Bioactive Materials 4 (2019) 207–214

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: http://www.keaipublishing.com/biomat

# Effect of solution condition on hydroxyapatite formation in evaluating bioactivity of $B_2O_3$ containing 45S5 bioactive glasses

Xiaonan Lu, Jessica Kolzow, Roberto R. Chen, Jincheng Du*

*Department of Materials Science and Engineering, University of North Texas, Denton, TX, 76203, USA*

ARTICLE INFO

*Keywords:*
Bioactive glasses
Boron oxide
In vitro
Hydroxyapatite

ABSTRACT

The effects of testing solutions and conditions on hydroxyapatite (HAp) formation as a means of *in vitro* bioactivity evaluation of $B_2O_3$ containing 45S5 bioactive glasses were systematically investigated. Four glass samples prepared by the traditional melt and quench process, where $SiO_2$ in 45S5 was gradually replaced by $B_2O_3$ (up to 30%), were studied. Two solutions: the simulated body fluid (SBF) and $K_2HPO_4$ solutions were used as the medium for evaluating *in vitro* bioactivity through the formation of HAp on glass surface as a function of time. It was found that addition of boron oxide delayed the HAp formation in both SBF and $K_2HPO_4$ solutions, while the reaction between glass and the $K_2HPO_4$ solution is much faster as compared to SBF. In addition to the composition and medium effects, we also studied whether the solution treatments (e.g., adjusting to maintain a pH of 7.4, refreshing solution at certain time interval, and no disturbance during immersion) affect HAp formation. Fourier transform infrared spectrometer (FTIR) equipped with an attenuated total reflection (ATR) sampling technique and scanning electron microscopy (SEM) were conducted to identify HAp formation on glass powder surfaces and to observe HAp morphologies, respectively. The results show that refreshing solution every 24 h produced the fastest HAp formation for low boron-containing samples when SBF was used as testing solution, while no significant differences were observed when $K_2HPO_4$ solution was used. This study thus suggests the testing solutions and conditions play an important role on the *in vitro* bioactivity testing results and should be carefully considered when study materials with varying bioactivities.

## 1. Introduction

45S5 Bioglass® with a composition of 46.1$SiO_2$-24.4$Na_2O$-26.9CaO-2.6$P_2O_5$ in mol% was discovered by Prof. Larry Hench in 1969 [1,2]. Various clinical products were developed based on 45S5 Bioglass®, such as orthopedics products for trauma, arthroplasty and spine fusion, cranial-facial products for cranioplasty, general oral/dental defect and periodontal repair, and dental-maxillofacial-ENT products (e.g., toothpaste, pulp capping, sinus obliteration, repair of orbital floor fracture) [3]. After the discovery of 45S5, many new bioactive glass compositions have been developed, since the glass matrix can accommodate various elements while maintaining the glass character and properties [4,5]. This composition flexibility enables the possibility to introduce additional functional elements that can potentially benefits to human body [6] such as enhancement of osteo-growth by $Sr^{2+}$ [7–9], angiogenesis by $Cu^{2+}$ [10–12], antibacterial by $Ag^{+}$ [13]. Recently, $B_2O_3$ containing bioactive glasses have drawn attention due to its potential effects and consequential biomedical applications, such as osteogenesis [14–18], angiogenesis [19], soft tissue repair [20], supporting tissue infiltration [21], controllable glass dissolution [21,22], improving coating adhesion [23,24], widening the processing window [25] and improving mechanical properties [26]. Accurate evaluation of the compositional effect, in particular the amount of $B_2O_3$, on the *in vitro* and *in vivo* bioactivity becomes critical while designing bioactive glass compositions for these applications.

Bioactive material is defined as a material that stimulates beneficial responses from the living tissue, organisms or cells, by inducing the formation of hydroxyl apatite (HAp) through which the material bonds to the host tissue [4]. Inorganic glasses in certain compositions such as 45S5 or other bioactive glasses, following the initial dissolution, illicit formation of HAp on the glass surface. The ability to form HAp in biological environment thus presents a means of evaluating the bioactivity of materials. There are generally two ways to evaluate bioactivity: in vitro and *in vivo* methods. An *in vitro* method evaluates bioactivity by testing the material in controlled environment outside living organism, such as by immersing materials in solutions such as simulated body

Peer review under responsibility of KeAi Communications Co., Ltd.
* Corresponding author.
*E-mail address:* du@unt.edu (J. Du).

https://doi.org/10.1016/j.bioactmat.2019.05.002
Received 14 May 2019; Received in revised form 29 May 2019; Accepted 31 May 2019
Available online 05 June 2019

fluid (SBF) or cell cultures. *In vitro* method is in general more economical and easier to implement as compared to an *in vivo* method which requires living organisms such as animal models. Nevertheless, it was found that bioactivity evaluated *in vitro* is generally agreeable with *in vivo* bioactivity with a few exceptions [27,28]. However, many challenges exist in study dissolution and bioactivity of glass materials *in vitro* [29], where testing conditions (e.g., glass mass [30] or glass surface area [31] to solution volume ratio, particle size [32], SBF preparation [27], medium pH [33], ion concentration [34], buffer type [35] and solution replenishment frequency [36]) directly affect the final results and interpretation of the glass bioactivity. For instance, mixed results were found in terms of bioactivity of boron-containing silicate glasses. Several studies showed that replacing $SiO_2$ with $B_2O_3$ produced a more rapid conversion of the glass to HAp [21,22,32], while some studies have also shown that the addition of boron impeded the HAp formation *in vitro* [31,37–41]. Various testing conditions can be one of the main reasons that caused these non-conclusive results from these studies.

In this work, we adopted a unified evaluation protocol proposed by Macon et al. [29] for bioactivity evaluation of glasses. The main objective is to investigate solution effects on HAp formation of boron-containing glasses *in vitro* by using both SBF and $K_2HPO_4$ with three different treatments. The rest of paper is arranged as following: methodology of glass synthesis procedure, bioactivity evaluation details and characterizations will be reported first. Then the results on characterizations of original glass samples, FTIR spectra of samples after different solution treatments and HAp morphology observed by SEM are presented. This is followed by discussions and conclusions.

## 2. Methodology

### 2.1. Glass synthesis procedures

Compositions of the glasses studied in this paper are shown in Table 1. $SiO_2$ was gradually (10, 20 and 30%) replaced by $B_2O_3$. The glasses were prepared by thoroughly mixing analytical grade $H_3BO_3$, $NH_4H_2PO_4$, $SiO_2$, $NaCO_3$ and $CaCO_3$ chemicals before melting in an $Al_2O_3$ crucible at 1300 °C for 2 h in an electrical furnace (Deltech Furnaces). Molten glasses were poured onto a stainless plate and cooled to room temperature.

Powder glass samples were prepared by manually crushing the bulk glass of each composition, grinding with an alumina mortar and pestle, and sieving to 32–45 μm with stainless steel sieves. Glass powders were cleaned in deionized (DI) water and ethanol two times each in an ultrasonic cleanser, respectively. Cleaned glass powders were oven-dried (90 °C) overnight and pending for in vitro tests.

### 2.2. Bioactivity evaluations

0.02 mol/L $K_2HPO_4$ solution was prepared by dissolving reagent grade $K_2HPO_4{\cdot}3H_2O$ in DI water, where the starting pH was 9.10 at room temperature, following studies on other boron-containing glasses [42–44]. pH measurements were performed on a bench-top pH/mV meter (Sper Scientific) with an accuracy of ± 0.02 pH. Simulated body fluids (SBFs) were prepared according to a study of Kokubo and Takadama [27] by mixing reagent grade chemicals in the following order: NaCl (8.035 g), $NaHCO_3$ (0.355 g), KCl (0.225 g), $K_2HPO_4{\cdot}3H_2O$ (0.231 g), $MgCl_2{\cdot}6H_2O$ (0.311 g), 1 mol/L HCl (39 mL), $CaCl_2$ (0.292 g) and $Na_2SO_4$ (0.072 g) in DI water (700 mL) with a plastic beaker at 37 °C. After the chemicals completely dissolved, DI water was added up to 900 mL in total, and the pH of the solution was 1.5 ± 0.1 at the time. The fluid was buffered to a pH value of 7.40 at 36 ± 0.5 °C by slowly adding tris (trihydroxymethyl)-aminomethane (total 6.118 g) and drops of 1 mol/L HCl alternately in order to maintain a fluctuation of pH values between 7.40 and 7.45. After dissolving all tris(trihydroxymethyl)-aminomethane, the solution was filled with DI water up to 1 L at room temperature.

Powder samples were used for in vitro tests, following a unified evaluation proposed by Macon et al. [29]: each 75 ± 0.5 mg glass powder was put in a polypropylene tube (Corning Inc.) with a 50 mL solution at 37 ± 0.2 °C up to 10 days. The tubes were agitated at an interval time throughout the in vitro tests to prevent glass powders from sticking together. Three different solution treatments were conducted on each glass composition: 1) no refreshment or adjustment of solutions was performed (referred as “still treatment”); 2) solutions were adjusted with HCl drops every 24 h to maintain a pH value of 7.4 (referred at “adjusted treatment”); 3) solutions were refreshed every 24 h (referred as “refreshed treatment”). Glass powders were obtained at different time intervals, washed in ethanol and oven-dried (90 °C) overnight. Fig. 1 shows an outline of the experimental details.

### 2.3. Glass characterizations

Glass powders were characterized with high-resolution X-ray diffraction (XRD) on a Rigaku Ultima III with a scanning speed of 3°/min and a step of 0.03°/point. XRD pattern analysis was performed with JADE 9 software package.

Fourier transform infrared spectrometer (FTIR) equipped with an attenuated total reflection (ATR) sampling technique was conducted with a Nicolet 6700 spectrometer (Thermo Electron) at room temperature. A diamond substrate was used for the ATR sampling. A total of 32 scans for background and per sample were used with a resolution of 2 $cm^{-1}$. A commercial HAp powder (calcium phosphate tribasic) obtained from Fisher Scientific was taken as a reference material.

Scanning electron microscopy (SEM) was conducted on a FEI Quanta ESEM to observe surface morphology of SBF treated samples after Au–Pd coating.

## 3. Results

XRD patterns of the four glass samples obtained from the melt-quench process are shown in Fig. 2. 14B sample was partially crystallized as shown in Fig. 2, where the crystalline phases could be $Na_3Ca_6(PO_4)_5$ or hexagonal $Na_2Ca_4(PO_4)_2SiO_4$, as studied previously [41]. Fig. 3 presents photos of the glass samples. Even though no crystallization peaks were observed from XRD pattern of 9B, there are heterogeneous phases in 9B as visually observed from the sample (shown in Fig. 3 (c)). In our previous studies [39,41], it was also observed that addition of boron increases the crystallization tendency of both 45S5 and 55S4.3 bioactive glasses at low $SiO_2/B_2O_3$ substitution, while no crystallization was found at high substitution levels (> 50%). It was found that crystallization delayed the initial time of HAp formation on 45S5 but did not inhibit the formation of HAp [45].

Fig. 4 and Fig. 5 show the FTIR spectra of 0B and 5B after SBF immersion with three different solution treatments (still, adjusted and refreshed), along with a FTIR spectrum of a commercial HAp powder for reference. The appearance of a split phosphate P–O bending band (~560 and 600 $cm^{-1}$), P–O stretching band (~1015 $cm^{-1}$) and a carbonate band (~870 $cm^{-1}$) indicates the formation of HAp [46]. For both 0B and 5B samples, refreshing SBF every 24 h promotes the HAp formation on glass surface, while still and adjusted treatments exhibit

**Table 1**
Composition (mol%) of the glasses studied.

| Sample | Composition (mol%) | | | | |
|---|---|---|---|---|---|
| | $B_2O_3$ | $SiO_2$ | $Na_2O$ | CaO | $P_2O_5$ |
| 0B | 0 | 46.1 | 24.4 | 26.9 | 2.6 |
| 5B | 4.6 | 41.5 | 24.4 | 26.9 | 2.6 |
| 9B | 9.2 | 36.9 | 24.4 | 26.9 | 2.6 |
| 14B | 13.8 | 32.3 | 24.4 | 26.9 | 2.6 |

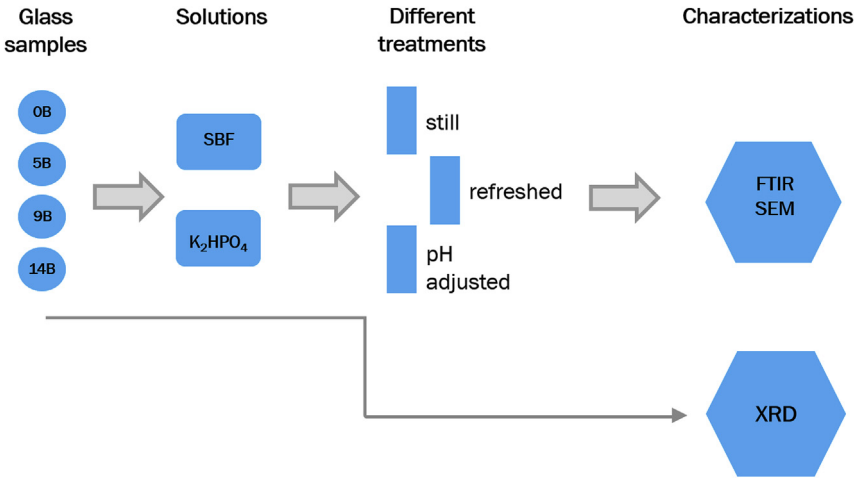


**Fig. 1.** Outline of the experimental details.

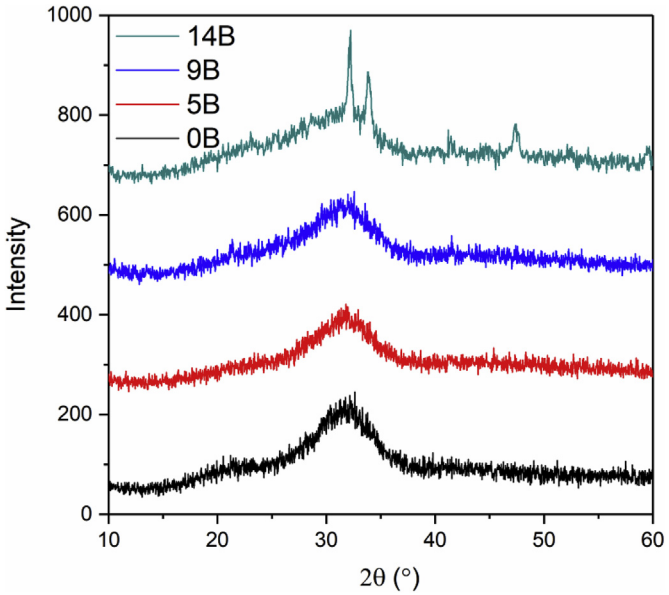


**Fig. 2.** XRD patterns of the prepared glass samples.

slower and similar HAp formation. However, for 9B and 14B samples, no HAp formation was identified by FTIR after SBF treatments for 10 days (shown in Fig. 6 and Fig. 7).

Fig. 8, Fig. 9, Fig. 10 and Fig. 11 show the FTIR spectra of 0B, 5B, 9B and 14B after $K_2HPO_4$ solution immersion with three different solution treatments, respectively. As compared to SBF treatments, HAp formation is much faster in $K_2HPO_4$ solution. Except 14B sample, HAp formation was identified by FTIR after 2 days of immersion for all samples among all the solution treatments. For 14B, HAp formation was not identified after 4 days of immersion for all treatments. Shorter sampling points are needed for $K_2HPO_4$ solution in order to observe difference between glass compositions and solution treatments.

SEM images of 0B after SBF immersion with three different solution treatments are shown in Fig. 12. Different HAp morphology was observed on glass surface after 7 days of SBF treatments. HAp formed after still treatment (Fig. 12 (a)) has a spherical feature in comparison with the other two treatments, which is consistent with a previous study [41]. For refreshed treatment, HAp precipitates have a needle-shaped or a flaky feature (Fig. 12 (c)); whereas, HAp formed after pH adjusted treatment ((Fig. 12 (b)) are more granular and no other distinguishable features were observed.

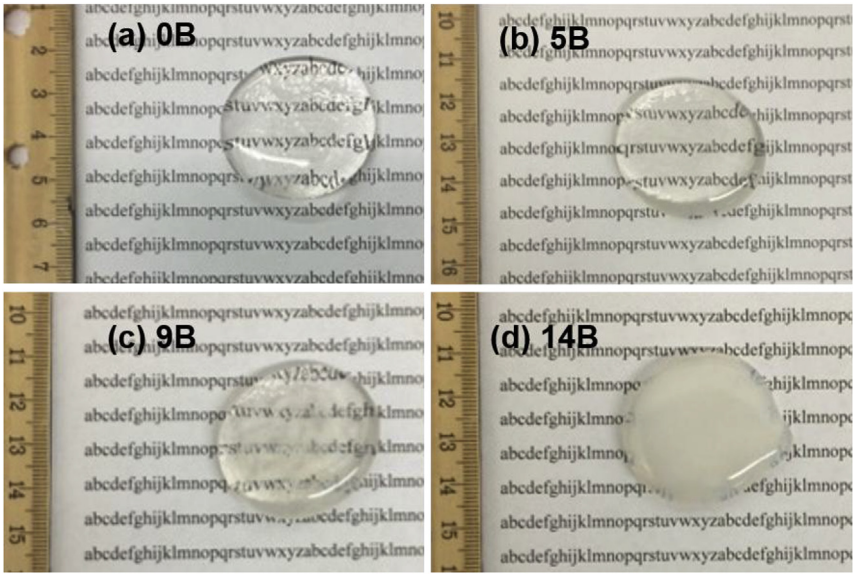


**Fig. 3.** Photos of the prepared glass samples.

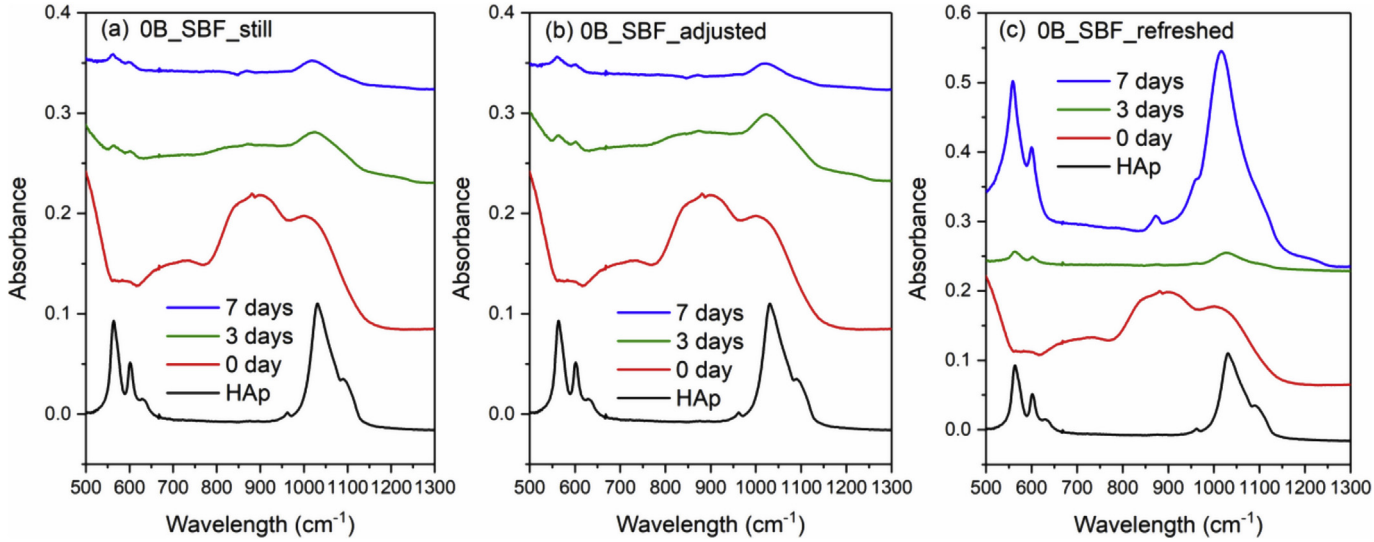


**Fig. 4.** FTIR spectra of the commercial HAp powder and 0B after SBF immersion with three different solution treatments, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

## 4. Discussions

Through this study, we found that different HAp growth behaviors and surface morphology during bioactivity testing when different solutions (SBF versus $K_2HPO_4$ solution) and testing conditions (e.g. static, refreshing, pH control) were used, suggesting complexity of the testing and calling for protocols to evaluate bioactivity of glass and glass-ceramic materials. The results indicate that solution chemistry has a significant effect on glass dissolution and HAp formation, and consequently the bioactivity. These results are in general consistent with those reported in the literature, where various testing conditions such as glass surface area to solution volume ratio [31], medium pH [33], ion concentration [34], buffer type [35] and solution replenishment frequency [36] were found to affect the final results and interpretation of the glass bioactivity. The mechanism of HAp formation [47,48] generally involves: 1) rapid cation exchange and creating silanol bonds (Si–OH) on the glass surface; 2) breaking Si–O–Si bonds caused by high local pH; 3) condensation of Si–OH groups near the glass surface and repolymerization of the silica-rich layer; 4) migration of $Ca^{2+}$ and $PO_4^{3-}$ through the silica-rich layer and from the solution, forming a film rich in amorphous $CaO–P_2O_5$ on the silica-rich layer; 5) incorporation of hydroxyls and carbonate from solution and crystallization of the $CaO–P_2O_5$ film to HAp. The Si–OH groups in $SiO_2$-rich layer were believed to provide nucleation sites for the apatite formation [49,50]; however, some studies have demonstrated that glasses without Si can form HAp in vitro as well [21,22,31,32,51–54]. Solution chemistry can greatly affect the concentration of the ions (particularly $Ca^{2+}$, $PO_4^{3-}$) critical for HAp formation, dissolution of the glasses, migration of ions from glass and solution, as well as nucleation and crystallization growth of HAp, leading to various results of glass bioactivity evaluations. For example, refreshing the test solutions would provide a constant level of critical ions for HAp formation hence lead to the highest HAp growth rate. This becomes critical when compositions that have low bioactivity are tested. Therefore, choosing the appropriate *in vitro* testing conditions is critical for evaluation and study the bioactivity of glass materials.

Very different conditions were used in the literature for bioactivity testing. For example, tests using the $K_2HPO_4$ solution were usually not

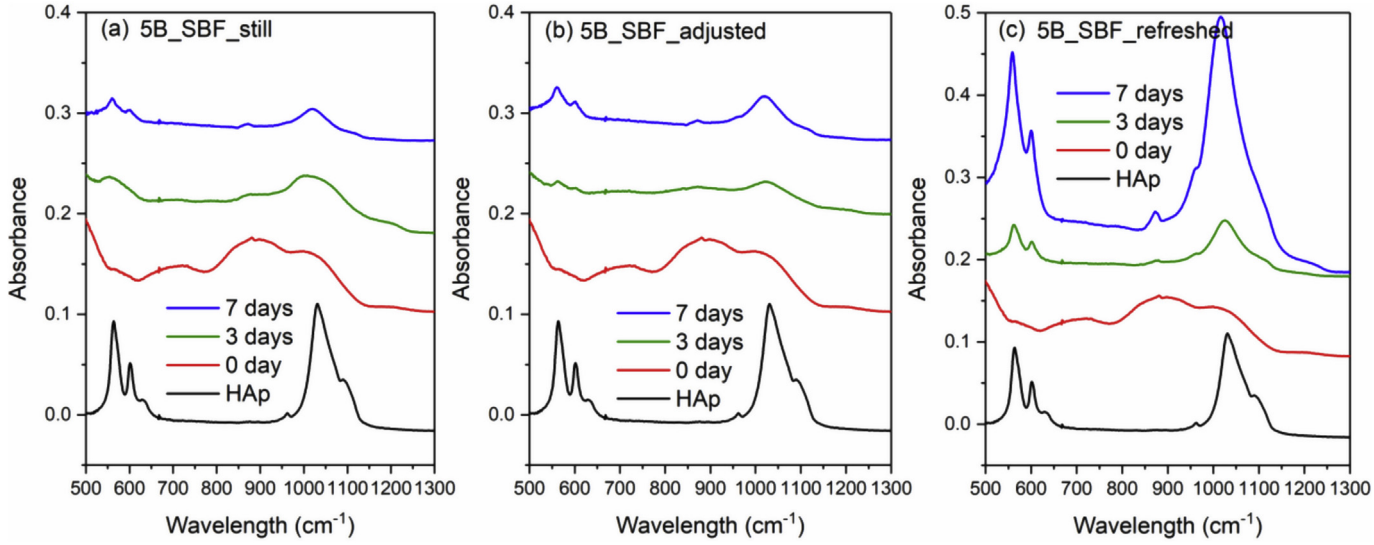


**Fig. 5.** FTIR spectra of the commercial HAp powder and 5B after SBF immersion with three different solution treatments, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

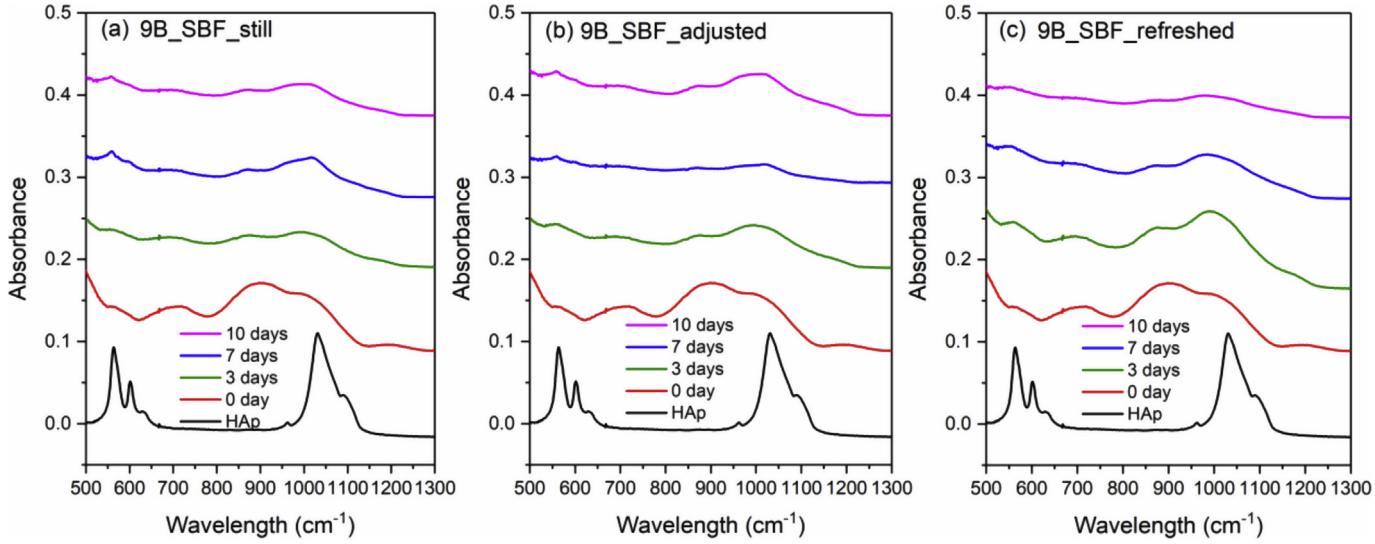


**Fig. 6.** FTIR spectra of the commercial HAp powder and 9B after SBF immersion with three different solution treatments, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

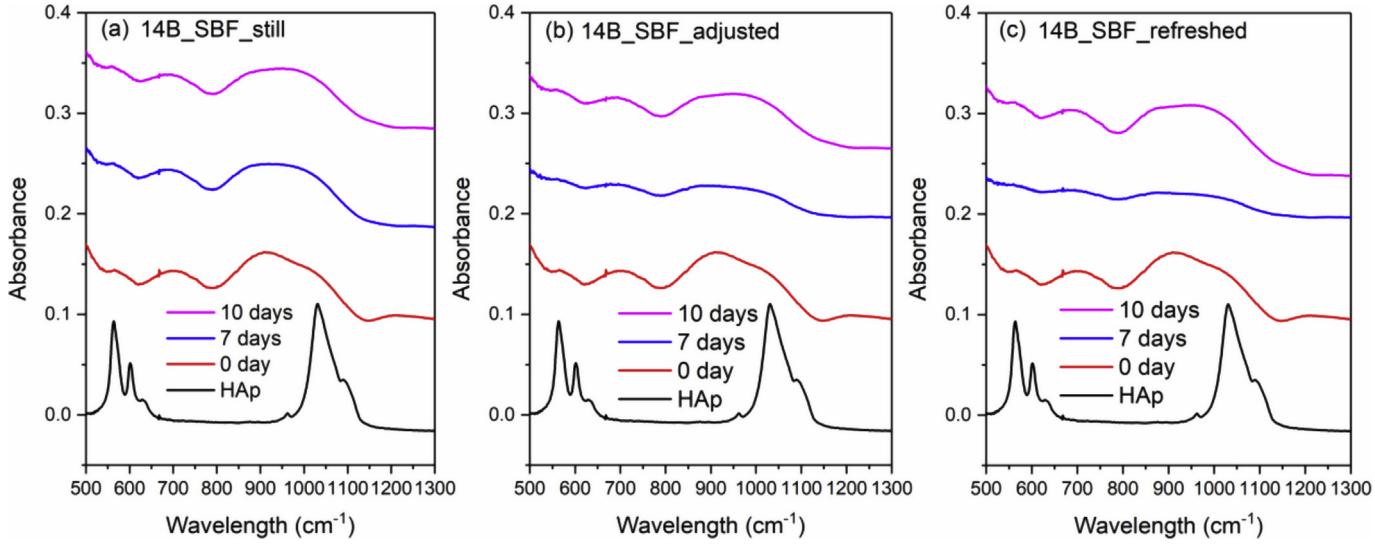


**Fig. 7.** FTIR spectra of the commercial HAp powder and 14B after SBF immersion with three different solution treatments, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

buffered while SBF contained TRIS buffer. It was found previously that the TRIS buffer in SBF distorts the assessment of glass-ceramic scaffold bioactivity, where TRIS buffer can increase the dissolving rate (by two times) of the glass and facilitate the HAp formation as compared to SBF solution without TRIS buffer [55]. As compared to SBF, however, HAp formation was found to be much faster in the $K_2HPO_4$ solution in our study. For instance, HAp formation of 9B was identified by FTIR after immersion in $K_2HPO_4$ solution for 2 days, while no HAp formation was found after SBF immersion for 10 days. This might be caused by the high concentration of $HPO_4^{2-}$ ions (1.0 mM in SBF [27] and 20 mM in $K_2HPO_4$ solution) and the high starting pH value (7.4 for SBF and 9.1 for $K_2HPO_4$ solution) of $K_2HPO_4$ solution, resulting a faster glass dissolution and HAp precipitation.

It was found that refreshing SBF every 24 h led to a much quicker HAp formation, while the 0B and 5B samples show the fastest HAp growth. The other two conditions, still or pH adjustment treatments, on the other hand, showed no significant effect on the speed of HAp formation. This indicates that ion concentrations in SBF, which provide source of phosphorus and calcium ions, has a far greater impact on HAp formation than the pH variation. On the other hand, no obvious trend was observed for the three treatments tested using the $K_2HPO_4$ solution. This might be due to the much higher phosphate concentration in $K_2HPO_4$ than SBF, suggesting that shorter sampling intervals are needed for $K_2HPO_4$ solution in order to observe differences between glass compositions and solution treatments.

Different HAp crystal morphologies were observed by SEM imaging for 45S5 samples treated in SBF solution with different conditions. Previously, it was found that the morphology of HAp formed depends on the types of immersion solutions [35,36,41]. In this work, even though the same medium (e.g., SBF) was used, different solution treatments were found to have a noticeable impact on the morphology of HAp formed as well, indicating that ion concentrations in solution and pH can also greatly affect HAp nucleation and formation mechanisms. Surface analyzing tests are desired for better study the effect of solution treatments on the composition of calcium phosphate precipitates (e.g., Ca/P ratio) formed. For example, the increased pH of the SBF during *in vitro* test can affect Ca/P molar ratios and chemical compositions of calcium phosphate precipitates [56]. $HCO_3^-$

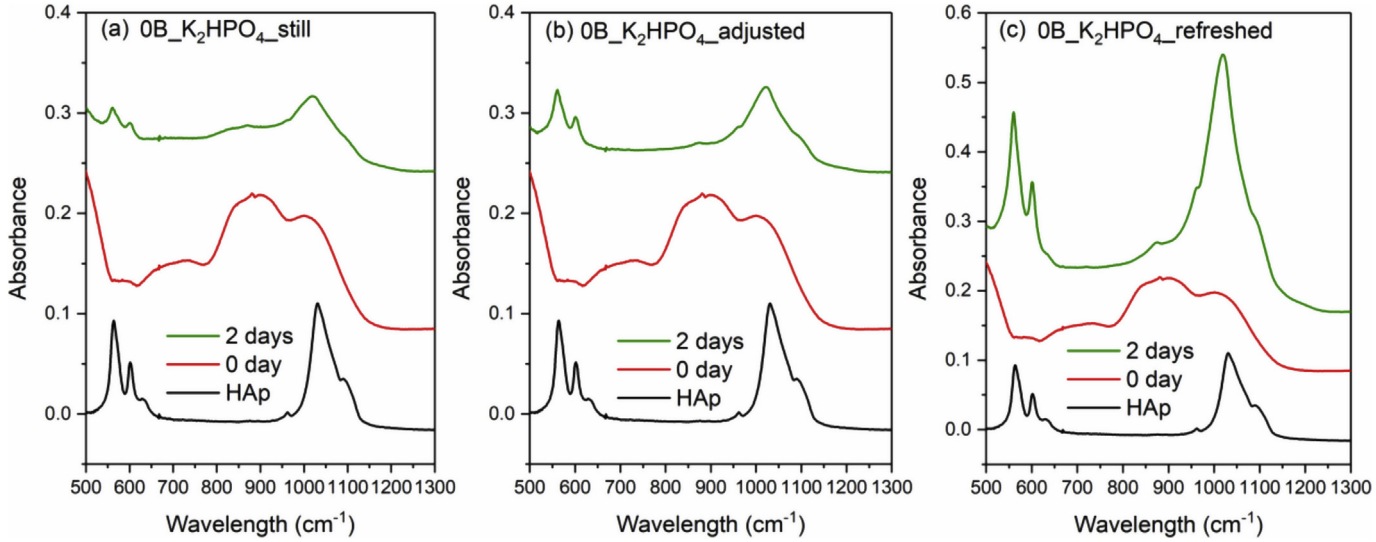


**Fig. 8.** FTIR spectra of the commercial HAp powder and 0B after $K_2HPO_4$ solution immersion with three different solution treatments, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

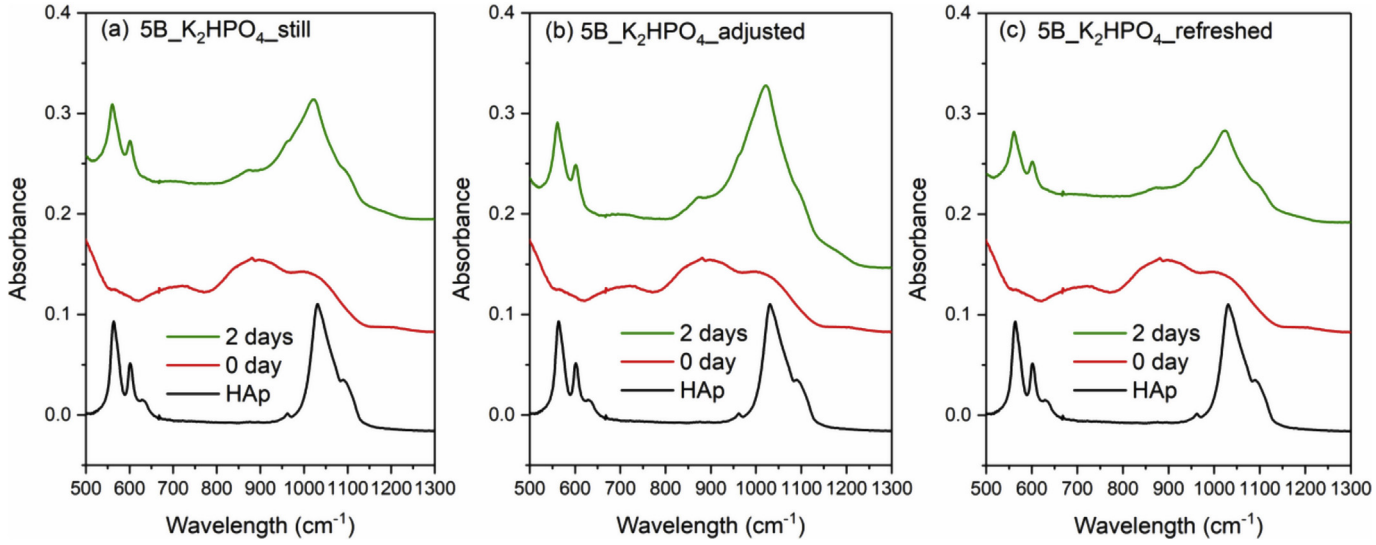


**Fig. 9.** FTIR spectra of the commercial HAp powder and 5B after $K_2HPO_4$ solution immersion with three different solution treatments, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

concentration in SBF can affect the thickness of calcium phosphate formed [34], as well as the heterogeneity and the crystal size of the calcium phosphate precipitates [57]. Additionally, it was found that chloride ions in TRIS-HCl butter solution were incorporated in the apatite formation during immersion tests, affecting the final composition of precipitates [46]. Future detailed compositional studies of formed HAp are desired for better understanding the apatite formation mechanism, which can benefit the improvement of *in vitro* bioactivity evaluation of glass materials, as well as designing new functional biomaterials (e.g., apatite formed matches bone or dentin tissue [58,59]).

## 5. Conclusions

The effects of three solution treatment conditions: still, pH adjusted, and refreshed for SBF and $K_2HPO_4$ solutions on HAp formation for *in vitro* bioactivity testing were studied on a series of boron oxide containing 45S5 bioactive glasses. It was found that, in general, substituting $SiO_2$ with $B_2O_3$ (up to 30%) in 45S5 delayed the HAp formation in both SBF and $K_2HPO_4$ solutions. Refreshing SBF was found to lead to the fastest HAp formation for low boron-containing glasses, while refreshing solution was found to have no significant differences on the results while using the $K_2HPO_4$ solution. It was also observed that HAp formation is much faster in $K_2HPO_4$ solution as compared to SBF. Additionally, different morphologies of HAp precipitates were observed by SEM on the glass surface of 45S5 after immersion in SBF for 7 days, indicating that ion concentrations and solution pH can affect HAp nucleation and formation mechanisms. These results provide insights on understanding the test conditions on *in vitro* bioactivity testing and how to better evaluate novel bioactive glasses with ever increasing composition domains. Further studies to find out the HAp composition differences would be beneficial to understand the formation mechanisms and how the solution composition affect HAp crystal nucleation and growth.

## Acknowledgments

We acknowledge funding support of the NSF Ceramics program (project #1508001) and JK acknowledges support of the NSF REU

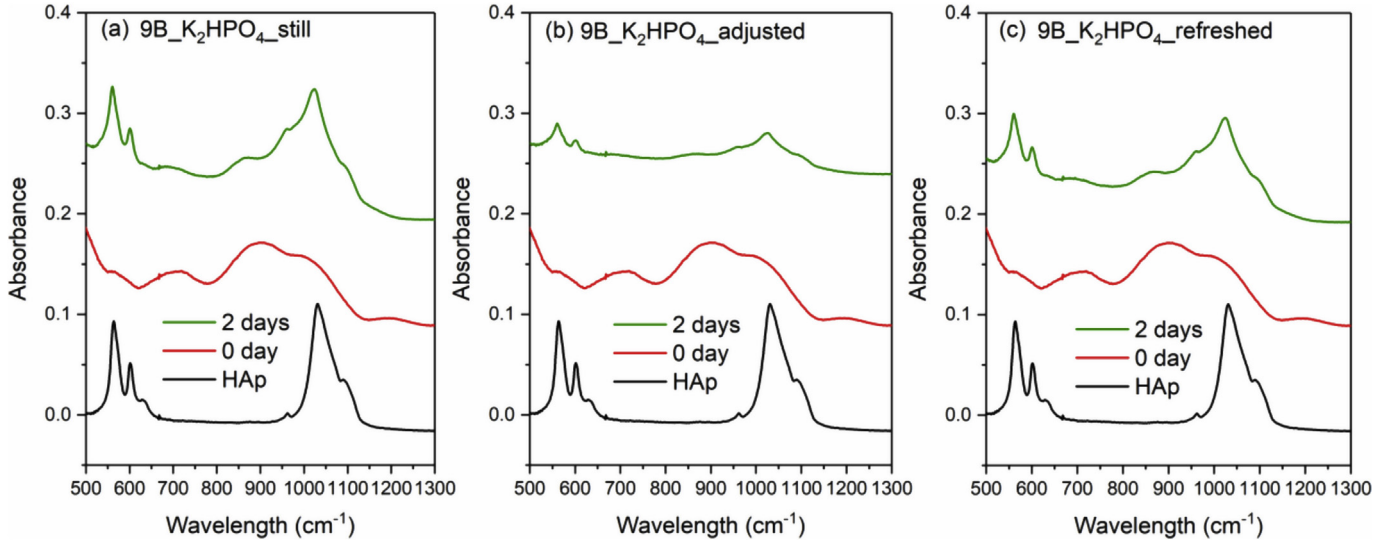


**Fig. 10.** FTIR spectra of the commercial HAp powder and 9B after $K_2HPO_4$ solution immersion with three different solution treatments, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

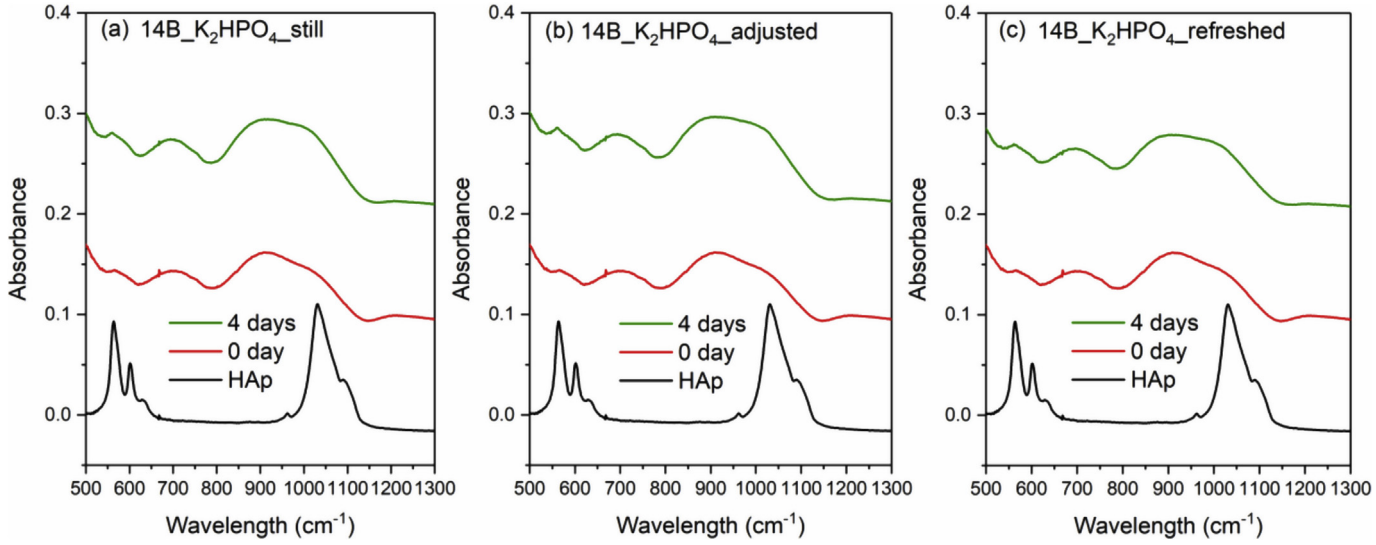


**Fig. 11.** FTIR spectra of the commercial HAp powder and 14B after $K_2HPO_4$ solution immersion with three different solution treatments, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

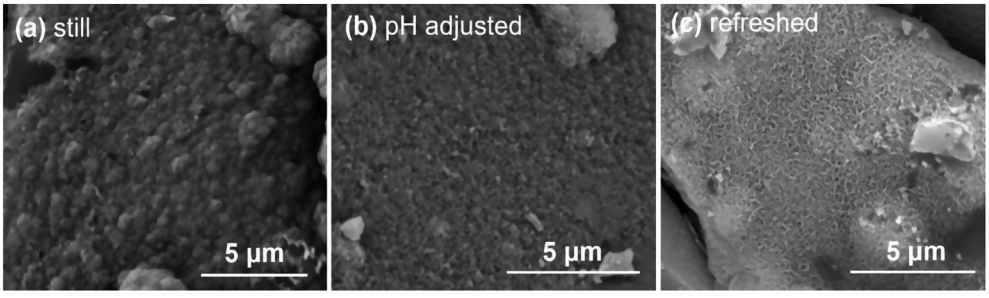


**Fig. 12.** SEM images of 0B after SBF immersion with three different solution treatments for 7 days, where (a), (b) and (c) is still, adjusted and refreshed, respectively.

program (project #1461048). SEM, XRD and FTIR experiments were conducted at the Materials Research Facility (MRF), a shared research facility for multidimensional fabrication and characterization at University of North Texas (UNT).

## References

[1] L.L. Hench, R.J. Splinter, W.C. Allen, T.K. Greenlee, Bonding mechanisms at the interface of ceramic prosthetic materials, J. Biomed. Mater. Res. 5 (1971) 117–141.
[2] L.L. Hench, The story of Bioglass, J. Mater. Sci. Mater. Med. 17 (2006) 967–978.
[3] L.L. Hench, Chronology of bioactive glass development and clinical applications, New J. Glass Ceram. 03 (2013) 67–73.

[4] J.R. Jones, Reprint of: review of bioactive glass: from Hench to hybrids, Acta Biomater. 23 (2015) S53–S82.
[5] S.M. Rabiee, N. Nazparvar, M. Azizian, D. Vashaee, L. Tayebi, Effect of ion substitution on properties of bioactive glasses: a review, Ceram. Int. 41 (2015) 7241–7251.
[6] G. Kaur, O.P. Pandey, K. Singh, D. Homa, B. Scott, G. Pickrell, A review of bioactive glasses: their structure, properties, fabrication and apatite formation, J. Biomed. Mater. Res. A 102 (2014) 254–274.
[7] M.D. O'Donnell, R.G. Hill, M.D.O. Donnell, R.G. Hill, Influence of strontium and the importance of glass chemistry and structure when designing bioactive glasses for bone regeneration, Acta Biomater. 6 (2010) 2382–2385.
[8] M. Tian, F. Chen, W. Song, Y. Song, Y. Chen, C. Wan, X. Yu, X. Zhang, In vivo study of porous strontium-doped calcium polyphosphate scaffolds for bone substitute applications, J. Mater. Sci. Mater. Med. 20 (2009) 1505–1512.
[9] N.J. Lakhkar, E.A. Abou Neel, V. Salih, J.C. Knowles, Strontium oxide doped quaternary glasses: effect on structure, degradation and cytocompatibility, J. Mater. Sci. Mater. Med. 20 (2009) 1339–1346.
[10] K.S. Raju, G. Alessandri, M. Ziche, P.M. Gullino, Ceruloplasmin, copper ions, and angiogenesis, J. Natl. Cancer Inst. 69 (1982) 1183–1188.
[11] M.N. Rahaman, D.E. Day, B. Sonny Bal, Q. Fu, S.B. Jung, L.F. Bonewald, A.P. Tomsia, Bioactive glass in tissue engineering, Acta Biomater. 7 (2011) 2355–2373.
[12] C. Stähli, M. James-Bhasin, A. Hoppe, A.R. Boccaccini, S.N. Nazhat, Effect of ion release from Cu-doped 45S5 Bioglass® on 3D endothelial cell morphogenesis, Acta Biomater. 19 (2015) 15–22.
[13] M. Bellantone, N.J. Coleman, L.L. Hench, Bacteriostatic action of a novel four-component bioactive glass, J. Biomed. Mater. Res. 51 (2000) 484–490.
[14] L.A. Haro Durand, A. Góngora, J.M. Porto López, A.R. Boccaccini, M.P. Zago, A. Baldi, A. Gorustovich, In vitro endothelial cell response to ionic dissolution products from boron-doped bioactive glass in the $SiO_2$-CaO-$P_2O_5$-$Na_2O$ system, J. Mater. Chem. B. 2 (2014) 7620–7630.
[15] A.A. Gorustovich, J.M.P. López, M.B. Guglielmotti, R.L. Cabrini, Biological performance of boron-modified bioactive glass particles implanted in rat tibia bone marrow, Biomed. Mater. 1 (2006) 100–105.
[16] X. Chen, Y. Zhao, S. Geng, R.J. Miron, Q. Zhang, C. Wu, Y. Zhang, In vivo experimental study on bone regeneration in critical bone defects using PIB nanogels/boron-containing mesoporous bioactive glass composite scaffold, Int. J. Nanomed. 10 (2015) 839–846.
[17] P. Balasubramanian, T. Büttner, V. Miguez Pacheco, A.R. Boccaccini, Boron-containing bioactive glasses in bone and soft tissue engineering, J. Eur. Ceram. Soc. 38 (2018) 855–869.
[18] C. Wu, R. Miron, A. Sculean, S. Kaskel, T. Doert, R. Schulze, Y. Zhang, Proliferation, differentiation and gene expression of osteoblasts in boron-containing associated with dexamethasone deliver from mesoporous bioactive glass scaffolds, Biomaterials 32 (2011) 7068–7078.
[19] L.A. Haro Durand, G.E. Vargas, N.M. Romero, R. Vera-Mesones, J.M. Porto-López, A.R. Boccaccini, M.P. Zago, A. Baldi, A. Gorustovich, Angiogenic effects of ionic dissolution products released from a boron-doped 45S5 bioactive glass, J. Mater. Chem. B. 3 (2015) 1142–1148.
[20] L.M. Marquardt, D. Day, S.E. Sakiyama-Elbert, A.B. Harkins, Effects of borate-based bioactive glass on neuron viability and neurite extension, J. Biomed. Mater. Res. A 102 (2014) 2767–2775.
[21] Q. Fu, M.N. Rahaman, H. Fu, X. Liu, B.S. Bal, L.F. Bonewald, K. Kuroki, R.F. Brown, H. Fu, X. Liu, Silicate, borosilicate, and borate bioactive glass scaffolds with controllable degradation rate for bone tissue engineering applications. I. Preparation and in vitro degradation, J. Biomed. Mater. Res. A 95A (2010) 164–171.
[22] A. Yao, D. Wang, W. Huang, Q. Fu, M.N. Rahaman, D.E. Day, In vitro bioactive characteristics of borate-based glasses with controllable degradation behavior, J. Am. Ceram. Soc. 90 (2007) 303–306.
[23] R.K. Brow, S.K. Saha, J.I. Goldstein, Interfacial reactions between titanium and borate glass, MRS Online Proc. Libr. Arch. 314 (1993) 77–81.
[24] L. Peddi, R.K. Brow, R.F. Brown, Bioactive borate glass coatings for titanium alloys, J. Mater. Sci. Mater. Med. 19 (2008) 3145–3152.
[25] O. Rodriguez, D.J. Curran, M. Papini, L.M. Placek, A.W. Wren, E.H. Schemitsch, P. Zalzal, M.R. Towler, Characterization of silica-based and borate-based, titanium-containing bioactive glasses for coating metallic implants, J. Non-Cryst. Solids 433 (2016) 95–102.
[26] K. Xie, L.L. Zhang, X. Yang, X. Wang, G. Yang, L.L. Zhang, Y. He, J. Fu, Z. Gou, H. Shao, Y. He, J. Fu, Z. Gou, Preparation and characterization of low temperature heat-treated 45S5 bioactive glass-ceramic analogues, Biomed. Glas. 1 (2015) 80–92.
[27] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 27 (2006) 2907–2915.
[28] A.A. Zadpoor, Relationship between in vitro apatite-forming ability measured using simulated body fluid and in vivo bioactivity of biomaterials, Mater. Sci. Eng. C 35 (2014) 134–143.
[29] A.L. Macon, T.B. Kim, E.M. Valliant, K. Goetschius, R.K. Brow, D.E. Day, A. Hoppe, A.R. Boccaccini, I.Y. Kim, C. Ohtsuki, T. Kokubo, A. Osaka, M. Vallet-Regi, D. Arcos, L. Fraile, A.J. Salinas, A.V. Teixeira, Y. Vueva, R.M. Almeida, M. Miola, C. Vitale-Brovarone, E. Verne, W. Holand, J.R. Jones, A.L.B. Maçon, T.B. Kim, E.M. Valliant, K. Goetschius, R.K. Brow, D.E. Day, A. Hoppe, A.R. Boccaccini, I.Y. Kim, C. Ohtsuki, T. Kokubo, A. Osaka, M. Vallet-Regí, D. Arcos, L. Fraile, A.J. Salinas, A.V. Teixeira, Y. Vueva, R.M. Almeida, M. Miola, C. Vitale-Brovarone, E. Verné, W. Höland, J.R. Jones, A unified in vitro evaluation for apatite-forming ability of bioactive glasses and their variants, J. Mater. Sci. Mater. Med. 26 (2015) 115.
[30] J.R. Jones, P. Sepulveda, L.L. Hench, Dose-dependent behavior of bioactive glass dissolution, J. Biomed. Mater. Res. 58 (2001) 720–726.
[31] Manupriya, K.S. Thind, G. Sharma, K. Singh, V. Rajendran, S. Aravindan, Soluble borate glasses: in vitro analysis, J. Am. Ceram. Soc. 90 (2007) 467–471.
[32] W. Huang, D.E. Day, K. Kittiratanapiboon, M.N. Rahaman, Kinetics and mechanisms of the conversion of silicate (45S5), borate, and borosilicate glasses to hydroxyapatite in dilute phosphate solutions, J. Mater. Sci. Mater. Med. 17 (2006) 583–596.
[33] L. Bingel, D. Groh, N. Karpukhina, D.S. Brauer, Influence of dissolution medium pH on ion release and apatite formation of Bioglass® 45S5, Mater. Lett. 143 (2015) 279–282.
[34] E.I. Dorozhkina, S.V. Dorozhkin, Surface mineralisation of hydroxyapatite in modified simulated body fluid (mSBF) with higher amounts of hydrogencarbonate ions, Colloids Surfaces A Physicochem. Eng. Asp. 210 (2002) 41–48.
[35] S. Jalota, S.B. Bhaduri, A.C. Tas, Effect of carbonate content and buffer type on calcium phosphate formation in SBF solutions, J. Mater. Sci. Mater. Med. 17 (2006) 697–707.
[36] L. Varila, S. Fagerlund, T. Lehtonen, J. Tuominen, L. Hupa, Surface reactions of bioactive glasses in buffered solutions, J. Eur. Ceram. Soc. 32 (2012) 2757–2763.
[37] Y. Ebisawa, T. Kokubo, K. Ohura, T. Yamamuro, Bioactivity of CaO·$SiO_2$-based glasses:in vitro evaluation, J. Mater. Sci. Mater. Med. 1 (1990) 239–244.
[38] R. Hill, An alternative view of the degradation of bioglass, J. Mater. Sci. Lett. 15 (1996) 1122–1125.
[39] X. Lu, L. Deng, P.H. Kuo, M. Ren, I. Buterbaugh, J. Du, Effects of boron oxide substitution on the structure and bioactivity of SrO-containing bioactive glasses, J. Mater. Sci. 52 (2017) 8793–8811.
[40] Y. Yu, M. Edén, Structure–composition relationships of bioactive borophosphosilicate glasses probed by multinuclear $^{11}B$, $^{29}Si$, and $^{31}P$ solid state NMR, RSC Adv. 6 (2016) 101288–101303.
[41] X. Lu, L. Deng, C. Huntley, M. Ren, P.H. Kuo, T. Thomas, J. Chen, J. Du, Mixed network former effect on structure, physical properties, and bioactivity of 45S5 bioactive glasses: an integrated experimental and molecular dynamics simulation study, J. Phys. Chem. B 122 (2018) 2564–2577.
[42] W. Huang, D.E. Day, M.N. Rahaman, Conversion of tetranary borate glasses to phosphate compounds in aqueous phosphate solution, J. Am. Ceram. Soc. 91 (2008) 1898–1904.
[43] W. Huang, D.E. Day, M.N. Rahaman, Comparison of the formation of calcium and barium phosphates by the conversion of borate glass in dilute phosphate solution at near room temperature, J. Am. Ceram. Soc. 90 (2007) 838–844.
[44] W. Liang, M.N. Rahaman, D.E. Day, N.W. Marion, G.C. Riley, J.J. Mao, Bioactive borate glass scaffold for bone tissue engineering, J. Non-Cryst. Solids 354 (2008) 1690–1696.
[45] O.P. Filho, G.P. Latorre, L.L. Hench, O. Peitl Filho, G.P. Latorre, L.L. Hench, Effect of crystallization on apatite-layer formation of bioactive glass 45S5, J. Biomed. Mater. Res. 30 (1996) 509–514.
[46] G. Kirste, J. Brandt-Slowik, C. Bocker, M. Steinert, R. Geiss, D.S. Brauer, Effect of chloride ions in tris buffer solution on bioactive glass apatite mineralisation, Int. J. Appl. Glass Sci. (2017) 1–12.
[47] L.L. Hench, Bioceramics: from concept to clinic, J. Am. Ceram. Soc. 74 (1991) 1487–1510.
[48] A.E. Clark, C.G. Pantano, L.L. Hench, Auger spectroscopic analysis of bioglass corrosion films, J. Am. Ceram. Soc. 59 (1976) 37–39.
[49] L.L. Hench, J. Wilson, An Introduction to Bioceramics, World Scientific Publishing Co. Pte. Ltd., Singapore, 1993.
[50] D.S. Brauer, N. Karpukhina, M.D. O'Donnell, R. V Law, R.G. Hill, Fluoride-containing bioactive glasses: effect of glass design and structure on degradation, pH and apatite formation in simulated body fluid, Acta Biomater. 6 (2010) 3275–3282.
[51] A.M. Abdelghany, The elusory role of low level doping transition metals in lead silicate glasses, Siliconindia 2 (2010) 179–184.
[52] K.S. Manupriya, K. Thind, V. Singh, G. Kumar, D.P. Sharma, D.P. Singh, Compositional dependence of in-vitro bioactivity in sodium calcium borate glasses, J. Phys. Chem. Solids 70 (2009) 1137–1141.
[53] S. Cai, G.H. Xu, X.Z. Yu, W.J. Zhang, Z.Y. Xiao, K.D. Yao, Fabrication and biological characteristics of beta-tricalcium phosphate porous ceramic scaffolds reinforced with calcium phosphate glass, J. Mater. Sci. Mater. Med. 20 (2009) 351–358.
[54] A. Saranti, I. Koutselas, M.A. Karakassides, Bioactive glasses in the system CaO–$B_2O_3$–$P_2O_5$: preparation, structural study and in vitro evaluation, J. Non-Cryst. Solids 352 (2006) 390–398.
[55] D. Rohanová, A.R. Boccaccini, D.M. Yunos, D. Horkavcová, I. Březovská, A. Helebrant, TRIS buffer in simulated body fluid distorts the assessment of glass-ceramic scaffold bioactivity, Acta Biomater. 7 (2011) 2623–2630.
[56] J. Li, H. Liao, M. Sjostrom, Characterization of calcium phosphates precipitated from simulated body fluid of different buffering capacities, Biomaterials 18 (1997) 743–747.
[57] F. Barrere, C.A. Van Blitterswijk, K. De Groot, P. Layrolle, Influence of ionic strength and carbonate on the Ca-P coating formation from SBF × 5 solution, Biomaterials 23 (2002) 1921–1930.
[58] B. Wopenka, J.D. Pasteris, A mineralogical perspective on the apatite in bone, Mater. Sci. Eng. C 25 (2005) 131–143.
[59] S. V Dorozhkin, M. Epple, Biological and medical significance of calcium phosphates, Angew. Chem. Int. Ed. 41 (2002) 3130–3146.