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Mechanical properties of bioactive glass (13-93) scaffolds fabricated by robotic deposition for structural bone repair

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Abstract

There is a need to develop synthetic scaffolds for repairing large defects in load-bearing bones. Bioactive glasses have attractive properties as a scaffold material for bone repair, but data on their mechanical properties are limited. The objective of the present study was to comprehensively evaluate the mechanical properties of strong porous scaffolds of silicate 13-93 bioactive glass fabricated by robocasting. As-fabricated scaffolds with a grid-like microstructure (porosity = 47%; filament diameter = 330 μm ; pore width = 300) were tested in compressive and flexural loading to determine their strength, elastic modulus, Weibull modulus, fatigue resistance, and fracture toughness. Scaffolds were also tested in compression after they were immersed in simulated body fluid (SBF) in vitro or implanted in a rat subcutaneous model in vivo. As fabricated, the scaffolds had a strength = 86 ± 9 MPa, elastic modulus = 13 ± 2 GPa, and a Weibull modulus = 12 when tested in compression. In flexural loading, the strength, elastic modulus, and Weibull modulus were 11 ± 3 MPa, 13 ± 2 GPa, and 6, respectively. In compression, the as-fabricated scaffolds had a mean fatigue life of $\sim 10^6$ cycles when tested in air at room temperature or in phosphate-buffered saline at 37 °C under cyclic stresses of 1–10 MPa or 2–20 MPa. The compressive strength of the scaffolds decreased markedly during the first 2 weeks of immersion in SBF or implantation in vivo, but more slowly thereafter. The brittle mechanical response of the scaffolds in vitro changed to an elasto-plastic response after implantation for longer than 2–4 weeks in vivo. In addition to providing critically needed data for designing bioactive glass scaffolds, the results are promising for the application of these strong porous scaffolds in loaded bone repair.

1. Introduction

Contained bone defects are repairable with commercially-available, osteoconductive and osteoinductive filler materials [1, 2]. However, no ideal biological solution exists to reconstitute structural bone loss, such as segmental defects in the limbs. The available treatments used to repair large bone defects, such as bone allografts, autografts, porous metals, and bone cement, have limitations related to costs, availability, longevity, donor site

morbidity, and uncertain healing to host bone. Consequently, there is a great need for porous biocompatible implants that can replicate the strength, morphology, porosity, bioactivity, and load-bearing ability of living bone.

Scaffolds made of synthetic and natural polymers such as poly(lactic acid), poly(glycolic acid), polycaprolactone, and collagen degrade in vivo, and are replaced by new bone matrix synthesized by tissue-forming cells [3, 4]. These materials have proven useful for filling contained bone defects, but their use in structural bone repair is challenging because of their inherently low strength [5, 6]. Calcium phosphate bioceramics such as hydroxyapatite (HA), beta-tricalcium phosphate (β -TCP), and biphasic calcium phosphate (BCP) are logical bone repair materials since they are composed of the same ions as the mineral constituent of bone. However, synthetic HA degrades too slowly to allow osseous repair, while porous β -TCP scaffolds are typically not strong enough to survive physiologic loading.

Bioactive glasses have attractive properties as a scaffold material for bone repair. In vivo, bioactive glass converts to hydroxyapatite (HA), the main mineral constituent of bone, which promotes osseous healing [7–9]. Calcium ions and soluble silicon released during the bioactive glass conversion further promote osteogenesis [10, 11] and activate osteogenic gene expression [12, 13]. Bioactive glass can be doped, during manufacture, with trace amounts of elements such as copper (Cu), zinc (Zn), and strontium (Sr) that are known to promote angiogenesis and healthy bone growth [9, 14]. As the bioactive glass degrades during conversion to HA in vivo, those elements are released at therapeutically acceptable rates.

Most previous studies have targeted glass compositions, such as silicate 45S5 and 13-93, and three-dimensional (3D) scaffold architectures with relatively low-strength, such as compressive strengths in the range of values reported for human trabecular bone [15–18]. Attempts have been made to prepare glass or glass–ceramic scaffolds with higher strength using methods such as sintering particles that were compacted with a pore-forming phase [19] and unidirectional freezing of suspensions [20]. However, the range of pore sizes and the interconnectivity of the pores were difficult to control, which could limit the capacity of the scaffolds to support bone infiltration. Recent studies have shown that silicate 13-93 and 6P53B glass scaffolds fabricated by robocasting, a solid freeform fabrication technique, have compressive strengths comparable to those of human cortical bone [21–25] as well as a highly interconnected porous microstructure that is known to be favorable for supporting bone infiltration. Those strong porous bioactive glass scaffolds could provide promising implants for loaded bone repair.

Although the mechanical properties of bioactive glass scaffolds have been widely reported in the literature, most studies have focused on evaluating the strength and elastic modulus in compression of the as-fabricated scaffolds or scaffolds that were immersed in an aqueous phosphate solution such as a simulated body fluid (SBF) [15]. Load-bearing bones, such as long limb bones, are subjected to multiple loading modes as well as cyclic loading. Consequently, the development of bioactive glass scaffolds for repairing structural bone loss requires a comprehensive evaluation of their mechanical response. As the bioactive glass converts to HA, its properties change with time. Data for the time-dependent mechanical response in vitro and in vivo are also critically important for the design of bioactive glass scaffolds for loaded bone repair.

The objective of the present study was to comprehensively characterize the mechanical response of strong porous scaffolds of silicate 13-93 bioactive glass which were fabricated with a grid-like microstructure by robocasting. The selection of 13-93 glass was based on its proven bioactivity [9] and on our previous studies which showed that scaffolds of this glass

can be created with compressive strengths comparable to cortical bone by solid freeform fabrication techniques [21–23]. The strength, elastic modulus, Weibull modulus in compression and flexure, fatigue resistance and fracture toughness of the as-fabricated scaffolds were evaluated. The mechanical response in compression was also evaluated as a function of immersion time of the scaffolds in SBF in vitro and implantation time in rat subcutaneous sites in vivo.

2. Materials and methods

2.1 Fabrication of bioactive glass scaffolds

Melt-derived bioactive glass frits with the composition designated 13-93 (53SiO₂, 6Na₂O, 12K₂O, 5MgO, 20CaO, 4P₂O₅, wt %), provided by Mo-Sci Corp., Rolla, MO, USA, were crushed in a steel shatterbox (8500 Shatterbox®, Spex SamplePrep LLC., Metuchen, NJ, USA) to form particles of size smaller than ~50 µm. Then the particles were ground for 2 h in an attrition mill using water as the liquid medium and ZrO₂ grinding media (3 mm in diameter) to give particles of size ~1 µm. A slurry (ink) for robotic deposition (robocasting) was prepared using a solution with reverse thermal behavior, as described in detail elsewhere [25]. Briefly, the glass particles (40 vol %) were mixed with a 20 wt % aqueous Pluronic® F-127 solution, previously cooled in a refrigerator, using a planetary centrifugal mixer (ARE-310; THINKY, Laguna Hills, CA, USA) and placed for 12 h in a refrigerator at 10 °C.

The ink was warmed to room temperature, loaded into a robotic deposition device (RoboCAD 3.0, 3-D Inks, Stillwater, OK, USA) and deposited on an Al₂O₃ substrate immersed in a reservoir of lamp oil (Florasense, Charleston, SC, USA). Scaffolds with a grid-like microstructure were formed by extruding the ink through a 410 µm nozzle (EFD Precision Tips, East Providence, RI, USA) with a line spacing (center-to-center distance between the filaments) of 910 µm. After forming, the scaffolds were dried for 24 h in air at room temperature, heated slowly (0.5 °C/min with a few isothermal holds) to 600 °C in flowing O₂ gas to burn out the processing additives, and sintered in air for 1 h at 700 °C (heating rate = 5 °C/min) to densify the glass filaments.

Scaffolds were coated with Au/Pd and examined in a scanning electron microscope (SEM) (S-4700; Hitachi, Tokyo, Japan) at an accelerating voltage of 15 kV and a working distance of 12 mm. Quantification of the microstructure (average pore width and glass filament diameter) was performed using image analysis (ImageJ; National Institutes of Health, USA), while the volume of the macropores in the scaffold was measured using the Archimedes method according to ASTM C830-00.

2.2 Mechanical testing in compression and flexure

The as-fabricated scaffolds were tested in compression and flexure using an Instron testing machine (Model 5881; Norwood, MA, USA). The compressive strength of scaffolds with a cubic shape (6 mm × 6 mm × 6 mm) was measured at a cross-head speed of 0.5 mm/min, using a 10 kN load cell. The deformation of the sample was determined from the movement of the cross-head. Prior to testing, the contact surfaces of the scaffolds were ground using a surface grinder (FSG-618, Chevalier Machinery Inc., Santa Fe Springs, CA, USA) to produce parallel surfaces. The elastic modulus was determined from the linear region of the stress vs. strain response. The load was applied to the samples in the z direction of the as-formed scaffolds, perpendicular to the plane of deposition (xy plane) shown in Fig. 1a. This loading direction was used because it would match the compressive loading direction of the scaffolds in a segmental bone defect.

Four-point flexural testing was performed on a fully articulated fixture (outer span = 20 mm; inner span = 10 mm) at a crosshead speed of 0.2 mm/min, using a 2 kN load cell. The stress was applied in the z direction of the scaffolds (the same direction used for the compression tests). The as-fabricated scaffolds (3 mm × 5 mm × 25 mm) were tested according to the procedure described in ASTM C1674-11, and the flexural strength was determined from the equation

$$\sigma = \frac{3Pl}{4bd^2} \quad (1)$$

where P is the applied load, l is the outer span, and b is the sample width and d is the thickness of the sample. The flexural modulus of the samples was determined from the stress and the deflection recorded using a linear variable differential transformer (LVDT) at the mid-span of the sample.

Thirty samples each were tested in compression and in flexure, and the strength and modulus were expressed as a mean ± standard deviation (SD). The Weibull modulus in each loading mode was determined according to ASTM C1239-07 by fitting the strength data with the equation

$$\ln \ln \left(\frac{1}{1 - P_f} \right) = m \ln \left(\frac{\sigma}{\sigma_o} \right) \quad (2)$$

where P_f is the probability of failure at a stress σ , and σ_o is the Weibull scale parameter determined from the intercept of the fit to the data and Weibull modulus, m . The value σ_o is also the stress at which the probability of failure is 63%. P_f was evaluated using the equation

$$P_f = \frac{i - 0.5}{n} \quad (3)$$

where n is the total number of specimens tested and i is the specimen rank in ascending order of failure stresses.

After testing, the fractured surfaces of samples with the highest and lowest flexural strengths were coated with Au/Pd, and examined in the SEM (Hitachi; S-4700). Particular attention was paid to the lower region of the fractured surface (which was subjected to a tensile stress during the four-point bending test) and the upper region of the fractured surface (subjected to a compressive stress) to determine differences in the morphology of the fractured surfaces.

2.3. Fracture toughness testing

The fracture toughness of the as-fabricated scaffolds was measured by the single-edge notched beam (SENB) technique using samples of size 3 mm × 5 mm × 25 mm according to the procedure described in ASTM C1421-10. A notch of width <100 μm and depth of ~1.5 mm was machined at the midpoint of the 3 millimeter-wide plane using a dicing saw (ACCU-CUT 5200, AREMCO Products Inc., Ossining, NY). The notch depth (a few times the cell size of the scaffold) was chosen to satisfy the conditions for applicability of linear elastic fracture mechanics (K-dominance at the crack tip) [26]. The fracture toughness K_{IC} was determined using the equation [26]

$$K_{IC} = \sigma \sqrt{\pi a} Y F(a/H) \quad (4)$$

where σ is the fracture stress, a is the depth of the notch, H is the thickness of the sample, $F(a/H)$ is a geometrical factor [27], and the dimensionless parameter Y is ~ 1 for the microstructure of the samples tested [26]. Five samples were tested in four-point bending as described earlier, and the fracture toughness was determined as an average $\pm$ SD.

2.4 Fatigue testing

Fatigue testing of the as-fabricated scaffolds was performed in cyclic compression using an ElectroForce 3330 testing system (Bose Corp., Eden Prairie, MN, USA). Samples (6 mm $\times$ 6 mm $\times$ 6 mm) were tested in air at room temperature and in phosphate-buffered saline (PBS) at 37 °C using load-control actuation, at a frequency of 5 Hz. Three cyclic compressive stresses of 1–10 MPa, 2–20 MPa and 3–30 MPa were used in the study, with the minimum to maximum stress ratio kept constant at 0.1. The tests were conducted until failure or until 10^6 cycles were reached. The fatigue testing conditions were based on the estimated number of gait cycles (10^6) in one year for patients after joint replacement [28] and the recommended stress levels (10–15 MPa) in ASTM F2118-10. Six samples were tested at each cyclic stress. The fatigue life (mean $\pm$ SD) was determined using a logarithmic transformation of the cycles to failure as recommended in ASTM F2118-10. Statistical analysis of the mean fatigue life was performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test; differences were considered significant for $p < 0.05$.

2.5 Degradation of compressive strength in vitro and in vivo

The degradation of the compressive strength and elastic modulus of the scaffolds (6 mm $\times$ 6 mm $\times$ 6 mm) in vitro and in vivo was evaluated in compression using the testing conditions described earlier. The samples were tested after immersion in SBF at 37 ± 0.5 °C in an incubator (Model CCC 0.5d, Boekel Industries Inc., Feasterville, PA) or after implantation in rat subcutaneous sites for 2, 4, 6 and 12 weeks. For each time point, six samples were tested, and the compressive strength (average $\pm$ SD) was plotted as a function of time. In vitro, the ratio of the scaffold mass to the SBF volume was kept constant at 1 g per 100 cm³, and the SBF was not changed during the immersion period. The samples were tested immediately after removal from the SBF (without drying).

A rat subcutaneous model was used for the in vivo study based on the ease of the implantation surgery and the need to implant scaffolds with an appropriate size and shape for subsequent mechanical testing. Six-month old male Sprague Dawley rats were used. All animal experimental procedures were approved by the Animal Care and Use Committee, Missouri University of Science and Technology, in compliance with the NIH Guide for Care and Use of Laboratory Animals. The rats were anesthetized with a combination of ketamine (50 mg/kg) and xylazine (4 mg/kg). Six scaffolds were implanted into subcutaneous pockets at six sites in the dorsum of each rat. After each implantation period, the animals were sacrificed by CO₂ inhalation. The samples were harvested, wrapped in gauze wetted with PBS, and tested.

After immersion in SBF or implantation in rat subcutaneous sites, the thickness of the converted layer of the glass filaments in the scaffolds, composed mainly of a porous HA-like material, was measured. The samples were freeze-dried (Freezezone 4.5; Labconco Corp., Kansas City, MO, USA), mounted in epoxy resin, sectioned, and polished. The polished cross sections were coated with carbon and examined in an SEM (Hitachi; S-4700) in the backscattered electron (BSE) mode at an accelerating voltage of 15 kV and a working distance of 13 mm. At least 15 filaments were examined and the thickness of the converted layer was determined as an average $\pm$ SD.

3. Results

3.1 Mechanical properties of as-fabricated scaffolds

The as-fabricated scaffolds had a uniform grid-like microstructure (Fig. 1), with glass filaments of diameter $330 \pm 10 \mu\text{m}$, a pore width of $300 \pm 10 \mu\text{m}$ in the plane of deposition (xy plane) and a pore width of $150 \pm 10 \mu\text{m}$ in the direction of deposition (z direction). The glass filaments were almost fully dense; the density of the glass phase of the scaffolds, as determined by the Archimedes method, was $98 \pm 1\%$ of the density of the bulk glass (2.65 g/cm^3) formed by melting and casting. In addition, the glass filaments appeared to be well bonded to those in the adjacent plane. The total open porosity of the scaffolds, as determined by the Archimedes method was $47 \pm 1\%$.

The mechanical properties of the as-fabricated scaffolds in compression and in flexure are summarized in Table I. In compression, the scaffolds had a strength $= 86 \pm 9 \text{ MPa}$ and an elastic modulus $= 13 \pm 2 \text{ GPa}$, whereas the flexural strength and flexural modulus were $11 \pm 3 \text{ MPa}$ and $13 \pm 2 \text{ GPa}$, respectively. The fracture toughness of the scaffolds was $0.48 \pm 0.04 \text{ MPa}\cdot\text{m}^{1/2}$.

Figure 2 shows Weibull plots of the compressive and flexural strength data. The plots were approximately linear over most of the stress range, but deviations from a linear relationship were apparent at the low and high stress values. Least-squares fitting of a straight line through the data points gave a Weibull modulus of 12 in compression and 6 in flexure. For each loading mode, an alternative might be to fit two straight lines through the strength data, one for the lower strength values, and the other for the remaining values. However, SEM images of the fractured surfaces of scaffolds with a higher flexural strength (15 MPa) and a lower flexural strength (8 MPa) showed no marked difference in fracture morphology (Fig. 3). Features such as hackle lines (indicated by arrows) and smooth mirror areas (starred symbols) [30] were found on the fractured surfaces of both samples. The fractured glass filaments near the top region (Fig. 3c, f) of the sample (subjected to a compressive stress during four-point bending) showed cantilever curls (curve lines indicated by arrowheads), typically found near the region of compression in flexural testing of brittle solids [30]. However, the origin of failure, typically found in the tensile region (near the lower region in four-point bending) was difficult to determine.

The fatigue life of the as-fabricated scaffolds when tested in compression in air and in PBS is shown in Fig. 4. Of the 6 samples tested under each condition, the number that survived the 10^6 cycles of testing is also indicated. In air, all 6 samples tested under a cyclic stress of 1–10 MPa survived, showing that the samples had a fatigue life greater than 10^6 cycles. As the cyclic stress amplitude was increased to 2–20 MPa and 3–30 MPa, 4 and 3 samples, respectively, survived the 10^6 cycles of testing. Although the mean fatigue life decreased with the increase in the stress amplitude, the difference was not significant.

Under a cyclic stress of 1–10 MPa, testing in PBS did not have a significant effect on the mean fatigue life when compared to the samples tested in air; 5 samples survived the 10^6 cycles of testing. However, testing under the higher cyclic stresses of 2–20 MPa and 3–30 MPa resulted in a significant decrease in the mean fatigue life. In PBS, the mean fatigue life was $10^{5.9}$, $10^{5.3}$, and $10^{4.2}$ cycles at the cyclic stresses of 1–10, 2–20 and 3–30 MPa, respectively. Together, the data indicated that for the cyclic stresses used, the mean fatigue life was independent of the stress when the samples were tested in air, whereas testing in PBS resulted in a significant reduction in the mean fatigue life with increasing stress.

3.2 Degradation of compressive strength in vitro and in vivo

The compressive strength and elastic modulus of the scaffolds after immersion in SBF in vitro or implantation in rat subcutaneous sites in vivo are shown in Fig. 5 as a function of immersion time or implantation time. The strength and modulus decreased rapidly during the first 2 weeks but more slowly thereafter. This trend was independent of the in vitro or the in vivo environment, but the decrease in vivo was larger than that in vitro. The strength decreased from the as-fabricated value of 86 ± 9 MPa to 58 ± 5 MPa after 2 weeks in SBF and to 35 ± 4 MPa after the same time in vivo (Fig. 5a). After 12 weeks, the strength of the scaffolds immersed in SBF was 52 ± 10 MPa, whereas the strength of the scaffolds implanted in vivo was 16 ± 4 MPa. The elastic modulus of the scaffolds decreased from the as-fabricated value of 13 ± 2 GPa to 11 ± 1 GP after 2 weeks in SBF and to 6 ± 2 GPa after 2 weeks in vivo (Fig. 5b). The modulus of the scaffolds was 9 ± 2 GPa and 2 ± 1 GPa, respectively, after 12 weeks in SBF and in vivo.

Figure 6 shows the mechanical response of the scaffolds in compression after they were immersed in SBF or implanted in rat subcutaneous sites. (The curves were shifted arbitrarily along the x-axis to maintain clarity). After an initial transient, the scaffolds that were immersed in SBF showed a mechanical response typical of porous brittle materials; the stress increased almost linearly with the deformation, followed by failure (Fig. 6a). At failure, the scaffolds fractured into several pieces. In comparison, the scaffolds that were implanted in vivo showed a markedly different response: the brittle response changed to an elasto-plastic response after 2–4 weeks (Fig. 6b). Instead of fracturing into several pieces, the samples maintained their integrity after the mechanical test and became more deformable (Fig. 7). SEM backscattered electron images of the cross-section of the filaments showed that a surface layer of the glass was converted after immersion in vitro or implantation in vivo (Fig. 8a, b). The thickness of the converted layer formed in vivo was larger than that formed in vitro.

4. Discussion

The present study provided a comprehensive evaluation of the mechanical properties of strong porous bioactive glass (13–93) scaffolds with a well-controlled microstructure which were fabricated by robocasting. The mechanical properties of the as-fabricated scaffolds in the relevant loading modes of compression and flexure were evaluated, as well as the fatigue resistance and fracture toughness. Since the mechanical properties of bioactive glass change as the glass converts to HA, the mechanical response of the as-fabricated scaffolds in compression was compared with their response after immersion in SBF in vitro or after implantation in a rat subcutaneous model in vivo.

4.1 Mechanical properties of as-fabricated bioactive glass scaffolds

The as-fabricated scaffolds with a uniform grid-like microstructure (porosity = $47 \pm 1\%$; filament diameter ~ 330 μm ; pore width ~ 300 μm in the xy plane) had a compressive strength of 86 ± 9 MPa when tested in the z direction, a value that is close to the lower end of the values reported for human cortical bone (100–150 MPa). Modifications of this uniform microstructure, as described in previous work [21–23], have resulted in compressive strengths similar to human cortical bone. The elastic modulus of the as-fabricated scaffolds was 13 ± 2 GPa, which is in the range of values reported for cortical bone (10–20 GPa).

The Weibull modulus determined from the strength data for a large number of identical samples (typically 20–30 or more) is commonly used as a measure of the mechanical reliability or the probability of failure of brittle materials [30]. The mechanical response of

brittle materials is sensitive to microstructural flaws such as pores and microcracks. Commonly, the strength and Weibull modulus are used to evaluate the probability of failure of brittle materials under a given stress. The Weibull modulus of dense or nearly dense ceramics and glasses has been reported in the range 5–20 [31]. Data for the Weibull modulus of bioactive glasses are limited. The Weibull modulus of porous bioceramic scaffolds, such as HA, beta-tricalcium phosphate (β -TCP), and calcium polyphosphate (CPP), has been reported in the range 3–10 for testing in compression [15, 32–34].

In this study, the Weibull modulus of the scaffolds in compression was 12. A possible reason for this high value is the uniform microstructure composed of dense glass filaments (relative density = $98 \pm 1\%$) that are mainly free from large flaws. Figure 9 shows a comparison of the Weibull plots for the bioactive glass scaffolds in this study with plots taken from the literature for calcium phosphate scaffolds tested in compression [32, 33]. Under the same allowable failure probabilities, the bioactive glass scaffolds showed a compressive failure strength that is higher than that for the HA scaffolds, and far higher than that for the β -TCP scaffolds. Based on the strength and the Weibull modulus data, when subjected to a compressive stress of 50 MPa, the failure probability of the bioactive glass scaffolds, P_f , is equal to 10^{-3} (1 in 1000 scaffolds is predicted to fail). The average stress on a hip stem is reported as 3–11 MPa [35, 36], well below the stress (50 MPa) for a failure probability of 10^{-3} .

The flexural modulus (13 ± 2 GPa) of the bioactive glass scaffolds measured in four-point bending was similar to the elastic modulus in compression. However, the flexural strength (11 ± 3 MPa) was only 10–15% of the compressive strength, and the Weibull modulus in flexure was half that in compression. The flexural strength of HA scaffolds with a porosity of 30–50% has been reported in the range 2–12 MPa [37]. In comparison, human cortical bone has a flexural strength comparable to its compressive strength (Table I).

Finite element analysis of a cubic scaffold (2 mm in length) of HA or β -TCP with a grid-like microstructure (filament diameter = 220 μm ; pore width = 80 μm in the deposition plane and 120 μm in the z direction) showed that under an applied compressive load of 250 N (stress = 63 MPa), the maximum tensile stress in the scaffold was ~ 80 MPa, generated at the middle of the unsupported filaments. In comparison, under an applied tensile load of 250 N (stress = 63 MPa), the maximum tensile stress was almost 4 times higher (~ 400 MPa), generated around the joint of two neighboring filaments [38]. The analysis indicated that when applying the same load on a grid-like microstructure, the maximum local tensile stress generated in the glass filaments was much higher in tensile loading than in compressive loading, and the distribution of the stress was very different between the two loading modes [38]. Since brittle materials such as glass typically fail in tension, and assuming that the scaffolds failed when the maximum local tensile stress exceeded the tensile strength of the glass filament, then the compressive strength is expected to be far superior to the tensile strength [38]. The flexural strength found in this study (11 ± 3 MPa) was much lower than the compressive strength (86 ± 9 MPa), which is in general agreement with the predictions of the finite element modeling.

One approach to enhance the flexural strength of the bioactive glass scaffolds is through microstructural modification. As described earlier, the scaffolds used in the present study had a uniform grid-like microstructure. Preliminary results of our ongoing research showed that the flexural strength of the bioactive glass scaffolds can be markedly improved by creating a “gradient” microstructure, similar to the topography of human long bone. The “gradient” microstructure consisted of a more porous inner region with the same grid-like microstructure used in the present study (porosity $\sim 50\%$), and an outer region with a grid-

like microstructure of lower porosity (15–20%) in which the glass filaments had the same diameter but were more closely spaced.

The fracture toughness of the bioactive glass scaffolds used in the present study ($0.48 \pm 0.04 \text{ MPa}\cdot\text{m}^{1/2}$) is much lower than the value for human cortical bone ($2\text{--}12 \text{ MPa}\cdot\text{m}^{1/2}$) [39], but in the range of values for dense glass ($0.5\text{--}1 \text{ MPa}\cdot\text{m}^{1/2}$), porous HA ($0.3 \text{ MPa}\cdot\text{m}^{1/2}$) [40], and porous phosphate glass–ceramics ($0.2\text{--}0.6 \text{ MPa}\cdot\text{m}^{1/2}$) [41]. The toughness of weak bioactive glass or bioceramic scaffolds (compressive strength = $1\text{--}20 \text{ MPa}$) can be improved by coating or infiltrating the porous scaffold with a biodegradable synthetic polymer [32, 42, 43]. However, a polymer coating might reduce the bioactivity of the scaffolds, particularly at early implantation times when the coating limits the interaction of cells and tissues with the bioactive glass surface.

Because long limb bones undergo cyclic loading, the fatigue resistance of scaffolds designed for bone substitution is relevant. The results of the present study provide for the first time, information on fatigue resistance of strong porous bioactive glass scaffolds. When the bioactive glass scaffolds were tested in air at room temperature, the fatigue life decreased as the maximum cyclic compressive stress was increased from 10 MPa to 30 MPa , but the decrease was not significant. However, in PBS at 37°C , the mean fatigue life showed a significant decrease from $10^{5.9}$ cycles to $10^{4.2}$ cycles for the same increase in the stress amplitude. The observed trend for the effect of stress and aqueous environment on the fatigue of silicate 13–93 bioactive glass scaffolds is compatible with the stress corrosion crack growth mechanisms for bioactive and conventional silicate glasses [44] which is generally attributed to the stress corrosion of Si–O–Si bonds at the crack tip [45]. In normal walking, the compressive load on the human femur is estimated to be smaller than 4 times the body weight [46]. Assuming a uniform load distribution, a femoral bone cross-sectional area of $\sim 4 \text{ cm}^2$ [47], and a body weight of 70 kg , the stress on an implant in a segmental femoral defect is $<2 \text{ MPa}$. The results of the present study therefore indicate that the bioactive glass scaffolds have excellent fatigue resistance at stresses far higher than normal physiological stresses.

4.2 Degradation of compressive strength in vitro and in vivo

The strength of bioactive glass scaffolds decreases with time as the glass converts to HA; typically the conversion starts at the surface and moves inward [48]. To account for the strength degradation observed in the present study, the thickness of the converted layer of the bioactive glass filaments in the scaffolds was measured from SEM images of the cross sections (Fig. 8). The thickness was measured as a function of immersion time in SBF or implantation time in rat subcutaneous sites. As described in previous studies [8, 9], the converted layer in silicate bioactive glass consists of an HA-like outer layer and a thin SiO_2 -rich inner layer. As shown in Fig. 8, at 6 weeks, the converted layer in vivo ($34 \pm 6 \mu\text{m}$) was much larger than that in vitro ($\sim 5 \mu\text{m}$). The faster conversion in vivo has been explained in terms of the more dynamic environment and the presence of electrolytes, proteins and biological polymers in vivo [49, 50].

As shown in Fig. 10a, the thickness y of the converted layer in vivo increased with time t , and can be well fitted by a parabolic growth equation

$$y^2 = kt \quad (5)$$

where k is constant, equal to $12.7 \mu\text{m}^2/\text{week}$. The basic load-bearing units of the porous scaffolds are the glass filaments of the grid-like microstructure. The newly formed HA-like layer and SiO_2 -rich layer formed as a result of the glass conversion were porous and presumably much weaker than the glass phase [51]. Therefore the decrease in strength was

expected to correlate with the reduction in the diameter (or cross sectional area) of the unconverted glass filaments. Using this assumption, the compressive strength σ_t of the scaffold at time t has been described in terms of a power-law relation [52, 53]:

$$\sigma_t = \sigma_o \left(1 - \frac{y}{a}\right)^n \quad (6)$$

where $\sigma_o = 86$ MPa is the compressive strength of the as-fabricated scaffold, a is the radius of the glass filaments, y is the thickness of the converted layer, and n is a fitting parameter. Substituting for y from Equation (5), and the radius of the glass filaments ($a = 165 \mu\text{m}$) measured in the present study, Equation (6) gives

$$\sigma_t = 86 \left(1 - \frac{12.7 \sqrt{t}}{165}\right)^n \quad (7)$$

The best fit to the compressive strength data of the scaffolds implanted in vivo gave $n = 6.1$ (Fig. 10b). These results show that the degradation of the compressive strength of the bioactive glass scaffolds in vivo is predictable if the conversion rate of the bioactive glass to HA is known.

A challenge in the design of bioactive glass scaffolds for the regeneration of loaded bone is to match the degradation of scaffold mechanical properties with the rate of new bone infiltration. We are currently studying the rate of new bone formation in osseous defects implanted with these scaffolds with grid-like microstructure. By combining those bone infiltration data with the results for the mechanical degradation of the scaffolds obtained in this study, the overall mechanical strength of the implants could be predicted as a function of implantation time.

5. Conclusions

Scaffolds of 13–93 bioactive glass with a grid-like microstructure (porosity = 47%; pore width = 300 μm in the xy plane and 150 in the z direction) were fabricated by robocasting. When tested in the z direction, the scaffolds had a compressive strength of 86 ± 9 MPa, elastic modulus of 13 ± 2 GPa, and a Weibull modulus of 12. In flexural loading (load applied in the z direction), the strength, elastic modulus, and Weibull modulus were 11 ± 3 MPa, 13 ± 2 GPa, and 6, respectively. When tested in compression in air at room temperature or in phosphate-buffered saline at 37 °C, the as-fabricated scaffolds had a mean fatigue life of $\sim 10^6$ cycles under cyclic stresses of 1–10 MPa or 2–20 MPa. When compared to similar scaffolds immersed in a simulated body fluid (SBF) in vitro, scaffolds implanted in rat subcutaneous sites in vivo showed a more rapid degradation in compressive strength as a result of faster conversion to HA in vivo. Whereas the scaffolds showed a brittle mechanical response after immersion for up to 12 weeks in SBF, the response changed from brittle to elasto–plastic within 2–4 weeks of implantation in rat subcutaneous sites. The degradation of the compressive strength of the scaffolds as a function of time in vivo can be predicted from the conversion of the bioactive glass to hydroxyapatite. In addition to providing critically needed data for designing bioactive glass scaffolds, the results are promising for the application of these strong porous scaffolds in loaded bone repair.

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Fig. 1. SEM images of silicate 13-93 bioactive glass scaffolds prepared by robotic deposition (robocasting): (a) plane of deposition (xy plane); (b) perpendicular to the deposition plane (z direction).

Fig. 2. Weibull plots of (a) compressive strength and (b) flexural strength for silicate 13-93 bioactive glass scaffolds with a grid-like microstructure. (Inset: optical images showing the shape of the samples used in each test).

Fig. 3.

SEM of images of the fractured surfaces of bioactive glass scaffolds tested in flexure (four-point bending): (a–c) scaffold with a flexural strength of 15 MPa and (d–f) scaffold with flexural strength of 8 MPa. Higher magnification images of the fractured surface near the bottom surface of the sample (in tension during the test) and near the top surface (compression) are shown in (b, e) and (c, f), respectively. Arrows point to the hackle lines; stars show the mirror area and arrowheads point to the cantilever curls.

Fig. 4.

Fatigue life (average number of cycles to failure) of 13-93 bioactive glass scaffolds tested in air and in phosphate-buffered saline (PBS) under cyclic compressive stresses. The stresses shown are the maximum applied stress in the cyclic loading. (*significant difference between groups, $p < 0.05$). The number in the bar indicates the number of samples that survived 10^6 cycles when the test was terminated.

Fig. 5.

(a) Compressive strength and (b) elastic modulus as a function of time for 13-93 bioactive glass scaffolds after immersion of the scaffolds in simulated body fluid (SBF) in vitro and after implantation in rat subcutaneous sites in vivo for the times shown.

Fig. 6. Mechanical response (compressive stress vs. deformation) of 13-93 bioactive glass scaffolds after immersion of the scaffolds in simulated body fluid (SBF) in vitro and after implantation in rat subcutaneous sites in vivo for the times shown.

Fig. 7. Optical micrograph showing 13-93 bioactive glass scaffolds after implantation in rat subcutaneous sites for 4 weeks: (a) prior to mechanical testing; (b) after mechanical testing in compression. The scaffolds became highly deformable after the mechanical testing.

Fig. 8.

SEM backscattered electron images showed the cross-sections of the glass filament in 13-93 bioactive glass scaffolds after (a) implantation for 6 weeks in vivo and (b) after immersion for the same time in SBF in vitro. (c) Sketch showing the parameters of the converted glass filament: a = initial radius of the glass filament; y = thickness of the converted layer at time t .

Fig. 9.

Weibull plots of the compressive and flexural strength data from the present study for bioactive 13-93 glass (BG). For comparison, Weibull plots of the compressive strength data from the literature [32, 33] for beta-tricalcium phosphate (β -TCP) and hydroxyapatite (HA) scaffolds fabricated by robocasting are also shown for comparison (dashed lines).

Fig. 10.

(a) Data for the thickness of the converted layer of the bioactive glass filaments as a function of implantation time in rat subcutaneous sites in vivo; the data can be fitted by a parabolic curve (Equation 5); (b) data for the compressive strength of the bioactive glass scaffolds as a function of implantation time in vivo; the curve shows the predicted compressive strength (Equation 7) based on the conversion data in (a).

Mechanical properties of as-fabricated 13–93 bioactive glass scaffolds with a grid-like microstructure in compression and flexure (four-point bending). The load was applied in the z direction, perpendicular to the plane of deposition (xy plane) of the scaffolds.

Table I

	Compression			Flexure		
	Strength (MPa)	Elastic modulus (GPa)	Weibull modulus	Strength (MPa)	Flexural modulus (GPa)	Weibull modulus
						Fracture toughness (MPa·m ^{1/2})
Scaffold	86 ± 9	13 ± 2	12	11 ± 3	13 ± 2	6
Cortical bone [15, 29]	100–150	10–20		135–193	9–16	
Trabecular bone [15]	2–12	0.1–5		10–20		
						0.1–0.8