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Surface reactions of bioactive glasses in buffered solutions

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Abstract

In vitro bioactivity of glasses is usually measured in buffered solutions whereby a formation of a hydroxyapatite layer on the surface is taken as an indication of the bioactivity. In this work we compare the layer formation on three glasses in simulated body fluid, Tris buffer solution, sodium phosphate buffered saline and osteoblast medium. Two of the glasses are known bioactive glasses, 45S5 (45 wt.% SiO_2) and S53P4 (53 wt.% SiO_2), while the third is an experimental composition with a higher silica content (68 wt.% SiO_2). Plates of the glasses were immersed in the solutions at 37 °C for different times up to two weeks. The results showed clear differences between the layer developments on the three glasses in the different solutions. The results indicated that the relative order of the reactivity depended on the solution. Thus, results gained in different solutions for different glasses cannot be directly compared.

Keywords: Bioactive glass; Surfaces; Chemical properties; Corrosion; Apatite

1. Introduction

Bioactive glasses show the ability to bond to bone tissue. When implanting bioactive glass into the body, partial dissolution of ions as well as breakdown of the silica network occurs. The reaction steps leading to formation of a SiO_2-rich layer and a hydroxycarbonate apatite layer on the surface of the glass have been described in detail by Hench.[1] Subsequently, biological mechanisms allow chemical bonding between the apatite layer and the surrounding tissue.

Bioactivity of glass is confirmed in the biological environment, *in vivo*. The reactions described by Hench[1] occur also in aqueous solutions. In this work the reactions of two known bioactive glasses, 45S5 and S53P4 and a novel glass, S68, were studied in four buffered solutions. S68 was developed to obtain slowly resorbable glass fibers, which guide bone growth in medical devices.[2] The glasses were immersed in simulated body fluid (SBF), Tris buffer solution (Tris), sodium phosphate buffered saline (Na-PBS) and osteoblast medium (OBM). The goal was to assess the impact of the composition of both the solution and the glass on the surface reactions of the glass.

SBF and Tris are commonly used in studying the reactions of bioactive glasses. Both are aqueous solutions buffered with tris(hydroxymethyl)aminomethane/HCl and SBF contains in addition similar inorganic components as blood plasma. Na-PBS is a phosphate buffered solution, in which the degradation of biodegradable polymers is studied. Na-PBS has also been used in *in vitro* studies of composite materials of bioactive glasses and polymers.[3,4] Na-PBS has been found to give faster rate of calcium phosphate formation on the glass than SBF or Tris.[5] OBM is a cell culture medium that is used to investigate the interactions of osteoblasts with bioactive glasses *in vitro*. In this work the impact of a cell-free OBM solution on the reactions of the glasses was studied. Immersion of bioactive glasses in cell culture media has been found to retard the dissolution of the glass.[6,7]

Only one of the solutions, SBF, contains both calcium and phosphate ions. Thus, in SBF the hydroxyapatite can form also solely from the solution, while in the other solutions dissolution and precipitation of calcium ions from the glass is a prerequisite for hydroxyapatite formation. The impact of different immersion solutions on the dissolution behavior of bioactive materials has been compared in several studies.[5–9]

Rohanová et al.[8] and Hlváĉ et al.[9] studied the impact of Tris buffer on the dissolution behavior of glass–ceramics. Tris buffer was reported to form a soluble complex with Ca^{2+} ions, leading

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Table 1
Composition of the glasses in wt.%.

Glass	SiO_2	NaO_2	CaO	P_2O_5	B_2O_3	MgO
45S5	45	24.5	24.5	6	–	–
S53P4	53	23	20	4	–	–
S68	67.8	14	9	1.5	2.3	5.4

to suppressed deposition of a hydroxyapatite (HAp) layer on the material surface.[9] The HAp layer forms a diffusion barrier for ions dissolving from the glass. As Tris buffer causes a suppressed formation of HAp on the surface, the leaching rate of ions from the material is not decreased with time as much as in solutions without Tris.

Fagerlund et al.[5] studied the effect of sodium phosphate buffered saline (Na-PBS) on the dissolution of four bioactive glasses, and observed that the dissolution reactions of glass in Na-PBS differed significantly from the reactions in Tris and SBF on two points: (1) the buffering capacity of Na-PBS is lower, resulting in a higher pH increase when alkali and alkaline earth ions dissolve. The increased pH causes a higher silica release and an increased calcium phosphate precipitation. (2) Na-PBS has a high concentration of phosphorus, resulting in a fast formation of calcium phosphate on the surface. The formation of the calcium phosphate layer retards the further dissolution process.

Bioactive glasses are used increasingly in composite materials with biodegradable polymers. As the reactions of the composites are studied often in Na-PBS, the behavior of bioactive glass alone in Na-PBS should be understood. In this paper the dissolution and layer development on three glasses in different buffered solutions are discussed.

2. Experimental

Bioactive glasses 45S5 and S53P4 were prepared from analytical grade chemicals by the melting procedure described by Arstila et al.[10] S68 was obtained from Vivoxid Ltd. and it was manufactured at the Glafo Glass Research Institute (Växjö, Sweden). The glass compositions are presented in Table 1.

SBF was prepared according to the procedure by Kokubo and Takadama.[11] Tris buffer solution was made by adding tris(hydroxymethyl)aminomethane/HCl to deionized water. Na-PBS was prepared by dissolving sodium chloride, monosodium phosphate and disodium phosphate into deionized water. The osteoblast medium was prepared according to the recipe described by Alm et al.[12] The ion concentrations in blood plasma, SBF, Tris, and Na-PBS are presented in Table 2. The initial pH of SBF, Tris and Na-PBS was adjusted to 7.4, while the initial pH of OBM was 7.96.

Glass plates of 20 mm × 15 mm × 1.5 mm were cut using a low speed diamond saw and polished with water polishing papers to 2500 grit. In the last polishing step, ethanol was used in order to avoid reaction of the glass surface with water. Since all glass plates were manufactured in the same way, the surface roughness of the plates was comparable. The samples were placed diagonally in 20 ml cylindrical polystyrene containers. The glass plates were immersed in SBF, Tris and Na-PBS at 37 °C for 4 h, 24 h, 72 h, one week and two weeks, with an SA/V ratio of 0.4 cm^{-1}. 45S5 and S53P4 were immersed in OBM for 24 h and 72 h. S68 was not studied in OBM. The solutions in the two-week tests were replenished after the first week of immersion. This was to investigate the impact of solution replenishment and also to avoid possible bacterial growth. At least two parallel tests were carried out for all time points, and for a few time points four or six parallel tests were made.

Table 2
Ion concentrations of blood plasma and buffer solutions (mmol/l).

Ion	Blood plasma	SBF	TRIS	Na-PBS
Na^+	142	142	–	156.2
K^+	5	5	–	–
Mg^{2+}	1.5	1.5	–	–
Ca^{2+}	2.5	2.5	–	–
Cl^-	103	147.8	45	100.9
HCO_3^-	27	4.2	–	–
HPO_4^{2-}	1	1	–	24.9
SO_4^{2-}	0.5	0.5	–	–
$H_2PO_4^-$	–	–	–	5.5

The pH of the solution was measured at each time point. The glass plates were dried at 60 °C for at least 1 h, and thereafter they were weighed. The surface and the cross section of the glass plates were examined using scanning electron microscope (SEM, LEO 1530) combined with energy dispersive X-ray analysis (EDX, Thermo Scientific UltraDry). The ion concentrations of the solutions from the one-week tests were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 5300 DV).

3. Results

3.1. pH of the immersion solutions

Fig. 1 shows the pH of SBF, Tris and Na-PBS after immersion of the glasses. Standard deviations are shown for the immersion times with more than two parallel samples. As only minor changes in the pH of OBM were measured, the values are not presented here. S68 affected the pH of the solutions very little and only the pH values at one and two weeks are presented in Fig. 1. The two other glasses increased clearly the pH of the solutions. The increase was, however, dependent on the composition

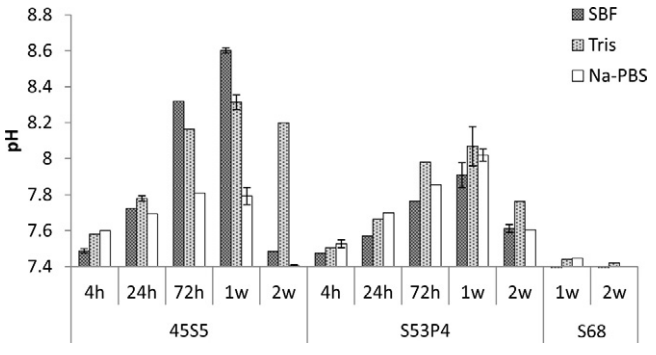

Fig. 1. pH of the solutions after immersing the glasses for 4 h to 2 weeks.

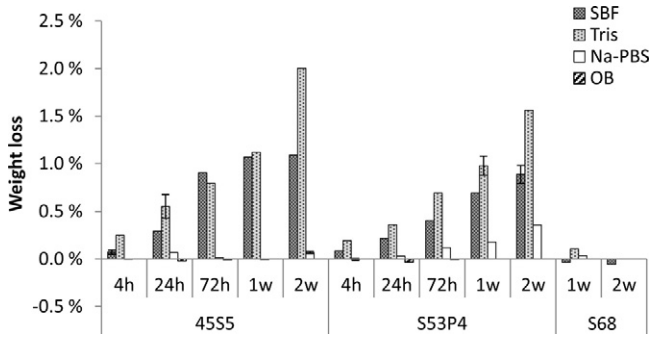

Fig. 2. Weight loss after immersion of the glasses.

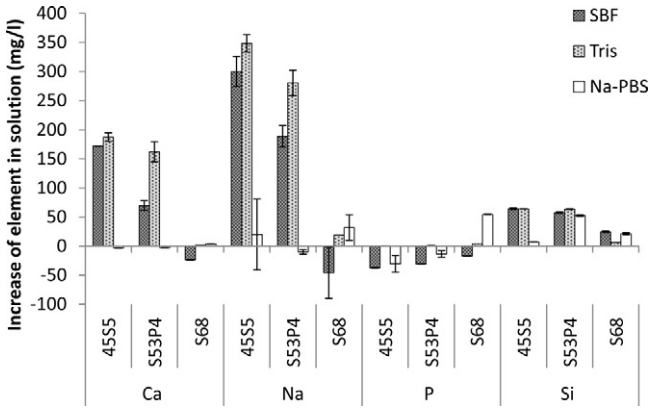

Fig. 3. ICP-OES data on element concentrations of the solutions at one week of immersion of the glasses.

of the glass and the solution. For 45S5 the order of the pH increase from the highest to the lowest was: SBF, Tris and Na-PBS, while for S53P4 the order was: Tris, Na-PBS and SBF. The differences between the pH values of the solutions were smaller for S53P4 than for 45S5. During the second week, after solution replenishment, the pH increased the most in Tris.

For solutions with equal buffering capacity, a higher pH increase indicates higher reactivity of the glass. The solutions have, however, differing buffering capacities and, thus, the order of reactivity cannot solely be confirmed from pH measurements.

3.2. Weight changes of the glass plates

The weight change is a sum of dissolution of the glass (weight loss) and HAp formation (weight gain). Fig. 2 presents the average net weight loss of the glasses after immersion in SBF, Tris, Na-PBS and OBM. Standard deviations are shown for immersion times with more than two parallel samples.

S68 showed negligible weight changes in all solutions, with values shifting above and below the initial weight. In the osteoblast medium only minor changes in the weight of the glass plates were observed. S53P4 showed consistent weight loss in the other solutions, with the highest value in Tris and the lowest in Na-PBS. The weight loss of 45S5 followed approximately the same trend as S53P4 with the highest weight loss in Tris. The weight loss of the bioactive glasses, especially 45S5 in Na-PBS was very small or insignificant. As the pH values did indicate dissolution of the glasses in Na-PBS, the small changes in weight could be a result of an equal amount of elements dissolving from the glass and HAp forming on the surface.

After the second week of immersion the weight loss was clearly greatest in Tris, thus confirming the pH results, according to which the highest changes were measured in Tris.

3.3. Changes in ion concentration

ICP-OES analyses were done on SBF, Tris and Na-PBS before and after one week of immersion. The increases in the ion concentrations after the one-week immersion in these solutions are shown in Fig. 3. No ion concentration data was measured for the samples immersed in OBM.

Generally, an increase in the concentration of calcium, sodium and silicon in the solutions was measured after the one-week immersion. The decrease in phosphorous in SBF and Na-PBS can be correlated with precipitation of hydroxyapatite on the glass surface. Phosphorous dissolved from the glass has been consumed completely and also phosphorous from the solution has precipitated.

The increase in especially sodium and calcium concentrations in Na-PBS was very low compared to the increase in their concentrations in Tris or SBF. The dissolution of the glasses in Na-PBS seemed therefore retarded. Generally, after immersion of 45S5 and S53P4 the ion concentrations were slightly higher in Tris than in SBF.

S68 caused only minor changes in the ion concentrations of the solutions. An increase in the silicon content was, however, measured in SBF and Na-PBS. After immersion in SBF, a small consumption of calcium and phosphorous could be observed, thus suggesting some formation of calcium phosphate on the glass surface.

3.4. Glass surfaces

The glass plates were analyzed using SEM–EDX both on the cross sections and the surfaces to give the thickness and composition of the reaction layers.

3.5. Reaction layers

SEM micrographs of the cross-sections of the glass plates showed silica-rich layers and calcium phosphate (CaP) layers on the glass surfaces. The calcium to phosphate (Ca/P) molar ratios in the CaP layers corresponded to the Ca/P-ratio in hydroxyapatite. The layer thicknesses were measured for all samples from the SEM micrographs. In the cross-sections of the glasses immersed in osteoblast medium no reaction layers could be detected.

In Fig. 4 the thickness of the silica-rich layers in the glasses after immersion in SBF, Tris and Na-PBS is shown. No silica-rich layers were observed in S68. S53P4 formed the thickest silica-rich layer in Tris, whereas 45S5 formed the thickest silica-rich layer in SBF. The silica-rich layers formed in the bioactive glasses in the three solutions were quite similar after 4 h.

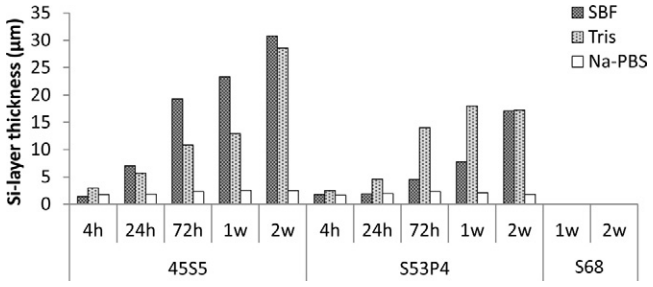

Fig. 4. Thicknesses of the silica-rich layers.

However, after 4 h, the layers in glasses immersed in Na-PBS grew very little in thickness with time.

Fig. 5 shows the thickness of the calcium phosphate layer on 45S5, S53P4 and S68. It should be observed that the thickness was measured for the layer that covered the surface evenly; sporadic local ball-like accumulations of CaP were not taken into account. These accumulations existed especially on plates immersed in SBF. Both 45S5 and S53P4 developed CaP layers already after 4 h in Na-PBS. The CaP layer formed, thus, quickly in Na-PBS but, similarly to the silica-rich layer, it grew only slightly in thickness with time. At 4 h in Tris, S53P4 had not developed any CaP layer, while in SBF 45S5 had not developed a clear CaP layer. Although the layer on 45S5 was not observed in the cross-sectional SEM images, the SEM images of the surfaces showed CaP at 4 h in SBF. During the second week of immersion in the fresh solutions, the CaP layers in Tris grew by more than 50% in thickness, whereas the layers in SBF and Na-PBS did not grow any further. This result is in agreement with the pH and weight results, which also indicated a continued reaction after solution replenishment in Tris but not in the other solutions.

Although no silica-rich layer was observed in the S68-plates, calcium phosphate was observed on the surface of S68 immersed in SBF (Fig. 5). Fig. 6 shows an EDX line analysis of the surface of S68 immersed in SBF for 2 weeks. A calcium phosphate layer but no silica rich layer had formed on the glass surface. As SBF is saturated on calcium phosphate, CaP precipitation is likely, if a suitable nucleating site, such as the silica-rich layer, is available.

3.6. Surface morphology

In Fig. 7 the surface of a non-immersed S68 plate together with the plates immersed in SBF (2 weeks), Tris (1 week) and Na-PBS (1 week) are shown. S68 formed a calcium phosphate layer only in SBF. The calcium phosphate layer on S68 immersed in SBF had partly cracked off, and the structure of the glass underneath can be seen in Fig. 7. Although the results from the pH and weight measurements gave little indication of dissolution of the glass, the SEM images revealed changes in the surface appearance of S68 immersed in SBF, Tris and Na-PBS. The glass surface was rough and cavities had formed along the polishing trails, indicating partial dissolution of the glass.

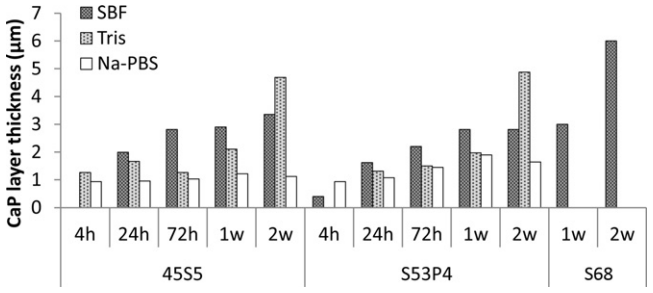

Fig. 5. Thicknesses of calcium phosphate layers.

The surface SEM micrographs revealed the rapid formation of calcium phosphate on bioactive glasses immersed in Na-PBS. Fig. 8 shows SEM micrographs of the surface of S53P4 at 24 h in SBF, Tris and Na-PBS. The glass plate immersed in Na-PBS had an even calcium phosphate layer on the surface, while the calcium phosphate formed in Tris and SBF did not cover the glass surface completely. This suggests that calcium phosphate formed quickly on the surface of 45S5 and S53P4 in Na-PBS, due to the high phosphorous content in the solution. S68 did however, not release a sufficient amount of calcium into the solution, required for calcium phosphate to precipitate on the glass surface.

According to the SEM micrographs, the shape of the calcium phosphate crystals on the glasses depended on the immersion solution (Fig. 9). The flake-like structures formed in SBF and Na-PBS appear to be larger than the ones formed in Tris.

Weight and pH measurements gave little indications of dissolution of the glasses in osteoblast medium. The cross sectional SEM images showed no reaction layers in the glass surfaces. Also the SEM micrographs of 45S5 before and after 72 h immersion in OBM showed only minor changes in the surface morphology (Fig. 10).

4. Discussion

The dissolution behavior of the bioactive glasses 45S5 and S53P4 depended on the composition of both the glass and the immersion solution. Table 3 shows the order of dissolution of 45S5 and S53P4 in SBF, Tris and Na-PBS according to the different analysis methods. According to pH measurements and the thickness of the silica rich layer, 45S5 dissolved the most in SBF and the least in Na-PBS. However, ion analyses of the solutions showed higher dissolution of 45S5 in Tris than in SBF. S53P4 showed quite consistent dissolution behavior according to all analyses with highest dissolution in Tris and lowest in Na-PBS. However, lower pH was measured for SBF than for Na-PBS, which can be explained by different buffering capacities of the

Table 3
A comparison of the effect of the buffer solutions on dissolution of bioactive glass according to different glass composition and analysis method (+++ indicates highest or thickest, + indicates lowest or thinnest) (wt.: weight loss, Si: silica rich layer thickness, ICP: ion concentration data).

Sol.	45S5				S53P4			
	pH	wt.	Si	ICP	pH	wt.	Si	ICP
SBF	+++	++	+++	++	+	++	++	++
Tris	++	++	++	+++	+++	+++	+++	+++
Na-PBS	+	+	+	+	++	+	+	+

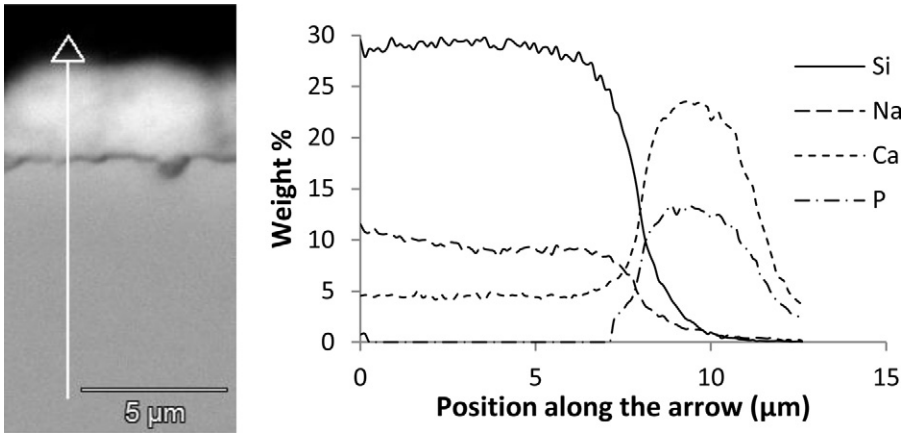

Fig. 6. SEM–EDX line analysis of Si, Na, Ca and P at the surface of S68 in SBF (2 w). A calcium phosphate layer has formed on the glass surface, but no silica rich layer.

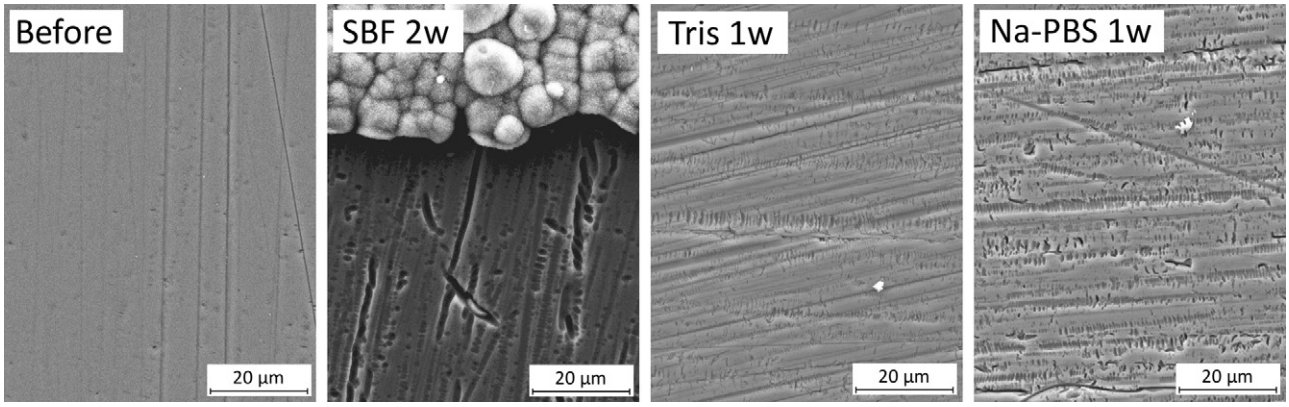

Fig. 7. Surfaces of S68 before and after immersion in SBF, Tris and Na-PBS. All surfaces after immersion are corroded. A calcium phosphate layer (partly cracked off here) is formed only in SBF.

solutions. The results of S53P4 correlate well with the results of Andersson and Kangasniemi, according to which the weight loss of S53P4 was greater in Tris than in SBF. They also observed that the silica rich layer was somewhat thicker in Tris than in SBF, but the calcium phosphate layer was thinner in Tris.[13]

In many studies the solution is replenished after certain time points, especially when longer time points are tested. The results of all analyses after solution replenishment show a clear trend. The glasses do not react much further in SBF or Na-PBS. However, in Tris the glasses continue to react.

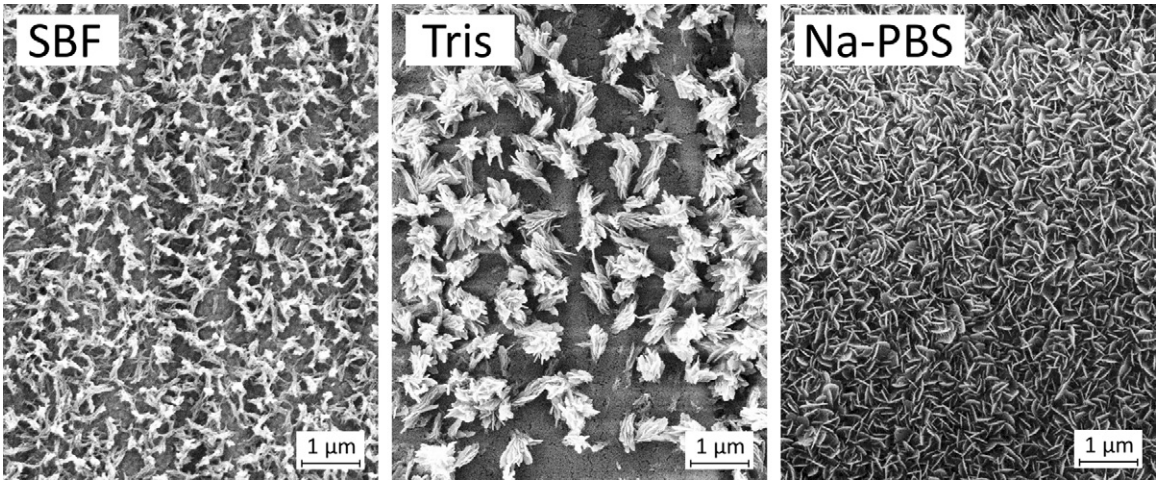

Fig. 8. SEM micrographs of surfaces of after immersion (24 h) of S53P4 in SBF, Tris and Na-PBS.

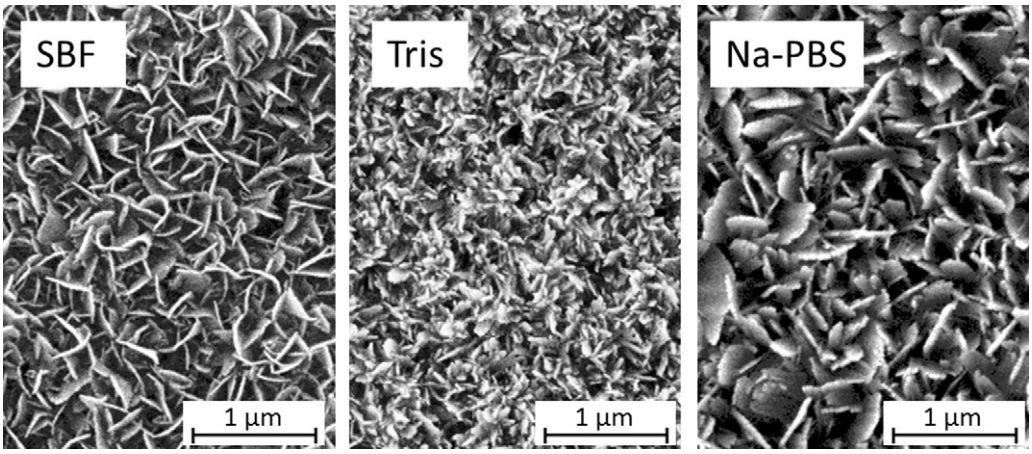

Fig. 9. Calcium phosphate on S53P4 at 2 weeks in SBF, 45S5 at 1 week in Tris, and S53P4 at 1 week in Na-PBS.

CaP layers formed quickly on glasses in Na-PBS. According to Fagerlund et al. the CaP layer forms rapidly in Na-PBS because of the high concentration of phosphorous in the solution.[5] Immediately when calcium ions dissolved from the glass precipitation of CaP commenced in Na-PBS. A further dissolution of the glasses was retarded. In general, the calcium phosphate layer forms a diffusion barrier, thus retarding the dissolution of the glass. In this work, the calcium phosphate layer formed in Na-PBS was the thinnest. However, it seemed to prevent diffusion of ions and thus further dissolution of the glass better than the CaP layers formed in the other solutions. These results suggest that a thicker calcium phosphate layer does not necessarily function as a better diffusion barrier. According to Hlváĉ et al. Tris buffer forms a complex with Ca^{2+} ions, which leads to a suppressed formation of a protective HAp layer.[9] The suppressed formation of HAp in the presence of Tris was noticed for the early time points in this study. Further, although the calcium phosphate layers formed in the presence of Tris were thicker than the ones formed in Na-PBS, they enabled diffusion of ions better. The CaP layer formed in Na-PBS was assumed to be denser than the layers formed in SBF and Tris. The calcium phosphate crystals formed in different solutions also differed in their appearance, with larger crystal-flakes formed in SBF and Na-PBS than in Tris. It should be further investigated how the morphology of the calcium phosphate affects the dissolution of the glass.

45S5 and S53P4 immersed in osteoblast medium showed very small pH and weight changes. ICP-OES was not conducted on OBM samples. The SEM micrographs showed little difference between the glass surfaces before and after immersion for both glasses and no reaction layers were detected. The dissolution of bioactive glasses was, thus, highly retarded in OBM. Other studies show similar results.[6,7] Mei et al.[6] reported that the presence of serum proteins causes a slower accumulation of calcium phosphate at the glass surface and forms a narrower calcium phosphate layer with lower concentrations of these elements.

No silica rich layer was formed on S68. However, a CaP layer formed on S68 immersed in SBF. Studies show that the ion exchange of alkali from the glass with hydrogen ions in the solution, dissolution of silica and especially silica repolymerization is retarded with increased silica content in the glass.[14] Due to the high silica content in S68, the ion dissolution was minor and silica repolymerization retarded, thus explaining that no silica-rich layer formed. CaP precipitation was observed only in SBF. As the release of calcium and

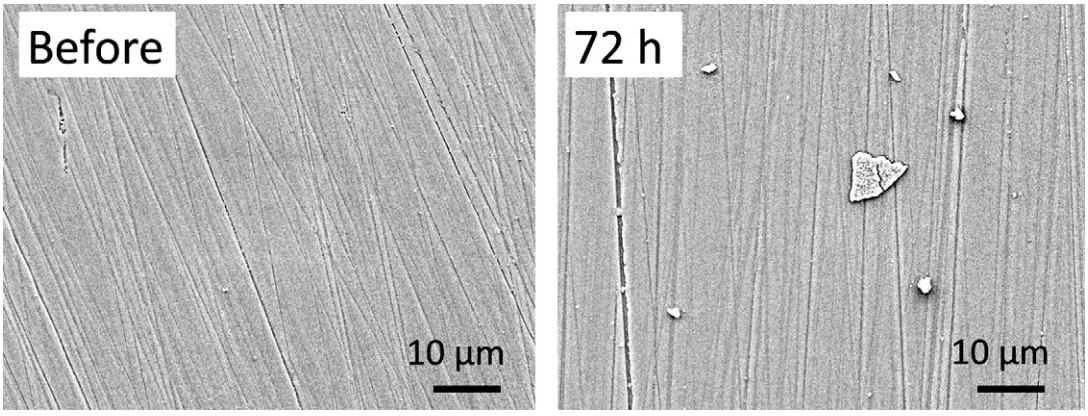

Fig. 10. Surface of 45S5 before and after immersion in OBM for 72 h.

phosphate ions from the glass into the solution was negligible, the calcium phosphate formed on S68 probably originated from calcium and phosphorous in the solution. The partial dissolution of S68, which was observed in the surface SEM micrographs, increased the surface roughness of the glass. A greater surface roughness provides more nucleation sites, thus, increasing the likeliness for CaP crystallization from SBF that is supersaturated on calcium phosphate. This CaP precipitation could be an indication of bone growth guiding capacity of S68.

5. Conclusions

The dissolution of bioactive glasses in SBF, Tris, Na-PBS and OBM clearly depended on the composition of the glass, the composition of the solution and solution replenishment. The dissolution was strongly retarded in osteoblast medium.

Generally, 45S5 showed higher dissolution than S53P4. S53P4 showed consistent dissolution behavior and dissolved the most in Tris. When replenishing the solution, the ion concentration was found to have a great impact. In SBF and Na-PBS the glasses did not react further as much as in Tris.

Glass S68 with high silica content showed signs of dissolution in all solutions and formed a calcium phosphate layer in SBF. Accordingly, the original content of the ions in the solution and the chemical durability of the glasses affected the layer structure formed.

In Na-PBS, the calcium phosphate layer formed rapidly on bioactive glasses. A further dissolution of the glasses was, however, retarded. The dissolution of the glasses was assumed to depend partly on the density of the calcium phosphate layer.

The *in vitro* bioactivity of glasses can be confirmed in various solutions buffered at physiological pH. However, the dissolution and layer formation on glasses in different solutions vary significantly and results obtained in different buffer solutions cannot be directly compared.

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