A Thesis Presented to

The Faculty of Alfred University

Investigation of Swelling and Antibacterial Properties of Hydrogel Composites Containing 45S5 Bioactive Glass

By

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In Partial Fulfillment of

the Requirements for

The Alfred University Honors Program

May 2021

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Acknowledgements

First, I would like to thank my advisor, Dr. Keenan, for providing so much support and encouragement throughout this whole process for my thesis, and my entire academic career so far. I would also like to thank PhD candidate, Sierra Kucko, and graduate student, Dutch Alles for their incredible effort, help, and support, especially when things did not always go as planned. Likewise, thank you to Dr. Wren and Dr. McGowan for being advisors for my honors thesis, on top all of their assistance and encouragement these past four years. Finally, I would like to thank my friends, and especially my family because without them I would not have been able to make it through my undergraduate career without their support and motivation.

Table of Contents

List of Tables

List of Figures

Pages

Abstract

The properties of 5 different compositions of hydrogels were investigated to determine the potential for future medical applications. Each batch consisted of an aqueous agent, which was water, a primary polymer, a secondary polymer, and an additive, which was 45S5 bioglass. The primary polymers used were either gelatin or polyvinyl alcohol (PVA) and the secondary polymers used were cellulose gum or anhydrous borax. The first of two tests used to investigate the properties of the hydrogels was swelling studies. Samples from each batch were placed in phosphate buffered saline (PBS) and left to incubate overnight. After 24 hours, the samples were observed and batches 1 and 2 had noticeable swelling while samples from batches 3, 4, and 5 had dissolved. The following day observations were made again and batches 1 and 2 had started to dissolve as well. Bacterial testing was then conducted with 3 samples for each batch being placed in LB agar and tested against *Escherichia coli* (*E. coli*). Batches 3, 4, and 5 all had varying sizes of inhibition zones and batches 1 and 2 showed no signs of inhibition of bacterial growth. Batches 3, 4, and 5 all had anhydrous borax as the secondary polymer while batches 1 and 2 had cellulose gum.

Introduction

Personal Statement

I found this thesis and research experiment to be very interesting and a valuable experience. This past summer I was accepted into a Research Experience for Undergrads and was very excited to be getting valuable lab research experience, however, due to Covid-19, the program was cancelled. Working on my thesis was basically my first time in a lab working on biomaterials research on my own, with the guidance of my advisor. I want to go into a career of research, so getting the chance to finally get some experience was very exciting.

I decided to research hydrogel-glass composites and asses their potential as a biomaterial for superficial wound repair. I investigated the swelling and antibacterial properties of 5 different compositions of hydrogels, seen in Table 1 in Materials and Methods. From experimentation and research, discussed in depth throughout the paper, I found that batch 5, containing polyvinyl alcohol (PVA), anhydrous borax, and 45S5 Bioglass had the most potential as a dressing for wound repair. This was due to its antibacterial activity and rigidity as a hydrogel. It dissolved within 24 hours in the swell studies, but that is not necessarily a bad thing given that it ideally would be used for superficial wound repair.

I think the most exciting part of this experience was getting inhibition zones with three of the batches of hydrogels, indicating that those three batches did have antibacterial properties. It was interesting to set the bacterial plates up and see successful results the following day, especially after a project that things did not always go as planned.

This research, and honestly this entire year, taught me how to be flexible and to make the best of what I have to work with. With the swell study, I expected the hydrogels to last up to 5-7

days in solution and to get 5-7 days' worth of data, so to see three of the batches totally dissolved within 24 hours and the other two batches had dissolved as well. On top of that, I had planned on doing ion release testing, to see what ions where release from the hydrogels and at what concentrations, however, out Inductively Coupled Plasma (ICP) machine, used to measure ions and elements in solution, was not properly working at the time, so this testing could not be conducted.

Even though things did not go as planned, I was still very thankful for what I was able to do in the lab and the results that I did get. Batch 5 showed great potential in just this preliminary research and I believe it is a good start to further research for a dressing for superficial wound repair. Since I plan on returning for graduate school at Alfred for Biomaterials Engineering as well, so this is something that I would like to continue researching and hopefully at the end of the next two years have a composition with the ideal characteristics to be able to produce an effective and efficient wound dressing. I would like to be able to do ion release testing, if the ICP is functioning properly again, do cell viability testing, to see how the hydrogels react with living cells, and other testing to investigate the structure and interactions of the components of the hydrogels on a molecular level.

Hydrogels

Hydrogels have been a big interest to biomaterial scientist for many years. The work of Wichterle and Lim in 1960 on crosslinked hydroxyethyl methacrylate hydrogels pioneered the research into hydrogels [1]. Hydrogels have a hydrophilic character and have the potential to be very biocompatible, which results in them being an ideal material for certain medical applications, especially superficial wound repair. Their hydrophilicity provides a suitable semiwet and three-dimensional environment that is good for molecular-level biological interactions. They also have antifouling properties, meaning that they provide an inert surface that does not allow the nonspecific absorption of proteins [2].

Hydrogels are made of a hydrophilic polymer network that can absorb 10-20% to thousands of times their dry weight in water [1]. The network made up of polymers can consist of synthetic, such as polyvinyl alcohol (PVA) or anhydrous borax, or naturally derived polymers, such as gelatin or cellulose. These hydrogels can be engineered to be chemically stable over a long period of time or to degrade, disintegrate, and dissolve over a specified amount of time as well [1].

Gelatin

Gelatin is a naturally derived polymer that is water soluble at 37°C, does not produce an immune response, and can react as both a base and an acid [3]. Gelatin forms high mechanical hydrogels and is a very viscous polymer solution that is fast to biodegrade [5]. Gelatin-based hydrogels are commonly used for contact lenses, matrices for tissue engineering, and drug delivery systems and new applications are currently being researched, such as restoring function

to intervertebral discs [3]. The mechanical and chemical properties of gelatin can also be modified by using different crosslinking agents.

PVA

PVA is one of the oldest synthetic polymers and one of the most frequently used. This is due to its ideal biocompatibility and it has been used for many biomedical applications, including wound dressing, wound management, drug delivery systems, artificial organs, and contact lenses[5]. One issue with PVA is that it does not have good elasticity due to a stiff membrane and has limited hydrophilicity. This lowers its potential when it alone is exclusively being used as a polymer for wound dressing [5]. However, PVA mixed with polysaccharides and other synthetic polymers have shown great potential for wound dressings, among other applications, because they are abundant, easily derived or modified, and frequently biocompatible [5].

Cellulose

Cellulose is the most abundant renewable resource on the earth currently. It can be found in plants and natural fibers such as cotton and linen [7]. It contains many hydroxyl groups which can be utilized to easily make hydrogels with different structures and properties [6]. Cellulose possesses excellent biocompatibility which has resulted in this polymer being widely used in biomedical applications [7]. Naturally derived cellulose on its own is not water soluble, however, cellulose-based hydrogels can be formed by properly crosslinking of cellulose ethers [7].

Anhydrous Borax

Borax contains boron, which plays a large role in the success of a recent preclinical level biomaterial designed for the treatment and recovery of muscle injuries. It is boron-loaded alginate hydrogel in which when the boron is released, it stimulates integrin proteins in the body, which then generates a correct formation of tissues [8].

Borax can also be used as a crosslinker with PVA in hydrogels. As previously mentioned, PVA can absorb water, but at the expense of its stability and mechanical properties, however, introducing borax as a crosslinker into the hydrogel composition can greatly improve the malleability of the hydrogel [9].

45S5 Glass

45S5 bioglass consists of 45% SiO_2, 24.5% CaO, 24.5% Na_2O and 6% P_2O_5 by weight percent. 45S5 bioglass has excellent bioactivity, able to deliver cells, and has controllable bioactivity [10]. These properties make bioglass ideal for tissue engineering. The glass release certain concentrations of soluble Si and Ca cations that dissolve in physiological fluids which promote tissue growth and vascularization [11]. 45S5 bioglass with a high specific surface area also has antimicrobial properties, and can chemically bond with soft tissues, which help efficiently and effectively accelerate the healing process [11].

Skin

Skin is the main exterior defense system that protects the body from such things as pathogen attacks, contaminations, infection, and the external environment. It also plays a vital role in maintaining homeostasis of the body be regulating body temperature and transmitting signals about the external environment, such as pain and heat. The skin can cover an area of 2 m^2 and it accounts for more than 10% of the total weight of an adult human [4].

Skin is made up of three different layers, the epidermis, the dermis, and the subcutaneous layer. The epidermis is responsible for the main barrier and providing protection to penetration and external invasion [4]. The dermis is in between the epidermis and the subcutaneous layers and is made up of a matrix of connective tissues which provide the skin wit elasticity and structure to be resistant to deformations. It also contains the majority of the blood vessels, which provide skin with oxygen and nutrients to survive, grow, and heal. The subcutaneous layer contains fat tissue that helps provide thermal isolation and mechanical protection to the body [4].

Wound Repair

A wound is defined as a break or defect in the skin due to physiochemical or thermal damage. Wound classification can be divided into acute and chronic. Acute wounds are from damage to the skin that can heal over a period of 8-12 weeks and are usually such things as burns or chemical injuries. Chronic wounds are wounds that need a long period of time, up to months, to heal and can leave serious scars. Some factors can affect chronic wound repair, such as diabetes, wound dryness, and infections.

Wound repair can be enhanced and helped with wound dressings. An ideal wound dressing is something that is wet due to a wet environment is best for healing with minimum

scarring or inflammation. A dressing with a high water content and permeable also allows for nutrients and oxygen to permeate through to the surface is ideal. Overall, an ideal wound dressing should be able to keep the wound site moist, permeable to gases, remove excess fluids, protect the wound from pathogens, infections, and contamination, stimulate growth, provide mechanical protection, are comfortable and easily removed and changed, biocompatible, and can help reduce pain [4].

Materials and Methods

Synthesis

5 different compositions of the hydrogel composites were made. The different compositions can be seen in Table I.

Table II. Compositions of the 5 batches of hydrogel composites.

Batch	Aqueous Agent	Primary Polymer	Secondary Polymer	Additive
Batch 1	25 mL of water	3g of gelatin	1.5g of cellulose gum	0.25g of 45S5 glass
Batch 2	25 mL of water	3g of gelatin	3g of cellulose gum	0.25g of 45S5 glass
Batch 3	25 mL of water	3g of gelatin	1.5g of anhydrous borax	0.25g of 45S5 glass
Batch 4	25 mL of water	3g gelatin	3g of anhydrous borax	0.25g of 45S5 glass
Batch 5	25 mL of water	3g of PVA	1.5g of anhydrous borax	0.25g of 45S5 glass

The 45S5 glass had to be ground down to a smaller size and were filtered to 25 uM in size. From there, the masses of each powder needed for each batch were measured out.

25 mL of water was placed in a beaker and then heated for 5 minutes. After 5 minutes the primary polymer, secondary polymer and then the 45S5 glass were added, in that order for every

batch. Once all the components were added they were mixed for 5 more minutes and then poured into a well plate and placed in the fridge to set overnight.

Swell Studies

3 different samples were tested for each batch for the swell studies. Each sample was first weighed, and the mass was recorded for an initial measurement. The samples were then put in their own petri dishes along with 10 mL of phosphate buffered saline (PBS). They were then placed in the Fisher Scientific incubator at 37.1°C for 24 hours. After 24 hours the samples were taken out and observed. If the hydrogels were not too dissolved, they were removed from solution and weighed, and their masses were recorded. This process continued every 24 hours until all the samples were dissolved.

The normalized change in weight percentage was then calculated for the batches that swelled and was plotted on a graph.

Bacterial Studies

20mL of LB agar was poured into 5 petri dishes and let to set and solidify. While the plates were setting, a diluted 5% solution of *E. coli* was prepared. This was done in a centrifuge tube with 950 uL of deionized (DI) water and 50 uL of concentrated stock solution of *E. coli* and the solution was vortexed for 30 seconds to mix.

Once the plates with the LB agar were set, three 8mm holes were removed in a triangular pattern from the agar from one plate and 3 samples from batch 1 were placed in the holes. It was

then covered with 5 mL of agar to create a thin layer over the samples. This process was repeated for the remaining plates and batches. The diluted bacteria solution was then used to coat the agar on each plate. Once an even coating was applied, the plates were placed in an incubator for 24 hours at 80°C and then moved to a fridge.

The plates were then observed and those with inhibition zones were measured. The inhibition zones were measured using ImageJ software. The inhibition zone was then calculated by subtracting the disc diameter from the total inhibition diameter. The average inhibition zones of the three samples for all 5 batches were then calculated and graphed.

Results

Swell Study

The swelling of the 5 different batches was examined after 24 hours of being incubated in PBS and figure 1, below, shows the results after the first day. Batches 1 and 2 swelled after 24 hours while batches 3 and 4 totally dissolved and batch 5 partially dissolved as well.

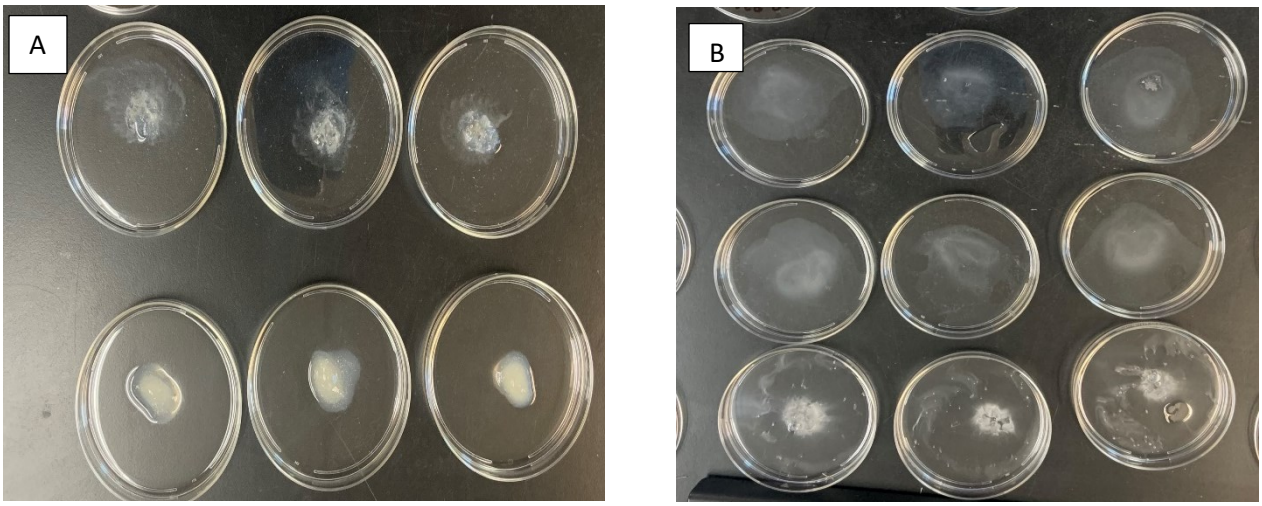

Figure 1. Hydrogels after 24hrs of incubating in PBS. A. Batch 1 is in the top row and batch 2 is the bottom row. B. Batch 3 is the top row, batch 4 is the middle row, and batch 5 is the bottom row.

Since samples from batches 1 and 2 swelled, the samples were removed from the solution and weighed, and their masses were recorded. Batch 1 was dissolved within 48 hours of the initial set up and Batch 2 was dissolved within 72 hours. With the data collected, the normalized change in weight percentage was calculated. The change was calculated using equation 1,

$$\Delta Wt\% = \frac{Day\ X\ Measurement}{Day\ 0\ Measurement} \text{ x } 100 \qquad \text{Equation 1}$$

where day X measurement is the mass measurement from that day and day 0 measurement is the initial measurement before it was placed in the PBS. The results can be seen below in figure 2.

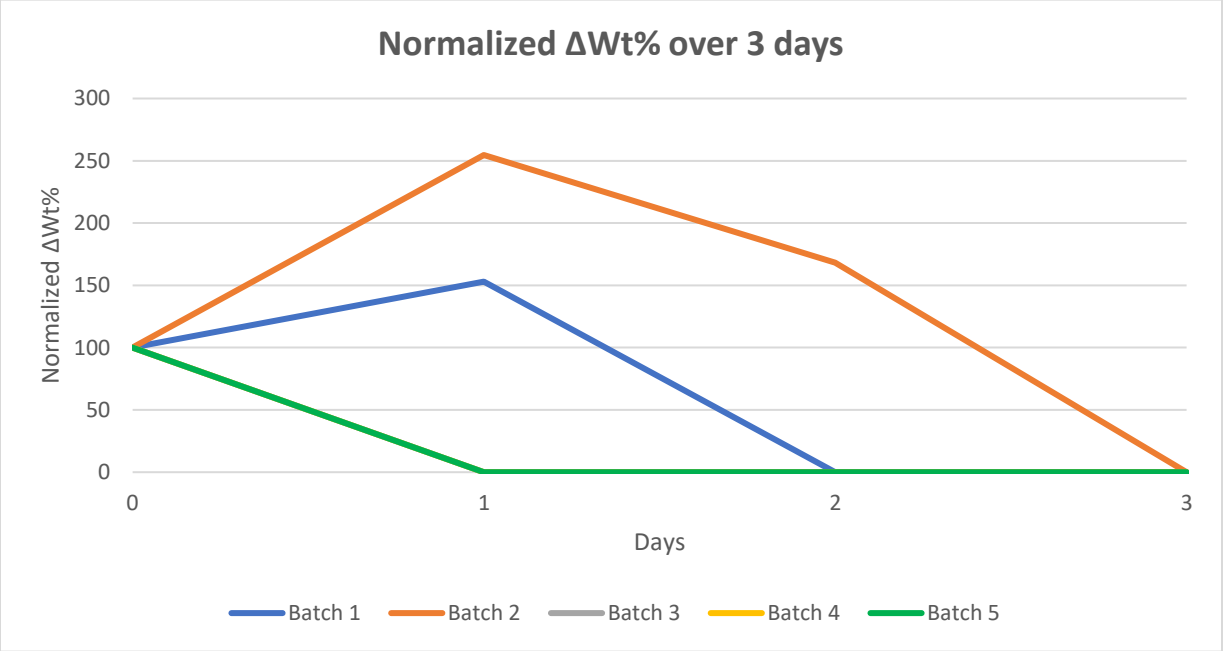

Figure 2. Normalized ΔWt% of the 5 batches over a 3-day period.

Bacterial Study

After swelling studies were conducted, the antibacterial properties of the hydrogels were investigated. Figure 4, below, shows the bacterial plates of batches 1-5. Batches 1 and 2 do not show any inhibition zones, but batches 3, 4, and 5 all had noticeable and measurable inhibition zones.

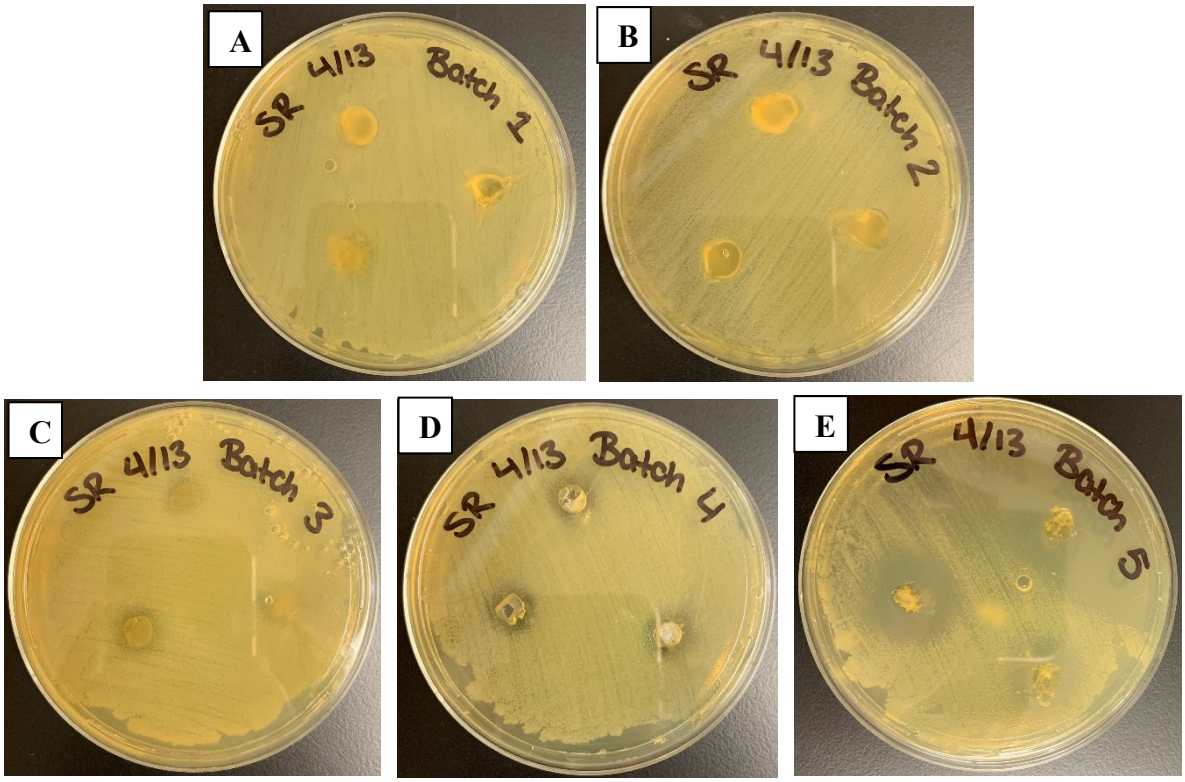

Figure 3. Bacterial plate results for all batches. A. Batch 1 with no inhibition zones. B. Batch 2 with no inhibition zones. C. Batch 3 with inhibition zones. D. Batch 4 with inhibition zones. E. Batch 5 with inhibition zones.

The inhibition zones of batches 3, 4, and 5 were measured and calculated using equation 2, below.

Inhibition Zone = Total Inhibition Diameter – Disc Diameter Equation 2

After the inhibition zones were calculated, the results were graphed and are shown in figure 5.

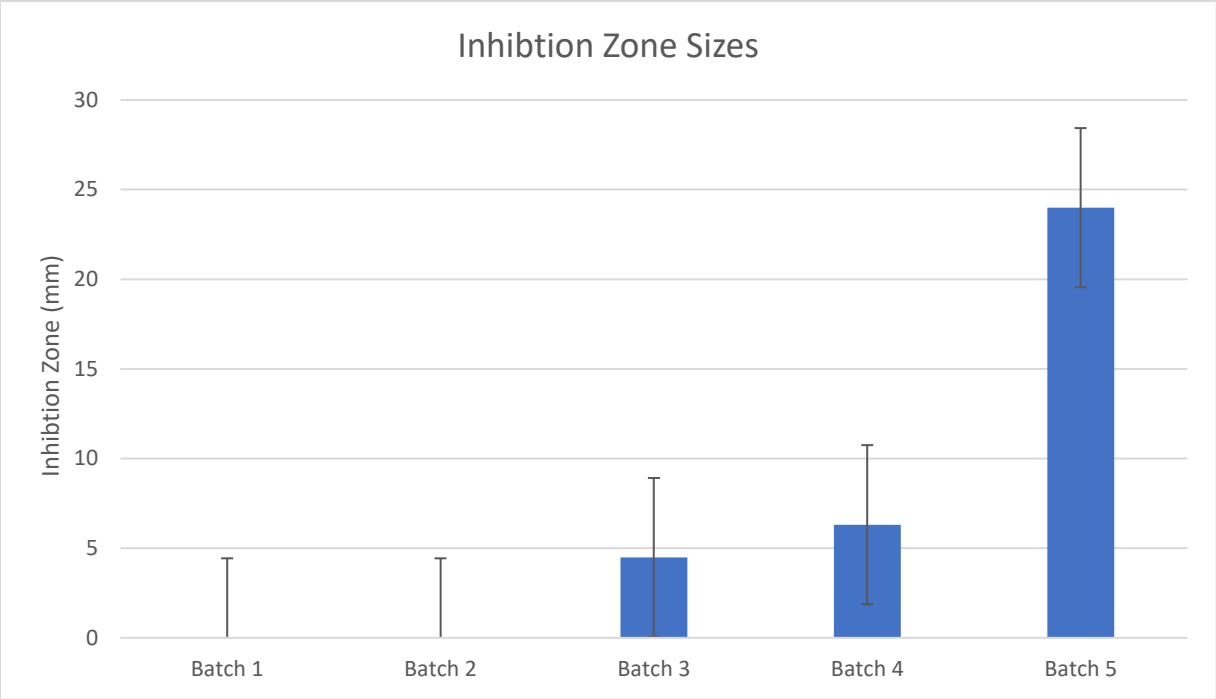

Figure 4. Inhibition zones for batches 1-5. Batch 3 had a standard deviation of 1.11, batch 2 of 0.95 and batch 3 of 13.08.

Batch 3 had an average inhibition zone of 4.48 cm ± 1.11cm, batch 4 had an average of 6.31 cm ± 0.95 cm, while batch 5 had an average of 23.99 cm ± 13.08 cm.

Discussion

Swell Study

Batches 1 and 2 had measurable swelling while batches 3, 4, and 5 dissolved in the PBS solution within 24 hours. The difference between the batches that swelled and the batches that immediately dissolves is that batches 1 and 2 contained cellulose as the secondary polymer while batches 3, 4, and 5 contained anhydrous borax as the secondary polymer. Cellulose is most likely the factor that resulted in batches swelling for one or two days and anhydrous borax is most likely responsible for the batches that dissolved withing 24 hours.

Cellulose is a naturally derived polymer which is composed of plenty of hydrophilic functional groups, including hydroxyl, carboxyl and aldehyde groups [11]. These functional groups result in numerous intermolecular and intramolecular hydrogen bonds in cellulose which, when used in a hydrogel, proved excellent mechanical performance and structure to the material[11]. Cellulose-based hydrogels have a 3D network that gives it its hydrophilic nature that allows the hydrogel to be able to swell by tens to hundreds of times in volume after absorbing large amounts of water [11].

As for the borax, it has been shown in previous studies to decrease swelling compared to samples that have not been loaded with borax [13]. In a study by Tantiwatcharothai S., et. al., their hydrogel loaded with borax was found to decrease swelling but improve the dimensional stability. This was due to the crosslinking between hydroxyl groups in basil seed gum used and the borax, which lead to a decrease in the hydroxyl groups available to interact with water [13]. It

has also been seen that hydrogels cross-linked with borax degrade rapidly in the initial time of the studies, which was seen within the first 24 hours with batches 3, 4, and 5 [14].

Bacterial Study

In the bacterial plates, batches 1 and 2 did not produce any inhibition zones, while batches 3, 4, and 5 did. As with the swell studies, it is thought that the secondary polymer plays a large role in this result. Batches 1 and 2 contain cellulose while batches 3, 4, and 5 contain anhydrous borax. Batch 5 also had an average inhibition zone of almost 4 times larger than either of the other inhibition zones, and this is most likely due to the PVA in the composition as well.

In a study by Cencetti C, et. al. the antimicrobial properties of silver-doped hydrogels crosslinked with PVA and borax produced similar results. The samples had high antimicrobial activity, however, it seemed to be independent of the silver content. All samples had similar silver content, just as batches 1-5 all had the same 45S5 Bioglass content, however, the ones with borax crosslinking, and especially the one with a high molecular weight of PVA seemed to enhance the antimicrobial activity [12]. To investigate it further, the samples were tested on their own, without a silver additive, and it was found that the samples did not show any antimicrobial activity [12]. This indicates that the borax and the PVA and borax compositions do not possess antimicrobial activity, however they enhance the microbial activity of the additive, which in this study was the 45S5 glass. The borax and gelatin composition for batches 3 and 4 relied on the borax to enhance the activity slightly while batch 5 utilized both the PVA and borax to enhance the activity of the bioglass significantly.

Future Work

Further research would ideally include ion release studies using the ICP and cell viability studies. The ion release would indicate what was released in solution during the swell studies and could give more accurate data about what caused or did not cause antibacterial activity in the hydrogel. This would also indicate which ions would most likely be released into the body if used for a wound dressing, and at which concentrations. That could be researched further to see if those concentrations are within safe levels for the body.

Cell viability studies would also give a better indication about which hydrogel composite is ideal for wound dressing. A hydrogel composition that enhanced and improved cell growth would be desired. This study would also be able to indicate which compositions should not be researched further if it harms and potentially kills living cells.

Conclusion

Batches 1 and 2 had the most ideal swelling and batches 3, 4, and 5 had ideal antibacterial activity with batch 5 having the largest inhibition zones. More research needs to be done to determine which composition is the best for wound dressing, however, right now Batch 5, composed of 25 mL of water, 3 g of PVA, 1.5 g of anhydrous borax, and 0.25 g of 45S5 Bioglass seems to show the most potential for a superficial wound dressing. This is due to its inhibition zone of 23.99 cm ± 13.08 cm, indicating that it helps enhance the antibacterial activity of the 45S5 Bioglass the best out of all the compositions. While in the swell study for this experiment, batch 5 dissolved within 24 hours, other research has indicated that borax actually improves structural stability and does not dissolve or degrade as quickly, so it can be further research with different concentrations to find a composition that holds its structure for longer.

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