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Characterisation of the surface structure and bioactivity of glass and glass ceramics using surface topography

Research Article

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Abstract: The present paper reports the results of the relationship between the surface topography, microstructure and the *in vitro* bioactivity of samples with and without fluorapatite content in simulated body fluid. Glasses and glass ceramics belonging to the Li_2O-SiO_2-CaO-P_2O_5-CaF_2 system were prepared by using conventional melting technique following by heat treatment to obtain glass ceramics. This current study demonstrates the benefits of combining two microscopic methods for better investigation of the surface structure. The formation of apatite layer on the surface and the increase in surface roughness proved that the glasses and glass ceramics with bioactive fluorapatite content could satisfy to the requirements for biomaterial applications. The results also showed that the roughness of apatite layer formed after immersion in body fluid on the surface of glasses with fluorapatite was more pronounced than that of equivalent glass ceramic samples cured under the same conditions.

Keywords: *Glass and glass ceramics • In vitro bioactivity • Surface chemistry • Surface morphology*

1. Introduction

Biomaterials are developed to replace or to repair the damaged hard and soft tissues. These synthetic organic or inorganic materials are successfully used as an alternative to the autogenous graft, eliminating thus the risk of infection from the donor. Among these materials, two groups of glasses have been found to be able to form a mechanically stable and biologically functional interface with bone. One group consisted of glasses in CaO-SiO_2-Na_2O system, with or without addition of phosphorus oxide. In 1991, Hench was the first who reported the existence of such glasses exhibiting the bone-bonding ability [1–3] and were designated as bioactive [4,5]. The glasses were registered under the name Bioglass®. A common characteristic of bioactive glasses and bioactive ceramics is a time-dependent, meaning the kinetics of surface modification that occurs after implantation. Surface modification is characterised by the formation of a biologically active hydroxycarbonate apatite (HCA) layer which provides the bonding interface with tissues. The HCA phase that forms on bioactive implants is chemically very similar to the mineral component of bone and other hard tissue in mammals. It is that equivalence which is responsible for interfacial bonding [1,6].

The second of bioglass materials are developed in SiO_2-Li_2O system with different addition of CaO and P_2O_5 expressed as fluorapatite ($Ca_5(PO_4)_3F$). Approaches to achieve enhanced mechanical and biochemical properties also include transformation of bioactive glasses into glass ceramics. In this technique, the glasses are subjected to thermal treatments which may affect the materials microstructures and hence their mechanical properties, but also their bioactivity. Many authors [7-15] have developed a large range of glasses and glass ceramics in the Li_2O-SiO_2 system. The applications of biomaterials depend on their phase composition,

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mechanical properties and microstructure, which depend mainly on original composition and by heat treatment. The best-known materials are the lithium disilicate glass ceramics in SiO_2-Al_2O_3-La_2O_3-MgO-ZnO-K_2O-Li_2O-P_2O_5 system, which excel in translucency and high strength (flexural strength: 300-400 MPa) and can be tailored by compressing [16]. High-strength and machinable glass-ceramics were formed in the ZnO-free SiO_2-Li_2O-Al_2O_3-K_2O-P_2O_5 system (flexural strength: 740.8 MPa, fracture toughness: 3.3 MPa $m^{1/2}$) [17]. Glass ceramics with optical properties were developed in the SiO_2-Li_2O-K_2O-ZnO-CaO-P_2O_5-F system. Microstructure of this glass ceramic type contains apatite crystals with needle-like morphology, like in natural teeth [18]. The glasses and glass-ceramics from the Li_2O-SiO_2-CaO-P_2O_5-F system dispose of hardness higher than that of teeth. Included with their optical properties (such as color and opalescence), which can be advanced and tailored by appropriate thermal treatment, it allows to use them in medicine, mainly in stomatology (as dental bridges, crowns or veneers) [1,19]. CaO, P_2O_5 and CaF_2 as nucleation agents are frequently added in small portions to lithium disilicate glasses, in which they initiate the internal nucleation through the phase separation [17,20,21]. However, when used in larger amounts, these oxides cannot yet be considered to provide nucleation sites. Therefore, the properties of the glass change, and nucleation data for both the basic and modified glasses are not comparable. The knowledge of the effect of these substance additions on mechanism and on crystallization kinetics provides the possibilities for influencing the properties of final materials by a controlled crystallization process. Consequently, it brings the possibility to optimize the composition and heat treatment history, which would lead to additional improvement of material properties. Until now, the usage of these materials is limited to the field of bioinert materials activity. The demonstration of bioactive properties which are initiated by CaO, P_2O_5, CaF_2 contents (expressed as fluorapatite, FA) brings about new application possibilities. It was found that adding fluorapatite enhances the bioactivity of glass ceramics, but on the contrary, the decrease in the annealing temperature decreases the biocompatibility. The biocompatibility and corrosion resistance of these implants are primarily determined by their constituent material and surface properties such as surface roughness, grain size, *etc*. [22]. Many of sophisticated equipments are now available to visualize biomaterials surfaces. One of the most versatile techniques is atomic force microscopy (AFM), which enables the production of three-dimensional (3-D) images of the samples topography at a nanometric scale. The AFM helps to detect the residues of surface reactions and individual surface defects [23]. Scanning electron microscopy (SEM) gives detailed information about the process of formation of apatite layer that occurred on the surface of glasses and glass ceramics materials. Other evidence to confirm the formation of apatite layer on the surface is the electron probe micro-analyser analysis (EPMA) that detects the presence of elements on the surface from parent samples and in the layer formed on the surface after assay in simulated body fluid (SBF). The purpose of the present study was to assess and discuss the influence of immersion time in SBF on the formation of calcium-phosphate layer on the surface of two sets of glasses and glass ceramic samples – without (G0, GC0) and with fluorapatite content, expressed as 14% (w/w) of P_2O_5 (G14, GC14). This can be an important factor in clarifying the mechanism of the biomineralization on the surface of sample, and in order to increase the biocompatibility of the implants.

2. Experimental procedure

2.1. Preparation of biomaterials

Glasses from the Li_2O-SiO_2-CaO-P_2O_5-CaF_2 system were prepared by a conventional melting technique from a mixture of raw materials in Pt crucible in a supercanthal furnace at 1450°C (2 h, 10°C min^{-1}) with intermediate grinding and with decarbonatation step (5 h at 950°C). In addition, two different glass compositions were selected for the comparison (Table 1). The compositions of these samples were chosen deliberately for better comparison of development of surface microstructure before and after assay in simulated body fluid (SBF). Pure lithium disilicate (shorthand LS_2) glass without P_2O_5 and CaF_2 content was prepared as a reference sample. The melts were quenched by pouring them onto a copper board and then placed in heated muffle furnace at 450°C. The muffle was switched off and glass samples were slowly cooled to ambient temperature. Such prepared glass samples were crushed into powder, homogenized, remelted and poured into copper moulds to form discs with precisely defined dimensions as listed above. Representative samples of glass ceramics were prepared by annealing or thermal treating of parent glasses under optimized regime in a muffle furnace at 600°C for 6 hours (heating rate 10°C min^{-1}) as reported in [7] to characterise the crystallisation course of different phases.

An important requirement for the samples to be observed by AFM is the flatness of the surface. Therefore, in the present study, the glass and glass ceramic samples without and with 14% (w/w) of P_2O_5 were polished by means ascending grades of wet

Table 1. Nominal composition of synthesized glasses (%).

	Li_2O	CO_2	SiO_2	CaF_2	CaO	P_2O_5
G0	25.00	25.00	50.00	0	0	0
G14	20.15	20.15	40.30	1.50	13.40	4.50

Table 2. Ion concentrations of SBF in comparison with those in human blood plasma [24].

Description	Ion Concentrations (10^{-3} mol L^{-1})							
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO^{3-}	HPO_4^{2-}	SO_4^{2-}
SBF	141.8	5.0	1.5	2.5	147.8	4.2	1.0	0.5
Human plasma	142.0	3.6-5.5	1.0	2.1-2.6	95.0-107.0	27.0	0.65-1.45	1.0

grinding papers (15 and 26 μm). Finally the samples were cut into cubes with size of 1.5 cm^2 by Precision Diamond Saw (Type VC-50).

2.2. In vitro test of bioactivity in static regime

The essential condition for a biomaterial to bond with living bone is the formation of a surface apatite layer in the body environment. Bioactivity of obtained biomaterials was evaluated by examining the apatite formation on their surfaces in simulated body fluid (SBF), an acellular aqueous solution with inorganic ion composition almost equal to human blood plasma (Table 2) [24]. The SBF was prepared by dissolving the components NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 6H_2O$, Na_2SO_4, and buffering at pH 7.4 at 36.5°C with $(HOCH_2)_3CNH_2$ and 1 M HCl per litre of ultra pure water in a beaker according to the method developed by Kokubo *et al.* [25]. Sodium azide was added to inhibit bacterial growth. The calculated volumes of SBF (Eq. 1) were poured in the plastic containers and heated up to the temperature of 36.5°C ± 1°C.

$$V_S = \frac{S_a}{10} \quad (1)$$

V_S - volume of SBF (mL)
S_a - apparent surface area of specimen (mm^2).

The volume of the SBF for the *in vitro* tests of the samples is a critical parameter when they are deposited on rough surfaces of porous materials. A high value for the surface area to volume ratio slows down the bioactive performance of coatings by the rapid creation of a thin Ca/P layer. The surface area in contact with the fluid should be carefully estimated prior to the immersion in order to avoid this effect [1].

The polished samples with precisely defined dimensions as listed above were immersed in SBF at the human body temperature (36.5°C) in sterilized polyethylene bottles with fastened lids and were placed in incubation apparatus (Binder BD 115). Soaking period was 7 days under static regime.

After exposure in SBF the glass and glass ceramic samples were withdrawn from the incubator and rinsed gently with pure ethanol and distilled water. Then the samples were dried at ambient conditions inside a desiccator for further analysis.

2.3. Surface analysis

Scanning electron microscopy (SEM - TESLA BS 300 with digital unit TeScan) in conjunction with electron probe micro-analyser analysis (EPMA JEOL JXA-840A, EDS parameters- 15 KV, Takeoff Angle 40.0°) allowed us to investigate the morphology (pore size, shape and interconnectivity) and chemical constituents of the prepared glass and glass ceramic samples before and after immersion in SBF. The samples were mounted on aluminium stubs with double sided carbon tape, ion sputtered with a thin layer of gold for SEM and carbon coated for EPMA before analyses. The three-dimensional imaging and the analysis capabilities of Atomic force microscopy (AFM – Veeco CP-II with digital unit DiProScan) were used to investigate the surface roughness of all polished samples as a function of immersion time in SBF. The surface morphology was evaluated by atomic force microscopy (AFM) on an Auto Probe scanning probe microscope. The images were obtained using a golden silicon probe (resonance frequency, 320 Khz; tip radius, 10 nm).

Surface roughness values were expressed as root-mean-square roughness (R_{RMS}), which give a good general description of the structure of the actual surface. R_{RMS} (Eq. 2) is the root-mean-square deviation from the profile mean over the sampling area. From a statistical point of view, R_{RMS} is not only relative to the deviation from the profile to the centre line, but it is also sensitive to very large and small height values. The approximate formula is [26]:

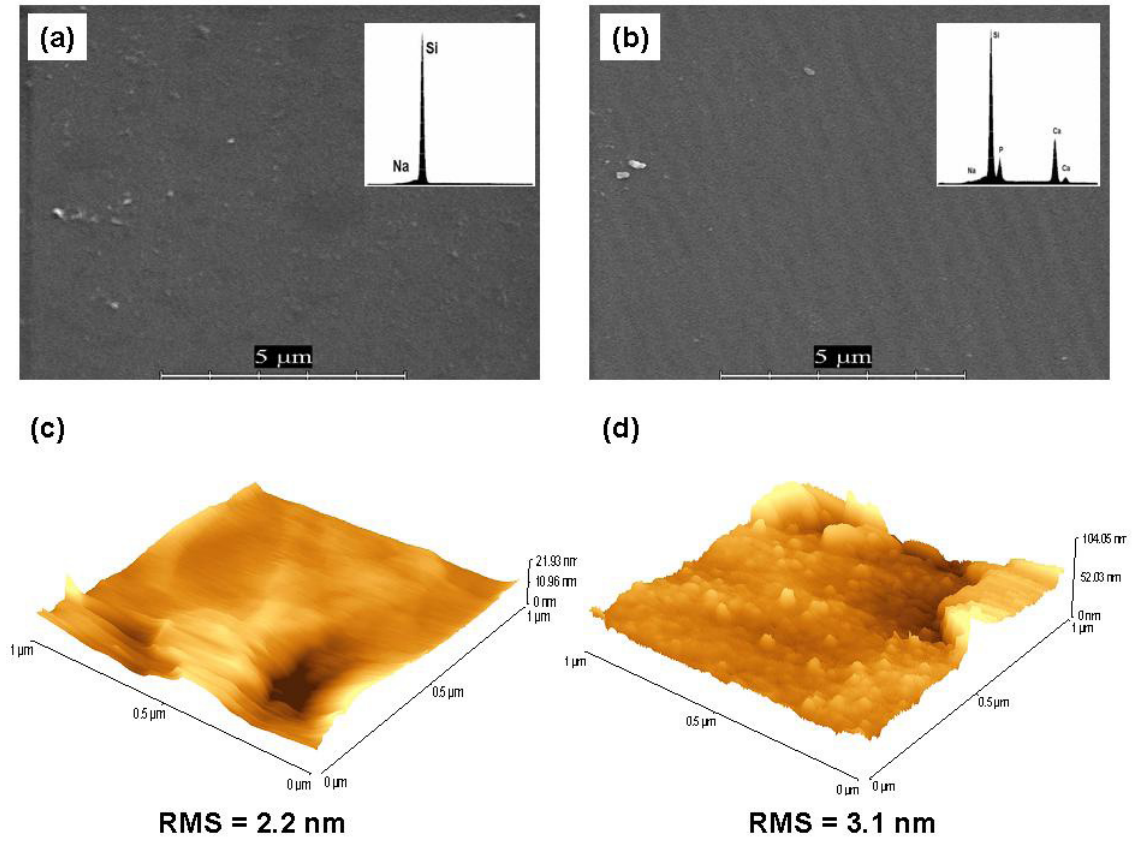

Figure 1. SEM - EPMA surface analysis (a, b) and AFM images (c, d) of glass samples (a, c – G0, b, d – G14) before immersion in SBF.

$$R_{RMS} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}[Z_i]^2} \qquad (2)$$

Zi - the *i*th dot of the distance to the centre line
n - the total number of sampling dots.

The values of root-mean-square roughness of the surface area of 1×1 µm in size were calculated with the Nova program package intended for processing AFM images.

3. Results and discussion

3.1. Surface characterisation of the obtained bioglasess before and after in vitro test

Figs. 1 and 2 show the SEM-EPMA and AFM microphotographs of glass samples (G0, G14) before and after 7-day immersion in SBF.

SEM images before immersion in biological fluid (Figs. 1a, 1b) revealed that the surfaces of both glass samples were smoother, uniform and homogeneous. Results from EPMA analysis (spectra in the right corner) show that surface of G0 sample is characterised by a dominant presence of silicon. The G14 sample is characterised also by a dominant presence of silicon with calcium and phosphorus originating from mixture of CaF_2 and $Ca_3(PO_4)_2$ in the batch with stoichiometric ratio corresponding to fluorapatite (FA).

Smooth surface morphology of glass samples changes after 7-day of immersion (Fig. 2). Observations from SEM examination and EPMA analyses suggest that a potential of bioactivity because of formation of bone-like apatite that is an essential condition for the bone bonding ability on their surfaces after exposure to SBF. It can be seen, that a few newly spherical clusters had formed on the surface of G0 sample in contact with SBF, suggesting apatite crystallites (Figs. 2a, 2b). The formed fragile and thick apatite layer grew by consuming calcium and phosphate ions from SBF [27,28]. More information on this layer can be drawn from the EPMA analyses (spectra in the right corner). The calculated average Ca/P atomic ratio was approximately 2.70 indicating the layer is deficient in phosphorous. In Fig. 2b it is evident that calcium phosphate layer is more pronounced in G14 sample and EPMA analysis showed that Ca/P ratio on the sample surface after 7-day

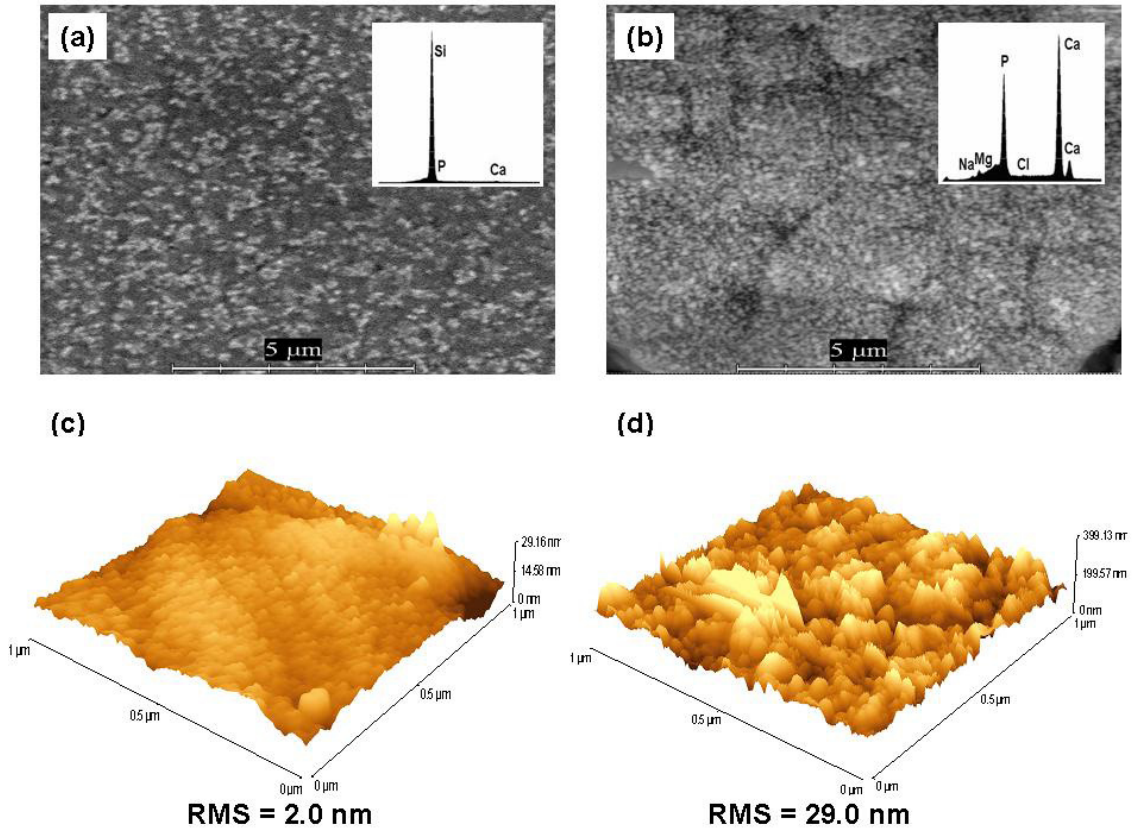

Figure 2. SEM - EPMA surface analysis (a, b) and AFM images (c, d) of glass samples (a, c – G0, b, d – G14) after 7-day immersion in SBF.

immersion in SBF reached values corresponding to that of bone apatite (~1.67) (Table 3). The rapid growth of the apatite layer on the surface of G14 sample can be assigned to the presence of CaO phase in the prepared material. The immersion of this glass sample in SBF leads to the breaking of Ca-O bonds and dissolution of this phase. This process creates an increase of Ca^{+} ion concentration in solution and consequently a supersaturated liquid develops, which accelerates the deposition mechanism and formation of the apatite layer [4]. This claim was confirmed by EPMA analysis, where the increased amounts of Ca and P may indicate the onset of the formation of an amorphous $CaO\text{-}P_2O_5$ rich layer. Besides these major elements, the presence of small amount of Na, Cl and Mg was detected. The above components were derived from SBF.

In order to clearly elucidate the effect of the $Ca_3(PO_4)_2$ addition and 7-day immersion in SBF on the surface roughness and the morphology of the samples, we performed an AFM analysis. Figs. 1c, 1d show AFM images (1×1 µm) of the G0 and G14 samples before immersion in SBF. The values of root-mean-square roughness (R_{RMS}) indicate that the presence of $Ca_3(PO_4)_2$ addition has initiated crystallisation, as reported by [7]. This study showed that the surface of G14 sample is therefore rougher than that of the G0 sample. The differences in roughness were not statistically significant (Figs. 1c,1d and 2c,2d), however, it was evident that there was an increase in the overall roughness when the G14 sample was immersed in SBF (Fig. 2d). This sample after 7-day immersion in SBF shows more rough surface structure than before immersion. Therefore, one can state that the surface roughness increased with the presence of CaF_2 and $Ca_3(PO_4)_2$ in the sample. The whole surface of G14 after a 7-day immersion is entirely covered by a layer of calcium phosphate (Fig. 2b). The high value of roughness is due to the growing layer of apatite, thus proving high bioactivity of glass containing $Ca_3(PO_4)_2$ addition.

3.2. Surface characterisation of the obtained glass ceramics before and after 7-day assay in SBF

Figs. 3 and 4 show the SEM-EPMA and AFM images of glass ceramic samples (GC0 and GC14), synthesized and thermally treated at 600°C for 6 hours, before and after 7-day immersion in SBF. As pictured, both samples

Table 3. Surface composition obtained by EPMA of glass samples before and after 7-day assay in SBF.

G0		
Element (Atom %)	Before assay	SBF assay
Si	32.95	27.45
Ca	–	2.84
P	–	1.05
Ca/P	–	2.70
G14		
Element (Atom %)	Before assay	SBF assay
Si	21.78	–
Ca	9.38	24.86
P	3.84	14.92
Ca/P	2.44	1.67

have different morphologies before soaking in SBF, depending on the chemical compositions and thermally treatment. The GC0 sample has a fine-grained surface morphology, compact and consists of one type of crystal (Fig. 3a). The GC14 sample showing that surface is looser and rougher with holes with irregular shapes and pores (Fig. 3b). EPMA analysis (spectra in the right corners) shows a dominant presence of silicon on the surface of both samples. Consequently, the chemical composition is related to that reported in Table 1. In addition to silicon the presence of calcium and phosphorus are detected on the surface of GC14 sample. This is due to the presence of fluorapatite crystals on the surface of glass ceramics.

As shown in Figs. 4a and 4b, SEM results indicate changes in the morphology and composition of the material surfaces after soaking in SBF. After a 7-day assay in SBF, both glass ceramics were covered with a newly formed layer, but the only sample totally covered by aggregates of apatite-like crystals is GC14 (Fig. 4b). From the presented SEM images it can also be seen that the prepared GC0 sample does not have a fully densified structure. These results confirm that the GC0 after 7-day immersion in SBF cannot induce the formation of apatite layer without the bioactive component. Some authors also postulate that the preparation of this structure could be assigned to the presence of silica in calcium phosphate structure and the temperature of thermal treatment [29]. Surface layer of the GC0 sample consisting of only flake-shaped particles partly spread out on the surface after a 7-day immersion in SBF.

The holes in the surface of the GC14 sample of the initial material have been reduced after immersion, suggesting the covering of the surface by another material. The surfaces were rough and fully covered by apatite granules due to the interaction of the surface of the glass ceramic materials with the SBF solution. The decrease in silicon concentration and increase in calcium and phosphorus concentrations are observed as the immersion time in SBF grows.

Another evidence to confirm the formation of apatite layer on the surface of glass ceramics sample was the EPMA results. Also the relative concentration of Na, Cl and Mg was detected. The above components were derived from SBF solution. Results obtained of the EPMA analysis for the glass ceramic surface before and after 7-day immersion in SBF are listed in Table 4.

After immersion in SBF, EPMA analysis revealed a decrease in the silicon and an increase in Ca and P peaks as well as other peaks corresponding to elements found in parent glass ceramics. The average Ca/P atomic ratio was calculated (Ca/P= 1.34). Peitl *et al*. [30] have found that glass ceramics with crystalline phases are less reactive than related glasses. In some case, the crystallisation can even turn a bioactive glass into an inert biomaterial.

The AFM observations (1×1 μm) corroborated with the previous findings with SEM-EPMA. As shown in Figs. 3c, 3d and 4c, 4d the results of surface topography glass ceramic samples after annealing at 600°C was similar with the glass samples (Figs. 1c,1d and 2c,2d) and evolved after a 7-day assay in SBF.

One can see that the differences in roughness before and after immersion in SBF were not statistically significant. The GC0 sample before immersion (Figs. 3a, 3c) showed uniform and homogeneous structure. After a 7-day immersion in SBF (Figs. 4a, 4c) showed that apatite film surface is more compact and the size of the globules is smaller, corresponding to lower roughness in both cases.

The GC14 sample after a 7-day immersion in SBF (Fig. 4d) showed more rough surface structure than before immersion (Fig. 3d). The surface roughness of the samples increased with the presence of CaF_2 and $Ca_3(PO_4)_2$ in sample. Also, one can see that the Ca/P deposits initially followed the topography of substrates but entirely masked the scratches as their size increased with time. The results cannot be compared directly with other glass ceramic samples because of differing composition. Schmidt presented in his PhD thesis [31] that the surface roughness depends on thermal history. On the other hand, increased roughness of the bioactive glass ceramic surface could result not only in a larger surface area for biomaterial-cell interactions, but also in an ideal topographical environment for enhanced cell attachment.

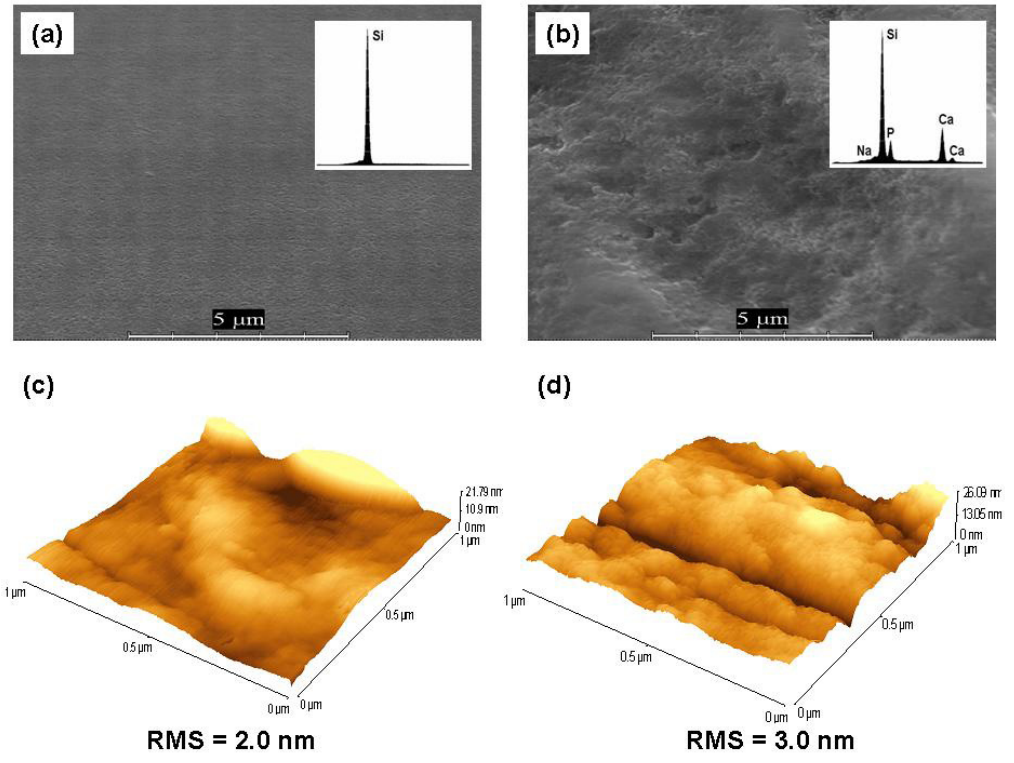

Figure 3. SEM - EPMA surface analysis (a, b) and AFM images (c, d) of glass ceramic samples (a, c – GC0, b, d – GC14) before immersion in SBF.

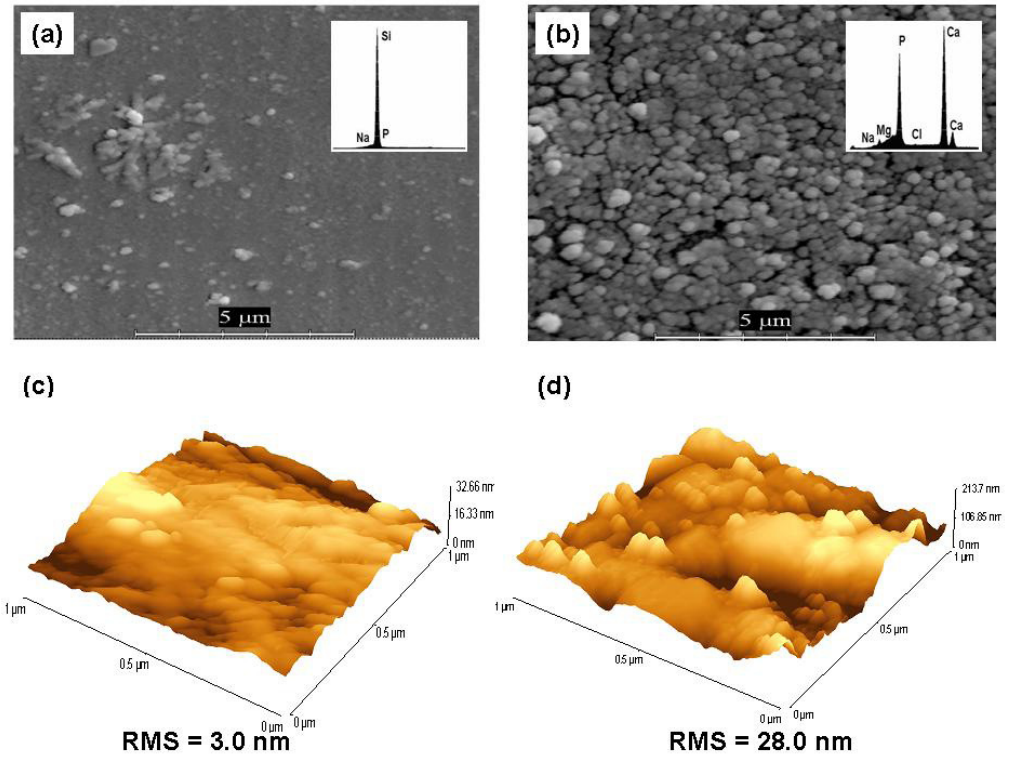

Figure 4. SEM - EPMA surface analysis (a, b) and AFM images (c, d) of glass ceramic samples (a, c – GC0, b, d – GC14) after 7-day immersion in SBF.

Table 4. Surface composition obtained by EPMA of glass ceramic samples before and after 7-day assay in SBF.

GC0		
Element (Atom %)	Before assay	SBF assay
Si	34.52	33.12
Ca	–	–
P	–	0.42
Ca/P	–	–
GC14		
Element (Atom %)	Before assay	SBF assay
Si	28.32	–
Ca	8.95	18.27
P	3.96	13.45
Ca/P	2.26	1.34

4. Conclusion

Bioactive glasses and glass ceramic samples in the system Li_2O-SiO_2-CaO-P_2O_5-CaF_2 were prepared by using a conventional melting technique following by heat treatment to obtain glass ceramics. Various technical methods like SEM, EPMA and AFM allowed us to determine the composition, properties and surface structure of materials before and after assay in SBF. The formation of apatite on the surface and the increase in surface roughness in the current study proved that the glass and glass ceramics with bioactive component (FA) content could satisfy the requirements for biomaterial applications. Furthermore, the formation of the Ca/P layer on the surface of the samples confirms that the samples exhibit a strong *in vitro* bioactivity that is supported by the bioactive component.

The bioactivity of samples was related to the phosphorus content. This element was indispensable for the apatite layer formation and therefore the bioactivity rate was higher when phosphorus was present in the composition. The results showed that the roughness of apatite layer formed after immersion in SBF on the surface of glasses with FA content was more pronounced than that of equivalent glass ceramic samples cured under the same conditions.

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