ROBOCASTING BIOGLASS BONE SCAFFOLDS FOR BONE TISSUE REGENERATION

By

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A Thesis

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ABSTRACT

Bioglass is a highly bioactive bioceramic that is composed of 46.1 mol% SiO_2, 26.9 mol% CaO, 24.4 mol% Na_2O, and 2.6 mol% P_2O_5. There are many applications of Bioglass, such as implants for bone regrowth and for correcting bone defects. Several methods for creating a bone scaffold for bone tissue regeneration have been reported, but the most conventional method is foam replication. However, foam replicated scaffolds have limitations in their mechanical strength. This report will focus on robocasting (direct ink writing) a designed Bioglass ink as an improved method of forming a bone scaffold. The printing ink consisted of varying amounts of 45S5 Bioglass powder, water and carboxymethyl cellulose to ensure the required rheological properties for the ease of ink extrusion and integrity of 3D structures after printing. Thermal treatment of the printed green body is required to remove the carboxymethyl cellulose at 400 °C. The debined scaffolds were then sintered to increase the mechanical strength and condense the microstructures. Sintered scaffolds were analyzed under a scanning electron microscope to study the microstructures. Biocompatibility and mechanical testing are required for future testing to determine if the printed scaffolds could be functional for bone tissue regeneration.

I. FORWARD

The idea for this project originated when I worked as a research assistant to Dr. Junjun Ding in the Nanomaterials and Manufacturing Lab during the summer of 2018 at Alfred University. During this time, I learned about different types of 3D-printing like fused deposition modeling, selective laser sintering, ink jet printing, and robocasting. In the Nanomaterials and Manufacturing Lab, I was able to gain experience with the fused deposition modeling printer, and a robocasting printer. I was also able to learn how to use different 3D modeling software, like Ultimaker Cura, and Sketch-up, which was a skill that I was really lacking before this position.

My assignment for my research assistant position involved editing a model for a robocasting mount and printing this mount through the fused deposition modeling printer. However, my main task was to print bone scaffolds intended for bone tissue regeneration by robocasting. This involved extensive research of literature (a review of this literature can be seen in the "Introduction" section) and trial and error in ink designs. Robocasting 45S5 Bioglass scaffolds was introduced to me by Dr. Junjun Ding, and this project became my undergraduate thesis.

I was very excited to work on this project because 3D-printing was something that I always wanted to experiment with, but never had the chance to. I also wanted to gain experience with 3D modeling software because I wanted to improve my abilities to model a part. This project allowed me to experiment with robocasting as well as gain confidence in my ability to model a part.

Another reason why I was excited for this project was because it had the potential to make a difference in society. Improving and refining the methods for bone scaffold fabrication has the opportunity improve the quality and production of a bone scaffold. Making a scaffold that easily forms and bonds to bone could help someone live a better life.

II. INTRODUCTION

Additive manufacturing, also called 3D-Printing, is a growing field that allows intricate parts to be made. There are many different types of 3D-Printing such as fused deposition modeling, stereolithography, selective laser sintering, and robocasting, just to name a few. Robocasting, also called direct ink writing, will be the focus of this thesis. Robocasting extrudes a viscous material from a syringe when a pressure is applied [1]. The material must have a high viscosity, allow for high particle loading, and have shear thinning qualities to be printed from the nozzle and retain the intended shape once printed. Once the ceramic part is printed it must be able to dry without cracking, and the binder must be able to burn out during thermal treatment.

Bioglass is a bioceramic, which includes bioactive ceramics, glasses, and glass-ceramics. For a material to be bioactive it must induce a beneficial response from the body, like bonding to tissue or bone [2]. Bioglass fits this definition, which is why it can be used as implants for teeth or to promote bone growth when there are defects in the bone. Bioglass 45S5 was developed by Larry Hench and is composed of 46.1 mol% SiO_2, 26.9 mol% CaO, 24.4 mol% Na_2O, and 2.6 mol% P_2O_5 and has been in use since 1985 [3, 4]. This type of Bioglass has a couple advantages when it comes to bone scaffold engineering; no other bioactive glass has out-performed Bioglass's biological performance, and Bioglass also bonds faster to bone than any other bioceramic [5].

Bioactive glasses like 45S5 Bioglass are surface reactive and attach to bone by chemically bonding to it. Not only must the Bioglass bond to the bone, but it also must promote bone growth. This starts with the formation of a hydroxycarbonate apatite (HCA) layer on the surface of the scaffold. This layer provides a bonding interface for the tissue and is similar to the mineral phase in bone. Bone-forming cells colonize on the HCA layer where these cells can crystallize, leading to the formation of new bone [6].

There are several methods to make a bone scaffold with Bioglass 45S5, the most conventional being foam replication. However, this technique has its limitations, mainly the scaffold's strength is not on par with that of cancellous bone. Robocasting techniques may be able to overcome some of the limitations previously mentioned. By tailoring the scaffold design, the scaffold's strength would increase, and pore size and volume can be controlled [7].

For a scaffold to be used in real-world applications, there are certain requirements. An ideal scaffold must be osteoconductive, which means it must be able to promote bone growth. A scaffold ideally would have a Youngs modulus of 15-20 GPa for cortical bone applications and a Youngs modulus of 0.1-2 Gpa for cancellous bone applications. The scaffold must have a compressive strength of 100-200 Mpa for cortical bone and 2-20 Mpa for cancellous bone. Since actual bone is porous, the scaffold must be too. Pore sizes need to be at least 100 μm in diameter, however diameters of 200-350 μm are optimal. The scaffold must also be able to degrade over time without harming the body [8].

45S5 Bioglass powder was used to fabricate a bone scaffold intended for bone tissue regeneration. Robocasting, an extrusion-based type of 3D-printing, was used to fabricate the bone scaffolds. Several scaffold designs were made by using 3D modeling software, and each design was compared when printed. Six different ink compositions were made with 45S5 Bioglass powder, carboxymethyl cellulose, and water, and had varying amounts of each. To be printed by robocasting, the ink must have shear thinning qualities to ensure the ease of printing and to maintain the mechanical integrity of the scaffold once printed. Viscosity measurements were performed to test for these characteristics. Once the scaffolds were printed, thermal treatment was used to burn out the carboxymethyl cellulose at 400 °C [7] and to densify the scaffold. A scanning electron microscope was used to analyze the microstructures of a sintered scaffold.

III. EXPERIMENTAL PROCEDURE

A. SCAFFOLD DESIGN

The scaffold was designed using Ultimaker Cura (Ultimaker, version 3.3.1). There were a couple of different infills used and the infill percentage varied. Figure 1 shows the infills used and their percentages. Design A used a 13% zigzag infill, design B used a 15% grid infill with a two-layer shell, and design C used a 17% grid infill. The scaffold design was adjusted as the experiment progressed, so each scaffold may have a slightly different design. The dimensions of the scaffolds were 3 cm x 3 cm x 0.5 cm with a 0.2 mm layer height.

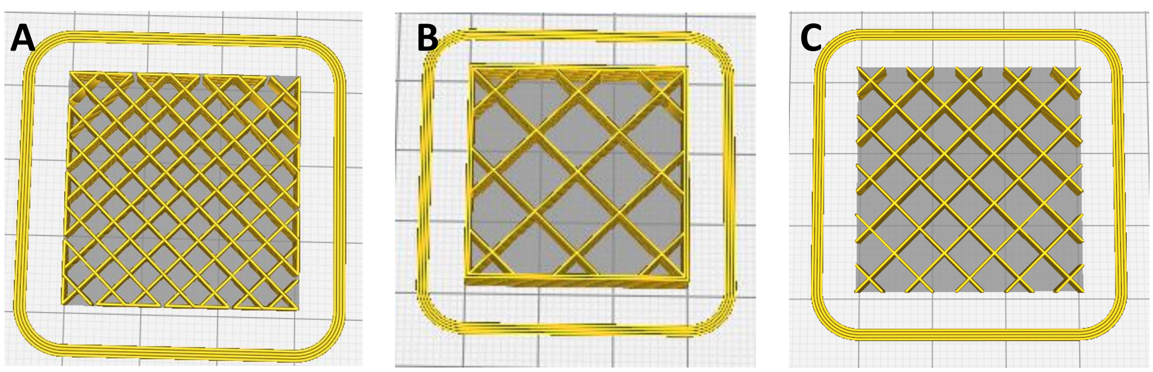

Figure 1: The different scaffold designs used. Design A used a 13% zigzag infill, design B used a 15% grid infill with a two-layer shell, and design C used a 17% grid infill.

B. INK FABRICATION

The 45S5 Bioglass powder was provided by the Biomaterials department at Alfred University. The composition of the 45S5 Bioglass was 46.1 mol% SiO_2, 26.9 mol% CaO, 24.4 mol% Na_2O, and 2.6 mol% P_2O_5. The particle size of the 45S5 powder ranged from 6-10 μm. Carboxymethyl cellulose (CMC, SIGMA-ALDRICH, MO) was used as a binder and as a dispersant.

Several ink compositions were used and can be seen in Table 1. Every ink composition had 45S5 Bioglass powder, CMC, and deionized water. For the rest of this report, an ink composition will be referred to their amount of 45S5 Bioglass powder in weight percent (wt%). Once the desired ratios of 45S5 Bioglass powder, CMC, and water are combined, the ink was mixed in a Retsch PM 200 (Retsch GmbH, Germany) planetary ball mill. The ink was ball milled for 1.5 hours at 250 rotations per minute.

Table 1: Ink Design

Amount of Bioglass (wt%)	Amount of CMC (wt%)	Amount of Water (wt%)	Scaffold Design Used (see Figure 1)
44.8	1	54.2	C
56.7	1.3	42	A
60	1	39	A
62.8	1.5	35.7	B
65	1	34	A
67.5	1.3	31.2	A

C. ROBOCASTING SCAFFOLDS

The scaffolds were printed through a modified Creality 3D (Creality, China) fused deposition modeling printer. The modification is shown in Figure 2. The ceramic ink is loaded into a syringe, which is then mounted onto the printer (see Figure 2). The ink was printed through a cone shaped nozzle with an inner diameter of 0.41 mm. The scaffold was generally printed at a speed of 2 mm/s, though this was adjusted depending on the ink's viscosity and how the scaffold was printing (for example, if there were breaks in the print, the printing speed should be lowered). The flow rate was adjusted based on the print (more viscous inks needed a higher flow rate). The scaffold was printed on masking tape for the ink to have more friction on the printing bed, preventing sliding. The printed scaffolds dried on the printing bed for at least one day before removing with a razor. The scaffolds were then dried out for at least another 2 days before thermal treatment.

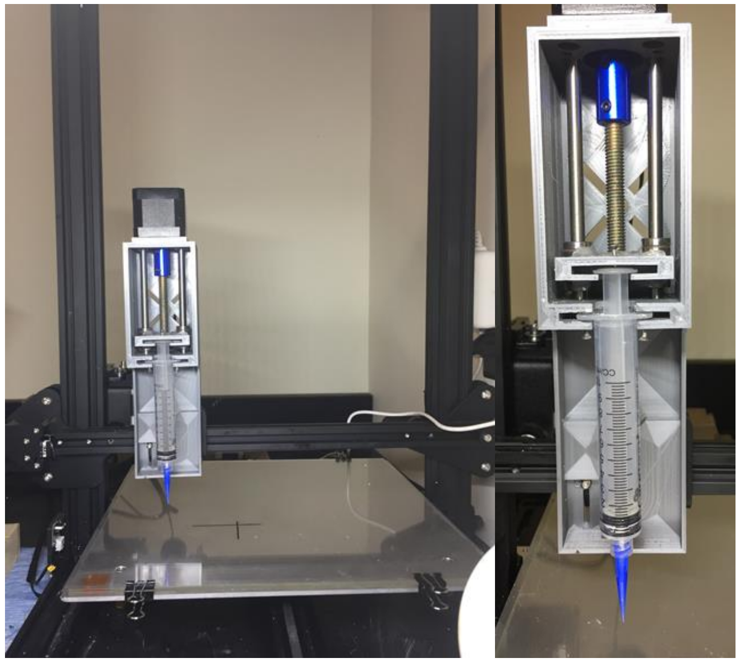

Figure 2: The modified fused deposition modeling printer used. The right-side picture shows a close-up of the extruder.

D. THERMAL ANALYSIS AND TREATMENT

Thermal analysis was performed on the 45S5 Bioglass powder using a SDT Q600 (TA Instruments, DE) thermogravimetric analysis (TGA). The thermal analysis started from room temperature and heated up to 1200 °C. A Thermo Scientific Lindenberg Blue M (Thermo Scientific, MA) tube furnace was used for thermal treatment of the printed green body scaffolds. The CMC was burned out at 400 °C for one hour with a heating rate of 5 °C/minute. After the burn out of CMC, the scaffold was sintered at 900 °C for one hour with a heating rate of 5 °C/minute.

E. SCANNING ELECTRON MICROSCOPE IMAGING

A JEOL JSM-6010PLUS/LA (JEOL, Japan) scanning electron microscope (SEM) was used to perform secondary electron (SE) imaging on a sintered and green body scaffold. Both samples were mounted on a carbon substrate and coated in gold to prevent charging.

F. RHEOLOGY MEASUREMENT

A Discovery HR-2 Hybrid (TA Instruments, DE) rheometer was used to test the viscosity of the 60 wt% 45S5 Bioglass ink. The same methods used in “Ink Fabrication” were used to prepare the ink for viscosity measurements.

IV. RESULTS AND DISCUSSION

A. SCAFFOLD DESIGN

There was trouble with the cross-sections during printing with the grid infills in designs B and C. The ink would accumulate at these cross-sections and cause breaks in the print, which can be seen in Figure 3. The zigzag infill in design A did not have this problem, or it was less prominent. Design B and C's extruded lines were further apart than in design A, which is due to the grid infill. This caused design B and C's extruded lines to collapse which then causes a break in the print and nonuniformity in the scaffold (see Figure 3). Because of design A's zigzag infill, the extruded lines were closer together and was able to support other extruded lines. The holes in design B and C's are much too big for a scaffold, where design A's holes are smaller.

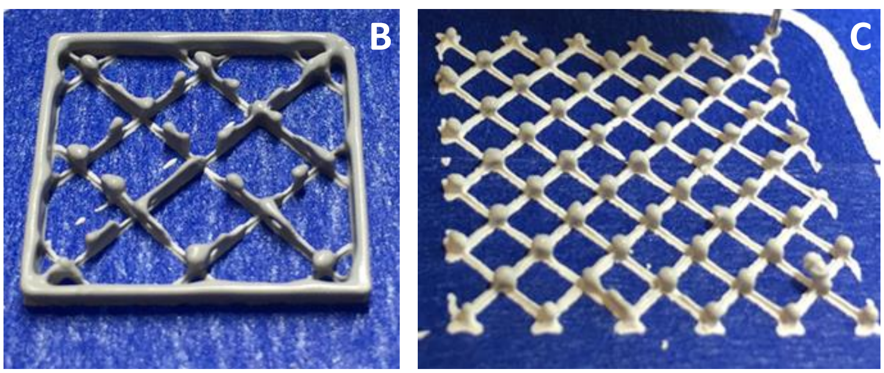

Figure 3: Design B (left) and Design C (right). Both show the accumulation of ink at cross-sections and B shows discontinuity in the extruded lines due to the accumulations and breaks in the printing process. The dimensions of the scaffold shown are 3 x 3 x 0.5 cm.

B. ROBOCASTING SCAFFOLDS

The scaffolds printed by robocasting are shown in Figure 4. Ink accumulated at the cross-sections of 44.8 wt% 45S5 and 62.8 wt% 45S5. This caused breaks in the printing process and resulted in a discontinuous extruded line. 56.7 wt% 45S5 did not print coherent lines and did not hold the structure of an extruded line. 60 wt% 45S5's extruded lines are easier to differentiate, but there are ink accumulations around the edges of the scaffold which eventually inhibited the print. 60 wt% 45S5 also had some if its holes filled in with due the unclogging of the printing nozzle which resulted in in spilling onto the scaffold. 65 wt% 45S5 also has clear and differentiable lines and does not have ink accumulations. However, 65 wt% 45S5 does have slumping, which causes the ink extrusions to fill in holes in the scaffold and morph together. 67.5 wt% 45S5 has the clearest extrusions and holes, but it still does experience some slumping that is also experienced in 65 wt% 45S5. Due to this slumping, the distance from the nozzle to the printing bed would have to be adjusted because the layer height would change.

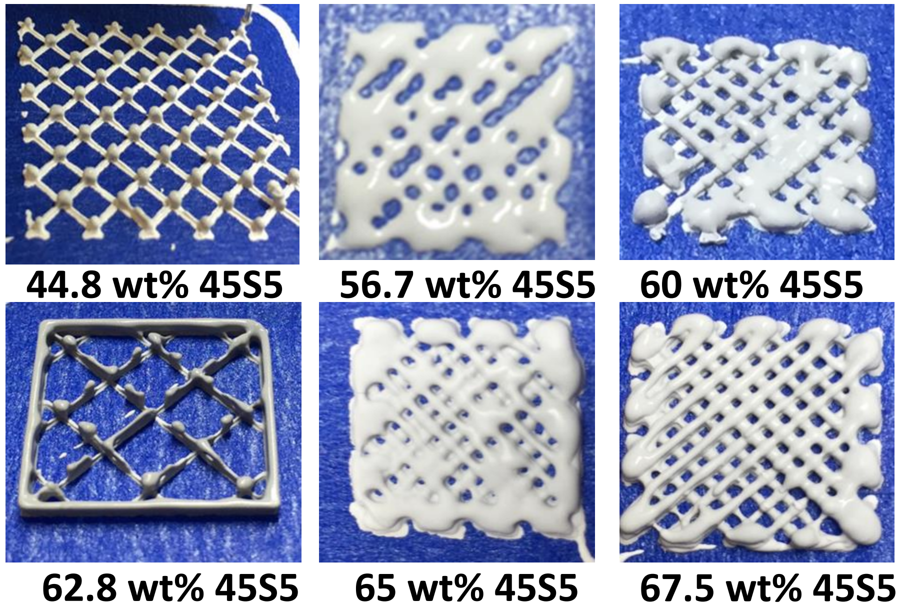

Figure 4: Scaffolds printed by robocasting. The amount of 45S5 Bioglass powder in wt% is under each image. The dimensions of the scaffold shown are 3 x 3 x 0.5 cm.

Scaffolds printed with a viscous ink had more structural integrity and held the intended shape of the scaffold better than those with lower viscosities. Figure 4 shows the progression of how an increase in viscosity effects the integrity of the scaffold. As the viscosity increases, the cross-sections and holes become more defined.

Clogging of the printing nozzle was an issue with all the scaffolds. When the extruder was not printing for a few minutes, the ink would dry very easily and clog the nozzle. This would also occur during printing too if ink was around the printing nozzle. If the nozzle clogged during a print, it would cause discontinuity in the extrusion and deform the shape of the scaffold.

Another issue during printing was the printer extruding ink when the printer head was traveling. When the printer head travels, it is supposed to move around without extruding ink. However, with the software and the printer used, the ink cannot retract the ink back into the nozzle or stop the ink from extruding. This caused the ink to build up around the edges of the scaffold (where the printer head travels). This accumulation of ink around the edges is most noticeable in the 60 wt% 45S5, 65 wt% 45S5, and 67.5 wt% 45S5 scaffolds (see Figure 4).

Removing the scaffold from the printing bed generally resulted in breaking the scaffold. Most scaffolds broke into very small pieces, but 67.5 wt% 45S5 had a greater amount of CMC and broke into bigger pieces. 62.8 wt% also had a greater amount of CMC but was not able to keep the structure upon removal and is most likely due to the scaffold design used.

C. THERMAL ANALYSIS

Figure 5 shows the results from the TGA in weight and heat flow. The weight vs temperature graph shows that the glass transition temperature (T_g) for the 45S5 Bioglass powder is ~670 °C. The heat flow vs temperature graph shows that the melting point for the 45S5 Bioglass powder is ~1100 °C.

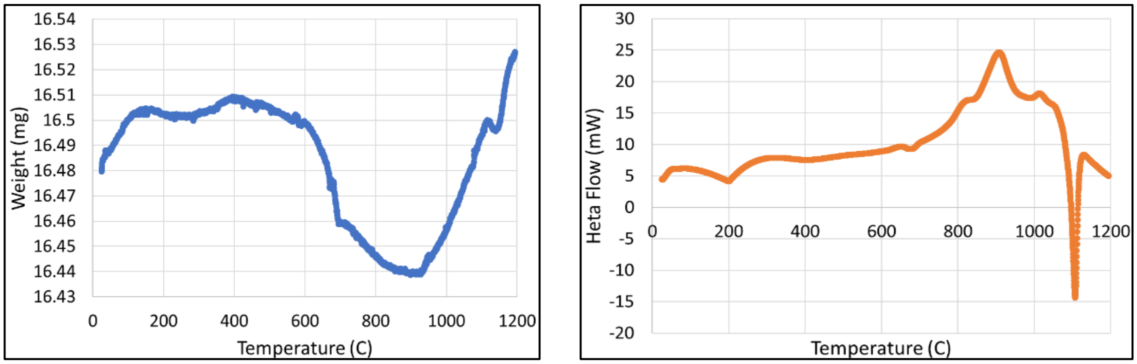

Figure 5: TGA of 45S5 Bioglass powder in weight vs temperature (left) and in heat flow vs temperature (right).

Figure 6 shows the 65 wt% 45S5 scaffold that was sintered at 900 °C (right) and the green body of the same scaffold (left). The sintered scaffold does not show many pores, which is most likely due to the high sintering temperature. Sintering at a lower temperature, under the T_g, may increase the porosity and create channels that are desired in a bone scaffold.

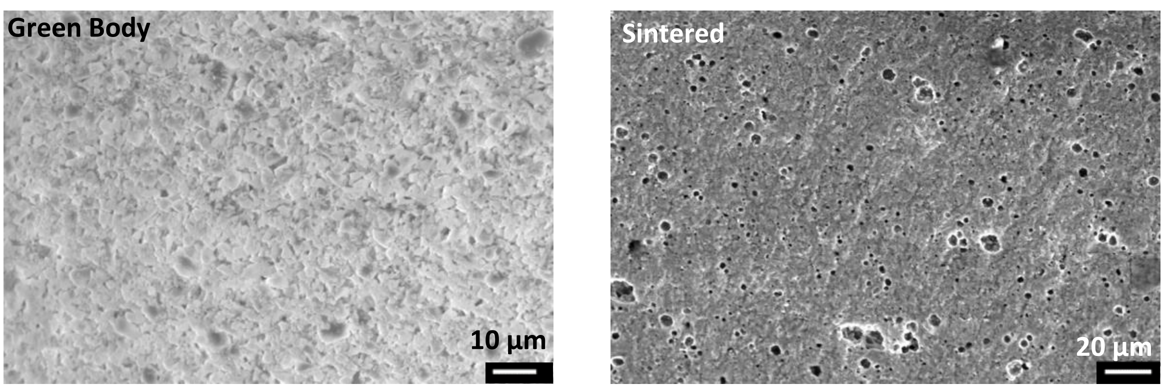

Figure 6: SEM images of the 65 wt% 45S5 scaffold at the green body stage after drying (left) and after sintering (right).

D. RHEOLOGY MEASURMENT

The viscosity of the 60 wt% 45S5 ink was measured, and the results are shown in Figure 7. The graph in Figure 7 shows that the 60 wt% 45S5 ink has shear thinning qualities which is optimal for ceramic printing. This demonstrates that the range of inks used in this experiment are within the correct viscosity range.

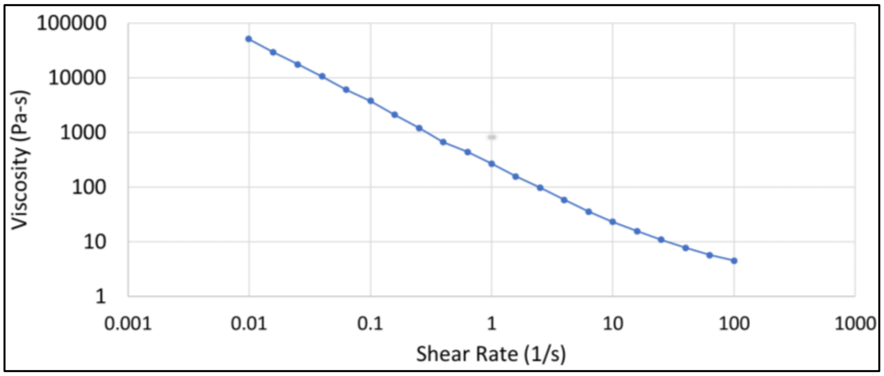

Figure 7: Viscosity measurements for 60 wt% 45S5 ink.

V. CONCLUSION

45S5 Bioglass scaffolds were printed by robocasting. The scaffold designs used included a 13% zigzag infill, a 15% grid infill with a two-layer shell, and a 17% grid infill. The 13% zigzag infill was the most successful for it had the least amount of ink accumulated at the ink cross-sections and had the least number of breaks in a print. Different amounts of 45S5 Bioglass was used in six different inks, ranging from 44.8 wt% - 67.5 wt% 45S5 Bioglass. The viscosity of an ink with 60 wt% 45S5 Bioglass was tested and showed shear thinning qualities that are required for ceramic printing. The inks with greater viscosities had clear and defined extrusions and holes. Lower viscosities had more ink accumulations at cross-sections and ink filling in holes. 67.5 wt% 45S5 had the most defined extrusions and holes and slumped the least. Removing the scaffolds from the printing bed resulted in breaking the scaffolds, though scaffolds with more CMC broke into larger pieces. A 65 wt% 45S5 scaffold piece was sintered at 900 °C. After sintering, the structure did not have many pores, but by lowering the sintering temperature the number of pores may increase.

VI. SUGGESTIONS FOR FUTURE WORK

Viscous inks had a greater success in printing a scaffold, with 67.5% 45S5 having the clearest cross-sections, holes, and extruded lines. However, this ink did have issues with clogging in the nozzle during printing, slumping in the scaffold, and breaking upon scaffold removal from the printing bed. Printing with an even more viscous ink (about 70 wt% 45S5) may solve some of these problems. With added 45S5 Bioglass powder or CMC to the ink composition, the scaffold may be able to hold its structure more firmly and slump less. With added CMC, the structure may not break upon removal from the printing bed because the addition of more CMC binder will increase the green body strength.

The porosity of the sintered scaffold was poor. The microstructure of the sintered scaffold did not have porous channels as normally seen in bone structures. A way to increase the number of pores is to reduce the sintering temperature. Reducing the sintering temperature to within 550-600 °C [9] may increase the number of pores and create a porous microstructure.

Biocompatibility testing is required to see if the printed scaffolds are functional. When emerged into simulated body fluid, the scaffold needs to be able to form a HCA layer. This layer is necessary for the regeneration of bone tissue. Mechanical testing of the scaffolds is also required to see how the strength of the scaffold compares to that of real bone. Compression stresses should be tested, and the elastic modulus of the bone scaffold should be compared to the elastic modulus of real bone.

VII. LITERATURE REFERENCES

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