Resin-based sealant containing sol-gel derived bioactive glass: ion release and biological response

Shiva Jafarnia[1], Alireza Valanezhad[1*], Shigeaki Abe[1], Sima Shahabi[2], Ikuya Watanabe[1]

[1]Department of Dental and Biomedical Materials Science, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan

[2]Department of Dental biomaterials, School of dentistry, Tehran University of Medical Sciences, North Kargar Street, Tehran 1439955991, Iran

Correspondence

Alireza Valanezhad: vala@nagasaki-u.ac.jp

Mailing Address: Department of Dental and Biomedical Materials Science,

Graduate School of Biomedical Sciences

Nagasaki University 1-7-1 Sakamoto, Nagasaki

852-8588, Japan

Tel: +81 95 819 7657

Fax: +81 95 819 7658

Declarations

Conflicts of interest/Competing interests

All authors declare that they have no potential conflict of interest.

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Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shiva Jafarnia, Alireza Valanezhad, Shigeaki Abe, Sima Shahabi and Ikuya Watanabe. The first draft of the manuscript was written by Shiva Jafarnia and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The principal author of this article is Shiva Jafarnia.

Consent to Participate (Ethics)

Not applicable.

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Not applicable.

Abstract

The aim of this study was to examine the effect of bioactive glass (BAG) loading on the ion release, pH changes and cell response of the experimental pit and fissure sealant containing sol-gel derived 58S BAG. The BAG and silica filler in different proportion were incorporated into a resin matrix with the final filler loading of 50% in all groups. The specimens were immersed in deionized water (pH 5.8) or lactic acid solution (pH 4.0) at different time points (6 h, 1, 7, 14, 30 and 45 days) for ion release and pH changes assessment. Biological properties were evaluated using MTS assay, alkaline phosphatase assays and alizarin red staining. In the deionized water, the BAG50% group pH reaches to 9.3 and in lactic acid solution to 4.31 after 45 days. The BAG filler percentage did not have a significant effect on ion release in the deionized water. However, ion release increased with the pH reduction, particularly in the BAG50% group. The BAG50% group showed less cytotoxicity and higher ALP activity and calcified nodule formation. The experimental pit and fissure sealant with 50% BAG filler loading exhibited an appropriate increase in pH elevation, ion release, cell proliferation and differentiation. Therefore, it can be concluded that this bioactive sealant has the potential to hinder secondary caries and can be used as a caries-inhibiting material.

KEYWORDS

bioactive glass, biocompatibility, caries inhibition, ion release, resin-based sealant

1. Introduction

Application of pit and fissure sealant is a conservative and cost-effective way to prevent occlusal caries which is one of the most common diseases in pediatric dentistry [1]. It has been reported that 90% of the permanent posterior teeth caries occurs in pit and fissures [2]. Occlusal surfaces are the best sites for food remnant and plaque accumulation because of their morphology which makes them the most susceptible surface for dental caries [3]. The reason for pit and fissure sealants application is to inhibit occlusal surface caries, especially on high-risk patients [4]. However, microleakage or detachment of part of the restoration causes secondary caries around the interface of the tooth and material which is one of the main limitations for the longevity of pit and fissure sealants [5, 6]. Finding a solution to overcome this shortcoming play an important role to improve the effectiveness of the restoration. Materials that are capable of calcium and phosphate release to inhibit secondary caries and induce remineralization can help to enhance the performance of the sealant [7].

Bioactive glass (BAG) is a well-known biologically active material with applications in dentistry such as dental hypersensitivity treatment, dentifrices, mouthwashes and bone regeneration for the dental implant. The first bioactive glass was introduced by Larry Hench [8]. The BAG materials undergo dissolution and degradation when in contact with the physiological fluid so it can have a controlled release of therapeutic ions like calcium and phosphate, which may lead to their precipitation and subsequently are able to convert to hydroxyapatite-like crystallites [9–11]. They have been used as bone regenerative materials in medical fields such as orthopedics [12]. In dentistry BAG also can be incorporated into a resin-based matrix as a restorative material which is a new approach [13]. Different methods are being used for BAG preparation; the traditional melt-quench method is more complicated compared to sol-gel procedures. In recent years, the sol-

gel technique has received more attention due to its simplicity and lower temperature needed for preparation. In addition, the final result is BAG with high purity which is important since even minor impurities in high temperature during melt-quench process may compromise the BAG bioactivity [14, 15]. There have been some studies examining the effect of BAG in dental adhesive or composite resin, however, the impact of BAG filler made by the sol-gel processing especially in a resin-based pit and fissure sealant material is still unknown. Bioactive glass materials made by sol-gel technique have nanoporous glass structure and high surface area which enhances ion release in comparison to melt derived glasses [16]. The sol-gel derived 58S BAG specifically showed higher potential of bioactivity and apatite formation compared to the conventional 45S5 BAG [17]. The preparation technique, high bioactivity potential and elemental composition of 58S BAG makes it a good choice for biomaterials application. Furthermore, the properties of the 58S BAG suggest that its incorporation into a resin matrix as a dental sealant would yield a material capable of substantially inhibiting secondary caries.

The objective of this study was to determine the effect of bioactive glass filler loading on the ion release, pH changes and biocompatibility of the new experimental pit and fissure sealant containing sol-gel derived 58S bioactive glass. The null hypothesis was that there would be no significant differences in properties mentioned above in different BAG loading of our experimental pit and fissure sealant.

2. Materials and methods

2.1 Synthesis and characterization of 58S BAG powder

The final components (mol %) of sol-gel derived 58S BAG were 58% SiO_2 – 38% CaO – 4% P_2O_5 [14]. For the 58S BAG powder preparation first 10.24 g tetraethyl orthosilicate (TEOS, Wako Pure Chemicals Industries, Osaka, Japan), 1.16 g triethyl phosphate (TEP, Wako Pure Chemicals

Industries), 7.02 g calcium nitrate tetrahydrate (Ca $(NO_3)_2.4H_2O$, Wako Pure Chemicals Industries) were mixed with 7.82 g deionized water and 1.30 g hydrochloric acid (2 M), then the mixture was stirred vigorously for 3 hours (h) at room temperature. The BAG sol was formed after hydrolysis and condensation reaction. The sol was poured in containers and kept at room temperature for 72 h to create a transparent gel. Then the prepared gel was kept at 60˚C for 24 h and then at 120˚C in an oven for an additional 24 h. Finally, the dried gel was transferred to sinter at 700˚C for 1 h, then cooled to ambient temperature followed by milling in a ball-mill machine (Pulverisette 7, Fritsch GmbH, Idar-Oberstein, Germany) with zirconia balls (5 mm in diameter) for 1 h at 500 rpm to obtain 58S bioactive glass powder. Mean particle size was determined by laser diffraction particle size analyzer, with the average particle size ranging from 0.05μm to 20μm. The 58S BAG powder phase was identified by a benchtop X-ray diffraction analysis (XRD, Miniflex 600, Rigaku Co., Tokyo, Japan) using Cu-K_α radiation ($\lambda = 1.5406$ Å) at 40 kV and 15 mA at a scanning rate of $2\theta = 1°$/min and in the angular range of 10˚ and 70˚. The chemical composition of the prepared 58S BAG powder was analyzed by energy-dispersive X-ray spectroscopy (EDS, FE-SEM, JSM-7500, JEOL, Tokyo, Japan) using an accelerating voltage of 40 kV.

2.2 Formulation of pit and fissure sealants

A resin matrix was prepared with a mixture of 49.5 wt% bisphenol A glycerolate dimethacrylate (Bis-GMA, Shin-Nakamura, Wakayama, Japan) and 49.5 wt% triethylene glycol dimethacrylate (TEGDMA, Wako Pure Chemicals Industries, Osaka, Japan) in a 1:1 mass ratio, then 0.3 wt% camphorquinone (CQ, Wako Pure Chemicals Industries) and 0.6 wt% ethyl-4-dimethylaminobenzoate (EDMAB, Sigma-Aldrich, St. Louis, MO, USA) as photoinitiator and accelerator were added, respectively. The resin was mixed with two types of fillers, silanized silica

and 58S BAG in different proportions, with the final proportion of filler in all groups being 50% in weight (Table 1).

2.3 Preparation of pit and fissure sealant blocks

To assess pH change and ion release properties of the experimental pit and fissure sealant, stainless-steel rectangular mould (12.0 × 2.0 × 2.0 mm^3) was used to make the specimens. The mould was filled with the pit and fissure sealant which was prepared as mentioned above and covered with a microscope slide glass. Each specimen was light cured for 40 s from each side with LED light curing unit (Delight series, Dental Corp, Korea). For each of the groups, 36 specimens were prepared (total=144).

2.4 Calcium and phosphate ion release

Pit and fissure sealant blocks were divided into two groups based on the immersion solution, which were deionized water pH=5.8 or 50 mmol/L lactic acid pH=4.0. Every three blocks were immersed in a 15 ml polypropylene tube (Thermo Fisher Scientific, Leicestershire, UK) containing 10 ml of one of the storage solutions and stored at 37˚C. For all the groups six different time points (6 h, 1, 7, 14, 30 and 45 days) were defined, three immersed specimens removed from the tube at each time and samples immersed in the fresh solution. Subsequently, the collected solutions at all time points were analyzed for Ca and P ions concentration. The ion release was measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Ultima 2, Horiba Jobin Yvon, Tokyo, Japan).

2.5 pH evaluation

To evaluate the alkalinizing ability of the experimental groups the pH values were measured for each collected solution after specimen immersion and subsequently used for pH evaluation using pH meter (Horiba, D-22, Ltd., Kyoto, Japan). Similar to ion release evaluation the pH changes

were measured at six different time points (6 h, 1, 7, 14, 30 and 45 days). The pH meter was calibrated at pH 4.0, 7.0 and 9.0 immediately before pH measurement. Besides, pH values of control solutions, deionized water and lactic acid without any samples were measured.

2.6 Cell culture

Preosteoblastic cell line MC3T3-E1 (Riken Cell Bank, Tokyo, Japan) cultured in α-minimum essential medium (α-MEM; GIBCO, Invitrogen TM, NY, USA) containing 10% fetal bovine serum (FBS, GIBCO) and 100 IU/ml penicillin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was maintained at 37˚C under a humidified 5% CO_2 air atmosphere. The culture medium was changed every 2 days until confluent. Thirty-five disks (5 mm diameter and 1 mm thickness) were prepared for each group. The discs were polished using diamond polishing paper up to #1000 grit under continuous distilled water. Specimen decontamination was done by soaking in 99.5% ethanol for ten minutes.

2.7 Cell proliferation (MTS assay)

To investigate the effect of the experimental pit and fissure sealant on cell proliferation, mitochondrial dehydrogenase activity was measured by MTS assay. Five discs of each group were placed in 96 well dishes, and 5×10^4 cells/ml of MC3T3-E1 cells were seeded on the specimen surface. They were incubated at 37˚C and 5% CO_2 for 3, 6, 9 and 14 days. At each incubation time points, 25 µl of MTS solution (CellTiter 96 Aqueous One Solution, Promega, USA) was added to each well. Plates were incubated for 3 h and absorbance was measured after mixing the media using a plate reader (Multiskan FC, Thermo Fisher, Scientific Inc.) at 492 nm.

2.8 Alkaline phosphatase (ALP) activity assessment

The MC3T3-E1 cells differentiation in direct contact with the specimens was evaluated by (ALP) activity determination. On each disk 5×10^4 cells/ml were immersed in differentiation medium

which was prepared using 50 µl/ml ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA), 10 mM β-glycerophosphate (Sigma-Aldrich) and 50 nM dexamethasone addition to the proliferation medium. Cells were seeded and incubated at 37˚C and 5% CO_2 for 7, 14 and 21 days. Differentiation medium was changed every 2 days. At each incubation period, all the wells were first washed with PBS and then the cells were suspended in 50 µl of NP-40 lysis buffer (Wako Pure Chemicals Industries) for 15 minutes. P-nitrophenyl phosphate (Wako Pure Chemicals Industries) was applied as the substrate reaction with each specimen for 30 minutes at 37ºC, with Stop Solution (0.2 M NaOH solution, Wako Pure Chemicals Industries) being added to stop the reaction. The optical density was determined the ALP activity using a plate reader at 405 nm.

2.9 Alizarin red staining

Alizarin red S staining experiment was used to assess mineralized matrix formation and cell calcification of the samples after 7, 14, 21 and 30 days. MC3T3-E1 cells were seeded into the well plates in direct contact with the specimen at a disk density of 5×10^4 cells/ml in differentiation medium. After each incubation period, cells were rinsed with PBS and fixed with 10% formaldehyde for 20 minutes. Subsequently, cells were washed with distilled water and stained with 1% alizarin red S (Sigma-Aldrich) for 15 minutes. After alizarin red S aspiration, cells were rinsed with distilled water 3 times. Calcium nodule formation and cell calcification ability of the samples were evaluated under an optical microscope.

2.10 Statistical analysis

The mean and standard deviation of results obtained for each indivdual test were calculated for all experimental groups. The data were analyzed using analysis of variance (ANOVA) to determine the significant difference among groups for each variable using GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA, USA) ($P < 0.05$). Tukey's multi-comparisons test was

used for post hoc evaluation of each data set.

3. Results

3.1 Characterization of 58S BAG powder

Figure 1 shows the phase and elemental analysis results of the 58S BAG. The XRD and EDS results confirmed that the prepared powder characteristics were similar to the literature [14]. According to the XRD pattern, it is clear that the prepared 58S BAG's phase structure is amorphous, and no crystalline phase can be assigned in the pattern. EDS spectra indicated that the measured area elemental composition of 58S BAG was 57% SiO_2 – 40% CaO – 3% P_2O_5.

3.2 Calcium and phosphate ion release

The cumulative Ca and P ions concentration in deionized water and lactic acid are plotted in Figure 2 and 3, respectively. It is evident that all groups release Ca and P during the entire 45 days experiment period. The cumulative concentration of P release is significantly less than Ca in both deionized water and lactic acid solution. It is shown in the Figure 2 that in under non-cariogenic conditions, BAG loading does not have a significant effect on the release of both Ca and P ions. When the pH of the solution was decreased to 4.0, both Ca and P ions release amount significantly increased. The BAG50% group showed the highest release of Ca and P ions (Figure 3).

3.3 pH changes

The measured pH values of the solutions obtained during the pH-change evaluation of all groups are illustrated in Figure 4. In deionized water, all groups caused a significant increase in pH, with the largest change being observed for the BAG50% group. The pH of the aqueous solution in the BAG50% group reaches 9.3 after 45 days, which was significantly higher than the Si50% and BAG12.5% groups (Figure 4a). The results showed that in non-cariogenic solution, BAG50% which had the highest amount of BAG filler, could change the solution pH from 5.8 to 9.3; however,

the Si50% group which contain only silica filler could elevate the pH from 5.8 to 6.5. In lactic acid solution, pH changes were lower than in deionized water. Figure 4b shows that in a cariogenic environment, BAG50% group could change the pH from 4.0 to 4.31; in contrast, the Si50% group exhibited almost no change after 45 days immersion, with the pH changing from 4 to 4.03. It is shown in Figure 4b that in cariogenic solution, BAG50% group showed the most alkalizing potential in comparison to other groups.

3.4 MTS biocompatibility

MTS assay results of MC3T3-E1 cells cultured in direct contact with the specimens after different incubation periods are shown in the Figure 5. At first, there was not a significant difference between the control group and BAG50%. As the incubation time was increased to 6 days, the level of cell growth on the BAG50% was significantly higher than the other groups ($p<0.0001$). The significant cell viability of the BAG50% becomes greater after 9 and 14 days. The findings of the MTS test indicated that the group with no BAG filler (Si50%) after 3 days showed good biocompatibility and the cytotoxicity of the samples increased after 6 and 9 days. The cell viability for the BAG12.5% almost remained steady during all four-incubation periods. The BAG37.5% group results were not significantly different between 3, 6 and 9 days, but its cell viability increased after 14 days.

3.5 ALP activity

ALP activity of cells cultured in direct contact with each specimen is shown in Figure 6. After 7 days of incubation, there was no statistical difference between groups ($p>0.05$). As the incubation time was prolonged to 14 and 21 days, the BAG50% group showed significantly higher ALP activity than all other groups ($p<0.0001$). Although BAG50% showed significantly higher ALP

activity among all samples, there was not a significant difference among BAG37.5%, BAG12.5% and Si50% groups after 7, 14 and 21 days.

3.6 Mineralized matrix formation

Calcium deposits for all groups after each incubation period are shown in Figure 7. The calcium deposits were stained using alizarin red S. The BAG50% group enhanced the calcium nodule formation more than BAG37.5% and BAG12.5% groups. The Si50% group showed no cell calcification for an incubation period of 30 days. The effect of the BAG50% group on cell calcification was most pronounced after 30 days, after which a layer of calcium nodules could be seen at the surface of the sample.

4. Discussion

Application of pit and fissure sealant has a principal role in preventive dentistry. Pit and fissure sealant materials are used to inhibit occlusal surface caries. Although they strongly bind to the enamel, there is a debate on their ability to prevent microleakage which will lead to secondary caries at the interface of the tooth and the restorative material [3, 18, 19]. More recently, in an attempt to find a solution to the problem, two strategies have been implemented. The first utilizes the ability of the sealant in the cariogenic environment to increase the pH, which can reduce tooth demineralization, while the second involves remineralizing the demineralized tooth structure [20, 21]. Accordingly, the aim of this study was to develop efficient pit and fissure sealant material containing a different proportion of bioactive glass and evaluating their calcium and phosphate ion release, pH changes, cytotoxicity and cell differentiation after storage after different time points.

When the oral pH is in the range of 4-5.5 (cariogenic zone), enamel dissolution and demineralization occurs [22, 23]. Generally, saliva tends to buffer the cariogenic pH. However, this is insufficient; hence, if the restorative material had the ability to release calcium and

phosphate ions, this will have a synergistic effect and the conditions can be shifted towards remineralization [24]. Moreover, after demineralization, if the tooth is exposed to a high concentration of Ca and P ions, they may locate on the enamel surface and ion precipitation will occur which will result in remineralization. Hence, it can be concluded that ion release in the cariogenic environment is essential to ensure the effectiveness of our restorative materials [25]. Consistent with the previous studies, calcium and phosphate ions in the materials such as toothpaste or mouthwashes are effective for tooth remineralization acceleration and to reduce demineralization [26, 27]. Restorative materials which are able to supply beneficial ions such as calcium may be capable of hindering the development of secondary caries, since the released ions can be precipitated on the tooth and inhibit demineralization [28, 29]. The concentration of Ca and P ions in deionized water and lactic acid solution are shown in Figures 2 and 3. In the non-cariogenic situation, which in this research is deionized water, the solution pH rises to almost 9.5 and the BAG in the sealant becomes stable, hence the release of ions is not dependent on BAG loading at this pH. On the contrary, the lactic acid solution with lower pH accelerates the dissolution of the BAG and the group with the highest loading of BAG showed a significant increase in ion release. As is demonstrated, the amount of P release in the cariogenic environment is almost ten times lower than the Ca release especially in lactic acid solution which is similar to their loading in the BAG compositions. Subsequently, exposure of the tooth to a high concentration of Ca and P ions in this environment can lead to ion precipitation at the demineralized area and inhibit caries progression. The ability of the material to neutralize and stabilize the pH is important, since it has an inhibitory effect on demineralization. It was shown in a previous study that there is always a gap at the interface between the sealant and tooth, which allows bacterial invasion; these bacteria produce acid and result in demineralization at the margin of restoration [30, 31]. Such

acidogenic bacteria ferment carbohydrate and generate organic acids, including lactic acid, formic acid and propionic acid; the acidic solution used in our study was lactic acid [32, 33]. When experimental pit and fissure sealant specimens in the present study were subjected to both cariogenic and non-cariogenic environments, the group containing only BAG filler had a greater pH increase (Figure 4). Although the pH was fluctuating across the time series, the ascending trend during the 45 days immersion was maintained, especially in the cariogenic solution. Previous studies showed that BAG has a biomimetic property; when it's in contact with solvents, it releases calcium phosphate and in an acidic environment can increase the pH, which is consistent with the result of this research [13, 34]. This pH increase is mainly the result of ion exchange and has an impact on microleakage inhibition and caries prevention in the marginal gaps.

Since dental materials are in contact with the oral environment, they need to be harmless for our body, and most particularly for hard tissue. However, there are always unreacted monomers that leach from resin-based dental materials, which can affect their biocompatibility [35, 36]. Biocompatibility of the experimental material utilized in this research was assessed using a culture of MC3T3-E1 cells in direct contact with the specimens. The use of a homogenous cell line related to hard tissue formation is the best way to investigate the effect of the experimental material on cell proliferation and differentiation. This cell line consists of osteoblast-like cells and is commonly used to evaluate hard tissue responses [37, 38]. MTS assay showed the mitochondrial activity of the cells after incubation on different time points. The results of this study showed that as the incubation period increased, the differences between groups with different BAG loading become more significant (Figure 5). The reason for insignificant cell proliferation during the first three days can be related to monomer release, which has an inhibitory effect on cell proliferation. The group with only BAG filler showed significantly better cell proliferation in comparison to

other groups after 6 days. This difference is most likely a result of Ca and P ion release, which may reduce the effect of unreacted monomers and enable cells to have better proliferation. Other work showed that bioactive glass itself is non-cytotoxic [39], which is consistent with the results of our study which indicated that when the proportion of BAG filler increase, the biocompatibility of the sealant improves.

ALP is an enzyme and representative mediator which is involved in different biological processes. There are different types of ALPs in the human body, including placenta, intestinal, germ cell and bone-type. In dentistry, bone-type ALP is involved in hard tissue formation and calcification. ALP is necessary for mineralization and is one of the markers related to the mineralizing ability of the osteoblastic cells [40–42]. The effects of the experimental materials on the mineralization potential of the MC3T3-E1 cells were tested by ALP activity investigation. The result suggested that the sealant with only BAG filler has a significantly better effect on cell proliferation (Figure 6). Increasing the ALP activity can elevate the mineralization effect of the material. Previous studies showed that the improvement of cell proliferation and differentiation ability of materials depends on the concentration of released Ca ions [43, 44]. The improved ALP activity of the BAG 50% group confirmed the results of the ICP data regarding the high Ca and P release of this group. The mineralized matrix formation is an important factor in the in-vitro cell differentiation properties [40]. The alizarin red S staining results presented in Figure 7 indicated that cells cultured in contact with BAG50% produced more calcified deposits and mineralized matrix. Therefore, the calcium-rich deposits observed in this test indicated that the BAG50% group has the potential to promote cell differentiation and mineralization over time, which is inconsistent with ALP test results.

5. Conclusion

The result of this study demonstrated that the incorporation of 50% sol-gel derived 58S BAG filler to resin matrix for pit and fissure sealant preparation makes the sealant bioactive. This experimental sealant showed favorable ion release in cariogenic environment and became stable in a non-cariogenic situation. In addition, BAG filler improved the sealant's biological characteristics, which makes this material suitable for cell proliferation and differentiation. Hence this pit and fissure sealant is a potential candidate to prevent occlusal surface caries in high-risk patients. However, further investigation is recommended to assess in situ mineralizing properties and mechanical aspects of this experimental pit and fissure sealant.

References

1. Weintraub, J.: Pit and fissure sealants in high-caries-risk individuals. J. Dent. Educ. 65, 1084 (2001)
2. Naaman, R., El-Housseiny, A.A., Alamoudi, N.: The use of pit and fissure sealants—A literature review. Dent. J. 5, 34 (2017)
3. Yang, S.-Y., Piao, Y.-Z., Kim, S.-M., Lee, Y.-K., Kim, K.-N., Kim, K.-M.: Acid neutralizing, mechanical and physical properties of pit and fissure sealants containing melt-derived 45S5 bioactive glass. Dent. Mater. 29, 1228–1235 (2013). https://doi.org/10.1016/j.dental.2013.09.007
4. Yang, S.-Y., Kwon, J.-S., Kim, K.-N., Kim, K.-M.: Enamel Surface with Pit and Fissure Sealant Containing 45S5 Bioactive Glass. J. Dent. Res. 95, 550–557 (2016). https://doi.org/10.1177/0022034515626116
5. Fontana, M., Platt, J.A., Eckert, G.J., González-Cabezas, C., Yoder, K., Zero, D.T., Ando, M., Soto-Rojas, A.E., Peters, M.C.: Monitoring of Sound and Carious Surfaces under Sealants over 44 Months. J. Dent. Res. 93, 1070–1075 (2014). https://doi.org/10.1177/0022034514551753
6. Hevinga, M., Opdam, N., Frencken, J., Bronkhorst, E., Truin, G.: Microleakage and sealant penetration in contaminated carious fissures. J. Dent. 35, 909—914 (2007). https://doi.org/10.1016/j.jdent.2007.09.001
7. Cheng, L., Weir, M.D., Xu, H.H.K., Antonucci, J.M., Kraigsley, A.M., Lin, N.J., Lin-Gibson, S., Zhou, X.: Antibacterial amorphous calcium phosphate nanocomposites with a quaternary ammonium dimethacrylate and silver nanoparticles. Dent. Mater. 28, 561–572 (2012). https://doi.org/10.1016/j.dental.2012.01.005
8. Hench, L.L., Splinter, R.J., Allen, W.C., Greenlee, T.K.: Bonding mechanisms at the interface of ceramic prosthetic materials. J. Biomed. Mater. Res. Part V 5. 117–141 (1971)
9. Wheeler, D.L., Stokes, K.E., Hoellrich, R.G., Chamberland, D.L., McLoughlin, S.W.: Effect of bioactive glass particle size on osseous regeneration of cancellous defects. J. Biomed. Mater. Res. 41, 527–533 (1998). https://doi.org/10.1002/(SICI)1097-4636(19980915)41:4<527::AID-JBM3>3.0.CO;2-E
10. Hench, L.L., Polak, J.M.: Third-generation biomedical materials. Science. 295, 1014–1017 (2002). https://doi.org/10.1126/science.1067404
11. Hench, L.L.: Bioceramics and the origin of life. J. Biomed. Mater. Res. 23, 685–703 (1989). https://doi.org/10.1002/jbm.820230703
12. Hench, L.L.: The story of Bioglass®. J. Mater. Sci. Mater. Med. 17, 967–978 (2006). https://doi.org/10.1007/s10856-006-0432-z
13. Al-Eesa, N.A., Johal, A., Hill, R.G., Wong, F.S.L.: Fluoride containing bioactive glass composite for orthodontic adhesives - Apatite formation properties. Dent. Mater. Off. Publ. Acad. Dent. Mater. 34, 1127–1133 (2018). https://doi.org/10.1016/j.dental.2018.04.009

14. Chen, J., Zeng, L., Chen, X., Liao, T., Zheng, J.: Preparation and characterization of bioactive glass tablets and evaluation of bioactivity and cytotoxicity in vitro. Bioact. Mater. 3, 315–321 (2018). https://doi.org/10.1016/j.bioactmat.2017.11.004
15. Li, R., Clark, A.E., Hench, L.L.: An investigation of bioactive glass powders by sol-gel processing. J. Appl. Biomater. 2, 231–239 (1991). https://doi.org/10.1002/jab.770020403
16. Mitchell, J.C., Musanje, L., Ferracane, J.L.: Biomimetic dentin desensitizer based on nano-structured bioactive glass. Dent. Mater. Off. Publ. Acad. Dent. Mater. 27, 386–393 (2011). https://doi.org/10.1016/j.dental.2010.11.019
17. Sepulveda, P., Jones, J.R., Hench, L.L.: Characterization of melt‐derived 45S5 and sol‐gel–derived 58S bioactive glasses. J. Biomed. Mater. Res. Off. J. Soc. Biomater. Jpn. Soc. Biomater. Aust. Soc. Biomater. Korean Soc. Biomater. 58, 734–740 (2001)
18. Chigira, H., Manabe, A., Hasegawa, T., Yukitani, W., Fujimitsu, T., Itoh, K., Hisamitsu, H., Wakumoto, S.: Efficacy of various commercial dentin bonding systems. Dent. Mater. 10, 363–368 (1994). https://doi.org/10.1016/0109-5641(94)90060-4
19. Geiger, S.B., Gulayev, S., Weiss, E.I.: Improving fissure sealant quality: mechanical preparation and filling level. J. Dent. 28, 407–412 (2000). https://doi.org/10.1016/s0300-5712(00)00016-6
20. Moreau, J.L., Sun, L., Chow, L.C., Xu, H.H.K.: Mechanical and acid neutralizing properties and bacteria inhibition of amorphous calcium phosphate dental nanocomposite. J. Biomed. Mater. Res. B Appl. Biomater. 98B, 80–88 (2011). https://doi.org/10.1002/jbm.b.31834
21. Itota, T., Nakatsuka, T., Tanaka, K., Tashiro, Y., McCabe, J.F., Yoshiyama, M.: Neutralizing effect by resin-based materials containing silane-coated glass fillers. Dent. Mater. J. 29, 362–368 (2010). https://doi.org/10.4012/dmj.2009-108
22. Dawes, C.: What is the critical pH and why does a tooth dissolve in acid? J. Can. Dent. Assoc. 69, 722–724 (2003)
23. Touger-Decker, R., van Loveren, C.: Sugars and dental caries. Am. J. Clin. Nutr. 78, 881S-892S (2003). https://doi.org/10.1093/ajcn/78.4.881S
24. Li, X., Wang, J., Joiner, A., Chang, J.: The remineralisation of enamel: a review of the literature. J. Dent. 42 Suppl 1, S12-20 (2014). https://doi.org/10.1016/S0300-5712(14)50003-6
25. Weir, M.D., Ruan, J., Zhang, N., Chow, L.C., Zhang, K., Chang, X., Bai, Y., Xu, H.H.K.: Effect of calcium phosphate nanocomposite on in vitro remineralization of human dentin lesions. Dent. Mater. Off. Publ. Acad. Dent. Mater. 33, 1033–1044 (2017). https://doi.org/10.1016/j.dental.2017.06.015
26. Cury, J.A., Francisco, S.B., Simões, G.S., Del Bel Cury, A.A., Tabchoury, C.P.M.: Effect of a Calcium Carbonate-Based Dentifrice on Enamel Demineralization in situ. Caries Res. 37, 194–199 (2003). https://doi.org/10.1159/000070444
27. Reynolds, E.C., Cai, F., Shen, P., Walker, G.D.: Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J. Dent. Res. 82, 206–211 (2003). https://doi.org/10.1177/154405910308200311

28. Zhang, K., Zhang, N., Weir, M.D., Reynolds, M.A., Bai, Y., Xu, H.H.K.: Bioactive Dental Composites and Bonding Agents Having Remineralizing and Antibacterial Characteristics. Dent. Clin. North Am. 61, 669–687 (2017). https://doi.org/10.1016/j.cden.2017.05.002
29. Davis, H.B., Gwinner, F., Mitchell, J.C., Ferracane, J.L.: Ion release from, and fluoride recharge of a composite with a fluoride-containing bioactive glass. Dent. Mater. Off. Publ. Acad. Dent. Mater. 30, 1187–1194 (2014). https://doi.org/10.1016/j.dental.2014.07.012
30. Cenci, M.S., Pereira-Cenci, T., Cury, J.A., Ten Cate, J.M.: Relationship between gap size and dentine secondary caries formation assessed in a microcosm biofilm model. Caries Res. 43, 97–102 (2009). https://doi.org/10.1159/000209341
31. Chacko, Y., Lakshminarayanan, L.: pH stabilizing properties of a posterior light cured resin composite: an in vivo study. Oper. Dent. 26, 219–222 (2001)
32. Featherstone, J.D.B.: The continuum of dental caries--evidence for a dynamic disease process. J. Dent. Res. 83 Spec No C, C39-42 (2004). https://doi.org/10.1177/154405910408301s08
33. Deng, D.M., ten Cate, J.M.: Demineralization of Dentin by Streptococcus mutans Biofilms Grown in the Constant Depth Film Fermentor. Caries Res. 38, 54–61 (2004). https://doi.org/10.1159/000073921
34. Stoor, P., Söderling, E., Salonen, J.I.: Antibacterial effects of a bioactive glass paste on oral microorganisms. Acta Odontol. Scand. 56, 161–165 (1998). https://doi.org/10.1080/000163598422901
35. Ferracane, J.L.: Elution of leachable components from composites. J. Oral Rehabil. 21, 441–452 (1994). https://doi.org/10.1111/j.1365-2842.1994.tb01158.x
36. Ferracane, J.L.: Hygroscopic and hydrolytic effects in dental polymer networks. Dent. Mater. Off. Publ. Acad. Dent. Mater. 22, 211–222 (2006). https://doi.org/10.1016/j.dental.2005.05.005
37. Matsumoto, T., Kawakami, M., Kuribayashi, K., Takenaka, T., Minamide, A., Tamaki, T.: Effects of sintered bovine bone on cell proliferation, collagen synthesis, and osteoblastic expression in MC3T3-E1 osteoblast-like cells. J. Orthop. Res. 17, 586–592 (1999). https://doi.org/10.1002/jor.1100170419
38. Ogata, K., Imazato, S., Ehara, A., Ebisu, S., Kinomoto, Y., Nakano, T., Umakoshi, Y.: Comparison of osteoblast responses to hydroxyapatite and hydroxyapatite/soluble calcium phosphate composites. J. Biomed. Mater. Res. A. 72A, 127–135 (2005). https://doi.org/10.1002/jbm.a.30146
39. Mortazavi, V., Nahrkhalaji, M.M., Fathi, M.H., Mousavi, S.B., Esfahani, B.N.: Antibacterial effects of sol-gel-derived bioactive glass nanoparticle on aerobic bacteria. J. Biomed. Mater. Res. A. 94, 160–168 (2010). https://doi.org/10.1002/jbm.a.32678
40. Wu, T., Xu, C., DU, R., Wen, Y., Chang, J., Huan, Z., Zhu, Y.: Effects of silicate-based composite material on the proliferation and mineralization behaviors of human dental pulp cells: An in vitro assessment. Dent. Mater. J. 37, 889–896 (2018). https://doi.org/10.4012/dmj.2017-328

41. Malaval, L., Liu, F., Roche, P., Aubin, J.E.: Kinetics of osteoprogenitor proliferation and osteoblast differentiation in vitro. J. Cell. Biochem. 74, 616–627 (1999)
42. Beck, G.R., Jr., Sullivan, E.C., Moran, E., Zerler, B.: Relationship between alkaline phosphatase levels, osteopontin expression, and mineralization in differentiating MC3T3-E1 osteoblasts. J. Cell. Biochem. 68, 269–280 (1998). https://doi.org/10.1002/(SICI)1097-4644(19980201)68:2<269::AID-JCB13>3.0.CO;2-A
43. Takita, T., Hayashi, M., Takeichi, O., Ogiso, B., Suzuki, N., Otsuka, K., Ito, K.: Effect of mineral trioxide aggregate on proliferation of cultured human dental pulp cells. Int. Endod. J. 39, 415–422 (2006). https://doi.org/10.1111/j.1365-2591.2006.01097.x
44. Rashid, F., Shiba, H., Mizuno, N., Mouri, Y., Fujita, T., Shinohara, H., Ogawa, T., Kawaguchi, H., Kurihara, H.: The effect of extracellular calcium ion on gene expression of bone-related proteins in human pulp cells. J. Endod. 29, 104–107 (2003). https://doi.org/10.1097/00004770-200302000-00004

Figure legends:

Figure 1. Characterization of 58S BAG powder prepared by sol-gel technique. (a) XRD pattern, (b) EDS analysis.

Figure 2. Cumulative calcium (Ca) and phosphate (P) ion release from BAG containing specimens in deionized water.

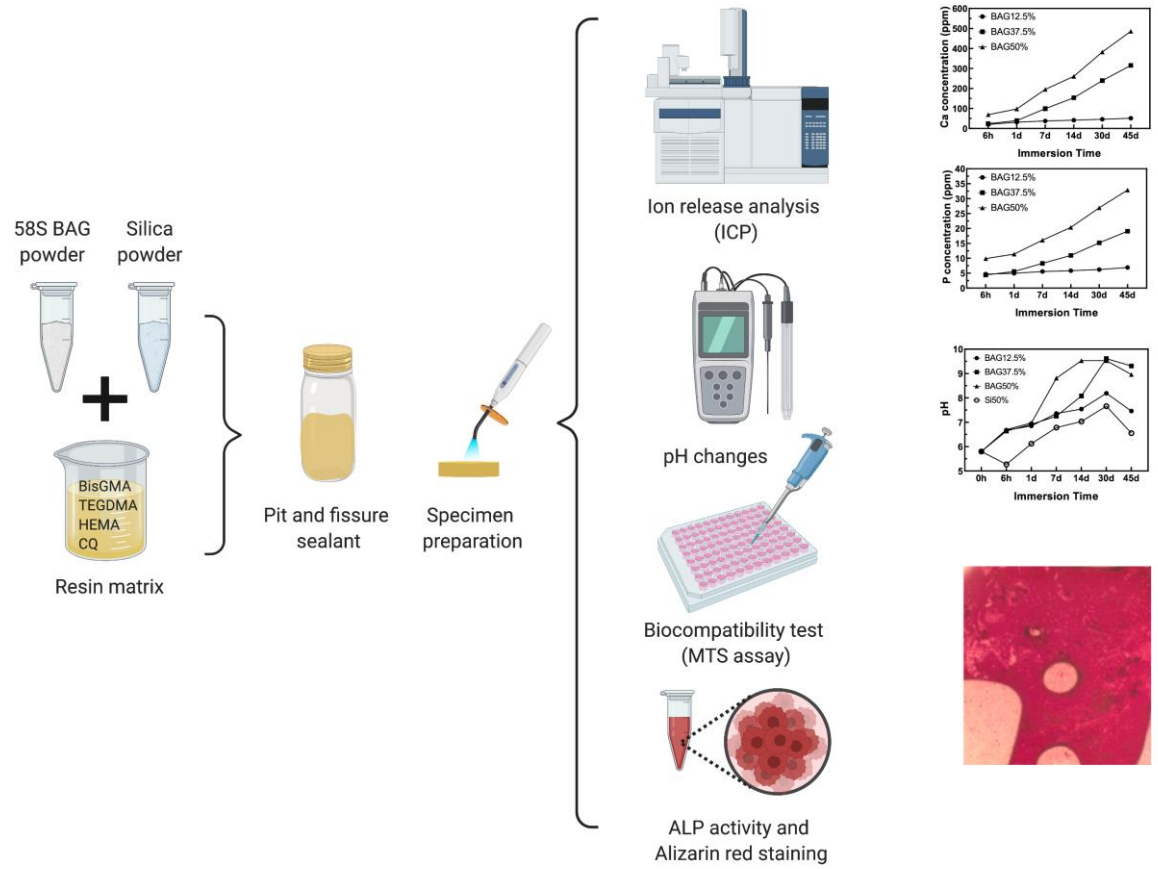
Figure 3. Cumulative calcium (Ca) and phosphate (P) ion release from BAG containing specimens in lactic acid solution.

Figure 4. pH of (a) deionized water and (b) lactic acid solution at six different time points after specimen immersion.

Figure 5. Mitochondrial dehydrogenase activities of cells cultured in direct contact with each specimen as determined by MTS assay. Similar lowercase letters indicate no statistically significant differences between groups at each incubation time ($p<0.05$).

Figure 6. ALP activities of the specimens cultured after 7, 14- and 21-days incubation period. Similar lowercase letters indicate no statistically significant differences between groups at each incubation time ($p<0.05$).

Figure 7. Alizarin red staining of each sample during 30 days incubation period.

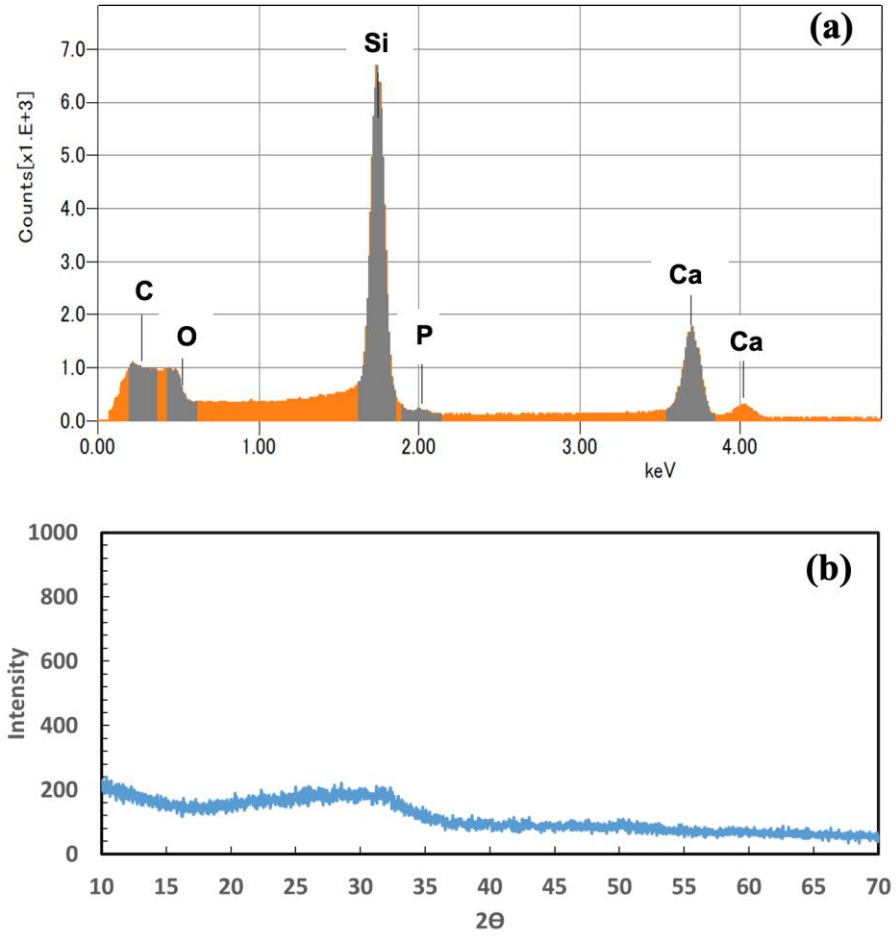


Graphical abstract

Table 1. Experimental group filler proportions.

Group	BAG 12.5%	BAG 37.5%	BAG 50%	Silica 50%
Resin matrix	50.0	50.0	50.0	50.0
58S BAG filler	12.5	37.5	50.0	0.0
Silica filler	37.5	12.5	0.0	50.0

Values are given in percentage weight



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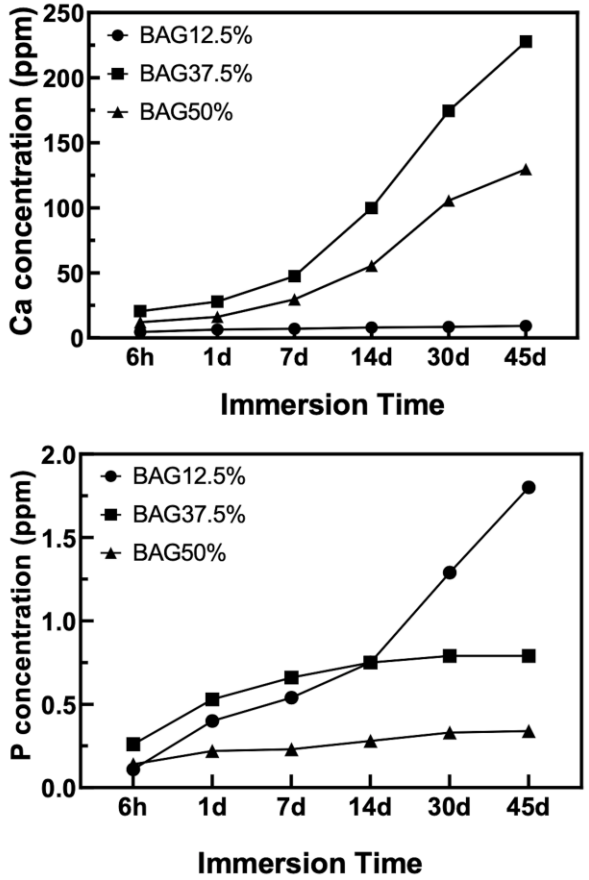


Figure 2. Cumulative calcium (Ca) and phosphate (P) ion release from BAG containing specimens in deionized water.

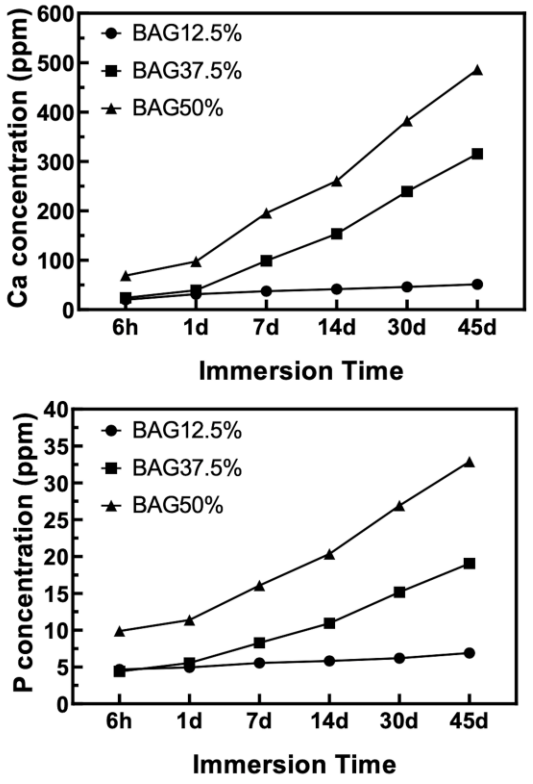


Figure 3. Cumulative calcium (Ca) and phosphate (P) ion release from BAG containing specimens in lactic acid solution.

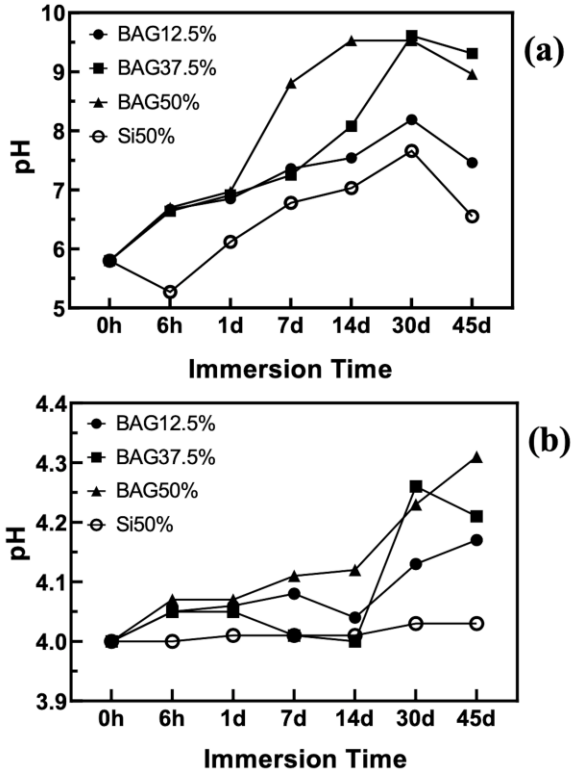
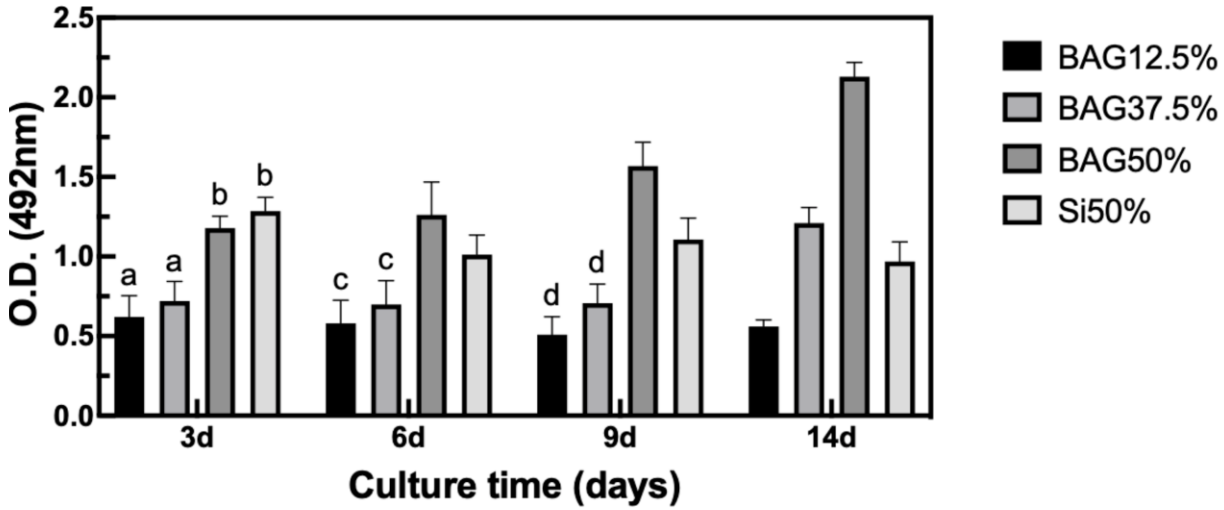


Figure 4. pH of (a) deionized water and (b) lactic acid solution at six different time points after specimen immersion.



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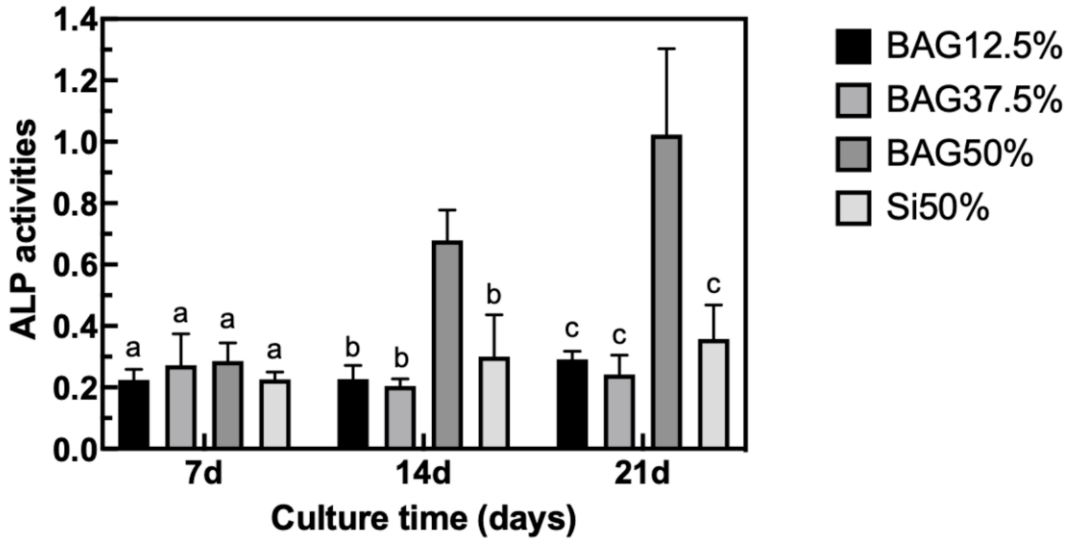

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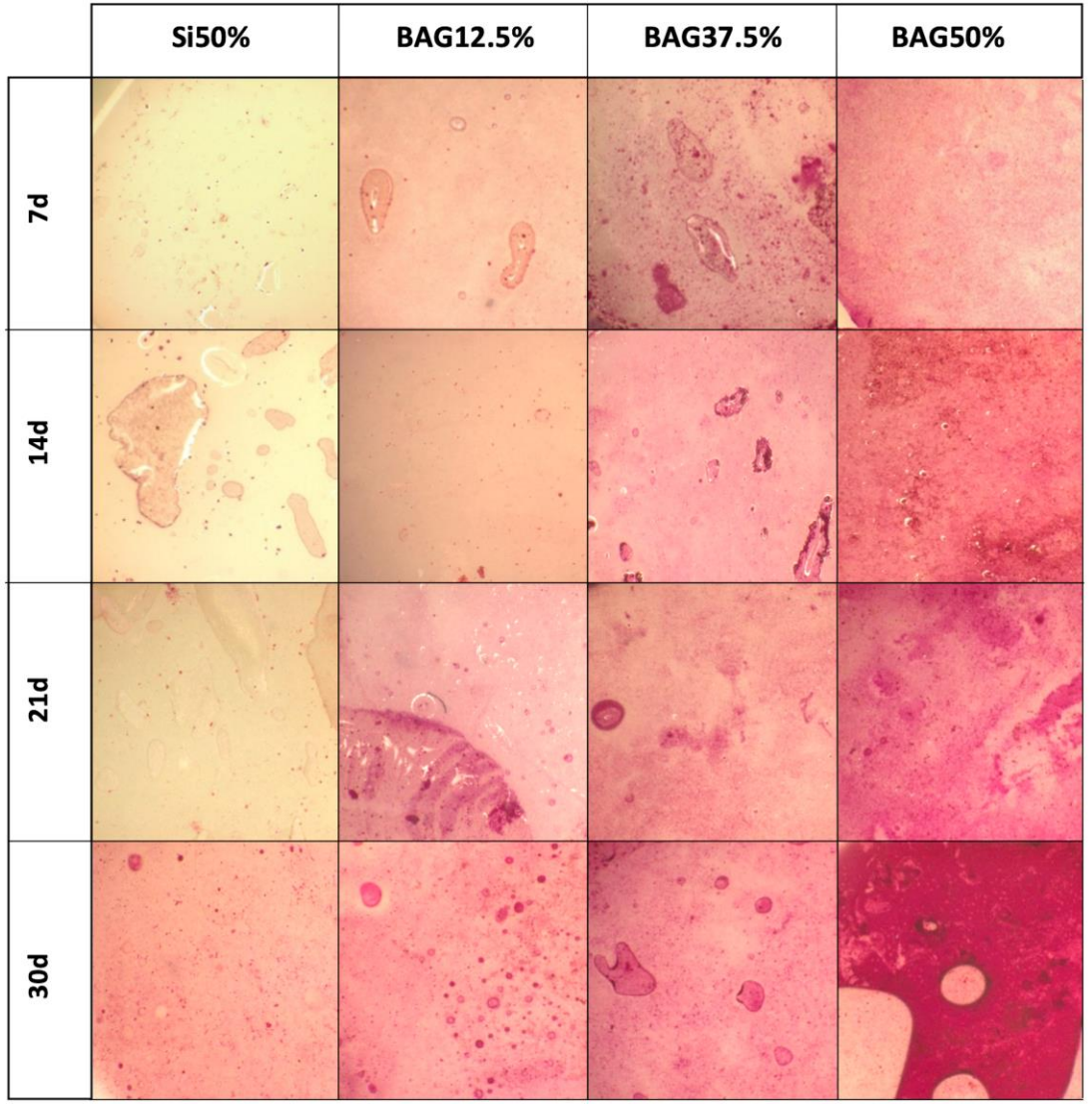

Figure 7. Alizarin red staining of each sample during 30 days incubation period.