate only are much smaller than the pore sizes (>100 μm) found to be favorable for promoting tissue infiltration into the interior of porous constructs [1].

4.3 Coarsening of camphene phase during thermal annealing

The thermodynamic driving force for the coarsening of the camphene phase and the attainment of a nearly circular cross section during annealing of the frozen constructs at 34°C (Fig. 4) is the reduction of the high surface area of the camphene dendrites. The microstructural observations and image analysis described earlier showed that the diameter (width) of the camphene in the frozen constructs and, hence, the diameter of the pores resulting from the camphene sublimation, increased with increasing annealing time (Figs. 4, 5). In order to provide information on the mechanism of the camphene phase coarsening, the growth kinetics

of the pores resulting from sublimation of the camphene were compared with the predictions of theoretical models.

The coarsening of a dilute concentration of spherical particles (or precipitates) in a solid or liquid medium has been analyzed by Lifshitz and Slyozov [38], and by Wagner [39], commonly referred to as the LSW theory. Coarsening is assumed to be controlled by diffusion through the medium (diffusion control) or by the interface reaction (dissolution of the particles in the medium or deposition of solute onto the particle surfaces). For a time-invariant (self-similar) particle size distribution, the theoretical distribution function for interface reaction-controlled coarsening has the form:

$$f(s, t) = \frac{3}{4} s \left(\frac{2}{2-s} \right)^5 \exp \left(\frac{-3s}{2-s} \right) \quad (0 < s < 2) \quad (2)$$

where $s = d/d^*$, d = particle radius, d^* = radius of particle that neither grows nor shrinks, and d^* is related to the average particle radius $\bar{d}$ by $\bar{d} = (8/9)d^*$. In the case of diffusion-controlled coarsening, the distribution function has the form:

$$f(s, t) = \frac{4}{9} s^2 \left(\frac{3}{3+s} \right)^{7/3} \left(\frac{3/2}{3/2-s} \right)^{1/3} \exp \left(\frac{-s}{3/2-s} \right) \quad (0 < s < 3/2) \quad (3)$$

and $\bar{d} = d^*$. The average radius of the particles increases with time according to:

$$\bar{d}^n - d_0^n = Kt \quad (4)$$

where $n = 2$ for interface reaction-controlled coarsening, and $n = 3$ for diffusion-controlled coarsening. While corrections have been made to the LSW theory to account for the effect of more concentrated systems, in the present work the LSW theory for a dilute concentration of particles is used.

Direct coalescence of particles has also been proposed as a mechanism for coarsening [40]. In this case, two or more particles interconnected by solid necks are assumed to coarsen by diffusive transport of matter occurring essentially in the neck regions. A statistical theory [41] for particle coarsening by coalescence also predicts a self-similar, time-invariant, particle size distribution function, but the distribution function has the form:

$$F(s, t) = 2.136 s^2 \exp(-0.712 s^3) \quad (5)$$

The average particle size, $\bar{d}$, is predicted to increase with time, t , according to Equation (4) with the exponent $n = 3$.

For the frozen constructs annealed for varying times at 34°C, Fig. 13a shows that a plot of the cumulative fraction of pores with diameters smaller than a certain size vs. the reduced pore size, s , falls on a single curve regardless of the annealing time, indicating a self-similar distribution for all annealing times (6–72 h). Furthermore, a comparison of the data for the pore size distribution with the predictions of the LSW theory (diffusion or reaction control)

and the coalescence model shows that the coalescence model provides the best fit to the data (Fig. 13b), indicating that direct coalescence of the interconnected camphene phase is the predominant mechanism of coarsening. Figure 14 shows that after a threshold annealing time (6 h), the growth of the pore diameters can be fitted by Equation (4), but a clear distinction between $n = 2$ and $n = 3$ cannot be made from the present data.

4.4 Microstructure and mechanical response of sintered scaffolds

The porosity and average pore diameter of the sintered scaffolds prepared from suspensions frozen at 3°C and annealed for 0–72 h at 34°C are summarized in Table II. Although all the constructs had a starting porosity of ~86% after the annealing step, sintering resulted in a markedly lower porosity, in the range 20–60% for annealing times of 0–72 h. The fine pores between the particles in the glass network sintered rapidly by viscous flow sintering to produce a dense glass network. Later, viscous flow sintering also resulted in some reduction of the pore diameter of the large oriented pores and, hence, to further shrinkage of the scaffold. Since the rate of viscous flow sintering varies inversely as the pore radius, the constructs that were annealed for shorter times sintered faster because of their smaller pores, so the porosity of the sintered scaffolds decreased with shorter annealing time (Table II).

When tested in the directions parallel and perpendicular to the freezing (orientation) direction, the sintered scaffolds showed a brittle response characteristic of dense ceramics and glass, in which the compressive stress increased linearly with deformation, followed by catastrophic failure (Fig. 11). This response is markedly different from that of oriented hydroxyapatite and 13-93 bioactive glass scaffolds prepared by unidirectional freezing of aqueous-based suspensions which showed an ‘elastic-plastic’ response and a large strain for failure (>20%) [8,11]. The response is also qualitatively different from that of 13-93 bioactive glass scaffolds prepared by a polymer foam replication technique, in which numerous peaks and valleys were observed in the stress vs. deformation response after the initial elastic deformation [35].

4.5 Scaffolds for bone repair

The present study shows promising results for the potential use of these oriented bioactive glass scaffolds in the repair of large defects in load-bearing bones, such as segmental defects in long bones. Bone is generally composed of two types: cortical (or compact) bone, and trabecular (cancellous or spongy) bone. Cortical bone, found primarily in the shaft of long bones and as the outer shell around cancellous bone, has a porosity of 5–10%; the compressive strength and elastic modulus in the direction parallel to the orientation (long axis) has been reported in the range 120–150 MPa and 10–20 GPa, respectively. A wide range has been reported for the elastic modulus (0.1–5 GPa) and compressive strength (2–12 MPa) of cancellous bone [42, 43]. In particular, sintered constructs prepared in this work for annealing times of 12–24 h, with a porosity of ~50% and an average pore diameter of 60–90 µm have a compressive strength of 25–50 MPa, and elastic modulus of 7–10 GPa.

In another study [17], scaffolds of silicate 45S5 bioactive glass were prepared by freezing camphene-bases suspensions in a random manner at room temperature. The frozen constructs were not annealed, so pore sizes resulting from the camphene sublimation were <50 µm. Sintering for 3 h at 700–1100°C resulted in crystallization of the glass, with the formation of a crystalline $\text{Na}_2\text{O} \cdot 2\text{CaO} \cdot 3\text{SiO}_2$ phase, and limited densification of the resulting glass–ceramic phase below 1000°C. While a devitrified 45S5 glass is still bioactive, the rate of conversion to a hydroxyapatite-type material (i.e., its bioactive potential) is markedly reduced [7].

These constructs prepared in this work have the requisite pore characteristics for supporting tissue infiltration, as well as adequate mechanical properties for replacing load-bearing bones.

A detailed study of the *in vitro* and *in vivo* biological performance of these scaffolds is in progress, and the results will be described in a subsequent publication.

5. Conclusions

Oriented bioactive glass (13-93) scaffolds with promising microstructure and mechanical response for potential use in the repair of load-bearing bones were created using a method based on unidirectional freezing of camphene-based suspensions. Annealing the frozen constructs for 0–72 h at 34°C (slightly below the solidification temperature of the suspension) resulted in coarsening of the camphene crystals, which provided a method for controlling the pore diameter of the constructs (in the range 15–160 µm after sublimation of the camphene). The coarsening of the camphene crystals during the annealing step can be described by a diffusion-controlled coalescence model. Sintering resulted in a decrease in the porosity and the pore diameter, giving scaffolds with porosities of 20–60% and pore diameters of 6–120 µm for annealing times of 0–72 h. The sintered scaffolds had compressive strength and elastic modulus values in the freezing (orientation) direction which varied from 180 MPa and 25 GPa, respectively (porosity = 20%) to 16 MPa and 4 GPa (porosity = 60 %) which were 2–3 times larger than those measured in the direction perpendicular to the orientation direction.

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Fig. 1. Viscosity of camphene-based suspensions containing 10 vol% 13-93 bioactive glass particles vs. isostearic acid (dispersant) concentration (shear rate = 100 s^{-1}).

Fig. 2. Relative viscosity vs. volume fraction of 13-93 bioactive glass particles in camphene-based suspensions stabilized with 2 wt% isostearic acid (shear rate = 100 s^{-1}). The data are fitted with the modified Krieger-Dougherty equation (Equation 1).

Fig. 3. SEM images of 13-93 bioactive glass constructs prepared by unidirectional freezing of camphene-based suspensions (10 vol% particles) on a copper substrate at 3°C (a, b), and at -196°C (c, d). The sections are perpendicular (a, c) and parallel (b, d) to the freezing direction.

Fig. 4.

SEM images of the cross sections perpendicular (a, c, e) and parallel (b, d, f) to the freezing direction, for 13-93 bioactive glass constructs prepared by unidirectional freezing of camphene-based suspensions (10 vol% particles) at 3°C, and thermal annealing at 34°C for the times shown.

Fig. 5.

Average pore size (diameter) vs. annealing time for 13-93 bioactive glass constructs prepared by freezing camphene-based suspensions (10 vol% particles) at 3°C and thermal annealing at 34°C.

Fig. 6.

SEM images of the cross sections perpendicular (a, c, e) and parallel (b, d, f) to the freezing direction, for 13-93 bioactive glass constructs prepared by unidirectional freezing of camphene-based suspensions at 3°C, thermal annealing at 34°C for the times shown, and sintering for 1 h at 690°C.

Fig. 7. X-ray tomography images of sintered 13-93 bioactive glass constructs prepared by unidirectional freezing of camphene-based suspensions at 3°C and annealing for 24 h: (a) glass phase; (b) pore phase.

Fig. 8. Porosity and pore size vs. annealing time for sintered constructs of 13-93 bioactive glass prepared by unidirectional freezing of camphene-based suspensions at 3°C, thermal annealing at 34°C, and sintering for 1 h at 690°C.

Fig. 9.

Optical images of the cross sections of sintered 13-93 bioactive glass constructs, showing the interconnectivity of the pores. The constructs were prepared by unidirectional freezing of camphene-based suspensions at 3°C, annealing at 34°C for 6 h or 72 h, and sintering for 1 h at 690°C. The flat surface (a, c) and the circumferential surface (b, d) of cylindrical constructs were lightly dipped into a blood-like solution, dried, and sectioned. (Inset: surfaces of the cylindrical constructs after drying).

Fig. 10.

Compressive stress vs. deformation in the orientation (freezing) direction for sintered 13-93 bioactive glass constructs prepared by unidirectional freezing of camphene-based suspensions (10 vol% particles) at 3°C, annealing at 34°C for the times shown, and sintering for 1 h at 690°C.

Fig. 11.

Compressive stress vs. deformation for oriented constructs tested in directions parallel and perpendicular to the orientation direction, and for constructs with a random microstructure. The constructs were prepared by freezing camphene-based suspensions at 3°C, annealing for 24 h at 34°C, and sintering for 1 h at 690°C.

Fig. 12.
Schematic of the growth of camphene dendrites during unidirectional freezing of camphene-based suspensions.

Fig. 13.

(a) Cumulative fraction of pores vs. normalized pore diameter, for constructs prepared by freezing at 0°C and -196°C, and annealing at 34°C for 6, 12, 24, and 72 h. (b) Pore size distribution data vs. normalized pore diameter for the constructs described in (a), compared with the predictions of the LSW model for interface- and diffusion-controlled coarsening, and a coalescence model.

Fig. 14.

Data for the average pore diameter vs. annealing time at 34°C for constructs prepared by freezing at 0°C and -196°C, and annealing at 34°C for 12, 24, and 72 h. The data are fitted using Equation (4) for $n = 3$ and $n = 2$.

Table I
Summary of porous constructs fabricated by freezing of camphene-based suspensions.

Material	Particle size (μm)	Freezing/annealing conditions	Freezing directionality	Pore size (μm)	Porosity (%)	Ref.
13-93 glass	1–2	3°C; –196°C/34°C (0–72 h)	Unidirectional	10–160	20–60	Present work
45S5 glass	—	20 °C	No	10–40	53	17
Al ₂ O ₃	0.4	25°C; 0 °C	No/Yes	—	20–50	16
Al ₂ O ₃	0.3	20°C	No	50	>88	18
Al ₂ O ₃	0.3	3°C/35°C (24 h)	Unidirectional	102–210	57–83	19
CaP	—	3°C/32°C (1–3 days)	Unidirectional	122–166	62–65	20
HA	—	20°C	No	20–40	56–75	21
HA	—	0–35 °C	No	80–250	55–76	22
HA	—	34°C	NO	141–324	71–73	23
HA/TCP	—	4–30 °C	No	40–200	31–73	24
Ti	1–3	33°C	No	>100	49–63	25
ZrO ₂	0.4	0°C; –196°C	Unidirectional	15–30	65–83	26

Microstructural characteristics and mechanical properties in compression for 13-93 bioactive glass scaffolds prepared by unidirectional freezing of camphene-based suspensions at 3 °C, annealing at 34 °C for the times shown, and sintering for 1 h at 690 °C.

Table II

Annealing time (h)	Annealed construct		Sintered construct			
	Porosity (%)	Pore diameter or width (μm)	Porosity (%)	Pore diameter or width (μm)	Compressive strength* (MPa)	Elastic modulus (GPa)
0	86 ± 1	40 ± 5	19 ± 4	6 ± 2	180 ± 70	25 ± 5
6	86 ± 1	75 ± 25	37 ± 7	38 ± 10	66 ± 32	10 ± 3
12	86 ± 1	130 ± 40	46 ± 6	60 ± 20	53 ± 10	9 ± 3
24	86 ± 1	150 ± 50	52 ± 3	90 ± 30	27 ± 8	7 ± 3
					13 ± 3 ¹	2 ± 1 ¹
					16 ± 3 ²	3 ± 1 ²
72	86 ± 1	160 ± 50	59 ± 3	115 ± 50	16 ± 2	4 ± 1

* Parallel to the orientation direction unless stated otherwise;

¹ Perpendicular to the orientation direction;

² Scaffold with random microstructure.