Research Article

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Anti-biofilm activity of two novel, borate based, bioactive glass wound dressings

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Abstract: Bioactive glass was first developed in the late 1960's as a compound that would facilitate bone re-growth. In more recent years, this technology has been used to promote wound healing through its ability to stimulate soft tissue growth, angiogenesis, reduce inflammation, and prevent infection. Chronically infected wounds, which result from biofilm formation, affect millions of patients in the United States each year and cost billions of dollars to treat. The present studies demonstrate exposure of pre-formed biofilms to bioactive glass, under simulated body conditions, resulted in significant reduction in bacterial load. Additionally, specific therapeutically active metal ions such as copper and zinc were added to a borate bioactive glass formulation through a process of ion doping and found to further enhance the anti-biofilm activity. Based on the present findings, the antibiofilm agents released by borate bioactive glasses may prove effective to eradicate the biofilm infections that prevent healing in patients with chronic wounds.

Keywords: Biofilm, Bioactive Glass, Minimum Biofilm Eradication Concentration, MBEC Assay®, Chronic Wounds, Copper, Zinc, Borate Bioactive Glass

1 Introduction

Chronic wounds, which are largely thought to be caused at least in part a result of biofilm infection, affect millions of people in the United States and cost tens of billions of dollars to treat each year [1]. Wound healing is a complex process, including four main stages: haemostasis, inflam-

mation, proliferation and tissue remodelling [2]. When wounds become infected, the immune systems attempt to clear the infection and induce a hyper-inflammatory response, which disrupts the wound healing process [1, 3]. During an acute infection, the hyper-inflammatory state helps to clear the infection, allowing the healing process to proceed. However, biofilm infections are resistant to attacks from the immune system, and are able to persist despite this hyper-inflammatory state [1], resulting in the formation of chronic wounds. Biofilms are a sessile population of cells embedded within a self-produced extracellular matrix composed of DNA, protein and polysaccharide [4]. The biofilm matrix protects the organisms against a wide variety of antimicrobial mechanisms which could be natural via cellular intervention, or clinician induced by desiccation, oxidation or other biocide, antibiotics, metallic cations, or radiation [4].

In addition to being resistant to attacks from the immune system, it has been well established that microorganisms growing in biofilms are 100-1000 times more resistant to antimicrobial agents [5], which can result in the failure of antibiotic treatment to resolve chronic infections [1, 6]. As a result, while many commercially available wound dressings and topical treatments demonstrate anti-bacterial activity, they are often unable to successfully treat biofilm infected wounds [3, 6]. Traditional treatment strategies for chronically infected wounds include antibiotic therapies, and frequent debridement to remove biofilm mass; however, infections that persist despite these therapies often require surgical intervention such as debridement, or possibly amputation to be completely resolved [7]. Improved options to prevent and/or treat chronic biofilm infections could save billions of dollars in related health care costs, and dramatically improve patient outcomes [1].

Bioactive glass was developed in the late 1960's as a material that would bond to bone and promote healing [2]. Bioactive glasses are biocompatible water soluble materials that release their ionic constituents when placed in body fluids. Ions such as calcium and phosphorus react to form a calcium apatite material similar to the hydroxyapatite component of hard tissues such as bone and teeth in

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a basic pH environment that is beneficial for hard tissue formation [8, 9].

In recent years, there has been increasing interest in using bioactive glasses for soft tissue wound repair [9]. In soft tissue, such as the skin, the reduced pH prevents the formation of the calcium apatite material [10]. Instead, the free calcium promotes chemotaxis as a cell sensing receptor and recruiter to aid in the healing process [11]. In-vitro analysis of 45S5, 13-93B3, and GL1605 were studied in static and dynamic flow models where it was shown that the borate glasses (13-93B3 and GL1605) had higher ion release (weight loss) when compared to 45S5 [12, 13]. Ion release, especially alkali ions that increase the local pH may be beneficial to soft tissue healing. Increasing pH in soft tissue has been hypothesized to increase metabolic activity and proliferation rate of mammalian cells which could lead to faster healing [14].

Copper and zinc were selected as trace element additions to the GL1605 glass because they are co-factors to several biological processes necessary for tissue regeneration [15]. Implants composed of bioactive borate glass doped with copper, and copper + zinc, statistically increased angiogenesis over the 13-93B3 glass during a rat skin in-vivo evaluation [9, 16]. In addition to their inherent healing properties, bioactive glass doped with positively charged metal cations have been shown to provide antimicrobial properties, with copper and zinc having significant effect [2, 16, 17]. In addition to copper and zinc, other cations such as silver, cerium, and gallium have been studied as trace ions in bioactive glass for possible applications as antimicrobial agents [18].

Borate bioactive glasses doped with copper and made into fibers were studied in-vitro and in-vivo for applications in wound healing [19]. Cultures containing the supernates of borate bioactive glasses with Human Umbilical Vein Endothelial Cells (HUVEC) promoted cell migration, production of Vascular Endothelial Growth Factor (VEGF), endothelial tube formation [19]. In-vitro nerve cell studies on the 13-93B3 glass fibers showed that the bioactive borate glass increased the percentage of live neurons, suggesting a neuroprotective effect and prevention of overproliferation of cells (fibroblasts and glia) that can lead to fibrous scar tissues [20]. Aligned glass rods contained within a fibrin scaffold also promoted directionally oriented neurite growth which could have applications in nerve regeneration [20].

Several mechanisms for antimicrobial response of bioactive glass have been proposed [21–23] and they include increased pH, osmotic pressure, physical damage caused by cutting or piercing of organism cell wall, or the ionic composition of the local environment. However,

most of the previous work detailing antimicrobial activity for bioactive glasses focused on planktonic bacteria, or the inhibition of biofilm formation [24, 25]. Studies demonstrating effectiveness against pre-formed biofilms have been more limited. While these limited studies suggest that bioactive glass bandages could prevent acute wound infections, given that bacteria in a biofilm are more difficult to treat, it is not clear whether they would also be effective against chronically infected wounds. The present results demonstrate that borate based bioactive glass effectively eradicates pre-existing biofilms, using a variety of different micro-organisms, suggesting they may also aid in the resolution of chronically infected wounds [1].

A plethora of work has been completed on the bioactive glass family of materials, which covers thousands of compositional iterations, but there is still extremely important work to be completed on the compositions that are being used in medical products. The present work, focusing on three bioactive glass compositions currently being used for medical products, contributes to a growing body of evidence demonstrating that bioactive glass may contribute to improved hard and soft tissue wound healing through their ability to treat biofilm infections. The silica based bioactive glass 45S5 was first cleared for use by the United States Food and Drug Administration (USFDA) in 1985 for applications in middle ear prosthesis [26]. In 2016, the first bioactive glass wound care intervention containing borate based bioactive glass (13-93B3) was cleared by the USFDA for use on several clinical indications focussed around acute and chronic soft tissue wounds and injuries. The GL1605 bioactive borate glass, a variant of 13-93B3 with the addition of copper and zinc, was studied presently because of its use in veterinary market for the treatment of wounded animals; and in the human orthopedic market as a component to a bone grafting implant for bone void filling and spinal fusion that was USFDA cleared in 2018.

2 Materials and methods

2.1 Generation of the bioactive glass

The bioactive glasses (45S5, 13-93B3, and GL1605) were prepared by mixing reagent grade raw materials (Table 1) and melting in a platinum crucible until molten. The bioactive glasses used in this analysis were manufactured by Mo-Sci Corporation, Rolla, MO, USA, as follows: 13-93B3 (lot 115-11), 45S5 (lot 279-10), and GL1605 (lot 109-10). The molten glass was made into a fiber matrix composed of fibers (d50 of 2–4 μm) and microspheres (d50 of ~200 μm) by subject-

Table 1: Chemical composition of the bioactive glass wound dressings (%wt)

	SiO ₂	B ₂ O ₃	CaO	Na ₂ O	P ₂ O ₅	MgO	K ₂ O	ZnO	CuO
45S5 (target)	45.0	0	24.5	24.5	6.0	0	0	0	0
45S5 XRF Lot:279-10	44.9	0	23.9	25.4	5.8	0	0	0	0
13-93B3 (target)	0	53.0	20.0	6.0	4.0	5.0	12.0	0	0
13-93B3 XRF Lot:115-11	0	54.3	19.2	5.4	4.0	5.4	11.7	0	0
GL1605 (target)	0	51.6	20.0	6.0	4.0	5.0	12.0	1.0	0.4
GL1605 XRF Lot:109-10	0	51.4	19.8	6.4	4.0	5.1	11.9	1.0	0.4

ing the molten glass to a compressed air attenuation manufacturing process to both make the fibers and quench the glass. The chemical composition of each bioactive glass was verified by a multipoint standardized X-ray fluorescence analysis (Table 1) completed by Mo-Sci Corporation.

2.2 Biofilm challenge

Pre-formed biofilms were prepared and challenged with the three different bioactive glass formulations similar to previously described [6, 27]. Inoculums containing between 10⁵ and 10⁶ CFU/mL were prepared, in either tryptic soy broth (TSB; bacterial strains of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) or Sabouraud Dextrose Broth (SDB; *Candida albicans*), by diluting overnight cultures. Then, 150 µL was added to the appropriate wells of the MBEC Assay® P&G device and the peg lid was placed into the bottom plate. The entire device was placed on a rotary shaker (110rpm) and incubated for 24 hours at 37±2°C. Following the 24-hour incubation, planktonic micro-organisms were rinsed from the biofilms by dipping the peg lids in saline. The lids containing the pre-formed biofilms were then transferred to challenge plates, which were generated by placing two biopsy punches (8mm in diameter) of bioactive glass along with 190 µL of 60% human serum (in PBS) in each well. Untreated glass wool (Acros Organics; New Jersey, USA) was used as a negative control. The biofilms were then incubated at 37°C for 24 or 48 hours. After the challenge period, the adhered biomass and planktonic micro-organisms were recovered and quantified, as previously described [27] by serial dilution and spot plating.

2.3 Statistical analysis

The number of cells recovered after the designated challenge times were compared using a One Way ANOVA with a Tukey post test, using InStat (GraphPad). The various

treatments were compared for each challenge time, as well as the two different challenge times (24 and 48 hours) were compared for each treatment condition. For interpretation purposes in Figure 1: * indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001. The asterisks directly above the bars indicate a comparison to the untreated control. When the asterisks are above a line, the ends of the line indicate the two conditions that are being compared.

2.4 SEM fixation and visualization

After the challenge period, pegs were also fixed for Scanning Electron Microscopy (SEM) by incubating for 24 hours in 5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.5). Pegs were then broken off of the MBEC Assay® lid, sputter coated and visualized using a Hitachi S3700N Scanning Electron Microscope. Images of the biofilms before and after challenge testing are present in Figure 2.

3 Results

3.1 Recovery of microorganism after treatment with bioactive glass

We assayed the anti-biofilm activity of bioactive glass against four organisms, including: gram-negative bacteria (*P. aeruginosa* and *A. baumannii*), gram-positive bacteria (*S. aureus*), and fungi (*C. albicans*). These microbes were selected because they represent a diversity of organisms, and because they are a major cause of drug resistant nosocomial infections. Microbial recoveries from pre-formed biofilms treated with all three bioactive glasses were significantly (p<0.05) reduced, after both the 24- and 48-hour challenge, compared to the untreated control (Figure 1). In contrast, glass wool, which was used as a negative control, did not result in a reduction in the number of micro-organisms recovered. This suggests that the reduction in the number of cells recovered were a result of the

Figure 1: Ability of bioactive glass dressings to reduce and eliminate preformed biofilms. Mature, pre-formed, biofilms of *Pseudomonas aeruginosa* (Figure 1A), *Candida albicans* (Figure 1B), *Staphylococcus aureus* (Figure 1C), and *Acinetobacter baumannii* (Figure 1D), were grown on hydroxyapatite coated 96 peg lids. Lids were then rinsed in saline, and transferred to challenge plates containing bioactive glasses 13-93B3 (white bar), 45S5 (diagonal hatch), GL1605 (vertical lines), glass fiber controls (horizontal lines) and control biofilm cell counts in 60% human serum (grey bar). Lids were incubated in the challenge plate for 24 or 48 hours before the number of planktonic and adhered bacteria were quantified. Each bar represents the average Log_{10} value of the number of colony forming units recovered from each peg/well.

antimicrobial activity of the bioactive glasses, and not a result of mechanical disruption or physical damage caused by the glass fibers. Of the four organisms tested, *S. aureus* biofilms were the most resistant to the three bioactive glasses.

Borate based bioactive glasses dissolve faster than silica based bioactive glasses, therefore we hypothesized that the borate based bioactive glass would have stronger anti-biofilm activity [13]. To test this hypothesis, we compared the ability of bioactive glass 13-93B3 to 45S5. With the exception of one challenge conditions (*P. aeruginosa* biofilms after 24 hour challenge), 13-93B3 resulted in a larger reduction in the number of microbial cells recovered after all treatments. In addition 48 hour treatment of *P. aeruginosa*, *C. albicans*, and *A. baumannii* biofilms with bioactive glass 13-93B3 resulted in no detectable biofilm cells, while 45S5 did not reduce the number of cells to undetectable levels under any condition tested. These results suggest that borate based bioactive glass may have a more potent anti-biofilm activity than the silica based bioactive glass 45S5.

We hypothesized that the addition of anti-microbial ions (Zn^{2+} and Cu^{2+}), could further enhance the anti-biofilm activity of the bioactive glass. To test this hypothesis, we compared the ability of bioactive glass GL1605 to 13-93B3. In all cases, bioactive glass GL1605 had either similar, or larger reductions compared to 13-93B3. This was particularly true for *S. aureus* biofilms, where bioactive glass GL1605 was the only glass formulation to completely eradicate the preformed biofilms under any of the test conditions. Overall bioactive glass GL1605 was the most consistent anti-biofilm activity, against all four species tested. These results are consistent with the hypothesis that the addition of copper and zinc ions enhance the anti-biofilm activity of bioactive glass formulations.

Figure 2: Scanning Electron Microscopy analysis of biofilms following 48-hour challenge with bioactive glass and control glass fiber. Pre-formed biofilms were incubated for 48 hours in challenge plates containing the glass control (GC), 13-93B3, GL1605, and 4555 bioactive glasses in 60% human serum. Following the 48-hour challenge, the remaining biofilm was fixed with 5% glutaraldehyde and visualized using Scanning Electron Microscopy. Representative images were taken and compared to biofilms remaining after a mock treatment of 60% human serum. White arrows indicate cell like structures, black arrows indicate misshapen cell like structures that we interpret as dead cells or cell debris. Scale bars equal 5 micrometers.

3.2 Visualization of treated and untreated biofilms by Scanning Electron Microscopy

To better understand the effect of the borate based bioactive glass compounds (13-93B3 and GL1605) on the pre-formed biofilms, scanning electron microscopy was used to assess the state of the biofilm in both treated and untreated biofilms after the 48-hour challenge. For untreated biofilms, large areas of the pegs were covered with a solid layer of microbial cells (Figure 2A-D). Also, cells often appeared to be submerged into a smooth layer, which we interpret to be biofilm matrix (Figure 2A and D). *P. aeruginosa* cells had the expected rod shape, while *S. aureus* and *A. baumannii* cells appeared round or nearly round. As expected, the *Candida albicans* cells were much larger than the bacterial cells, and the biofilms contained a mix of round cells and hyphae.

In contrast, pegs that had been treated with the bioactive glass had dramatically reduced numbers of visible bacterial cells (Figure 2E-P), consistent with the reduced numbers of adhered bacteria recovered under these conditions (Figure 1). It was possible to visualize what appeared to be small localized clusters of cells on the pegs that contained bioactive glass treated biofilms from *C. albicans*, *S. aureus*, and *A. baumannii* (Figure 2H, 2J, 2L, 2O and 2P) as indicated by the white arrows. However these cells were limited in number and lacked the three dimensional structures typical of biofilms. In the case of *P. aeruginosa* biofilms, no obvious cells could be visualized after treatment with bioactive glass. Some rod-shaped structures were visible (Figure 2E) black arrow; however, they appeared flattened, and misshapen, compared to the cells from the untreated biofilm. While hyphae could still be visualized in treated *C. albicans* biofilms, no round cells, which comprised the majority of the untreated biofilm, were observed (Figure 2F

or 2N). The 45S5 glass (Figure 2J) did have some identifiable viable cells, but few in numbers. We were unable to visualize *S. aureus* biofilms after treatment with either 45S5 or 13-93B3 groups (Figures 2G or 2K). This is despite having 10^4 and 10^3 CFU/peg microbial recoveries, respectively (Figure 1C). One possible reason for this would be that the biofilms are not adhered to the pegs that well and so they did not survive the treatment followed by the fixation. Another possible reason could be that the surviving cells are buried within a biofilm matrix that does not show up well by SEM. Taken as a whole, our SEM results are consistent with the microbial recovery, with dramatic reductions in the number of cells present following treatment with bioactive glass.

4 Discussion

The antimicrobial effectiveness of the 13-93B3, GL1605, and 45S5 bioactive glasses is clearly varied by the organism in which it was challenged. To define a single mechanism is unlikely as these organisms are susceptible to several possible conditions that may cause cell death and previous work has identified the presence of multiple potential mechanisms [21]. The possible mechanisms identified by these bioactive glasses include increased pH, alkali release from the glasses, boron release from the borate glasses, trace element (copper and zinc) release from the GL1605 glass, and physical damage caused by the microstructure of the fiber. The results of the glass wool control showed no statistical effect on viable cells counts, therefore physical damage has been ruled out as a major cause of microbial biofilm reduction. Based on the present data, possible mechanisms for the reduction of each biofilm have been suggested.

Pseudomonas aeruginosa is a gram negative bacterium that is rod shaped and can affect both people and plants. *P. Aeruginosa* has already become multi-drug resistant which makes treating this organism with antibiotics such as Tobramycin increasingly difficult and extremely relevant to the present work [5]. Possible conditions include sepsis due to ecthyma gangrenosum in neutropenic patients, urinary tract infections in catheterized patients, and skin or soft tissue necrosis in people with burns or wounds [28]. *P. aeruginosa* has been shown to be susceptible to H_3BO_3 and even more so to $Na_2B_8O_{13} \cdot 4H_2O$ when trying to grow biofilm in-vitro, indicating that both boron and alkali are potential mechanisms for biofilm reduction [29]. Growth curves for *P. aeruginosa* at pH of 5 to 9

show a limited growth rate below a pH of 7, however a pH between 7 and 9 the growth curves were not affected [30].

The microbial recovery data present in Figure 1A shows that there is no planktonic cell recovery with the 45S5 glass at 24 hours and only a few recognizable cells at 48 hours while the biofilms at 24 and 48 hours are also significantly reduced. The possible mechanisms for 45S5 are increased pH and alkali release having the greatest contribution. The 13-93B3 and GL1605 glasses have less effect at 24 hours to kill the planktonic cells and the biofilm, but by 48 hours, do so to a non detectable level in the biofilms. This can be explained by the fact that while the overall dissolution for the borate glasses is faster than 45S5, the pH of ions released by the borate glasses rises more slowly than 45S5 do to the lower overall alkali content and presence of boron [13]. As the boron and alkali level increases along with pH for the 13-93B3 and GL1605 glasses, the overall reduction in identifiable organisms drops to zero by 48 hours. The copper and zinc being released from the GL1605 glass also appears to have an improved effect over 13-93B3 at 24 hours, but by 48 hours neither borate glass had viable recoverable cells.

Candida albicans is common yeast found in the human gut, but can survive outside the human body for long periods of time [31]. *C. albicans* is the most prevalent cause of fungi derived infections in humans and include the GI tract, mouth, and vagina. Like most organisms, humans can live with *C. albicans* with no complications, but given the right circumstances, it will become opportunistic [32]. *C. albicans* is known to be susceptible to boric acid as demonstrated during in-vitro zone of inhibition studies [33].

Based on the data in Figure 1B for *C. albicans*, it is clear that the most significant mechanism for planktonic and biofilm cell reduction was the boron dissolution, and to a lesser extent pH and alkali release. The 45S5 glass reduced cell recovery, likely due to increased pH and alkali release, but not nearly as much as the borate glasses. It is difficult to tell if the addition of the copper and zinc had much of an effect since the 24 hour recoveries of planktonic and biofilm cells for 13-93B3 and GL1605 glasses were at non-detectable levels.

Staphylococcus aureus is a gram positive, round shaped bacterium typically found in the upper respiratory tract or on the skin [34]. *S. aureus* is a common cause for skin and sinus infections and if left unchecked, can lead to osteomyelitis and sepsis which can be fatal [23]. In the U.S. alone, approximately 500,000 patients contract a *S. aureus* infection while in the hospital, and 50,000 deaths per year are associated with *S. aureus* infections [35, 36].

Staphylococcus aureus has been shown to be susceptible to H_3BO_3 and even more so to $Na_2B_8O_{13} \cdot 4H_2O$ when trying to grow biofilm in-vitro, indicating that both boron and alkali are potential mechanisms for biofilm reduction [29]. Copper containing mesoporous bioactive glass nanoparticles have been shown to be effective in-vitro against planktonic and preformed biofilms of *S. aureus* and *S. epidermidis* [37]. In-vivo evaluation of bone defects containing chronic osteomyelitis from multiple etiologies including *S. aureus* were successfully treated with S53P4 bioactive glass, which is a silicate bioactive glass similar to 45S5, had a 90% eradication rate at 21.8 months follow-up [23]. In-vitro, S53P4 was found to suppress *S. aureus* biofilm formation on titanium discs, and decreased particle size (higher surface area and higher ion release) was identified as most efficacious group studied [22].

Figure 1C clearly shows that *S. aureus* is the least susceptible microorganism of the four tested to the bioactive glasses. Only the GL1605 glass at 48 hours had a non-detectable cell count. The 13-93B3 glass did reduce the cell counts but there was little difference between 24 and 48 hours in the planktonic and the biofilm groups. 45S5 was similar in that at 24 hours there was reduction from controls, but then no statistical change between 24 and 48 hours. The most critical component was the copper and zinc that was released between 24 and 48 hours that reduced the recoverable cells to a non-detectable level. The alkali, boron, and pH all had a positive effect on reducing the *S. aureus*, but the copper and zinc release was likely the most potent mechanism.

Acinetobacter baumannii is a nearly round rod shaped bacterium that affects people with compromised immune systems, such as those with diabetes, heavy smokers, and people who use excessive amounts of alcohol [38]. *A. baumannii* is a pathogen with increasing multi-drug resistance, so new treatment options are imperative [39]. It can also survive on artificial surfaces for long periods of time making it a huge risk to hospitals and clinics. Interestingly, this organism is a well-established war-related pathogen initially discovered during the Korean War, is now one of the dominant Gram-negative bacteria affecting wounded soldiers from Iraq and Afghanistan [38].

The data in Figure 1D indicate that the borate glasses had the strongest effect on the reduction of the *A. baumannii* viable cells for planktonic and biofilm groups. The GL1605 glass has no viable cells detected in the 24 hour biofilm and planktonic groups, while the 13-93B3 had some viable cells remaining albeit relatively few. The 45S5 glass had a significant reduction at 24 hours, but hit a plateau at 48 hours with no significant reduction from 24 hours. Based on this data, the boron release and the cop-

per and zinc release appear to have made the most significant contribution to viable cell reduction of *A. baumannii* while increased pH and alkali release had an effect but not enough for complete reduction of viable cells.

Many publications often discuss antimicrobial and or antibiofilm effects of a material or chemical against a single strain of evasive microbe. It is important to understand that organisms can cohabitate the same biofilm, for instance *S. aureus* and *C. Albicans*, so a broad spectrum antimicrobial effect is important for clinical efficacy [36]. The study retrospectively reviewing bioactive glass S53P4 in chronic osteomyelitis had 10% of patients that did not overcome their chronic osteomyelitis, and these cases were documented having co-isolated infections with two or more pathogenic organisms [23].

5 Conclusions

The present work contributes to a growing body of evidence that bioactive glass may contribute to improved soft tissue wound healing. Specifically, our results demonstrate that borate based bioactive glass is capable of eradicating pre-formed biofilms, from a variety of clinically relevant gram-negative, gram positive, and fungi organisms. These results expand on the current body of anti-microbial knowledge that has focused primarily planktonic bacteria and preventing biofilm formation. This is an important distinction, as it is well established that pre-formed biofilms are much more difficult to treat than planktonic bacteria, and many commercially available wound dressings are effective against planktonic bacteria, but not pre-formed biofilms. At least one of the bioactive glasses tested was successful at reducing the viable biofilm cells to a non-detectable level, but the mechanism by which this occurred is believed to be differed by organism. Based on this analysis, the bioactive borate glasses had broader microbial efficacy than 45S5 glass, and the additional of copper and zinc in GL1605 proved to be critical for reducing the most resistant organism tested, *S. aureus*, to a non-detectable level.

Ethical approval: The conducted research is not related to either human or animals use

Conflict of Interests: There is no conflict of interest regarding the publication of this paper

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