A Thesis Presented to

The Faculty of Alfred University

OXIDATED ALGINATE COMPOSITES FOR ANTIMICROBIAL HARD TISSUE REPAIR

by

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ABSTRACT

Natural polymers such as alginate are highly desired in the medical field due to their excellent biocompatibility. However, like most materials, this pure alginate is far from perfect. In order to enhance and optimize this polymer for hard tissue repair, low viscosity alginate was oxidized using sodium periodate and two different solvents (DI water and a 1:1 water/ethanol mixture) and then composited with 45S5 Bioglass©. The two version of the oxidized sodium alginate composites were then compared based on their microstructures, antibacterial properties, and compression strength. Overall, it was found that the composited samples using the 1:1 water/ethanol solvent produced more favorable properties, especially in its ability for better gelation and mechanical properties. While more work needs to be done, the research conducted shows oxidized sodium alginate composites have the potential to be a safe and effective biomaterial for hard tissue repair.

I. Introduction

A. Preface

Biomedical materials are used to replace part of a living system or to function in intimate contact with living tissue. One of the most rapidly advancing fields in science today is tissue engineering, where the ultimate goal is to quickly induce tissue repair and growth within the damaged area.[1] This is often completed by taking advantage of tissue specific cells and signaling pathways along with biocompatible scaffold materials to guide the correct cell phenotype.[2] Specifically, this report will focus on scaffold materials for bone, which provide temporary support for cells, allowing them to adhere, develop, proliferate, and regenerate new tissue in order to regain native functionality. These scaffolds can be created with a wide variety of materials, ranging from derived proteins to synthetic plastics.[1,2] One such group of materials that will be discussed in detail are naturally derived hydrogels.

While research within this field encompasses all kinds of tissue found throughout the body, bone is particularly interesting due to its unique structure, highly dynamic environment, and diversity of functions. In general, bone is a porous structure composed of cells, blood vessels, and mineralized hydroxyapatite. Many people associate bone as just being a load bearing structure that allows us to move and support our weight. While this is correct, bone has much more functions that make it so diverse, such as sites for hematopoiesis, B-lymphopoiesis, electrolyte exchange, and more.[3] In addition, bone tissue is constantly in a state of resorption and remodeling though osteoclasts and osteoblasts, respectively. This bone remodeling process creates an interesting dynamic that must be considered when creating materials to aid in the bone repair process. In essence, bone remodeling and repair can be thought of as a coupling

mechanism. While osteoclasts dissipate the mineralized hydroxyapatite and outer matrix layer, osteoblasts work at the same rate to secrete osteoid in its place, which is a premature version of the finalized bone.[3,4] Therefore, the sheer number of hormones, proteins, and cell signaling mechanisms create a highly specialized and efficient process that allows bone to constantly adapt to mechanical load and strain.

Once the scaffold framework is constructed, it is used as a vehicle to deliver host cells. Additionally, these scaffolds can also be loaded with growth factors, bioactive glass particles, and other additives to help aid with the regeneration process or to enhance the mechanical properties of the scaffold. Ultimately, the goal is to regenerate the damaged tissue while the scaffold disintegrates naturally in the body. In the end, only human host tissue will remain and still be able to have native cell functionality.[1,2] Despite the large number and range of materials that can serve as scaffolds, this report will focus on oxidated alginate and its composites.

B. Alginate Polymers

Alginic acid, or alginate, is a polysaccharide that is naturally found in the cell walls of brown seaweed. Because it is derived from natural sources, alginate is a hydrophilic, easily extracted, and biocompatible material. While alginate and its derivatives have historically been used as materials for superficial wound dressings, it has caught the attention of scientists involved in tissue engineering.[5,6]

The general structure of alginate is composed of 1,4-linked β-d-mannuronic acid (M-block) and 1,4 α-l-guluronic acid (G-block) residues [6,7], which can be visualized in Figure 1.

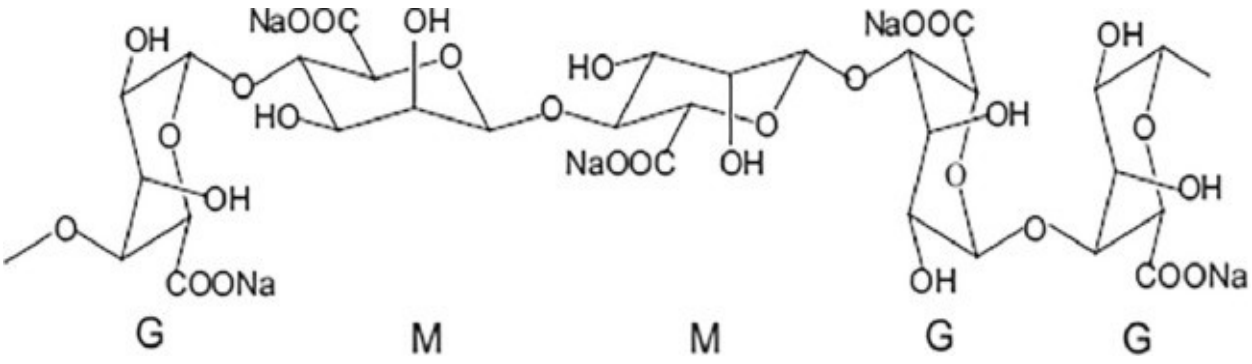

Figure 1: Molecular structure of 1,4-linked β-d-mannuronic acid M and 1,4 α-l-guluronic acid G residues that make up alginate.[8]

As seen in Figure 1, the alginate polysaccharide is composed of two types of monomers (M or G-blocks) that are linked together by oxygen. However, since alginate is a natural material, the exact composition of M-blocks to G-blocks can vary depending on the brown algae it was extracted from.[7,8] The variation of these groups can have a profound effect on the properties of alginate. For example, alginates that contain a higher percentage of G-blocks often produce stiff and inelastic hydrogels. Meanwhile, higher M-blocks produce the opposite, soft and elastic hydrogels.[8,9] Regardless of the monomer concentrations, pure alginate has several major disadvantages. First, it is not truly biodegradable in humans, due to the lack of the alginase enzyme. Second, pure alginate tends to cause cell clustering, since it is not a good promoter of cell migration or adhesion. Lastly, the chemical composition of pure alginate does not favor mechanical strength, an important factor when considering the stresses of the body. Luckily, there are many free hydroxyl and carboxyl groups distributed along oxanes, which create an easy way to chemically functionalize those groups.[7,8,9]

One such way of functionalizing these groups is through oxidation. This process, in essence, breaks the carbon-carbon bond and replaces the carbons and hydroxyls with two aldehyde groups in each oxidized monomer, as shown in Figure 2.

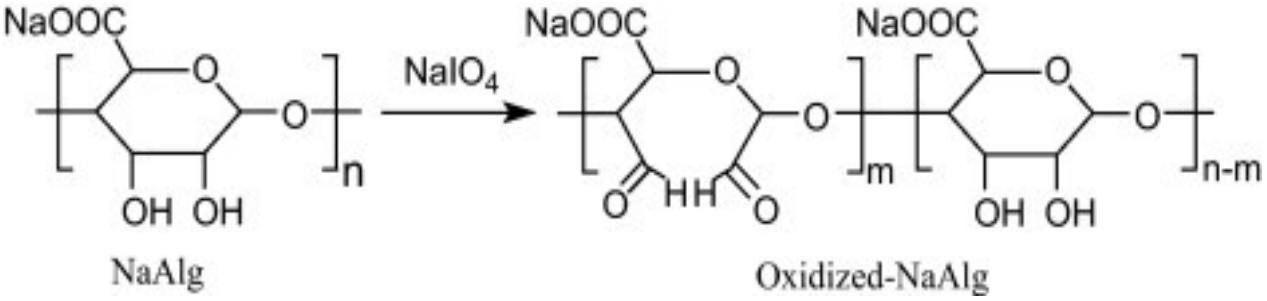

Figure 2: Oxidation reaction of sodium alginate using sodium periodate.[8]

Being able to oxidize alginate as shown in Figure 2 can drastically change the behavior of the polymer, creating more advantageous properties. The addition of aldehyde groups allows the oxidized alginate to have more reactive side groups and a faster degradation rate when compared to pure alginate.[8,10] Yet, oxidizing alginate does not provide a solution to all of the problems mentioned beforehand. One potential way to solve these problems is by compositing the oxidated alginate. Historically, alginate has been composited with many different items, such as other natural polymers (collagen, chitosan, gelatin), synthetic polymers (polylactide, polypyrrole), or inorganic materials such as hydroxyapatite.[7] However, this report will focus solely on the oxidated alginate composited with Bioglass© (45S5).

45S5 Bioglass© is a bioactive material that has great potential for wound healing. Specifically, it has been shown to be an excellent promoter of bone growth and osteogenesis. When composited with oxidized sodium alginate, the hydrogel then gains the ability to occlude wounds and stimulate blood vessel formation. Ultimately, the creation of an oxidized sodium alginate

hydrogel composited with 45S5 Bioglass© has the potential to be a useful material for hard tissue repair.[11]

C. Oxidated Sodium Alginate Composites in Literature

Current research regarding oxidated sodium alginate composites has been documented before. While the properties and benefits of pure alginate have been known about for a while, modifying and compositing alginate is still a relatively new area of study.

One such study was performed by Sarker et al.[13] by compositing oxidated alginate, gelatin, and 45S5 Bioglass©. According to this study, the addition of 45S5 Bioglass© increased the degree of crosslinking between the oxidated sodium alginate and gelatin. Ultimately, this created a hydrogel with increased mechanical properties, shorter gelation time, and more favorable release kinetics. It was also found that the amount of 45S5 Bioglass© controlled the degree of crosslinking within each hydrogel, making it possible to adjust the hydrogel to have the properties that are desired in specific situations. In addition, FTIR analysis also discovered that these hydrogels had hydroxyapatite (HA) deposition on the hydrogel's surface, further adding advantages to the composition of these hydrogels. However, cell viability studies found that the hydrogels not containing 45S5 Bioglass© were better at promoting stem cell differentiation and proliferation. While this study provided promising results, further research will have to be conducted on cell viability.[13]

Another similar study, conducted by Rottensteiner et al.,[14] compared the incorporation of 45S5 Bioglass© into oxidated sodium alginate/gelatin hydrogels. While most of the work done in this study followed the same guidelines, further research was conducted on the effects of these hydrogels *in vivo*. Results regarding *in vitro* testing were similar in both cases (considering

mechanical testing, cell viability, degree of crosslinking) when compared to their counterparts without 45S5 Bioglass©. *In vivo* testing showed no significant immune response and showed biodegradation four weeks after implantation. Interestingly enough, there was no observed difference in cell viability between the two types of hydrogels.[14]

II. Experimental Procedure

The research conducted for oxidated sodium alginate composites was conducted in three stages: synthesis, characterization, and material testing. The following methods, materials, and procedures were used and performed under the departments of the Kazuo Inamori School of Engineering at Alfred University.

A. Synthesis

The oxidation process for sodium alginate was most thoroughly reported by Balakrishnan et al [12] and was followed closely. Two batches of oxidized sodium alginate were created using low viscosity alginic acid. Group 1 was made with an aqueous solvent, while group 2 used a 1:1 DI water to ethanol solvent mixture. In both cases, a 4.0 wt% alginate-loaded mixture was created (8g alginic acid). 8g of alginic acid were then slowly added to each group and allowed to stir undisturbed for 2 hours.

Meanwhile, 5.0 mol% of sodium periodate ($NaIO_4$) was dissolved into DI water. Specifically, 0.53g of $NaIO_4$ and 50 ml of DI water were mixed until dissolved. At the end of the 2 hours stir time, the $NaIO_4$ solution was added dropwise to sodium alginate solution, and allowed to stir in a dark, cool environment (~25°C) for an additional 6 hours.

Next, two appropriate pieces of 3500 MWCO (molecular weight cut-off) dialysis tubing (Spectra/Por® 1) were cut and soaked in DI water until pliable. With one end clamped, the sodium alginate solution was transferred from the mixing beaker into the 3500 MWCO dialysis tubing. Once all the material was transferred, the open end was then clamped until the dialysis tubing was taunt. Both dialysis tubes were then placed in a 4L beaker and submerged in DI water. While under constant slow stirring, the DI water was replaced 3 separate times every 6 hours.

Once dialysis was completed, 25ml of solution was pipetted into petri dishes until all solution was distributed. The petri dishes were then placed in a -70°C freezer overnight (Isotemp, Fisher Scientific). After 24 hours, the petri dishes were removed from the freezer, and the lids were replaced with Kimwipes. Once each petri dish was ready, they were all placed into a freeze drier (FreeZone Triad®, Labconco) to lyophilize. The freeze drier was defrosted every 24 hours. Upon lyophilization, the dried polymer was scraped into centrifuge tubes, where they were kept in a desiccator (DryKeeper, Samplatec) until composites were ready to be made.

Before composites could be made, the 45S5 glass had to have a particle size of ~60µm, which was accomplished by using sieving dishes. Also, the oxidized alginate had to be dissolved in phosphate-buffered saline (PBS) in order to rehydrate. Throughout all samples, 0.1g of oxidized alginate was used. In the samples that used the aqueous solvent, a ratio of 1:1 polymer to PBS was used, while the ethanol/water solvent used a 1:2 ratio of polymer to PBS. All samples were then vortex mixed until dissolution and left overnight. Finally, the 45S5 glass was then added to each sample group as 0.5 wt%, 1.0 wt%, and 2.5 wt%. All samples were vortex mixed again until evenly distributed.

Lastly, 5 well silicone mats were placed on microscope slides, which were then placed inside a petri dish. The liquid composite was then pipetted into each well until all material was distributed. The petri dishes were then placed in a 37°C incubator until solidified. All samples were then stored in the desiccator indefinitely.

B. Characterization

All oxidized alginate composite samples were imaged using a Jeol JSM-6010 Plus/LA Scanning Electron Microscope (SEM). However, some preparation had to be done prior to imaging due to the low conductivity of the dried polymer samples. To accomplish this, carbon tape was placed on a small metallic stud, while the polymer stuck to the opposite side. Next, each sample was sputter coated (Cressington, 108 Sputter Coater) using silver nanoparticles in an argon atmosphere. Afterwards, samples were imaged and characterized using the SEM. Parameters that were kept constant throughout sample imaging included a working distance of 10 mm, spot size of 60 nm, and the use of secondary electron imaging only. In most cases, the voltage was kept at 15 kV. However, in several samples the voltage was reduced to 10 kV to try and reduce charge buildup and artifacts.

C. Material Testing

Compressive and bacterial testing were done on all samples to evaluate the mechanical and antibacterial properties of the oxidized alginate composites. Compression testing was a relatively straightforward procedure, which was done by placing a hydrogel sample in between two plates of a compression tester (Com-Ten Industries) until barely touching. A 40 lb load cell was used at a compression speed of 5. Note that water-treated samples were not tested due to their inability to form a solid hydrogel disc.

Bacterial testing was completed using gram positive and gram negative bacteria: *E. coli* and *S. aureus*, respectively. LB agar was used for *E. coli* bacteria tests while TS agar was used for *S. aureus* testing. Regardless, both types of agar were melted until completely viscous. 25 ml of the appropriate liquid agar was then dispensed into petri dishes. While the agar was solidifying, both bacterial strains were prepared. 20 µl of *E. coli* was mixed with 980 µl of DI water while 50 µl of *S. aureus* was mixed with 950µl of DI water. Upon agar solidification, disc samples were simply placed on top of the agar. However, samples that did not form discs were hydrated with 20 µl of DI water and pipetted into an agar well. A cotton-tipped applicator was then used to gently swab the surface of the agar with the bacteria in an even manner. Samples were then placed upside-down in a 37°C for 24 hours. Lastly, all samples were then imaged (Synbiosis, Protocol 3) and inhibition zones were measured 3 times each.

III. Results

A. Synthesis

Despite making the solvent the only variable, the synthesis process was noticeably different between the aqueous solvent and the 1:1 ethanol to water solvent. Observational differences were first noticed after the oxidizing agent ($NaIO_4$) was added to both mixtures, which can be seen in Figure 3.

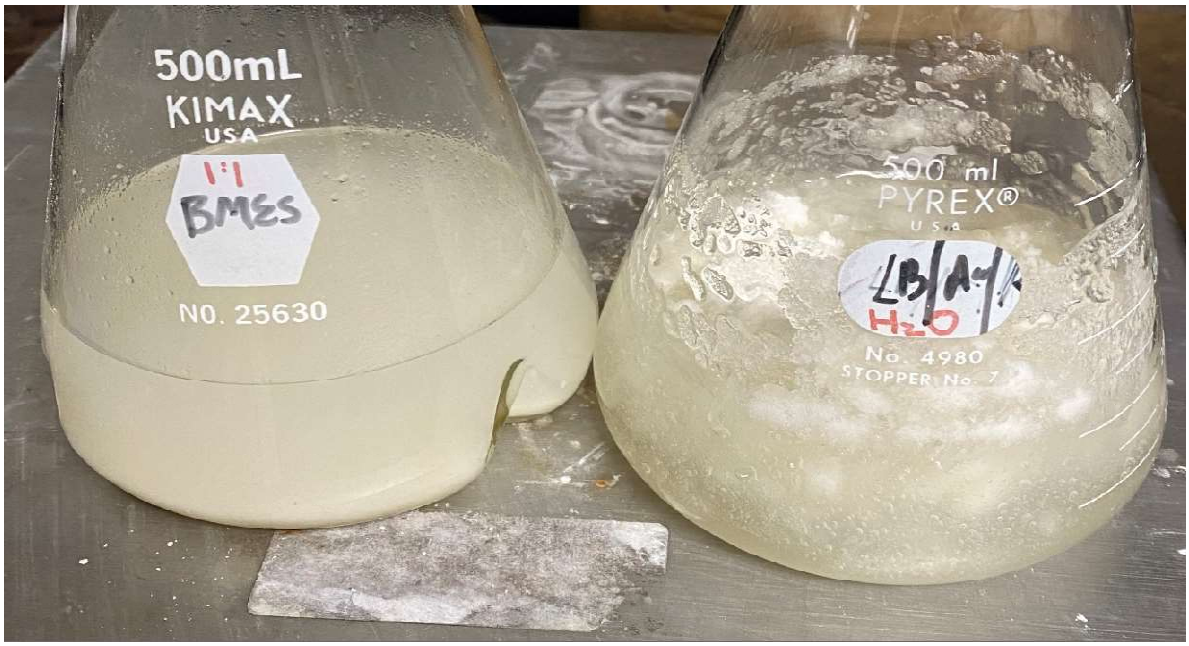

Figure 3: Alginate mixture after adding $NaIO_4$ to the 1:1 ethanol to water solvent (left) and aqueous solvent (right).

As seen in Figure 3, the alginate in the aqueous solution produced a much more viscous product that resulted in small coagulations of alginate. Meanwhile, the 1:1 ethanol to water solution produced a much more evenly distributed solution.

However, the highly viscous nature of the water-treated alginate seemed to have reversed by the time all samples were lyophilized. Figure 4 shows both groups of alginate after lyophilization.

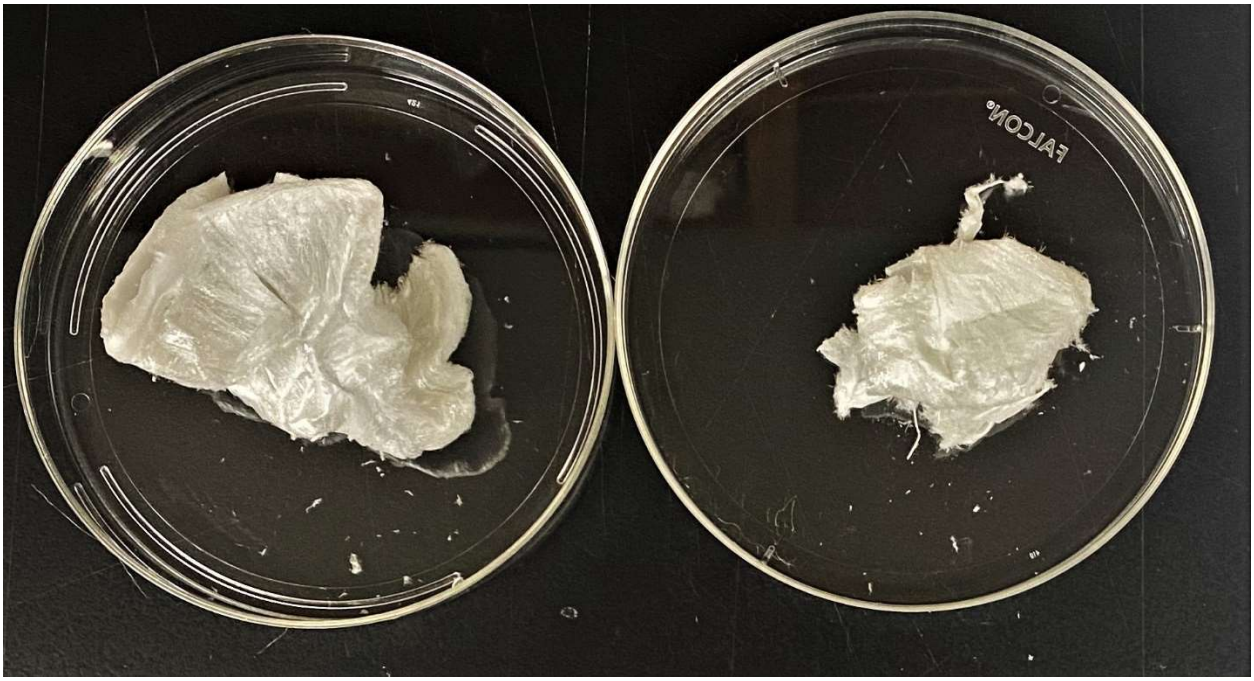

Figure 4: Oxidated alginate polymer post-lyophilization for ethanol-treated alginate (left) and water-treated alginate (right).

While both groups of oxidized alginate look similar in Figure 4, their observational properties were, in fact, noticeably different from each other. For example, the water-treated samples possessed much stiffer mechanical properties, which can be implied by its sharp, jagged corners as opposed to the smooth curves of the ethanol-treated oxidized alginate. Because of this, the ethanol-treated samples were considerably more ductile and fibrous.

Compositing both types of oxidated alginate with 45S5 Bioglass© once again showed considerable changes in properties. Surprisingly, the ethanol-treated polymer produced a much more viable structure, which can be seen in Figure 5.

Figure 5: Ethanol-treated oxidized alginate composited with 45S5 Bioglass©. Composites were created using 0.5 wt%, 1.0 wt%, and 2.5 wt%. Shown from left to right, respectively.

As shown in Figure 5, noticeable variation was present between 45S5 Bioglass© concentrations. As the concentration was increased, the hydrogel became stiffer and rigid. While glass coagulation was present in the 0.5 wt% samples, much more even dispersion was obtained in the 1.0 wt% and 2.5 wt% samples. Interestingly, the hydrogels containing 2.5 wt% 45S5 Bioglass© appeared to have holes and minor structural defects visible, which was not apparent in the other samples.

Meanwhile, the water-treated samples turned out very differently, and did not produce a useable hydrogel. Despite the same glass concentrations that were used for the ethanol-treated samples, all water-treated samples turned out the same on a macroscopic level. An example of a water-treated sample can be seen in Figure 6.

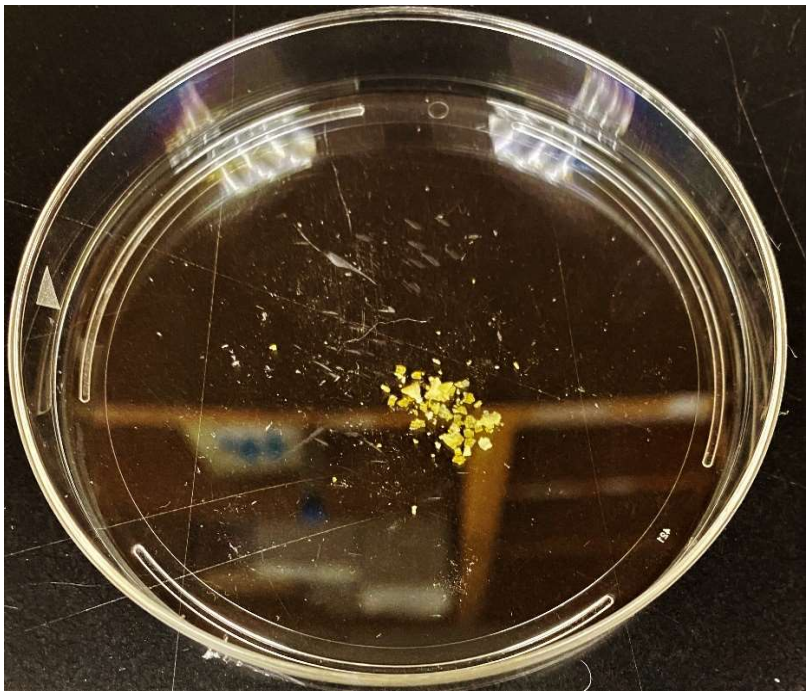

Figure 6: Water-treated oxidized alginate composited with 1.0 wt% 45S5 Bioglass©.

As shown in Figure 6, the water-treated samples were not able to retain any saturation, ultimately causing it to degrade to particles. Obviously, this is not a viable material for hard tissue repair. However, an interesting characteristic not present in the ethanol-treated samples is the yellowish hue present.

B. Material Characterization

Despite many macroscopic similarities between samples of the same solvent-treated groups, the microscopic characteristics and differences of the hydrogels were found to vary significantly between the variable concentrations of 45S5 Bioglass©. However, the microstructure of the basic oxidized alginate polymers was compared first, as seen in Figure 7.

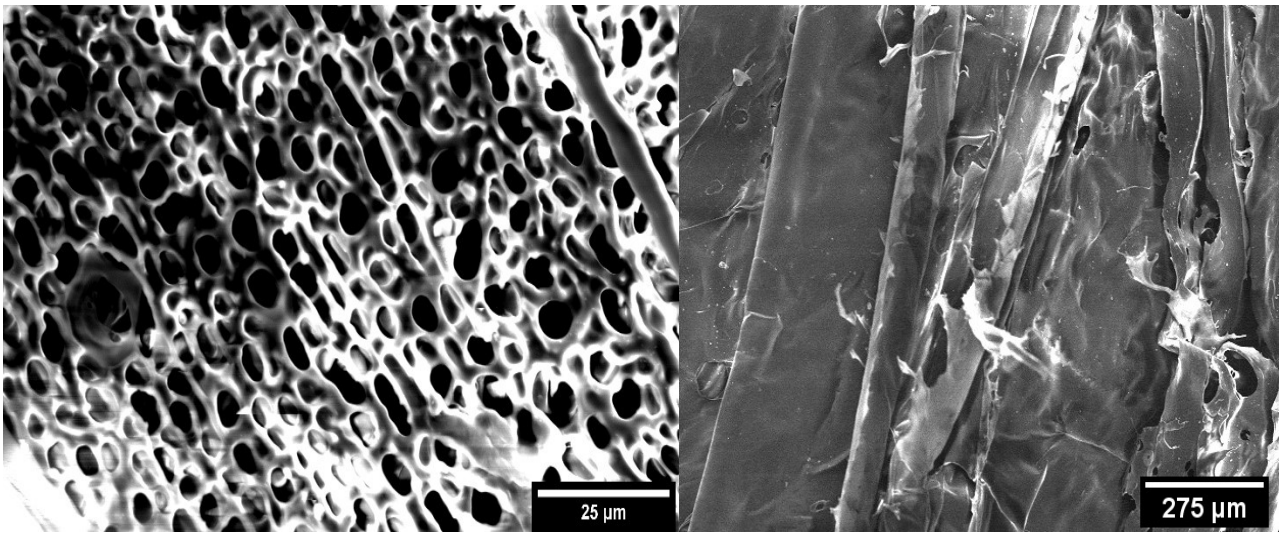

Figure 7: SEM images of oxidized ethanol-treated alginate (left) and oxidized water-treated alginate (right).

As seen in Figure 7, the microstructures of oxidized alginate varied greatly in response to the solvent used. When a 1:1 ethanol to water solvent mixture was used, the resulting polymer became a highly porous structure with a very low density. Meanwhile, the water-treated oxidized alginate appeared to be much more fibrous. While it is apparent that some holes are present, it appears that most holes that have tried to form ended up tearing or breaking apart. This can be seen most easily on the right side of the water-treated oxidized alginate in Figure 7.

Finally, the microstructures of the three variations of the separate composited solvent-treated oxidized alginate hydrogels were characterized and compared using SEM and EDS. Figure 8 shows the SEM images taken for the composited ethanol-treated oxidized alginate hydrogels.

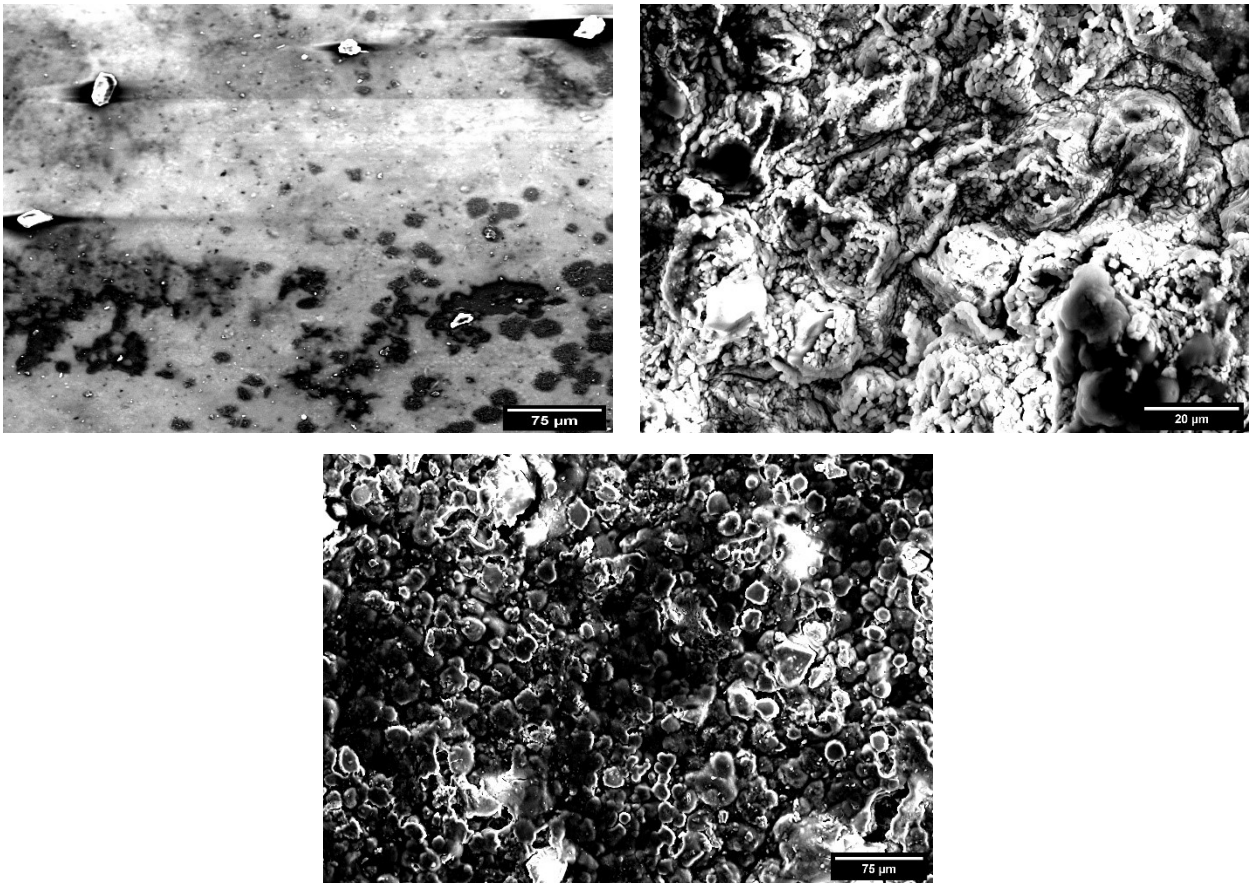

Figure 8: SEM images taken of ethanol-treated oxidized alginate composited with 0.5 wt% (top left), 1.0 wt% (top right), and 2.5 wt% (bottom) 45S5 Bioglass©.

As Figure 8 illustrates, the composited hydrogel consisting of 1.0 wt% 45S5 Bioglass© appears to have the most mechanically and chemically stable structure. Figure A2 provides EDS data to confirm the elemental surface concentrations present in each sample. For further comparison, Figure 9 shows the SEM images taken for the water-treated composited hydrogels.

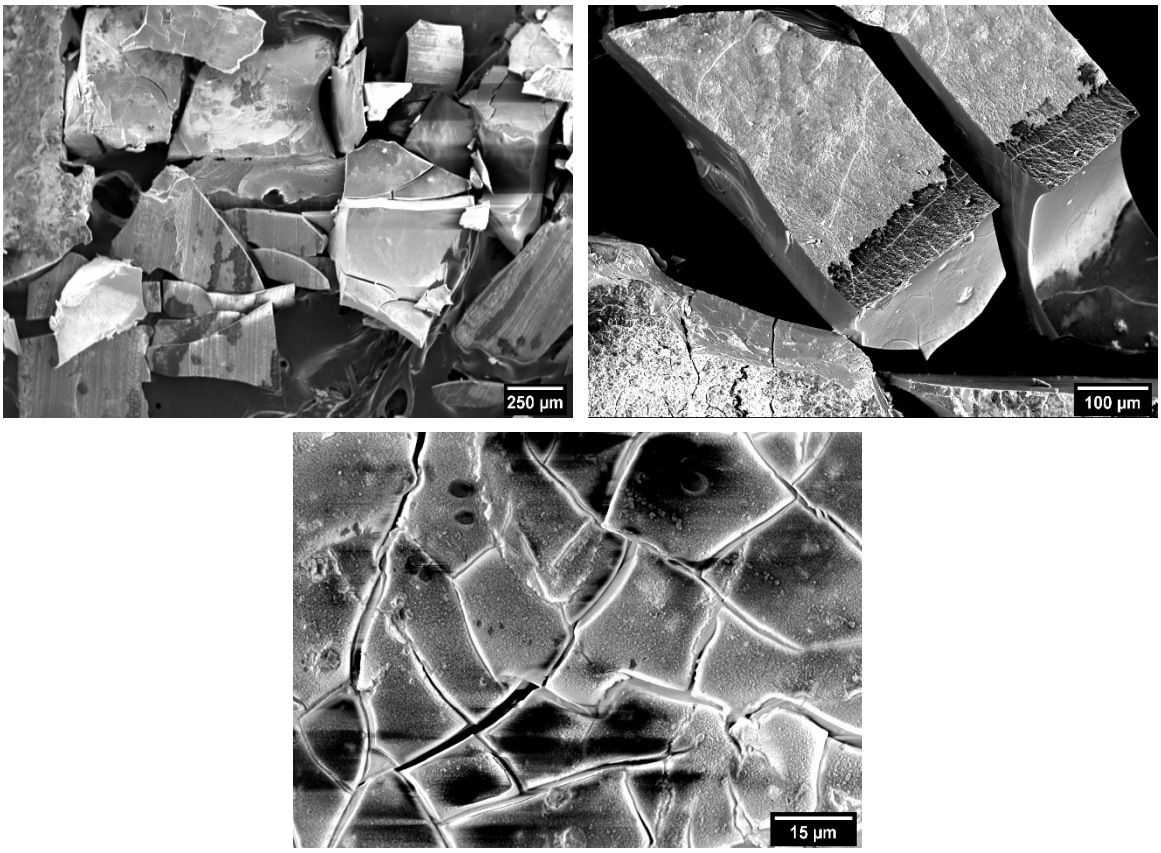

Figure 9: SEM images taken of water-treated oxidized alginate composited with 0.5 wt% (top left), 1.0 wt% (top right), and 2.5 wt% (bottom) 45S5 Bioglass©.

Unlike in Figure 8, the water-treated composited hydrogels did not appear to have significantly different microstructures when glass concentration was changed. For the most part, the resulting microstructures were in a particle form consisting of sharp, jagged grain boundaries. Figure A3 provides additional EDS data to ensure that the elemental surface concentrations in each sample are correct.

C. Compression and Bacterial Testing

As described in the experimental procedure, only ethanol-treated oxidized alginate composites could be tested due to the inability of water-treated samples to form solidified discs. Therefore,

all data related to compression testing will imply ethanol-treated samples. Figure 10 shows the stress-strain curve for oxidized alginate composited with 0.5 wt% Bioglass©.

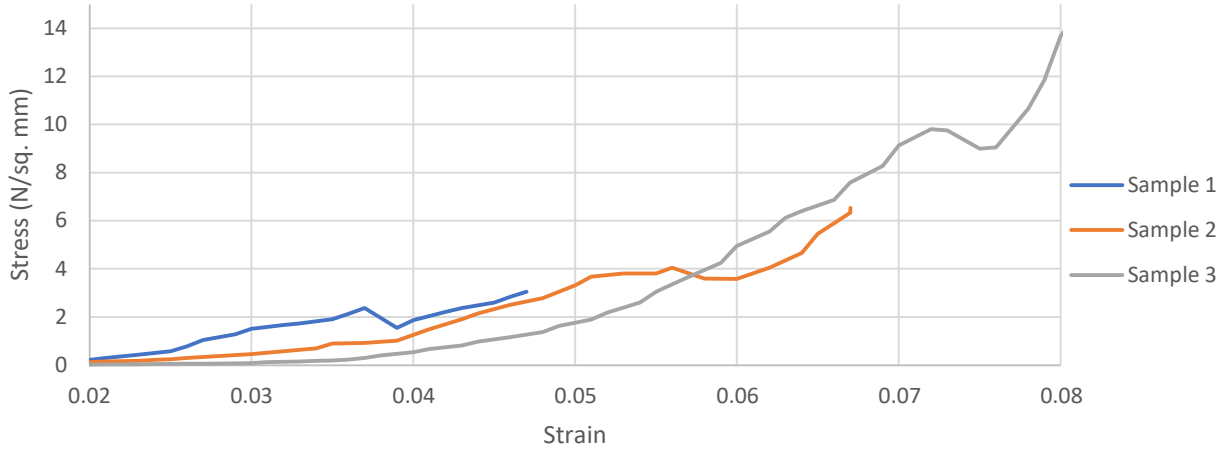

Figure 10: Stress-strain curve for oxidized alginate composited with 0.5 wt% Bioglass©.

As expected, Figure 10 shows weak stress-strain data typically seen in polymeric materials. While there is noticeable variability between samples, the average compression modulus was found to be 0.90 N/mm^2. Meanwhile, Figure 11 shows a similar stress-strain curve for oxidized alginate composited with 1.0 wt% Bioglass©.

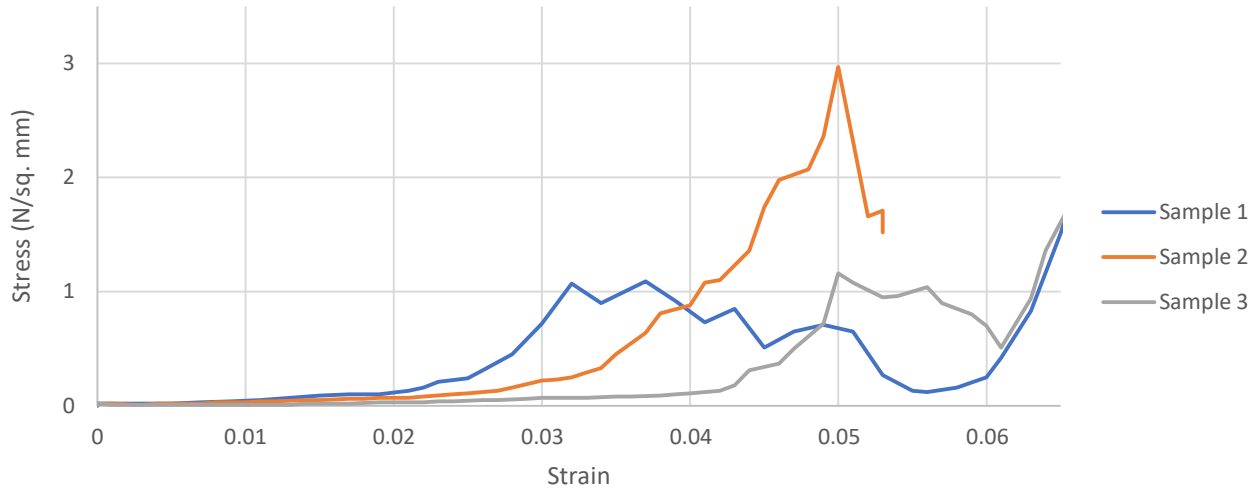

Figure 11: Stress-strain curve for oxidized alginate composited with 1.0 wt% Bioglass©.

Similarly, Figure 11 shows noticeable variation between samples. However, the average elastic compression modulus decreased to 0.39 N/mm^2. Lastly, the stress-strain curve for oxidized alginate composited with 2.5 wt% Bioglass© is shown below.

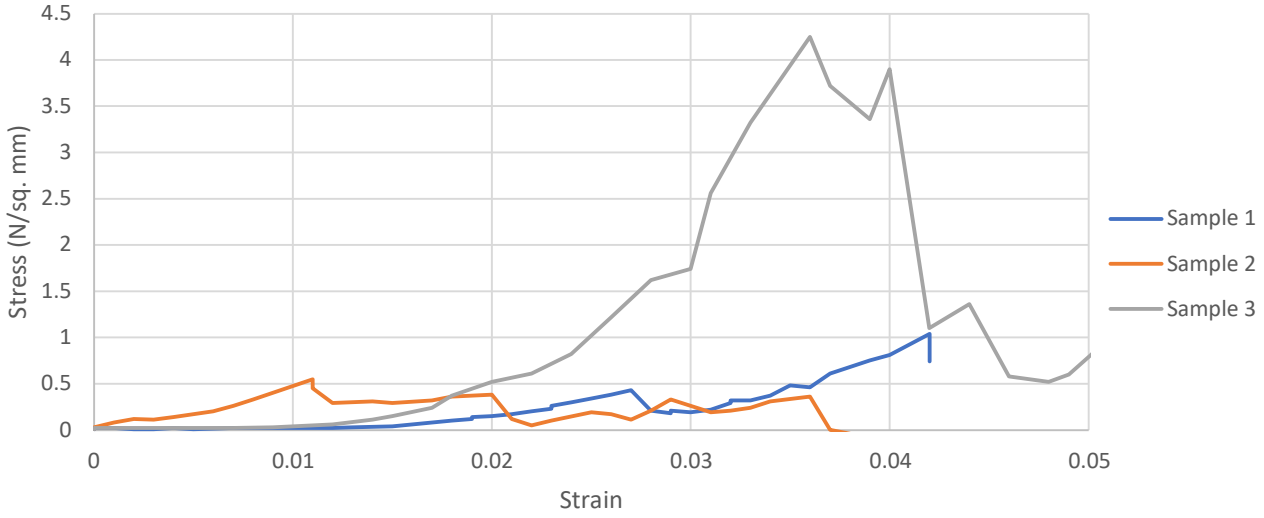

Figure 12: Stress-strain curve for oxidized alginate composited with 2.5 wt% Bioglass©.

Figure 12 shows much more variable elastic compression moduli due to sample 3. When averaged, the compression modulus was 0.70 N/mm^2

Results of the gram positive/negative bacteria tests were not as promising as originally thought. Figure 13 shows the results for ethanol-treated oxidized alginate composites while Figure 14 shows the same tests for water-treated oxidized alginate composites.

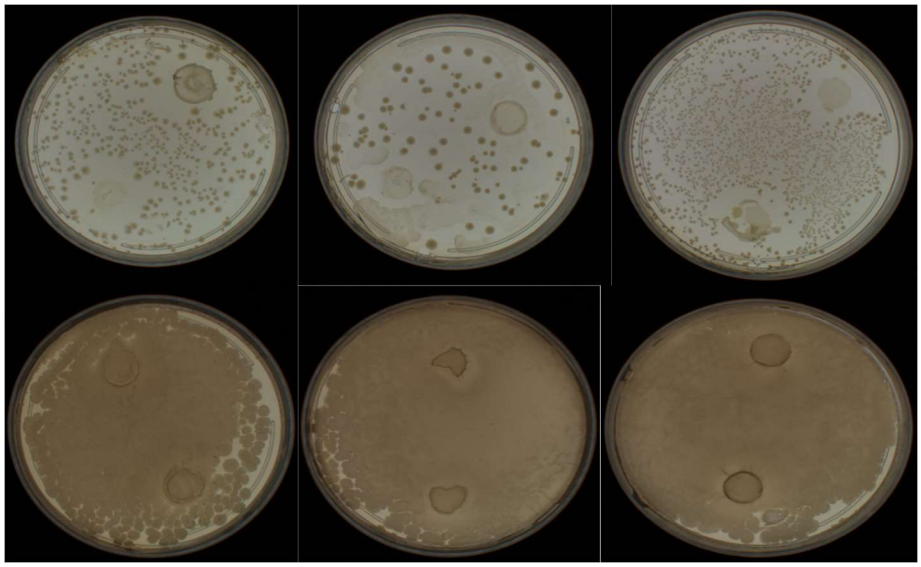

Figure 13: Bacteria tests for ethanol-treated oxidized alginate composites. Shown from left to right is 0.5 wt%, 1.0 wt%, and 2.5 wt% Bioglass© concentrations, respectively. Top row shows samples introduced to *E. coli* while the bottom row shows samples introduced to *S. aureus.*

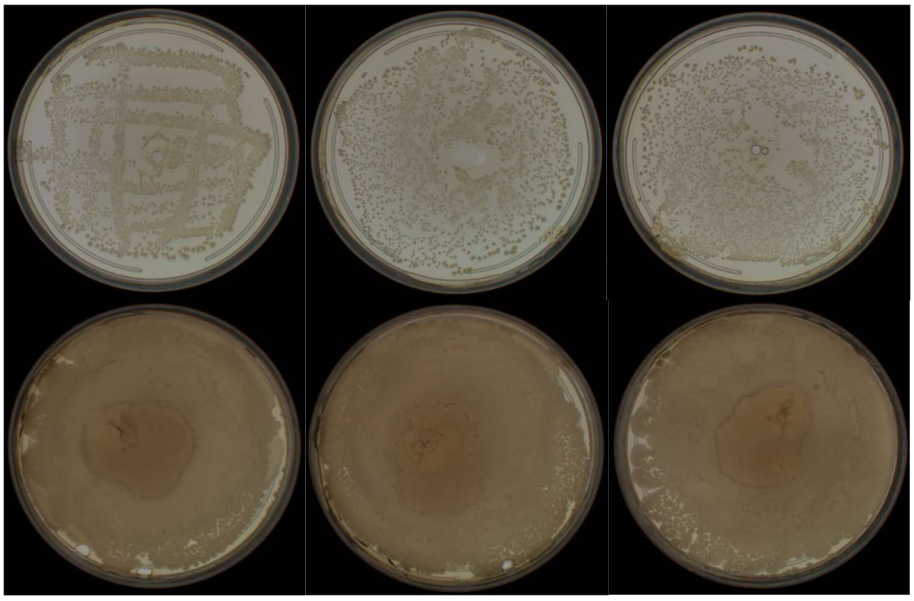

Figure 14: Bacteria tests for water-treated oxidized alginate composites. Shown from left to right is 0.5 wt%, 1.0 wt%, and 2.5 wt% Bioglass© concentrations, respectively. Top row shows samples introduced to *E. coli* while the bottom row shows samples introduced to *S. aureus.*

As shown in Figures 14 and 15, the incorporation of Bioglass©, independent of the solvent used, had no effect on gram positive bacteria such as *S. aureus* in this case. However, small inhibition zones can be seen in both figures with regard to *E. coli*. Figure 15 compares the inhibition zones between samples introduced to *E. coli*.

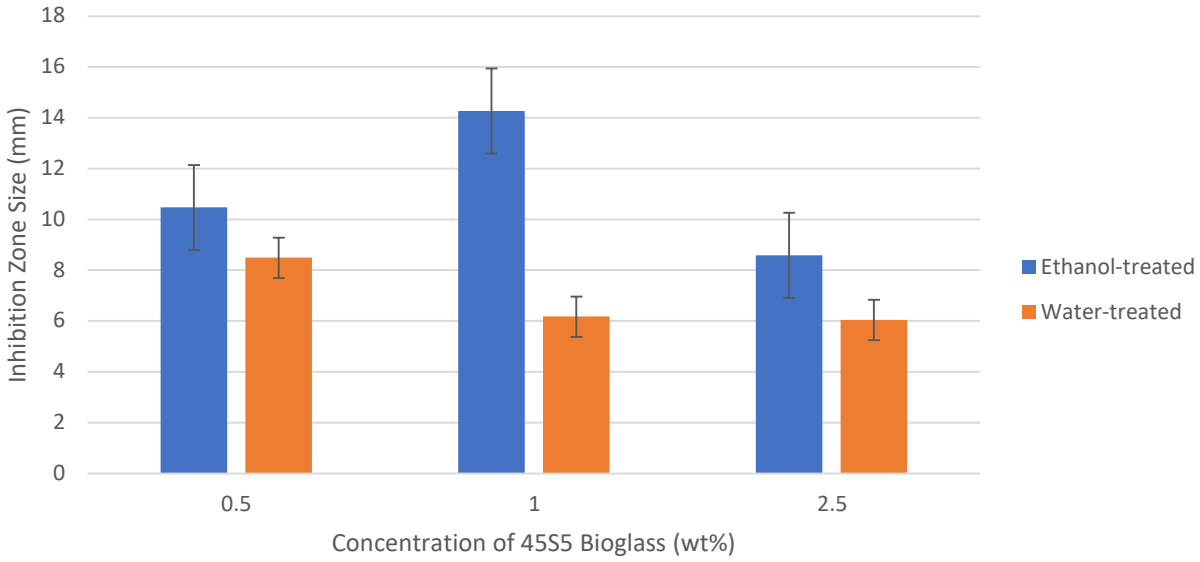

Figure 15: Average inhibition zones of composited hydrogels introduced to *E. coli.*

As shown in Figure 15, it is apparent that ethanol-treated hydrogels had better antimicrobial properties. Interestingly enough, the results of this test show that there is not a direct correlation between the inhibition zone size and the concentration of 45S5 Bioglass©. However, it should be noted that the results obtained for 0.5 wt% and 2.5 wt% 45S5 Bioglass© are not statistically significant.

IV. DISCUSSION

The research presented in this work shows interesting results regarding the properties and characteristics of oxidized alginate composites, especially when comparing the ethanol-treated group to the water-treated group. Based on the results that were obtained, it is obvious that carrying out the oxidation reaction yields much better results when using diluted ethanol as the solvent. The most logical reason behind this pertains to the fact that using a completely aqueous solvent would allow the alginate to gelate and swell too much. This not only decreases the yield and efficiency of the reaction, but also creates an environment that does not allow the alginate solution to mix properly. Ultimately, this creates a more sheet-like structure (Figure 7) that is much more brittle. Additionally, this type of structure makes it difficult for glass particles to adhere or bind to the oxidized alginate. If glass does bind, it mostly likely does not form a strong polymer-glass matrix. To verify the material, Figures A1, A2, and A3 show the EDS data collected using the SEM. While most of the elements and their concentrations match to what is expected, it is worth noting the appearance of silver and chlorine. The appearance of silver can be attributed to the sputter coating to increase conductivity during SEM imaging. However, chlorine should not be present at all. The most likely explanation to this is that the SEM mistook the elemental characteristics of another element for chlorine. Phosphorous and potassium share similar atomic weights, which would both be found in the elemental composition of 45S5 Bioglass©.

Compression testing yielded results that were unexpected. In a normal trend, as the glass concentration is increased, the elastic compression modulus increases as well. In this case, ethanol-treated oxidized alginate composited with 1.0 wt% Bioglass© had the lowest average elastic compression modulus of only 0.39 N/mm^2. The elastic compression modulus is a value

determined by the material's stiffness during elastic stress. Therefore, it would be expected that the lowest concentration of 45S5 Bioglass© would have the lowest elastic compression modulus, since more of the material is a hydrogel. Similarly, it would also be expected that the highest concentration of 45S5 Bioglass© would have the highest elastic compression modulus. Instead, ethanol-treated oxidized alginate composited with 0.5 wt% Bioglass© had the highest value of 0.90 N/mm^2. While this could be a result of having such a low sample size, it could also be a result of reaching optimal saturation.

Regarding bacterial testing, it was notable to see such contrast between gram positive and gram negative bacteria. Other literature has reported that 45S5 Bioglass© has exhibited strong antimicrobial properties against *E. coli* and *S. aureus.*[15] It is possible that the failure of the composited hydrogels to defend against *S. aureus* can be attributed to significant changes in the pH environment, or that the potency of the glass decreased during the compositing process. On a similar note, while the composited hydrogels did show better results for *E. coli*, only one of the groups was significantly different. While the data does seem to skew in favor of ethanol-treated samples, testing must be continued with a higher sample size in order to confirm this.

V. CONCLUSION

Based on the research that has been carried out, ethanol-treated oxidized alginate composites seem much more promising that its water-treated counterparts. Besides its ability to form basic hydrogels, the qualitative and quantitative data collected also favor the ethanol-treated composites. Compression testing showed a minimum elastic compression modulus of 0.39 N/mm^2 for hydrogels containing 1.0 wt% 45S5 Bioglass© and a maximum elastic compression

modulus of 0.90 N/mm^2 for hydrogels containing 0.5 wt% 45S5 Bioglass©. Lastly, ethanol-treated hydrogels exhibited better antimicrobial properties, despite only one group being statistically significant. While additional research is required, the preliminary findings conclude that ethanol-treated oxidized alginate composites do have the potential for hard tissue repair materials.

VI. SUGGESTIONS FOR FUTURE WORK

While the research presented shows interesting results regarding the usage of oxidized alginate composites for biomedical applications, much more research needs to be carried out. As was shown, it is obvious that the solvent used plays a major role in the final material properties and characteristics. A completely different solvent could be used instead, or the ratio of ethanol to water could also be changed. Smaller changes to tweak the resulting properties could also include changing the amount of oxidizing agent, 45S5 Bioglass©, or the viscosity of alginate powder. The smallest changes during polymer synthesis can result in unexpected outcomes. Therefore, it is important to explore many of the variables encountered when creating a polymer composite for biomedical applications.

VII. APPENDIX

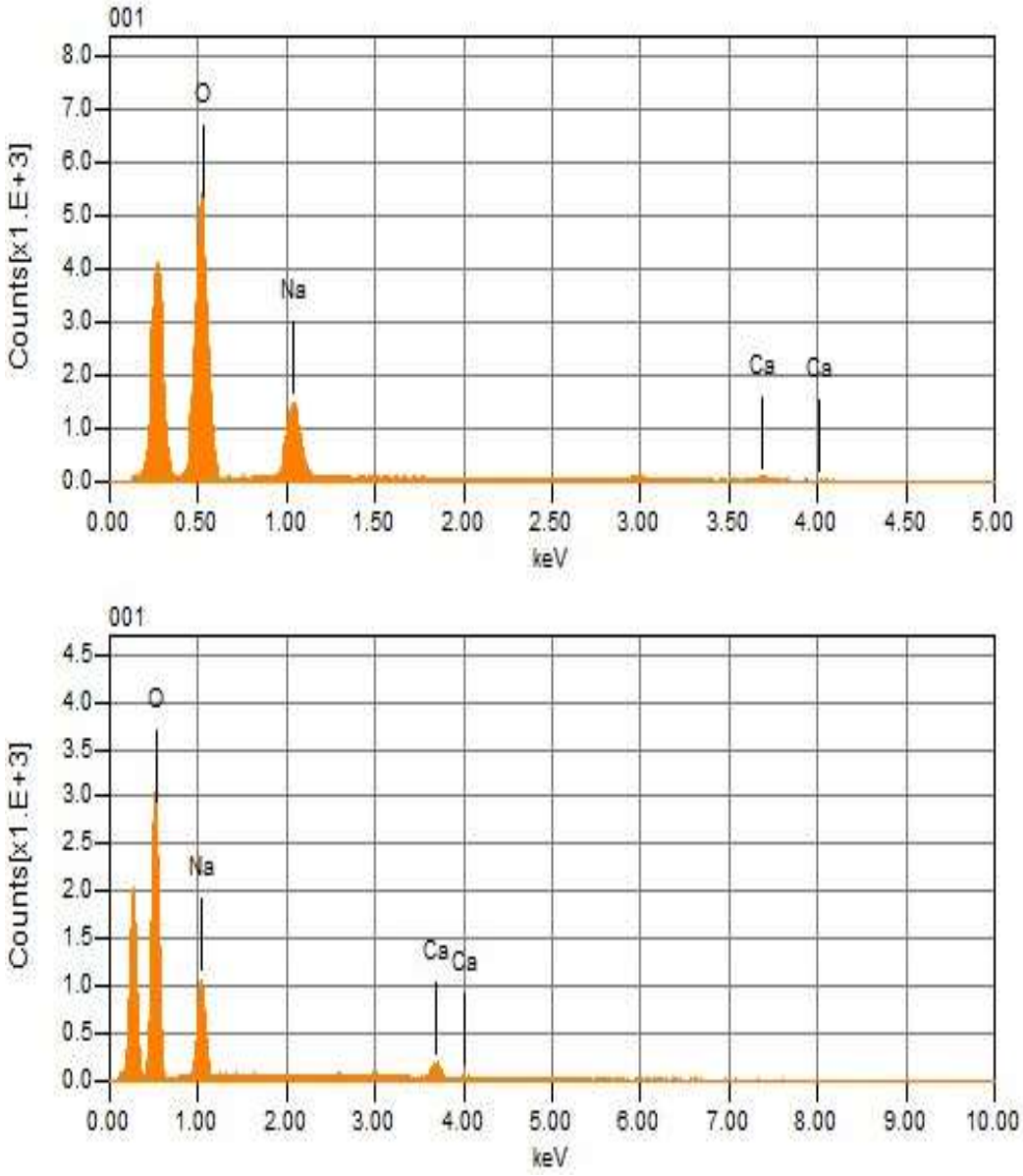

Figure A1: EDS elemental surface analysis of water-treated oxidized alginate (top) and ethanol-treated oxidized alginate (bottom)

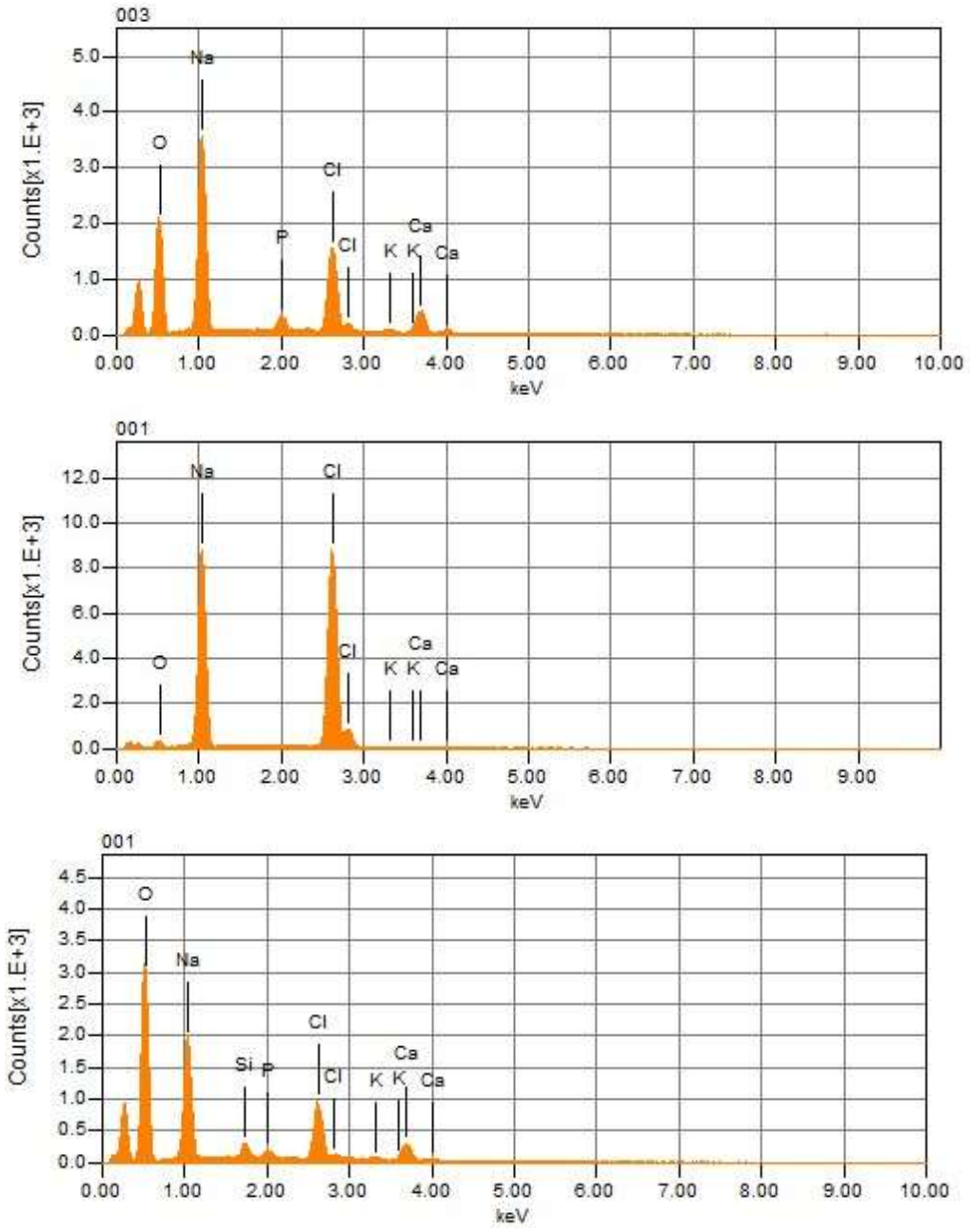

Figure A2: EDS elemental surface analysis of ethanol-treated oxidized alginate with 0.5 wt% Bioglass© (top), 1.0 wt% Bioglass© (middle), and 2.5 wt% Bioglass© (bottom).

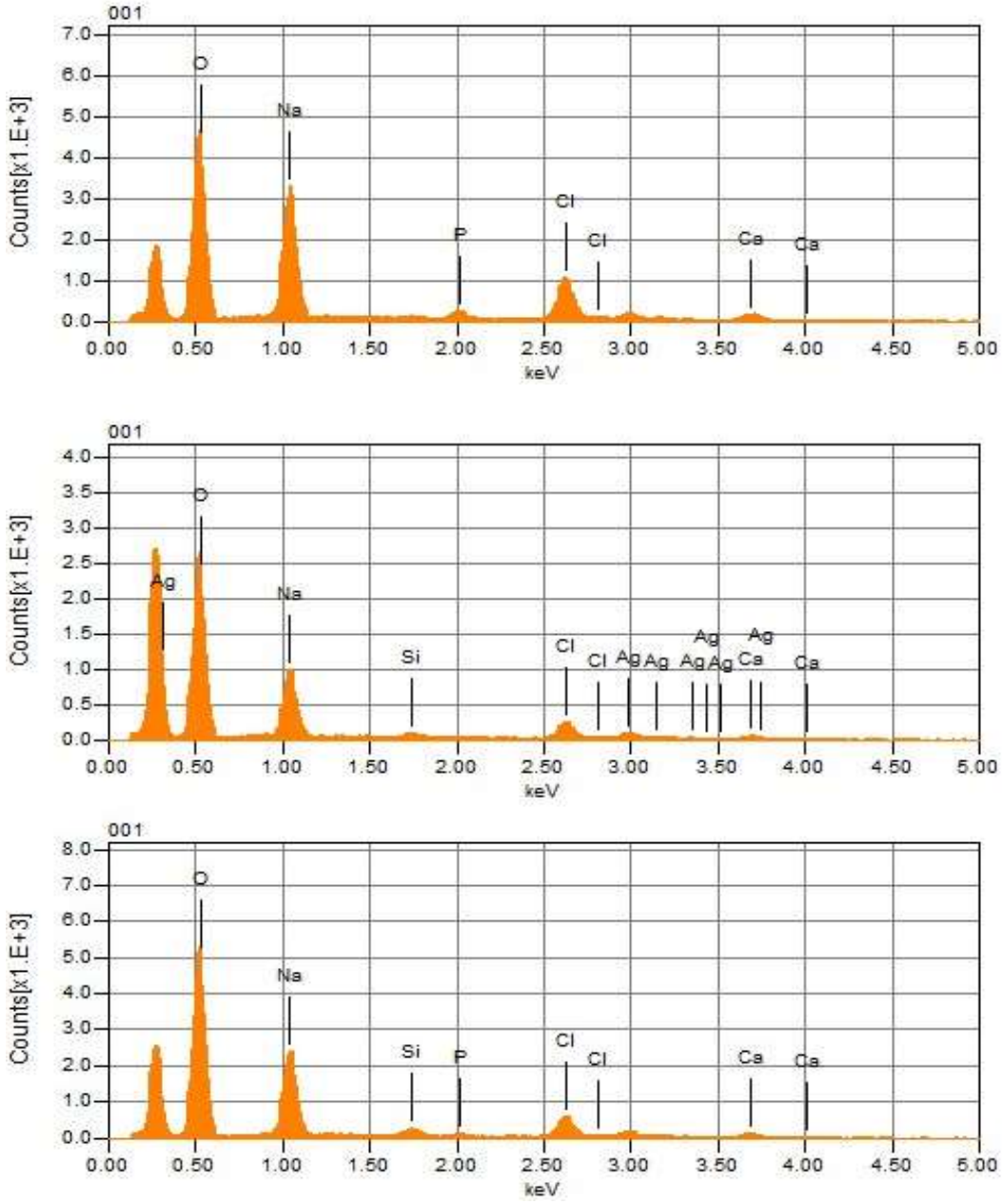

Figure A3: EDS elemental surface analysis of water-treated oxidized alginate with 0.5 wt% Bioglass© (top), 1.0 wt% Bioglass© (middle), and 2.5 wt% Bioglass© (bottom).

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