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BIOACTIVE GLASS CERAMIC CONTAINING ENDODONTIC SEALER
GUTTAFLOW BIOSEAL

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Abstract

Aim of the study was to evaluate apatite-forming ability and ion dissolution of bioactive glass-ceramic (BGC) particles from novel polydimethylsiloxane (PDMS) based endodontic sealer Guttaflow Bioseal. Firstly, water sorption and solubility were determined for Guttaflow 2 and Guttaflow Bioseal (GB), the latter containing BGC filler particles. Mineral precipitations in simulated body fluid (SBF) were observed with SEM/EDX. Ion-release from the sealer was determined with inductively coupled plasma optical emission spectrometry (ICP-OES) in Tris-buffer solution. Change of pH was measured also after 14 days. The obtained data was statistically analyzed with Tukey's HSD test ($p < 0.05$). GB exhibited significantly higher water sorption and solubility in comparison with Guttaflow 2. Surface structure exposed particles of BGC in the PDMS matrix. The BGC particles (size of 20–40 μm) indicated to consist of $\text{CaO-SiO}_2\text{-Na}_2\text{O-ZrO}_2\text{-P}_2\text{O}_5$. Morphologically spherical Ca/P precipitation formed after 3 days in the SBF on the sealer surface. Ca/P ratio of the precipitation ranged in 1.20–1.65 indicating transformation to hydroxyapatite (HA). The pH of the immersion solution rose gradually.

Keywords: Bioactive glass-ceramic, Endodontic sealer, Guttaflow Bioseal, Ion-release, Apatite-forming ability

Original paper

**DISSOLUTION AND MINERALIZATION CHARACTERIZATION OF
BIOACTIVE GLASS CERAMIC CONTAINING ENDODONTIC SEALER
GUTTAFLOW BIOSEAL**

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INTRODUCTION

Over the past decade, several new generation endodontic sealers have been introduced to the profession. The research and development of materials of this kind should always focus on clinically significant issues; eliminating infections and preventing recontamination of pathogens. Endodontic therapy is based on three fundamental principles: removing infectious biofilm, disinfection with irrigation protocols and obturation of the root canal. Endodontic sealers are expected to function as a plug in the periapical area of root canal system together with the core material. They are supposed to bond both to intraradicular dentin and to the over-laying post-and-core material.

Several studies have shown that hydraulic calcium-silicate based cements are capable to induce biomineralization when immersed in human plasmalike solutions (1,2). Biocompatibility of calcium-silicate based materials has been demonstrated and they have potential as endodontic materials (3). Also some specifically modified silicate glasses, such as the so-called bioactive glasses (BG) are known to induce both biomineralization and provide antibacterial effects upon their gradual dissolution.

BGs have been tested as antimicrobial root canal fillers in several studies (4-7). The antimicrobial effects are attributed to an increase of the pH due to changes in the ion concentrations of the interfacial solution around the dissolving BG. Furthermore, continuous release of ionic species from the glass over a critical time period is assumed to be essential for the antimicrobial effect (8). Antimicrobial effects of certain BGs have been demonstrated against 29 species of aerobic microbes including pathogens related to endodontic infections (9).

Biomineralization and formation of hydroxyapatite (HA) begin with release of ions, especially calcium ions from the inorganic calcium-silicate particles and continue with formation of Si-OH groups at the material's surface. The Si-OH groups on the surface function as ideal site for nucleation of HA. HA, *i.e.* $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ precipitates first as an amorphous layer with the stoichiometric ratio Ca/P 1.67 and later crystallized into carbonated hydroxyapatite (CHA). HA precipitation is favored by an increase in the pH of the surrounding solution.

The increase of the pH of the interfacial solution to around 7.9 and above around the dissolving BG has been shown to induce not only bacteriostatic effects but also be successful in treatment of chronic osteomyelitis (10). Similar bacteriostatic effects have been reported also when using bioactive glass particles as a component in cranioplasty composite implants (11,12).

The combination of adequate physical properties containing silicone-based sealer and biointeractive bioactive glass-ceramic (BGC) particles is a prospective option to traditional sealers. Silicone-based root canal sealers also show advantages against mineral trioxide aggregate (MTA) cements in handiness and clinical application. In the current scientific literature the evidence for Guttaflow Bioseal's (GB) bioactive properties are minor whereas MTA's are known to be highly bioactive. This study explores in detail the potential of GB, a new endodontic sealer containing BGC particles to induce biomineralization and local environment typical for BG with antibacterial effects. The aim was to characterize apatite forming ability, the influence of the BGC on the surface structure and examine ion dissolution patterns of the endodontic sealer GB more widely that is

currently available in the scientific literature. Most of the data in similar studies are now limited to concern only calcium ion release. In addition, change of pH in the surrounding media, water sorption and solubility was measured for GB.

MATERIALS AND METHODS

Materials

Polydimethylsiloxane (PDMS) based endodontic sealer Guttaflow Bioseal containing BGC was tested for water sorption and solubility, surface structure, ion release, changes in the pH of the immersion solution and mineral precipitation on the sealer surface. Sealer Guttaflow 2 without BGC filler particles was used as a control material in the water sorption and solubility tests (Table 1).

Water sorption and solubility

Disc shaped specimens ($n=5$) were prepared by filling circular stainless steel molds (thickness 1.0 ± 0.1 mm diameter 150 ± 0.1 mm) with slight excess of the sealer material on inert glass plates and covered with 50 μm thick Mylar sheet. The molds were covered with another Mylar sheet and pressed together with glass plates to remove the excess sealer material. The molds between the glass plates were transferred for curing at 37.0°C for 60 min.

The disc-formed test specimens were then removed from molds and excess material from the periphery of the specimens was removed with surgical knife and silicon carbide grinding paper (FEPA #1000) and grinding machine (Struers LaboPol 21, Struers, Rodovre, Denmark). The test specimens were transferred to a desiccator and maintained at 37.0°C in an oven. After 22 h, the specimens were moved into another desiccator at 23.0°C for 2 h and then weighted to an accuracy of 0.1 mg. This two-step dehydrating cycle at 37.0 and 23.0°C was repeated until a constant mass (m_1) was obtained (difference in mass not more than 0.1 mg in any 24 h period).

Thereafter, the test specimens were immersed in 20 mL of ion exchanged distilled water at 37.0°C for seven days to determine water sorption. After the period of water immersion, the test specimens were washed with distilled water and dried from visible moisture with absorbent paper. Water saturated mass, m_2 was recorded after 1 min to an accuracy of 0.1 mg. After this, the specimens were reconditioned to constant mass (m_3) in the desiccators using the same cycle as mentioned above.

Apparent water sorption, W_{sp} was calculated by using equation $W_{sp} = \frac{m_2 - m_1}{m_1} \times 100\%$. The solubility of the specimens, W_{sl} was calculated by using equation $W_{sl} = \frac{m_2 - m_3}{m_1} \times 100\%$. Thus, both W_{sp} and W_{sl} are related to the original weight of the samples. The net water uptake was calculated by the sum of apparent water sorption and solubility. The data were normally distributed and verified with normal quantile plot and shapiro-wilk test.

Inorganic filler morphology and elemental analysis

Sealer disks ($n=6$) were prepared by filling Guttaflow Bioseal and Guttaflow 2 in stainless steel molds (5.0 ± 0.1 mm diameter 1.0 ± 0.1 mm thickness) and placed in desiccator at 23.0°C for 24 h. The morphology and elemental composition of the disks was studied with SEM/EDXA. Before the SEM/EDX analysis (PHENOM

ProX model no. 800–07334 serial no. MVE0204441079, Eindhoven, the Netherlands), the specimens were coated with gold-palladium (Quorum SC7620-15-119 Mini Sputter Coater, Lewes, UK). Firstly, the BGC particles were imaged using an accelerating voltage of 10 kV and thereafter, elemental mapping of the surfaces was performed using 15 kV and expose time of three s.

Biom mineralization test

Disk shaped specimens were prepared by filling circular stainless steel molds (5.0 ± 0.1 mm diameter 1.0 ± 0.1 mm thickness) as described above. One liter of Kokubo's simulated body fluid (SBF) was prepared which inorganic ion concentration is corresponding to human plasma was prepared for comparing the capability of the two sealers to induce biom mineralization (13). The specimens were immersed in 20 ± 1 mL SBF at 36.7°C in Grant's OLS200 linear shaking water bath using 60 rounds per second for 3, 7, 14, 21 and 28 days. Before immersion the media was filtered.

After the predetermined test periods, the specimens ($n=5$) were removed from the SBF and dried from visible moisture with absorbent paper and stored in desiccator at room temperature for 24 h. Thereafter the samples were placed on aluminum stubs and their edges were treated with carbon glue to obtain conductivity. Then, the specimens were coated with carbon and the changes in the surface morphology and composition were studied using SEM/EDX analysis (PHENOM ProX model no.800-07334).

The analyses were carried out using an accelerating voltage of 15 kV and an exposure time of three s with the magnification of $8,000\times$ for taking images of the total top surface and the magnification of $4,000\times$ images showing surface structure in more detail. X-ray diffraction (XRD) was performed in order to investigate the crystal structure of the formed biom mineralization after certain immersion times. The XRD measurements were carried out on a Bruker D8 Discover instrument equipped with a Cu $K\alpha$ X-ray source and scintillator point detector. The samples were scanned in the $20\text{--}60^\circ$ 2θ range, with an increment of 0.04° at a scan speed of 20 s per point.

Ion dissolution and pH change

Twelve specimens of Guttaflow Bioseal were prepared using circular stainless steel molds (15.0 ± 0.1 mm diameter 1.0 ± 0.1 mm thickness) and placed in desiccator at 23.0°C for 24 h. The top surface of half of the number of the specimens ($n=6$) was ground with silicon carbide grinding paper (grit P1000, FEPA) to remove the PDMS membrane covering the BGC particles. Half of the number of the specimens were left as such. Thus, some of the BGC particles in the surface layer were covered by the PDMS membrane.

Tris buffered solution (50 mM) was prepared as the dissolution medium. The pH of the buffer solution (Trizma base, Sigma-Aldrich, Darmstadt, Germany, $pK_a=8.06$ at 25°C) was adjusted with 1 M HCl to 7.4 at 35°C . Firstly, the specimens were weighed individually. Later, when analyzing the test results, the masses of each specimens was normalized to the average mass, *i.e.* 400 mg. The specimens were immersed in 20 mL Tris-buffer and kept for 1, 3, 7 and 14 days in an orbital incubator under a shaking speed of 100 rpm at 37°C . After the desired immersion time, the pH of the Tris-buffer was determined

at 35.0°C and the specimens were collected and washed with deionized water. The pH change induced by the specimens immersed for longer periods of time was also monitored. The ion concentrations in the Tris-buffer after the immersion were analyzed with an inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 5300 DV, Waltham, MA, USA).

The data of ion release and pH change were both normally distributed with equal variance and therefore Tukey's honestly significant difference test was used to compare means between the tested groups.

RESULTS

Water sorption and solubility

The Guttaflow Bioseal and Guttaflow 2 endodontic sealers differed significantly in the percentages of apparent water sorption ($p < 0.05$) and solubility ($p < 0.05$). The higher apparent water sorption and solubility was seen in GB, reaching 2.64% for water sorption and 1.38% for solubility after seven days (Table 2). The control material's (GF2) apparent water sorption (0.03%) and solubility (0.41%) were significantly lower. Correspondingly, the net water uptake of GB (4.02%), which is the sum of apparent water sorption and solubility was much higher compared to the uptake of GF2 (0.44%).

Inorganic filler morphology and elemental composition

SEM examination of Guttaflow Bioseal revealed BGC particles with sharp edges embedded in PDMS matrix on the surface of the sealer (Fig. 1). The particles could be distinguished even without grinding the surface. Particulate size of BGC varied in the range of 20–40 μm . The elemental analysis of BGC particles suggest that the composition is $\text{CaO-SiO}_2\text{-Na}_2\text{O-ZrO}_2\text{-P}_2\text{O}_5$ (Table 3).

Biom mineralization test

Guttaflow Bioseal formed globular like Ca/P precipitations with ratio of 1.20 on the sealer surface already after 3 days of immersion (Fig. 2a). By that time the weight percentage of calcium ions was 18.55% and 5.76% for silicate-ions (Table 4). In 7 days' time point the incorporation of Si-OH groups to sealer surface raised percentage of Si-ions to highest value 15.92% and the Ca/P ratio was 1.26 (Fig. 2b). By 14 days' timepoint Si-ions percentage began to decrease and Ca-ions to rose reciprocally due to nucleation (Fig. 2c). After 21 days, the percentage of Si (3.17%) was lowest observed with Ca/P ratio of 1.28 (Fig. 2d). The trend was preserved until 28 days' timepoint where weight percentage of Ca reached highest value of 23.34% and 8.45% for Si the ratio of Ca/P rose gradually to 1.65 in 28 days' timepoint (Fig. 2d). The morphology of precipitations resembles the structure of HA.

XRD was performed on spots where biom mineralization had occurred after 3, 14 and 28 days in SBF solution (Fig. 3a). Three major phases were identified: baddeleyite (ZrO_2 , JCPDS card number: 00-037-1484), trizirconium oxide (Zr_3O , JCPDS card number: 01-074-1282), and calcite (CaCO_3 , JCPDS card number: 00-005-0586). These phases originate more likely from the filler materials inside the GB matrix rather than from the biom mineralization. However, as indicated by the arrows in Fig. 3a, a minor phase also appears after longer immersion times in SBF (especially after 28 days). A higher magnification of the 20–40° 2θ range

(Fig. 3b) indicates that this phase is composed of HA ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$, JCPDS card number: 01-076-0694).

Dissolution and pH change

Guttaflow Bioseal test specimens with ground surface (0.315 mmol/L) released significantly more Ca ions ($p < 0.05$) in comparison with non-ground specimens (0.125 mmol/L). Any statistically significant difference on ion release of Na, P, and Si was not found between the two groups ($p > 0.10$). Trends of dissolution profiles and pH change are shown in Fig. 4 during 14 days. The specimens with non-ground surface is presented with continuous line and ground samples with dashed lines. The pH of the media did not differ statistically ($p > 0.10$) between the two groups. Initial pH 7.4 rose gradually to 7.84.

DISCUSSION

BGs gradually dissolve when exposed to aqueous media. The degree of dissolution depends on the ratio of the surface area of the glass in contact with a certain volume of solution. The endodontic sealers tested in this study consisted of a hydrophobic PMDS matrix with and without embedded particles of BGC.

If PDMS totally covers the BGC particles, their dissolution reactions providing the desired effects of antimicrobial and biomineralization are hindered. Interestingly, it was found from the micrographs that some of the BG particles were without covering membrane of PMDS even without intentionally grinding the surface for exposing the particles.

Clinically it is an important observation because in root canal the sealer is as sprayed from the syringe and it was now shown that ion release and related phenomena can occur without exposing the BGC particles. However grounded samples released significantly greater amount of calcium which may relate to aforesaid circumstances even though any difference was not detected relating to the release of Na, Si and P ions. Reason for having the outermost BGC particles to become spontaneously exposed may be due to large difference of hydrophilicity of BGC particles in comparison to the hydrophobicity of the PMDS matrix.

This study showed that sealer Guttaflow Bioseal released Ca and Si ions in surrounding aqueous solution and formed finally HA-like morphological structures on the surface with Ca/P ratio close to that of crystallized HA. However, the Ca/P ratio levelled off rather slow. It has been noted by Kokubo and Takadama that SEM observation can be used to observe material formation on the surface but is insufficient alone to identify that the precipitation is apatite (13). XRD analysis confirmed the formation of HA (especially after longer immersion times). The rather weak intensities of the HA reflections are indicative of a thin HA layer, which also results in strong interfering signals from the crystalline filler materials inside the underlying matrix. Biomineralization requires excess of mineral forming ions to be present in the solution and in this case, the ions originated from the dissolution of BGC particles and from the SBF. Biomineralization and morphology was as found also in previous studies with similar experimental design whereas BGC particle characterization and ionic dissolution for Na, Si and P was not measured (14).

In this study, the ion-release was measured by using ICP-OES whereas many of similar studies on fast setting calcium-silicate based sealers have used EDTA titration method. Guttaflow Bioseal's calcium release reached similar levels with other bioactive properties possessing endodontic materials (15-18). Although Ca ion release is strongly related to bioactivity most of the studies in current scientific literature ignores the release of other ions which also have biological interest.

Different BG compositions used as bioactive fillers in composites are known to have different dissolution behaviours (15). Sealer Guttaflow Bioseal showed low number of BGC particles in the PDMS matrix, but despite of that, biomineralization, and more importantly increase of pH to the bacteriostatic level was seen. Therefore, it can be assumed that the sealer could have beneficial effects also *in vivo* conditions where ratio of surface area/volume favors the emerging local alkalinity. Nonetheless that there has been some discussion about disadvantageous impacts on cell viability, there are lack of evidence between the relation of alkaline micro environmental pH and cytotoxicity of root canal sealers (16). However GB has shown good biocompatibility in recent study (17). It is important to note that Gandolfi *et al.* reported somewhat opposite findings regarding to the remarkably alkaline testing media (18,19). Tris-buffered solution (pKa=8.06) which was used in this study instead of distilled water as media may explain the significantly lower increase in pH.

Elemental composition of the sealer showed BGC to be predominantly calcium-silicate glass alongside traces of sodium and phosphate with particulate size of 20–40 μm . Particulate size has been proved to have effect on biomineralization; nanoparticles have been shown to perform better than microparticles in inducing biomineralization (19,20). Paying regard to this fact, there might be still potential for improving the performance of bioactive properties. The composition of the glass based on SEM/EDX suggests that the glass type is CaO-SiO₂-Na₂O-ZrO₂-P₂O₅. It must be noted that EDX analysis is sensitive when performed on micro particles with different shapes and sizes. Some minor elements which were not detected on every particles were disregarded as error and therefore the total wt% is slightly less than 100%.

The control material Guttaflow 2, which does not contain BGC but has similar PDMS matrix has been distinguished for its minimal water sorption and solubility (21). This study confirmed this and the considerably higher water sorption for the Guttaflow Bioseal which was likely due to presence of BGC particles in the matrix. There may have been microscopic gaps between the BG particles and PMDS matrix which absorbed water and caused the higher water sorption. On the other hand, dissolution of BGC during the water sorption-solubility test may have lowered the weight of the specimens which resulted in in higher solubility values than that of control material.

CONCLUSION

Guttaflow Bioseal had exposed particles of BGC on the sealer surface even when the material was not intentionally ground from its surface. Exposed particles of BGC caused higher water sorption and solubility to the GB compared to the control material of Guttaflow 2. GB released ions from the BGC to the surrounding media where the pH increased to level of 7.9 which relates to feasible antimicrobial effects *in vivo*. Biomineralization was confirmed on the surface of sealer GB with the Ca/P ratio and the XRD data suggesting that the precipitation

is HA. Further studies are needed to evaluate the clinical impact of desired properties because apatite-forming ability is not directly related to bone-bonding ability.

CONFLICTS OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Table 1 Sealer materials and their composition as specified by the manufacturer

Materials	Manufacturer	LOT number	Composition
Guttaflow 2	Colténe Whaledent AG, Alstatten Switzerland	G91385	Gutta-percha powder, polydimethylsiloxane, platinum catalyst, zirconium dioxide, microsilver (preservative), coloring
Guttaflow Bioseal	Colténe Whaledent AG, Alstatten Switzerland	G66355 H00606	Gutta-percha powder, polydimethylsiloxane, platinum catalyst, zirconium dioxide, silver (preservative), coloring, bioactive glass ceramic

Table 2 Water sorption and solubility exhibited by Guttaflow 2 and Guttaflow Bioseal

Material	Solubility (%)	Apparent Water Sorption (%)	Net Water Uptake (%)
Guttaflow 2	0.03±0.01	0.41±0.03	0.44±0.03
Guttaflow Bioseal	2.64±0.11	1.38±0.24	4.02±0.24

Values presented are means ± standard deviations (N=10)

Net water uptake is the sum of apparent water sorption and solubility

Table 3 Average elemental composition of BGC particles in Figure 1

Element	Mean of all particles (wt%)
Si	24.2 (±0.5)
O	30.5 (±3.0)
Ca	19.9 (±1.0)
Na	8.9 (±1.3)
Zr	9.4 (±0.53)
P	1.2 (±0.05)

Table 4 The atomic ratios of the Ca/P on the surface of the test specimen in SBF for 3, 7, 14, 21 and 28 days

Period	Day 3	Day 7	Day 14	Day 21	Day 28
Ca/P ratio	1.20	1.26	1.28	1.35	1.65

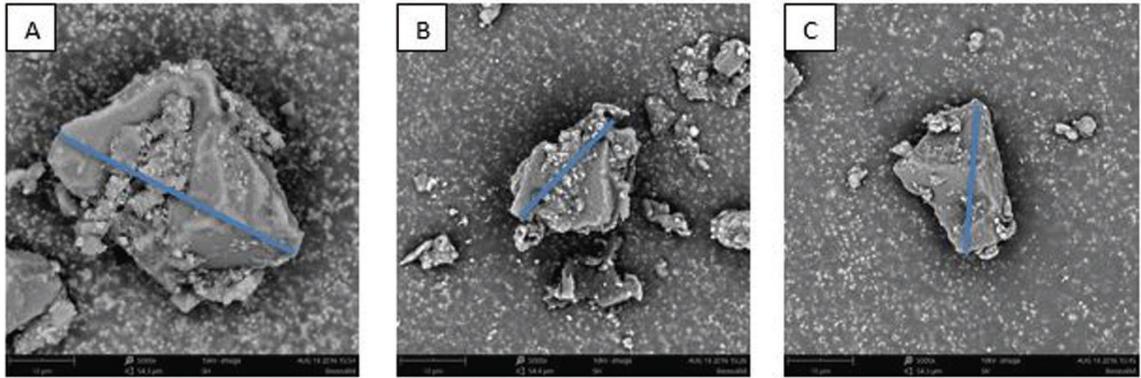


Fig. 1 Morphology of bioglass particles on the surface of Guttaflo Bioseal. Blue line showing diameter of particle A 40 μm , particle B 22 μm , particle C 25 μm ($\pm 2 \mu\text{m}$).

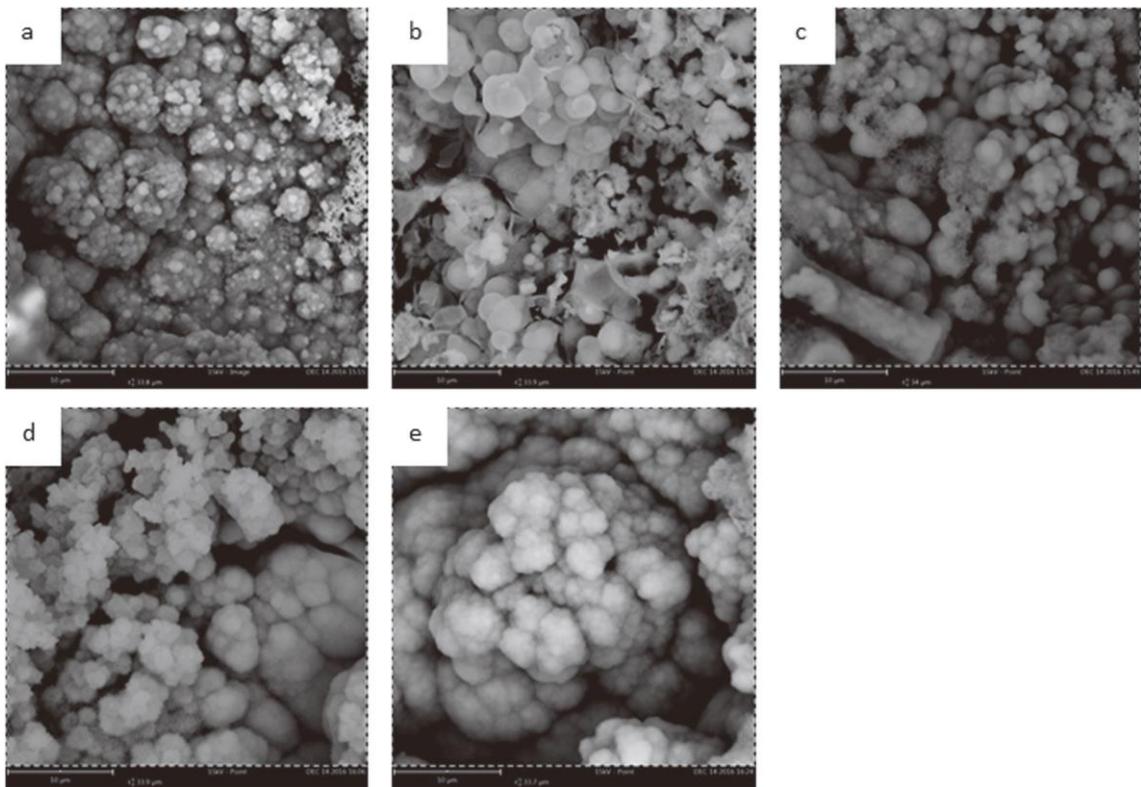


Fig. 2 SEM micrographs of mineral precipitations at 3 (a), 7 (b), 14 (c), 21 (d), 28 (e) days' time point in magnifications of 8,000 \times .

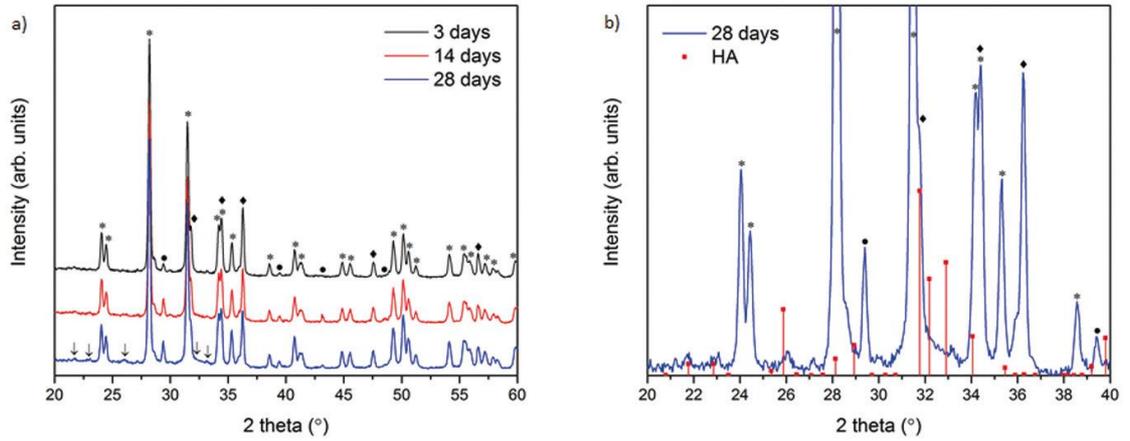


Fig. 3 a) XRD patterns measured on spots where biomineralization had occurred after immersion in SBF for different times. The symbols indicate reflections from ZrO₂ (*), Zr₃O (◆), and CaCO₃ (●), respectively. The arrows (↓) indicate reflections from HA (Ca₅(PO₄)₃OH). b) A magnification of the 20–40° 2θ range for the 28 days' sample, including the reference pattern for HA (JCPDS card number: 01-076-0694).

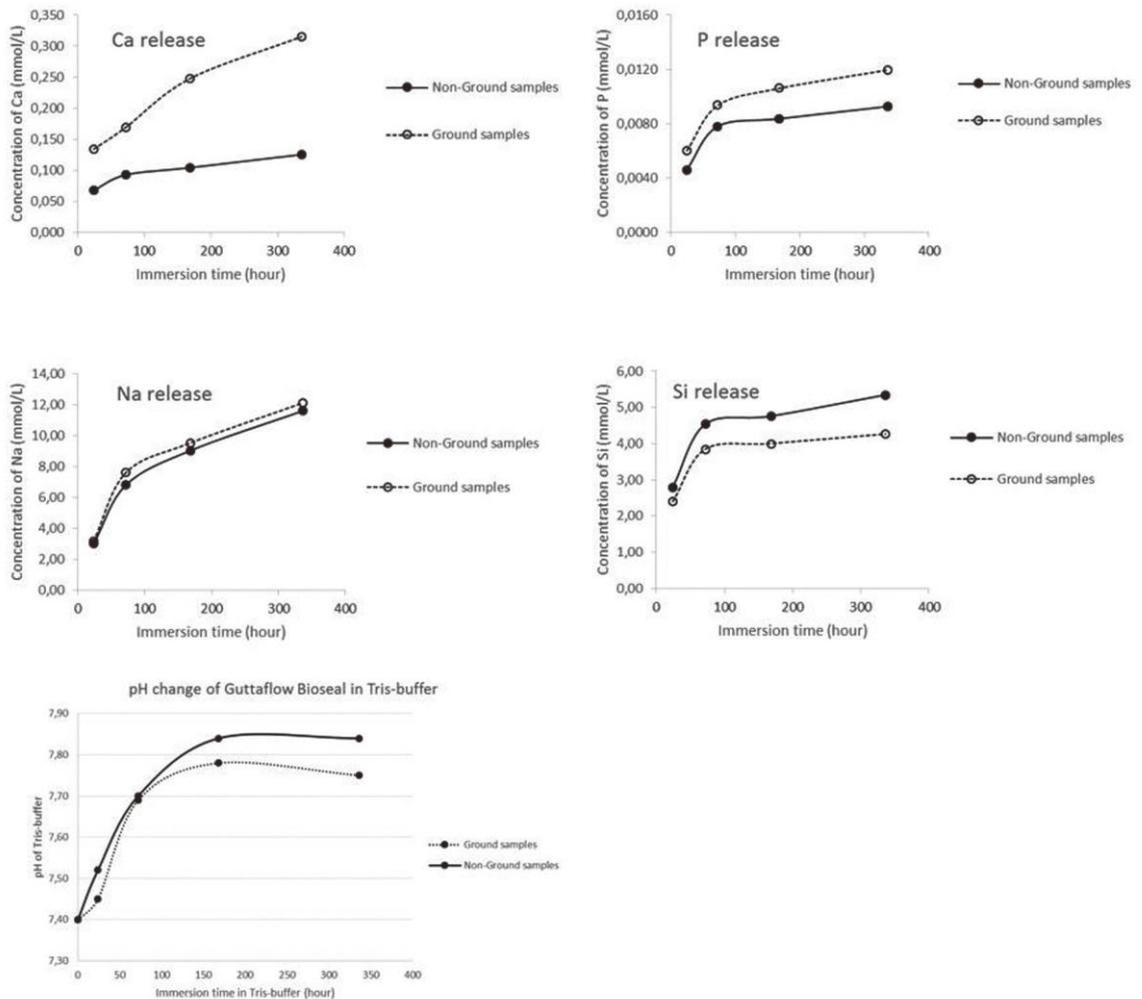


Fig. 4 Dissolution profile of ion release and pH change in 20 mL Tris-buffer during 14 days. Immersion time in hours is shown in X-axis, ion concentrations (mmol/L) and pH change is shown in Y-axis. Continuous line is presenting the average values for nonground samples and dash-line for ground samples. (Please note: The ion concentration plotted in the figure was normalized against a standard mass of the sealer sample of 400 mg to minimize the effect of mass variation of the samples.)