ABSTRACT
The pore geometry of bone scaffolds, intended for use in bone repair or replacement, is one of the most important parameters in bone tissue engineering. It affects not only the mechanical properties of the scaffolds but also the amount of bone regeneration after implantation. Scaffolds with five different architectures and four porosity levels were fabricated using borate bioactive glass (13–93B3) using the selective laser sintering (SLS) process. The pore size of the scaffolds varied from 400 to 1300 μm. The compressive strength of the scaffolds varied from 1.7 to 15.5 MPa for porosities ranging from 60 to 30%, respectively, for the different architectures. Scaffolds were soaked in a simulated body fluid (SBF) for one week, followed by measurements in the variation in mechanical properties and the amount of bioactive glass surface conversion. In-vitro tests were conducted on the scaffolds with different architectures to investigate the preferential cell proliferation. The MTT labeling experiments were conducted on the scaffolds and the MTT formazan was extracted at intervals of 2, 4, and 6 days. The results indicated that the scaffolds which mimic trabecular bone architecture have higher cell proliferation compared to those with traditional lattice structures.

1. INTRODUCTION
The discovery of Bioglass® by Prof. Hench in 1969 has led to active research interest in the field of bioactive materials in the past four decades [1, 2]. The bioactive materials convert to Hydroxyapatite (HA), the main mineral constituent of bone, when exposed to body fluids thereby integrating with the surrounding tissue. Recently, interest has been focused on developing bioactive glasses as they offer excellent biological characteristics when compared to glass-ceramics or ceramics. Borate based bioactive glasses not only bond to the surrounding hard tissue but are also known to bond with soft tissues [3, 4]. Table 1 shows the compositions of the borate based 13-93B3 bioactive glass in comparison to the silicate based 13-93 bioactive glass. The 45S5 glass composition is also included for comparison. The molar concentration of SiO₂ in 13-93 glass is replaced by B₂O₃ in the 13-93B3 glass. The borate glass is chemically less durable when compared to the silicate glass and therefore, it degrades at a faster rate and allows for faster, but controlled bone formation in comparison to the silicate glass [3-5].

|   | Table 1. Compositions (in wt.%) of 13-93 and 13-93B3 glasses compared to 45S5 glass. |
|---|---|---|---|---|---|---|
|   | SiO₂ | P₂O₅ | CaO | MgO | Na₂O | K₂O | B₂O₃ |
| 45S5 | 45 | 6 | 24.5 | - | 24.5 | - | - |
| 13-93 | 53 | 4 | 20 | 5 | 6 | 12 | - |
| 13-93B3 | - | 3.7 | 18.5 | 4.6 | 5.5 | 11.1 | 56.6 |

Recently, 13-93B3 glass scaffolds, with ~50% porosity, were fabricated with an organic based paste composition using the Robocasting technique [6, 7]. Although porosity and pore size can be controlled using Robocasting, the process has limited control over the pore architecture when fabricating porous parts because of the layer-by-layer filament deposition. In comparison, powder based additive manufacturing (AM) techniques like the selective laser sintering (SLS) process provide flexibility in fabricating scaffolds with complex pore architectures as they do not require support structures during part fabrication. Therefore, the SLS process provides an opportunity to investigate the effects of porosity and pore architecture on the structural and cell support properties of the scaffolds. There have been some articles in the literature wherein researchers have proposed techniques to develop CAD models for scaffolds which closely mimic the human trabecular bone architecture [8-10]. However, fabricating bioceramic scaffolds with such a complex architecture is still a challenge since not all AM techniques can be used to fabricate them at required pore sizes (~100 – 300 μm) [11]. Also, only limited work has been done thus far to compare bioceramic scaffolds with such complex architectures and with traditional lattice structures, considering the aspects of both manufacturability and ability to support cell growth.

In our previous work, we have shown that silicate based 13-93 bioactive glass scaffolds made by the SLS process provide good mechanical properties and preferable surface morphology for cell proliferation [12-14]. In the current work, we investigated the effects of pore architecture and porosity on the cell proliferation and mechanical properties of the scaffolds. Five different architectures were considered and the scaffolds were fabricated with each of these architectures at four designed porosity levels. The sintered scaffolds were immersed in simulated body fluid (SBF) for one week and the effects of architecture and porosity on the compressive resistance of the scaffolds were studied. The scaffolds with different
2. MATERIALS AND METHODS

2.1. Fabrication of scaffolds

13-93 (silicate) and 13-93B3 (borate) bioactive glasses (prepared by Mo-Sci Corp., Rolla, MO) were used in this research. The average size of the glass particles was measured ~12 µm (d_{50}). Particle size distributions were obtained using a laser diffraction-based particle size analyzer (S3500, MicrOTrac Inc., Largo, FL). The bioactive glass particles were mixed with stearic acid as the binder (C_{18}H_{36}O_{2}, grade HS, Acros Organics, Morris Plains, NJ) and dry ball-milled for 8 hrs with ZrO_{2} milling media to obtain the feedstock powder for the SLS machine. The fabrication experiments were carried out on a commercial DTM Sinterstation 2000 machine. The effect of SLS parameters on fabricating scaffolds using stearic acid binder and bioactive glass powder was investigated in our previous work [14], and the same set of parameters (laser power ~ 5 W, scan speed ~ 508 mm/s, scan spacing ~ 0.23 mm, layer thickness ~ 76.2 µm, 15% binder content) were adopted for the current study.

2.2. Post-processing and scaffold assessment

The fabricated green parts were post-processed in a three-stage programmable air furnace (Vulcan Benchtop Furnace, York, PA). The following heat treatment schedule was used for this study: de-binding heating rate of 0.1°C/min to 550°C, then increasing the heating rate to 1°C/min until a final sintering temperature of 570°C with a 1 hour hold, and then turning off the furnace to allow cooling down to room temperature. Optical microscopy was used to measure the pore sizes of the sintered scaffolds. Archimedes method was used to measure the apparent porosity of the sintered scaffolds. Cubic shaped parts measuring 10 mm in length were used for the purpose of measuring porosity, and parts measuring 5 mm in length were used for the purpose of mechanical testing and the SBF study. A cross-head speed of 0.5 mm/min was used on a mechanical load frame (Instron 4469 UTM, Norwood, MA) to determine the compressive strengths of the parts. Scans were run from 20 values ranging from 10° to 80° using Cu Kα radiation (λ = 0.154056 nm) for powder X-ray diffraction (XRD) analysis (Philips X-Pert, Westborough, MA) on the as-received bioactive glass powders, sintered scaffolds, and also on the dried scaffolds after removing them from the SBF to determine the changes in the crystalline/amorphous nature of the material.

2.3. SBF tests

The SBF solution was prepared using the Kokubo method [15]. All the samples were ultrasonically cleaned three times using ethanol and then dried in an oven overnight before being kept in the SBF solution (100 ml of solution was used for 1 g of the scaffold for soaking). The SBF solution container with scaffolds was kept in an incubator maintained at 37°C. All the compression tests were conducted on wet scaffolds to provide realistic data on the degradation of the scaffolds. Scanning electron microscopy (SEM) (S-570, Hitachi Co., Tokyo, Japan) was used to analyze the surface morphology of the scaffolds.

2.4. Cell proliferation tests

The established MLO-A5 line of mouse late-osteoblast, early-osteocyte cells was used as a model for the in vitro tests of cell growth [16]. Prior to cell-seeding, the discs were ultrasonically cleaned in ethyl alcohol twice for 10 min each, blotted, and dry-heat sterilized overnight at 250°C. The MLO-A5 cells were cultured in phenol red free alpha-MEM medium supplemented with 5% fetal bovine serum and 5% new born calf serum (serum and media purchased from Life Technologies, Grand Island, NY). Scaffolds were placed on a Teflon sheet and seeded with 80,000 MLO-A5 cells suspended in 40 µl of complete medium. After a 2 h incubation to allow cell attachment, the scaffolds with attached cells were transferred to a 12-well plate with 2 ml of complete medium per well and incubated at 37°C in a humidified atmosphere of 5% CO_{2}. The cell-seeded scaffolds were placed in a medium containing the tetrazolium salt MTT (50 µg per 250 µl of medium) for the last 4 h of incubation to permit visualization of metabolically active cells. The samples were rinsed with phosphate buffered saline (PBS) at the conclusion of the incubation and blotted dry. The insoluble purple formazan, the product of mitochondrial MTT metabolism, was then extracted from the scaffolds with 2.0 ml of ethyl alcohol and measured at a wavelength of 570 nm using a spectrophotometer (Evolution 300 UV-Vis, Thermo Scientific, Bannockburn, IL). Scaffolds with cubic architecture, one of the five architectures considered in this study, were used as control samples for comparison of the absorbed intensities of the extracts. The scaffolds were cut to a thickness of 2 mm (minimum of two rows of repeatable units) so that the effect of architecture could be investigated.

2.5. Statistical analysis

Five samples in each set were used for compressive testing of the sintered and dry scaffolds and the results were reported as a mean ± one standard deviation (± SD). Three samples in each set were used for compression testing of the SBF soaked scaffolds and the results were reported as mean ± SD. Three samples in each set (2, 4, and 6 days) were used for cell proliferation tests and the absorbance data collected from the spectrophotometer is reported as mean ± SD. Analysis for differences in absorbance after the incubation period and among different architectures was performed using one-way ANOVA with Tukey’s post hoc test. Differences were considered significant for P < 0.05.

3. RESULTS

The CAD models of the repeatable units of the five architectures considered in this work are shown in Fig. 1(a). The first, second, and third unit cubes are termed cubic, spherical, and X architectures, respectively. The porosity in these unit cubes is a function of the size of the unit cell and the diameter of the struts. These three architectures are the typical and frequently used designs in most of the AM techniques to
manufacture scaffolds. The fourth and fifth are “diamond” and “gyroid” architectures, which were intended to mimic the trabecular bone architecture. The surface was generated using open source software K3DSurf v0.6.2 [17]. The surface generated was modeled into a volume using Siemens NX software and then converted to a .STL file for fabrication using the SLS machine. The fabricated scaffolds after binder burnout and sintering are shown in Fig. 1(b) as representative specimens for each of the architectures in the same order as shown in Fig. 1(a). The scaffolds with each of these architectures were designed at 80, 70, 60, and 50% porosity levels. Fig 1(c) (i through iv) shows the distribution of pore size among cubic architecture scaffolds at different porosities (66%, 54%, 40%, and 34% respectively). The measured apparent porosities using Archimedes principle were observed on an average ~17% lower when compared to the porosities of their CAD models for all architectures. Therefore, it is noted that the difference in porosity factor should be accounted for while fabricating porous parts using the SLS process.

3.1.Effect of architecture and porosity on strength

Figure 2 shows the variation in the compressive strengths of the scaffolds made with different architectures at porosity levels from ~30% to ~65%. The scaffolds with a cubic architecture and a porosity of ~33% provided the highest compressive strength among all of the scaffolds. The scaffolds with the X architecture showed the lowest compression strength (~4.5 MPa at ~35% porosity). Out of the two architectures designed to closely mimic trabecular bone, the gyroid offers a higher compressive strength than the diamond architecture because of the relatively thick struts (~1.3 mm for gyroid compared to ~0.9 mm for diamond) and their wavy nature. The compressive strengths of the scaffolds at the low porosity level (~30%) are near the high end of the range of compressive strengths for a human trabecular bone (~2 to ~12 MPa), whereas the strengths measured for the scaffolds at the high porosity level (~60%) are near the low range of the strength of human trabecular bone [18]. Though the scaffold with cubic architecture provides the highest compressive strength, its rate of strength reduction with respect to increase in porosity is the fastest among all the scaffolds with different architectures. The scaffolds with the spherical architecture exhibited the smallest reduction in compressive strength. The other three architectures, namely, X, diamond, and gyroid, have a similar rate of reduction in compressive strength with respect to porosity.

3.2.Finite element analysis prediction

Finite element analysis (FEA) was performed to predict the compressive strengths of the scaffolds using commercial software Abaqus. For this purpose, the part shrinkage and porosity reduction were incorporated into the CAD models. The scaffolds were modeled with 3D tetrahedral elements with sizes ~0.1 mm. The total number of elements for each of the models was kept in the range of 300,000 to 350,000. The ultimate strength of the 13-93B3 material was determined experimentally by fabricating a solid dense cylinder and performing compressive tests. The density of the solid dense cylinder was calculated (2.164 g/cc) and considered as the material’s theoretical density for the purpose of FEA. A Poisson’s ratio of 0.2 was assumed, which is typical for a bioceramic [19]. A 1.2% strain failure criterion was adopted to determine the point of specimen failure, which is typical for a porous bioactive glass specimen. The results in Fig. 3 show that FEA can achieve a reasonable estimate of the compressive strengths of the scaffolds fabricated with bioactive glass using the SLS process. Though predictions for cubic and spherical architectures are only shown considering the space limitations, the predictions of all the architectures are reasonably accurate.
Thus FEA could help in determining the appropriate architecture for an implant and the type of material (bioactive glass, glass-ceramic, etc.) to use for repair of a trabecular bone based on the predicted compressive strength.

3.3. Effect of SBF immersion on strength

The scaffolds were mechanically tested in a wet state after immersion in SBF for one week. The compressive strength measured for each of the scaffolds was about 2 MPa or slightly less, which is near the low end of the range of compressive strength (2 – 12 MPa) of a human trabecular bone [18]. The only exceptions were the with cubic and spherical architecture scaffolds with porosities of ~33% and ~32%, whose strengths were measured ~4 MPa. The reduction in strengths of the scaffolds with different architectures from ‘as-sintered dry state’ to ‘wet state’ after immersion in SBF for one week is shown in Fig. 4. Among the scaffolds with an average porosity of ~62%, irrespective of the architecture, the reduction in strength in the wet scaffolds is more than ~90% when compared to the as-sintered, dry scaffolds.

After testing, the scaffolds were dried at room temperature, sputter coated with gold-palladium, and investigated using SEM. Specifically, SEM was used to look for any crystal-like formations on the surface, which were typically formed within one week after immersion of the scaffolds’ in SBF. Figure 5 shows SEM images of a representative “X” architecture scaffold taken out of the SBF after 7 days. The optical images of the changed surface morphology of the SLS scaffold before and after immersion are shown in Fig. 5(a). Figure 5(b) shows the fracture surface of the scaffold. The reacted surface of the scaffold with SBF can be clearly distinguished with an unconverted 13-93B3 glass core as indicated in the image. A higher magnification image indicating the rounded HA-like crystal formations, unlike needle-like crystal formations on a 13-93 glass, on the surface of the scaffold after reacting with the SBF is shown in Fig. 5(c) [5, 14].

3.4. Effect of architecture on cell proliferation

Figure 6(a) shows a schematic of the static cell seeding on the scaffolds and how they are kept in a 12-well cell culture plate. At the time of cell seeding, all of the cell suspension volume applied to the scaffolds adhered to the samples since no suspension was observed on the teflon sheet after the scaffolds were aseptically transferred to the 12-well plate. The scaffolds with cubic architecture were considered as controls for this experiment as this particular architecture was studied in our previous work and they showed a better cell density when compared to the commercially available BD CaP scaffolds [13]. Optical images of cell-seeded scaffolds with five different architectures incubated with MTT during the last 4 hours of
incubations of 4 and 6 days are shown in Fig. 6(b). The relative intensity of purple formazan staining on these scaffolds increased with the duration of incubation, which is an indication of metabolically active cells undergoing vigorous growth on the scaffolds. The absorbance of the amount of formazan product of mitochondrial metabolism that was recovered from the cell-seeded scaffolds labeled with MTT is shown in Fig. 7. There was no significant difference in the cell densities among the scaffolds with different architectures after 2 days of incubation. The difference in cell density on the scaffolds is clearly visible for the gyroid and diamond architectures when compared to the cubic, spherical and X architectures after 4 days of incubation. The scaffolds with the diamond architecture were completely covered with metabolically active cells, at least on the surface of the scaffolds after 6 days of incubation.

![Figure 6](image)

**Figure 6.** (a) Schematic of the cell seeding on the scaffold with at least two rows of repeatable units. The cell seeded scaffold is later transferred to a 12-well cell culture plate, (b) Optical images after the MTT labeling of MLO-A5 cells on the five architectures of scaffolds after cell culture intervals of 4 (top row) and 6 days (bottom row).

![Figure 7](image)

**Figure 7.** The absorbance values which represent the measurement of cell growth plotted for all the scaffolds after 2, 4, and 6 days of incubation. Diamond and gyroid architectures show a better overall cell proliferation compared to the other architectures.

The difference in compressive strength offered by the five architectures is significant at lower porosities and amongst them, the cubic architecture offers the highest compressive strength (see Fig. 2). It can also be observed that the cubic scaffold has the faster decrease in its compressive strength with increasing porosity in comparison to the spherical and other scaffolds. The difference in the strengths of cubic and spherical scaffolds at ~32% porosity is ~6 MPa and at ~62% porosity is ~1 MPa. The diamond and gyroid scaffolds have a similar rate of reduction in strength with respect to increase in porosity and the difference in strength also follows a similar pattern to spherical and cubic scaffolds (~4 MPa difference at ~32% porosity and almost no difference at ~62% porosity). The X scaffold has the lowest strength at ~32% porosity because of the struts oriented at an angle of 45° in its design. The compressive strength of scaffolds with all designs at the porosity of 65% or more is ~3 MPa or less, which is either equal to or less than the lowest strength of trabecular bone. This indicates that the effect of architecture is not significant at the higher porosities from the strength perspective in repairing trabecular bones using bioceramics, although it may be significant for cell proliferation and new tissue regeneration.

In our current study, we have compared cell proliferation on scaffolds with different architectures by fabricating them with similar pore sizes and porosities and investigate the effectiveness of different architectures with the scaffolds made with bioactive glass using the SLS process. Though there was no significant difference in the density of the metabolically active cells on the scaffolds after 2 days of incubation, marked differences among the scaffold types in the amounts of formazan recovered was observed after 4 and 6 days of incubation (Fig. 7). After 4 days of incubation, the scaffolds with diamond and gyroid scaffolds offered significantly higher cell proliferation when compared to the X scaffolds. After 6 days of incubation, the gap in cell proliferation further widened with the diamond scaffold being the architecture that was significantly higher than the cubic, spherical, and X scaffolds. Also, the significance in the rate of cell growth from 2 to 4 days and from 4 to 6 days is also indicated in Fig. 7. The results indicate that the diamond and gyroid architectures offer a sustained cell proliferation for all the 6 days of incubation. This could be because of the effective inflow of nutrients owing to the gyroid and diamond architectures which mimic the trabecular bone.

### 4. CONCLUSIONS

Our study has shown that among the borate-based bioactive glass scaffolds fabricated by the selective laser sintering (SLS) process, the cubic pore architecture provides the highest compressive strength at lower porosities (< 40%). However, scaffolds with the cubic architecture also exhibit the highest rate of reduction in the compressive strength with increased porosity among all the architectures considered. The effect of different architectures at higher porosities (> 65%) is not significant from a strength retention perspective for bone repair because all the scaffolds offer low compressive strengths, which are less than the lower range of the compressive strength of human trabecular bone. Finite element analysis was found to provide a fair estimate of the compressive strengths of the scaffolds fabricated using the SLS process. The simulated body fluid tests indicate that the gyroid and diamond architectures, which mimic trabecular bones, have higher percentages of reduction in the compressive strengths in comparison to the...
traditional lattice structures because of their tortuosity and permeability. The in vitro tests further confirm the effectiveness of the gyroid and diamond architectures in providing sustained cell proliferation for the entire 6 days of incubation compared to the other scaffold designs which could not provide significant cell growth after 4 days. The study has shown that the SLS process could be used to fabricate scaffolds with controlled rates of strength degradation and bone regeneration by selecting appropriate architecture and bioactive glass in repairing a specific region of the trabecular bone.

8. ACKNOWLEDGMENTS
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9. REFERENCES