Antibacterial effect of a new bioactive glass on cariogenic bacteria

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Abstract

Objectives: *To investigate the antibacterial effect of a new bioactive glasses on cariogenic bacteria.*

Methods and materials: *A newly developed bioactive glass named Huaxi bioactive glass-ceramic (HX-BGC) and its antibacterial effect was investigated. 3M^{TM} Specialty Glass 19933 (SG) was used as the positive control while deionized water was used as a negative control. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of HX-BGC and SG against four cariogenic bacteria (Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilius, Lactobacillus rhamnosus) were determined by a microdilution method. Subsequently, bacterial growth and acid production were assessed by co-culture the four cariogenic bacteria species with HX-BGC or SG at different concentrations anaerobically at 37°C. Optical density (at 660nm) was assessed by a microplate reader and the acidity of the supernatant were measured by a pH electrode at 6, 12, 24, and 48h. Microbial kinetics, morphology and viability were evaluated by counting colony forming units (CFU), scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).*

Results: *The MBC of HX-BGC were 15.63mg/ml, 15.63mg/ml, 7.81mg/ml and 62.5mg/ml against S.mutans, S.sobrinus, L.acidophilus and L.rhamnosus, respectively. The optical density (7.8mg/ml) value of the bacterial suspensions did not change during the 48 hours of culture. The acidity of bacterial suspension kept steady at around 8.8. CFU indicated that HX-BGC had stronger antibacterial effect when compare with SG, which is consistent with SEM and CLSM results.*

Conclusions: *HX-BGC inhibits acid production and growth of cariogenic bacteria.*

1. Introduction

Dental caries is a biofilm-mediated disease that causes loss of minerals of hard tissues of the teeth. The cariogenic bacteria in biofilm produce acid for a prolonged time and create an acidic environment in the mouth, leading to demineralization of the teeth (Kutsch, 2014). To control oral microbial biofilms, antiseptics such as chlorhexidine and triclosan are commonly used. However, antibiotics are not used clinically to reduce cariogenic bacteria because these agents are usually designed to function at low concentrations through a defined high-affinity antimicrobial target – a set of circumstances that makes it comparatively easy for bacteria to develop resistance. As a result, excessive use of antibiotics can result in the development of bacterial resistance and alter oral and intestinal flora (Cheng, Li, He, & Zhou, 2015).

Bioactive materials are introduced for use in dental care because they can interact in some positive ways with the oral environment to protect dental tissues. Bioactive glass was initially introduced for treatment of bone defect. It was found to be able to bond with bone and stimulates bone growth with promising bioactivity and biocompatibility to form bonds to hard and soft tissues (Balamurugan et al., 2008; Lepparanta et al., 2008). The mechanism of bioactive glass in promoting bone regeneration is a stepwise process, which begins with calcium phosphate precipitation and then followed by bonelike apatite formation on the surface to bind to hard tissues (Stoor, Soderling, & Salonen, 1998; Zhang et al., 2010). Bioglass 45S5 was introduced into dentistry to treat dentine hypersensitivity at the beginning of this century (Forsback, Areva, & Salonen, 2004). The action of bioactive glass on tooth surface is similar to that on bone. Bioactive glass can mineralize dentin tubules to produce a long-term alleviation of tooth pain and hypersensitivity due to external stimuli. The remineralization process begins with dissolving the material in an aqueous solution and then raising the pH. High pH promotes precipitation of hydroxyapatite (HA), which is the main component of mineralized enamel and dentin. Calcium and phosphate ions in bioactive glass and saliva supply the substrate for mineralization (Jones, 2013). It is suggested that strontium (Sr) has caries-potentiating effect. The combination of Sr and fluoride (F) has been shown to improve the crystallinity of low-carbonated hydroxyapatite (HAp) which is very similar to enamel (Lippert & Hara, 2013). The antibacterial effect of Sr is not fully understood. A recent review article stated that two contradictory views on whether Sr has an inhibitory effect on bacteria, but they both suggested that the Sr^{2+} has synergistic effect with F^{-} in promoting antibacterial activity (Kargozar, Montazerian, Fiume, & Baino, 2019). Recently, a new bioactive glass is developed with

addition of strontium. However, the antibacterial effect of this bioactive glass is unknown. Thus, this study aimed to investigate the antibacterial effect of the new bioactive glass on the cariogenic bacteria.

2. Materials and Methods

2.1 Materials

2.1.1 Bioactive glasses products

Patent of the new bioactive glass powder used in this study, Huaxi bioactive glass-ceramic (HX-BGC), belongs to the West China Hospital of Stomatology, Sichuan University, China. The HX-BGC powder used in this study was provided by Dencare (Chongqing) Oral Care Co., Ltd. It has the following components: SiO_2 (12 wt% to 45 wt%), P_2O_5 (10 wt% to 35 wt%), CaO_2 (5 wt% to 48 wt%), Na_2O (5 wt% to 15 wt%), SrO (3.5 wt% to 4.9 wt%) and F (1.5 wt% to 2.1 wt%). The other bioactive glass, namely 3M^{TM} Specialty Glass 19933 (SG, 3M, Ceradyne, Inc., USA), which is an analogy of Bioglass 45S5 (SiO_2 45 wt%, CaO 24.5 wt%, Na_2O 24.5 wt%, and P_2O_5 6.0 wt%), was used as positive control.

2.1.2 Microorganisms

The microorganisms used in this study were *Streptococcus mutans* (*S.mutans*, ATCC 35668), *Sterptococcus sobrinus* (*S. sobrinus*, ATCC 33478), *Lactobacillus acidophilus* (*L. acidophilus*, ATCC 9224), and *Lactobacillus rhamnosus* (*L. rhamnosus*, ATCC 10863). These are amongst the most common cariogenic bacteria species in the human oral cavity.

2.2 Methods

2.2.1 Characterization

Both HX-BGC and SG powder were washed by ethanol for three times, followed by removing the ethanol and waiting for evaporation of remained ethanol. Then, the HX-BGC and SG powder was put onto two metal holders and coated with gold for 200s in a coating machine (MSP-2S, IXRF System, US). Subsequently, the powder was observed using Scanning Electron Microscopy (SU1510, Hitachi, Japan) to evaluate the morphology of the material. Energy Dispersive X-ray spectroscopy (EDS) was used to confirm the chemical elements. It was a stepwise procedure to conduct the analysis of the spectrum of the two study bioactive glasses.

2.2.2 MIC and MBC

There were 3 groups for testing: 1) HX-BGC suspension; 2) SG suspension (positive control); 3) deionized water (negative control). HX-BGC powder and SG powder were put into distilled water to form a suspension for the determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). For MIC determination, a microdilution method was chosen to dilute the suspension with twofold dilution for 10 times. The initial concentration of HX-BGC suspension and SG suspension was 250mg/ml. They were diluted to 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.63mg/ml, 7.81mg/ml, 3.91mg/ml, 1.95mg/ml, 0.98mg/ml and 0.49mg/ml. The four study bacteria strains were diluted with Brain Heart Infusion (BHI) broth to a concentration of 1.0×10^6 CFU/ml to form bacterial suspensions. A 96-well microplate was used to assemble the samples and each well was filled with 200μl of study suspension or water using a pipette, followed by adding 10μl of prepared bacterial suspension in each well. The four species of pure culture bacteria were cultured together with bioactive glasses for 48 hours at 37 ℃, while the bacteria strains in deionized water served as the negative control group. The MIC results were observed by naked eyes after 48-hour incubation. After completing the MIC test, growth tests of bacterial colonies were carried out on the HX-BGC and SG suspensions of each of the study concentrations for MBC testing (Xu et al., 2015). A 10μl suspension from each well of each group in the microplate was applied on the agar plate and the colonies were checked after incubating for 48 hours in an anaerobic incubator (Figure 1). The procedure was conducted in triplicate.

2.2.3 Bacterial growth and acid-productivity

Four species of pure culture bacteria (*S. mutans, S. sobrinus, L. acidophilus, L. rhamnosus*) were used and eluted in 4 test tubes individually with BHI broth. The tubes were put into a centrifuge at 3500 r/min for 15 min. Then the 4 species of bacteria were collected and diluted with BHI broth at a concentration of 1.0×10^8 CFU/ml to form a bacterial suspension. The production of a bacteria mixture was carried out later. BHI was prepared in advance with 5% sucrose. After confirming the MBC results, 3 concentrations of HX-BGC (BGC) and 2 concentration of SG were chosen to add into the BHI broth culture medium, with pure BHI broth acting as control. The groups were: 1) BGC1 medium (3.9mg/ml); 2) BGC2 medium (5.85mg/ml); 3) BGC3 medium (7.8mg/ml); 4) SG1 medium (7.8mg/ml); 5) SG2 medium (11.7mg/ml); 6) BHI1 (pH=7.7) medium; 7) BHI2 (pH=9) medium; 8) BHI3 (pH=11) medium.

Optical density (OD) was used to measure bacterial growth. The prepared bacterial suspensions were added to each study group in a volume ratio of 1:20 (HX-BGC, SG and BHI). Each well was filled with 200μl of different media and 10μl of the bacterial mixture were added into each well. Anaerobic culturing of the samples was carried out at 37 ° C. The OD values (660 nm) of the suspensions was measured by a microplate reader (SpectraMax® M2, California, USA) at 0, 6, 12, 24, and 48h. The pH of the control group (BHI) was 7.7 and other two groups of BHI were initially set to the initial pH=9 and pH=11, inoculating the bacterial suspensions in the medium according to the reagent solution 1:20 (volume ratio). The bacteria were incubated anaerobically for 6, 12, 24, and 48h at 37 °C. There were 3 tubes in each group and the acidity of the supernatant in each tube was determined for each time point using a pH meter (ExStik® pH100, Massachusetts, USA).

2.2.4 Microbial morphology and viability

The morphology of the bacteria was assessed by SEM. HX-BGC and SG media were prepared to incubate with the mixture of 4 species of bacteria for 18h and the concentration was set as 7.8mg/ml. The bacterial suspension was set at a concentration of 1.0×10^8 CFU/ml and it was added into HX-BGC, SG and BHI media with a volume ratio of 1:10 for 18h anaerobic incubation. For SEM assessment, samples in each group were collected by centrifugation and washed with phosphate-buffered saline (PBS) three times, and then fixed in 2.5% glutaraldehyde for four hours.

The viability of the bacteria was assessed by confocal laser scanning microscopy (CLSM). Samples in each group were collected by centrifugation and washed with PBS three times. The treated suspensions in three groups were stained with the LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit (Invitrogen™, California, USA), according to the manufacturer's instruction. Each sample was stained with SYTO9 and propidium iodide (PI) at room temperature for 40 minutes in a dark room, followed by visualizing and capturing with a CLSM (Flouview FV 1000, Olympus, Tokyo, Japan) equipment. Live bacteria with intact cell membranes would be stained green, while dead cells with damaged membranes would be stained red. Eight cellular images from each sample were obtained and analyzed using an image analysis software (ImageJ, National Institutes of Health, Bethesda, MD, USA). The red-to-green ratio was calculated to denote the ratio of dead-to-live bacteria, respectively. A low ratio indicates a positive antimicrobial effect of the study agent.

2.2.5 Microbial kinetics

After incubation for 18h, 100μL of each sample was collected, mixed with 900μL BHI for serial 10-fold dilution three times and then sub-cultured onto BHI agar plates for a further 48h. Selective media agar plates of Mitis Salicarius and Rogosa were also used for *Mutans Streptococcus* species (*S.mutans* and *S. sobrinus*) and *Lactobacillus* species (*L. acidophilus* and *L. rhamnosus*) respectively. The number of bacteria in different agar plates was determined by counting the colony forming units (CFUs).

2.2.6 Statistical Analysis

Data obtained from the bacterial growth and acid-productivity assessments were analyzed by the Shapiro-Wilk normality test. Friedman's test was used to assess the variation of the optical density of bacteria in each medium during the 48h incubation period, as well as the pH of the various media. Data collected from microbial viability assessment were analyzed by the Shapiro-Wilk normality test. One-way ANOVA test was used to measure the area ratio of live/dead bacteria of the three groups with different treatments. All the assessments and analyses were conducted by IBM SPSS Statistics 25. The statistical significance level was set at 0.05.

3. Results

3.1 Characterization

The main difference between the two study bioactive glasses was the presence of strontium ions in HX-BGC. The morphology images and elements spectrums of HX-BGC and SG powder are shown in (Figure 1 and 2). Small particles of HX-BGC have similar shapes and sizes (range from 23.5 μm to 40.9 μm), clustering together to form larger particles, while SG particles have shaper edges with different sizes (range from 9.07 μm to 69.5 μm), adhering to each other. The details of elements in each bioactive glass are also listed in Table 1. The main elements of the two bioactive glasses (calcium, silicon and phosphorus) are in common while HX-BGC has strontium (around 1.6%).

3.2 MIC and MBC

It can be seen in Table 2 that the MBCs of the BGC against the four study cariogenic bacteria were lower than those of SG. Due to the turbidity induced by precipitation of BGC

and SG in the suspensions, determination of their MIC was not possible through the microdilution method.

3.3 Bacterial growth and acidity

As the data did not all follow normal distribution ($p<0.05$), non-parametric tests were performed. Friedman's test was used to assess the antibacterial effect of the different media. The line graph in Figure 3 illustrates the trend of bacterial growth in the different study groups. In groups BGC1, BGC2 and SG1, the optical density (OD) value gradually increased during the 48h cultivation period. On the contrary, the OD values in BGC3 and SG2 groups were nearly steady at the same period. For the control groups of BHI1 and BHI2, the bacteria grew rapidly between the 12h and 24h time points, while BHI3 group with an initial pH=11 did not display any bacteria growth. Results of the Friedman's test show that the OD values at the 5 time points were significantly different ($p<0.05$) in the BGC1, BGC2, SG1, BHI1 and BHI2 groups but not in the BGC3, SG2 and BHI3 groups.

The acidity of bacterial suspension is shown in Figure 4 which compares the pH value changes during 48h cultivation between different groups. It is obvious that the pH value of 3 groups (BGC1, BGC2 and SG1) decreased from 12h to 48h. BGC3 group and SG2 group have the identical variation tendency with the pH value, while the former has no statistical difference ($p>0.05$) and the latter had statistically significant difference ($p<0.05$) with just a marginal difference between 0h and 24h time point ($p=0.045$). BHI3 group had significantly higher pH values ($p<0.05$) but even pH values had slightly reduced, pH values were still high around 10 over time. On the contrary, there is a significant sharp drop in the pH of BHI1 and BHI2 groups (both $p<0.05$) from the 6h time-point.

3.4 Microbial morphology and viability

As shown in Figure 5, the bacteria amount decreased significantly in BGC and SG group after 18h anaerobic incubation when compared to that in control group. As seen in Fig. 5(c), only a small number of bacteria was attached to the BGC particles, while more bacteria adhered to the SG particles and also appeared in the culture medium. The area ratio of live/dead bacteria was detailed in Table 3. The lower the area ratio, the better the antibacterial effect of the agent. Since data followed normal distribution, one-way ANOVA was used in this multiple comparison analysis. The control group was significantly different ($p<0.01$) from the other two

groups treated with BGC and SG, though there was no significant difference between the two experimental groups.

3.5 Microbial kinetics

The number of cariogenic bacteria in each group was counted after incubation for another 48h. According to the blood agar incubation results, BGC was more effective in killing bacteria when compared to SG (Table 4). There was no bacterial growth in the Mitis Salicarius agar plates which aimed to incubate *Streptococcus* which means that BGC and SG had killed all the species of *Streptococcus* during the first 18h incubation. The CFU counts in the Rogosa agar plates show that *Lactobacillus* species growth was inhibited by BGC and SG when compared to that in the BHI group.

4. Discussion

Results of this study show that the antibacterial potential of HX-BGC is better than that of SG. HX-BGC has lower MBCs against different species of cariogenic bacteria than those of SG. In bacterial growth and acidity test, HX-BGC with lower concentration showed greater effect in totally inhibiting the growth of the study cariogenic bacteria during the 48h incubation period when compared to SG with a higher concentration. Moreover, BGC displayed greater bacterial inactivation effect than SG in the CFU counting test. SEM and CLSM observations showed both BGC and SG possess an antibacterial effect on cariogenic bacterial species, though SG performed poorer than BGC at the same concentration level.

Several methods used in this study are routine assessments in microbial investigations. MIC and MBC determination is a common method to assess the antibacterial potential of different anti-caries agents. A previous study found that the MIC and MBC of Bioglass 45S5 were 18.75mg/ml and 37.5mg/ml, which are similar to the results obtained in this study. It was found that at twice the concentration of MBC (2MBC) on planktonic bacteria, Bioglass 45S5 had a bactericidal effect on the biofilm of *S.mutans* (Xu et al., 2015). Another study (Munukka et al., 2008) revealed that 8 bioactive glasses (MBG0103, MBG0118, MBG0123, S53P4, 13-93, H2-02, CaPSiO, CaPSiO II) had a bacterial inhibitory effect at the concentration of 100mg/ml, and only S53P4 was effective at a lower concentration (50mg/ml). In the current study, HX-BGC powder and SG powder possess better antibacterial potential than the other bioactive glasses listed above. CFU counting method is commonly used in directly assessing

the viability of bacteria species. A previous study showed that *S.mutans* lost its viability when it was incubated with bioactive glass powder for a short period (Stoor et al., 1998). Confocal microscopy provides an intuitional observation to live and dead planktonic bacteria or biofilms. Lower live/dead ratios in groups treated with Bioglass 45S5 (2MBC) and combination groups (BAG+NaF, BAG+TCS) determined than that in control groups (Xu et al., 2015). In current study, MIC could not be assessed through traditional method due to the precipitation of the bioactive glasses on the bottom of the microplate. Therefore, a possible MIC was determined by choosing different concentrations of BGC and SG against the mixture of multi-species bacteria and obtaining the lowest the concentration which can inhibit its growth. Another limitation in this study is that only planktonic bacteria was used and assessed while the effect of the study BAGs on cariogenic biofilm was not evaluated.

The possible action of bioactive glass against cariogenic bacteria comes from two main mechanisms: pH elevation and presence of antibacterial ions. When bioactive glass contact with the aqueous medium, ions from the material such as sodium will be released and lead to an increase in pH, creating an alkaline environment which inhibits bacteria growth (Kim et al., 2018; Liu, Rawlinson, Hill, & Fortune, 2016). The antibacterial effect of bioactive glasses without any antibacterial ions is mainly explained by the elevation of pH of the medium, which is considered bacteriocidal (Siqueira et al., 2017; Stoor et al., 1998). Previous investigation has also reported that such antimicrobial properties have an effect on certain planktonic microorganisms (Galarraga-Vinueza et al., 2017). Two bioactive glasses (Glass 45S5 and Glass S53P4) displayed antibacterial activity against S.mutans due to the high pH value in the aqueous environment (Zhang et al., 2010). An earlier study found that the pH value of the medium increased in the first 10 minutes and *S.mutans* totally lost their viability after incubation with Glass S54P3 (Stoor et al., 1998). Bioglass 45S5 can elevate and maintain pH values at around 11-12 and has a higher antibacterial effect against S.mutans when compared with both 5% and 20% niobophosphate (Bauer et al., 2019). In the present study, there were three control groups of BHI with different initial pH value. When the initial pH value of BHI was higher than 9, the inhibition effect of BHI was mainly due to the highly alkaline aqueous environment. The SG2 group also maintained a high pH (around 9.1) value during the 48h incubation period and displayed a superior inhibition effect on cariogenic bacteria growth. Thus, the main reason of SG in suppressing bacterial growth is the elevation and maintenance of pH value. However, HX-BGC showed a bacterial inhibition effect at a lower pH (around 8.5) which indicates that its antibacterial effect may not be just due to pH rise.

The presence of antibacterial ions is another main reason for prohibiting bacteria growth. Many metal ions, such as silver, which possess antibacterial properties are doped into bioactive glass and then inhibit the growth of microorganisms (Kim et al., 2018). Ag-BGN@MSN is a type of silver-doped bioactive glass, which has been shown to be effective in prohibiting bacteria growth (Jung et al., 2019). For the bioactive glass HX-BGC used in this study, strontium can be a substitution of calcium due to its similar charge-to-size ratio to calcium. It is suggested that strontium in HX-BGC may work against cariogenic bacteria. A previous investigation showed that strontium-containing glass had a greater antibacterial effect on *S. mutans* than that of material without strontium (Guida, Towler, Wall, Hill, & Eramo, 2003). Another study also found that bacterial inhibition was enhanced with increasing proportion of strontium in the strontium-substituted bioactive glass (Liu et al., 2016). When strontium is combined with fluoride, it plays a beneficial role in the anti-caries process though it has been shown to have weak antibacterial activity (Dabsie, Gregoire, Sixou, & Sharrock, 2009). Adding specific concentration (with 15-25 mol%) of strontium to glass can significantly decrease the size of inhibition zone and the bactericidal effect of bioactive glass is mainly attributed to individual or combined effect of Sr^{2+}, Na^{+}, and Ca^{2+} (Li et al., 2016). For the study HX-BGC, the antimicrobial activity can be mainly due to the ions, especially strontium ion. Besides, an investigation has exhibited the potential of strontium on stabilizing the pH environment and slowing ion dissolution (Zhu, Zhu, He, Zhang, & Tao, 2013). Therefore, the BGC3 medium in this study maintained a high pH（around 8.8) during the whole period of anaerobic incubation. In summary, the possible reason why HX-BGC has better antibacterial effect than SG is the presence of antibacterial ion (Sr) in HX-BGC. The synergistic effect of pH elevation and antibacterial ion makes HX-BGC a better anti-bacterial agent.

According to the trends of OD value and pH value, groups BGC1 and BGC2 could inhibit the growth of bacteria during the first 6 hours and then the bacteria in BGC1 grew rapidly while those in BGC2 gradually increased. Meanwhile, the pH value of BGC1 and BGC2 started to decline from the 12-hour time point. Hence, these findings have revealed that the pH value of the media was not affected during 6h to 12h period, though bacteria growth occurred from the 6-hour time point. If HX-BGC at these two concentrations (3.9mg/ml and 5.85mg/ml) was added into the toothpaste, the effectiveness of bacterial inhibition will last for 6 hours and the pH of the environment in the oral cavity can be kept alkaline for nearly 12

hours. At the same time, the pH value of Group SG1 maintained a steady level, though the OD value of this group was gradually rising but with a very narrow range during the first 12h incubation. Brushing teeth twice daily with an interval of around 12 hours is a regular practice among most people. It is suggested that use of HX-BGC containing toothpaste with concentrations of 3.9mg/ml or 5.85mg/ml during tooth brushing can suppress the growth of bacteria during the whole day. It is also suggested to add SG powder at the concentration of 7.8mg/ml into toothpaste for daily use. Since the antibacterial effect of bioactive glasses is not very strong, experiments on the inhibition effect of HX-BGC on cariogenic biofilm was not performed in this study. It may be possible to improve the antibacterial properties of HX-BGC through doping some bactericidal components or adding some antibacterial material into the bioactive glass and pay more attention on the investigation of antibacterial effect on cariogenic biofilm in future studies.

5. Conclusion

HX-BGC shows an antibacterial effect on cariogenic bacteria by inhibiting acid production and suppressing the growth of bacteria.

Conflict of interest statement

This research is original. Authors declare no conflict of interest with respect to the authorship.

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Figures and captions

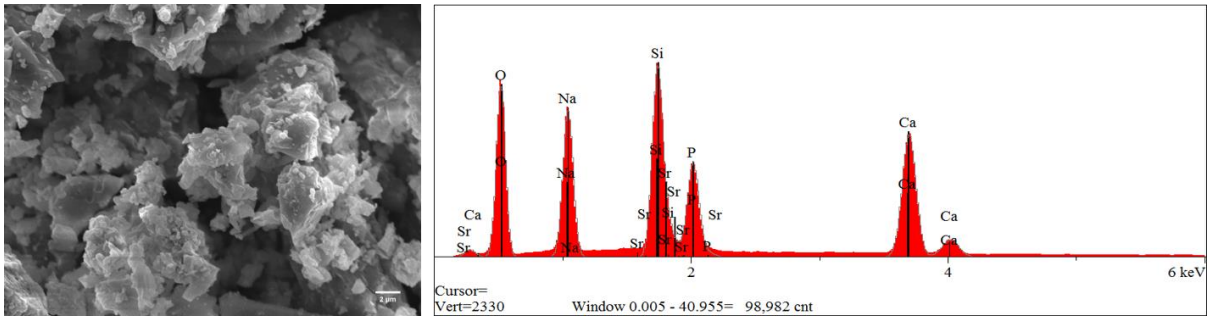

Figure 1. SEM image (3000×) of HX-BGC and EDS of HX-BGC

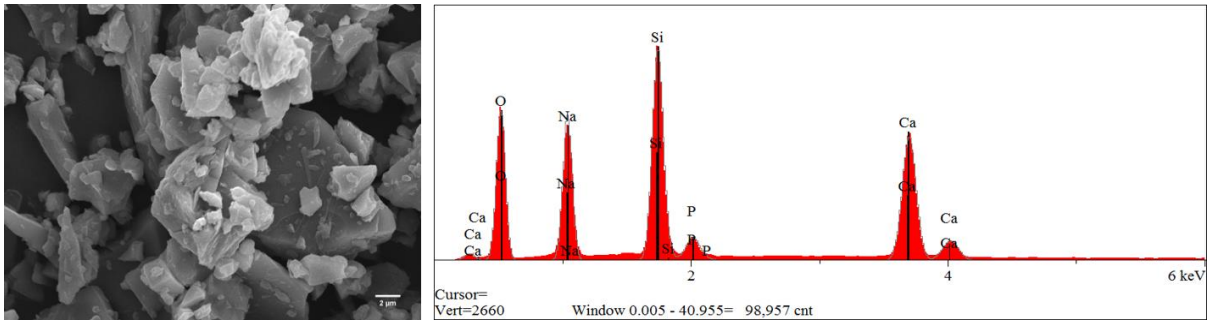

Figure 2. SEM image (3000×) of SG and EDS of SG

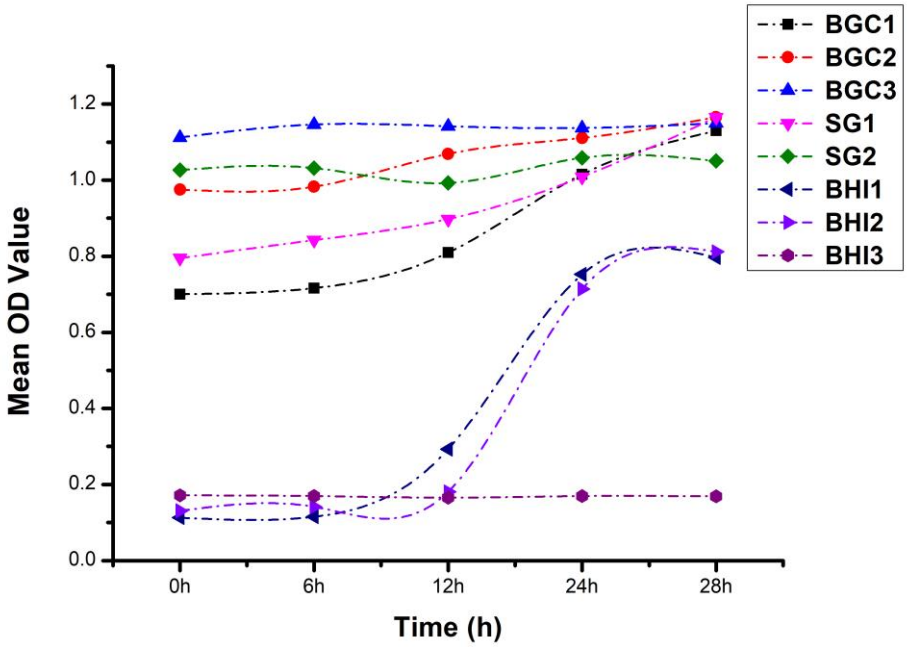

Figure 3. Mean Optical dentisty values of the study samples during the 48-hour incubation

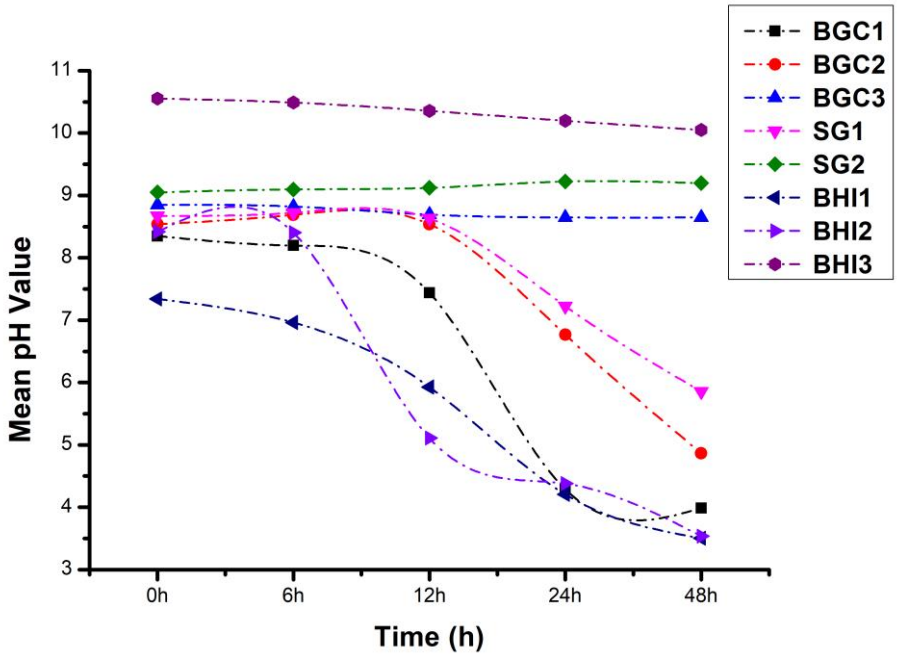

Figure 4. Mean of pH values of the study samples during the 48-hour incubation

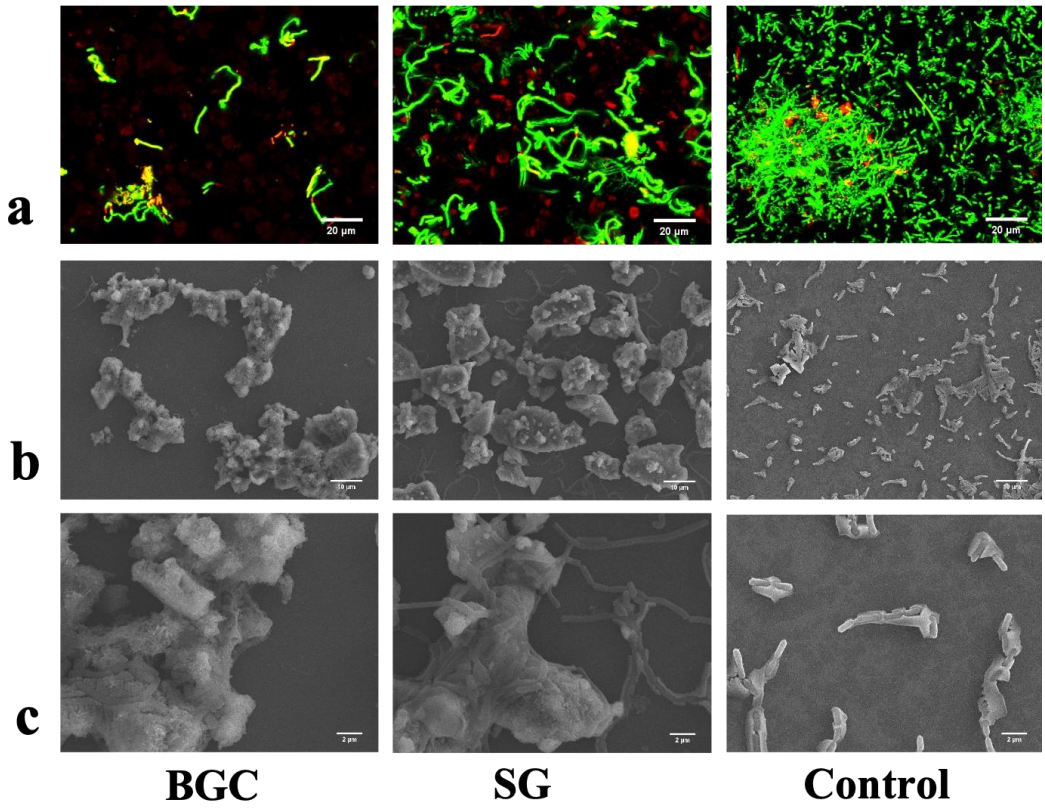

Figure 5. SEM/CLSM images of the bacteria: a) CLSM images of BGC, SG and control group; b) 1000× magnification images of BGC, SG and control group; c) 4000× magnification images of BGC, SG and control group

Tables

Table 1 Atomic percentage of the two study bioactive glasses (HX-BGC and SG)

	Atomic %	
Elements	**HX-BGC**	**SG**
O	45.39±0.24	46.72±0.21
Na	14.68±0.27	16.65±0.73
Si	13.40±0.38	18.89±0.27
P	8.86±0.12	3.09±0.07
Ca	16.07±0.26	14.66±0.99
Sr	1.59±0.04	-

Table 2 MBC of the two study bioactive glasses (HX-BGC and SG)

	MBC (mg/ml)	
Bacteria strains	**BGC**	**SG**
S. mutans (ATCC 35668)	15.63	31.25
S. sobrinus (ATCC 33478)	15.63	31.25
L. acidophilus (ATCC 9224)	7.81	31.25
L. rhamnosus (ATCC 10863)	62.5	125

Table 3 Ratio of live/dead bacteria in three experimental groups

Group	Area ratio of live/dead bacteria		P value	Multiple Comparison
	Mean	SD		
BGC (1)	0.18	0.10		
SG (2)	0.78	0.29	P<0.01	(1)=(2)<(3)
Control (3)	3.26	1.11		

Table 4 CFU results of HX-BGC and SG

Group	CFU/ml		
	Blood Agar Plates	Mitis Salicarius Agar Plates	Rogosa Agar Plates
HX-BGC	6.2×10^6	0	3.8×10^6
SG	1.5×10^7	0	9.4×10^6
BHI (control)	1.0×10^8	0.8×10^3	9.8×10^7