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Osteoblastic cells biomineralized on bioactive glass and glass-ceramics of the $SiO_2.Na_2O.CaO.K_2O.MgO.P_2O_5$ system modified with Al_2O_3 and B_2O_3

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A treatment study evaluates glass and glass-ceramics of the $Na_2O{\cdot}CaO{\cdot}K_2O{\cdot}MgO{\cdot}SiO_2{\cdot}P_2O_5$ system, modified with B_2O_3, Al_2O_3 or B_2O_3/Al_2O_3 up to 0.66. Both, bioactivity using simulated body fluid (SBF) immersion and mineral nodule formation using calvaria derived osteoblasts, were tested. The surface structure changes were examined by SEM-EDX line scan X-ray microanalysis and calcium deposition by the alizarin-red test. It was found that all compositions of glasses as well as glass-ceramics reacted with the SBF, allowing the development of a SiO_2 rich layer. In some cases the presence of a rich calcium and phosphorous layer was observed, that according to other studies, corresponds to the apatite layer identified in previous studies. This result suggests a bioactivity behavior, with the exception of the glass containing $B_2O_3/Al_2O_3 = 0.66$ and the glass-ceramic of the same composition with a 2nd heat treatment. The composition containing $B_2O_3/Al_2O_3 = 0.66$ showed the best biocompatibility with hydroxyapatite nodule formation on its surface, especially on the glass-ceramics.

Key words: Bioactive glass, osteoblast-like cells, in vitro biomineralization, Scanning Electron Microscopy (Line scan).

Introduction

During the last forty years, another revolution has occurred in the use of ceramics to improve the quality of life of humans. This revolution is the development of specially designed and synthesized ceramics for the repair and reconstruction of diseased or damaged parts of the skeleton. Ceramics used for this purpose are called bioceramics. Most clinical applications of bioceramics relate to the repair of the skeletal system, composed of bones, joints and teeth, and to augment both, hard and soft tissues [1-3].

The development of new technologies for implants has been oriented to the creation of active interfaces between implants and tissue, developing the concept of bioactive biomaterials, such as phosphated ceramics (Ca-P), active glasses (VP) and bioactive glass-ceramics [4-6]. All these materials have in common the generation of a hydroxyapatite carbonate layer equivalent from the structural and chemical point-of-view, to the mineral of those calcified tissues [7-11].

The basis of the bone bonding property of bioactive glasses is the chemical reactivity of the glass in body fluids. The interface reactions between a bioactive glass and bone, allows the development of SiO_2 rich and Ca and P rich layers. The silica rich layer is due to the Na+ exchange with H^+ or H_3O^+ from the simulated body fluid (SBF), this exchange is easy because Na^+ ions are not part of the glass network, they only modify it by forming non-bridging oxygen bonds, the release of network modifying ions is rapid for highly bioactive glasses. Migration of Ca^{+2} and $P_2O_5^{-3}$ groups to the surface through the SiO_2-rich layer and incorporation of soluble calcium and phosphorous from the solution generates the formation and growth of an amorphous $CaO\text{-}P_2O_5$ rich film, which was previously identified by other authors [12].

When it was first proposed, that glasses might be used as implant materials, the idea was slowly accepted but, they would develop on their surface, when in contact with body fluid and tissues, a reactive layer that, because of its gel-like structure, provided a compliant interface between the bulk glass and tissue [1]. This surface apatite layer consists of a carbonate-containing hydroxyapatite with small crystallites and/or defective structures. The compositional and structural characteristics of this apatite are similar to those of the apatite in natural bone. It is expected that a bone-producing cell, osteoblast or dentine, produced by odontoblast, would proliferate preferentially over fibroblasts on the layer. Consequently, fibrous tissue, which usually forms around foreign material, is not formed around bioactive glass, and the surrounding bone can grow directly on the

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surface apatite layer. When this occurs, a tight chemical bond forms between the surface apatite and the bone apatite, in order to reduce the interfacial energy [13].

Bioactive glasses are not strong enough to be used for load-bearing applications. One approach to solve this problem is to combine the glass with a fracture tough phase, such as a metal or a polymer, to produce a composite [13], also glass can be converted by heat treatment into glass-crystal composites containing various types of crystalline phases with controlled sizes and contents. The resultant glass-ceramics can exhibit superior properties to that of the parent glass and to sintered crystalline ceramics. To achieve the fine grained microstructure in a glass-ceramic, which favors the development of outstanding physical properties, it is necessary to ensure that the nucleation process of the crystals occurs within the body of the glass; one way to achieve this is with nucleation by oxide separation. This is caused by the formation of stable molecular structural groups and their enrichment in certain regions. As a rule, this will considerably facilitate crystal nucleation in the new phase, as the critical nucleus size can be reached with less than the full amount of the activation energy that would be necessary in case of a base glass of homogeneous composition.

Barrios de Arenas *et al*. [14, 15] showed that some glasses based on the $SiO_2.Na_2O.CaO.P_2O_5$ system and with controlled Al_2O_3 y B_2O_3 additions, allowed surface chemical reactions, resulting in the accumulation of calcium and phosphorous in the glass surface. They suggested that in modified bioactive glasses containing B_2O_3 and Al_2O_3, it is necessary to control their ratio due to their influence on the alumina stabilization effect, in order to allow the CaP deposition. Nevertheless, recently, Noris-Suárez *et al*. [16], showed cellular adhesion and proliferation of bone cells on glass surfaces of the system $SiO_2.Na_2O.\ CaO.K_2O.MgO.P_2O_5$ containing Al_2O_3 and B_2O_3, in 0.66 proportions.

The purpose of this investigation is to study the influence of different additions of B_2O_3/Al_2O_3 on glasses and glass-ceramics of the $SiO_2.Na_2O.CaO.K_2O.MgO.P_2O_5$ system. The apatite layer was determined by two in vitro tests; one of them with SBF and the other with osteoblast-like cells mineralization, analyzing the compositional changes through line scan compositional profiles.

Experimental procedure

Salts for glass and glass-ceramics synthesis were SiO_2, $CHNaO_3$, $CaCO_3$, P_2O_5, B_2O_3, Al_2O_3, all above 99% purity. Reagents and materials for cell culture and assays were purchased from the following sources: Dulbeco's Modified Eagle's Medium (DMEM), trypsin and fetal bovine serum (FBS) GIBCO, penicillin, streptomycin, dexametasona (DXM), ascorbic acid, β-glycerophosphate (β-GP) and collagenase, SIGMA, alizarin red, Merck. All other chemicals were of the highest quality grade available.

The experimental procedure, preparation and melt of each glass composition, has been described in a previous paper [14]. Glass compositions (Table 1) of the $Na_2O.K_2O.MgO.CaO.SiO_2.P_2O_5$ system, were prepared using partial substitution of Na_2O and CaO for K_2O and MgO respectively, from a previously studied $SiO_2.Na_2O.CaO.P_2O_5$ system [16], CaO/P_2O_5 in a proportion of 5 : 1 and different additions of Al_2O_3 and B_2O_3. The mixtures of the nominal compositions shown in Table 1 were melted in a platinum crucible for 6 h, at a heating rate of 5 ºC.minute^{-1}, a holding time of 60 minutes at 1000 ºC to eliminate carbonates and 1 h at 1450 ºC for glass homogenization. The melt was poured in to 500 ºC preheated graphite moulds with a cylindrical 10 mm diameter geometry. At least five pieces were prepared for each glass and their surfaces were polished with 4 to 3 μm diamond paste. Glass samples from each composition were submitted to 3 different crystallization cycles to obtain the glass-ceramics (Table 2), at 550 ºC for nucleation and 750 ºC for crystal growth with different holding times.

The immersion fluid: simulated body fluid (SBF) was prepared in the same way that had almost equal ion concentrations to those of human blood plasma [14, 17]. The samples immersed in permanently agitated SBF at 37 ºC, were taken out after 4 weeks, gently washed with acetone and embedded in cold epoxy resin. The cross-sections were polished down to 1 μm and then coated by a conductive carbon film to be analyzed by SEM-EDX. Some samples of glass-ceramics were submitted to XRD to identify the crystalline phases formed.

Table 2. Crystallization heat treatments

Cycle	Nucleation time (minutes)	Crystal growth time (minutes)
I	30	60
II	30	120
III	60	30

Table 1. Glass compositions synthesis (wt. %)

Glass	SiO_2	Na_2O	K_2O	CaO	MgO	P_2O_5	B_2O_3	Al_2O_3	B_2O_3/Al_2O_3
1	49	20	4	20	4	2	0	1	
2	49	20	4.3	20	4.2	2	0.5	0	
3	48.5	20	4.3	20	4.2	2	1	0	
4	48.5	20	4	20	4	2	1.5	0	
5	48	20	4	20	3.5	2	1	1.5	0.66

Osteoblasts were isolated from 12 calvaria of 2-3 day old neonatal rats as described in a previous article [16]. Briefly, the calvaria were dissected under aseptic conditions and freed from soft tissue except for periosteal coverings, followed by several phosphate-buffered (PBS) washes. Enzymatic digestion of tissue was obtained using 1 mg/ml collagenase, 0.125 % trypsin and 0.5 mM EDTA. Cells corresponding to the 3rd digestive extraction were seeded at a 6×10^5 cells/dish density in 100-mm diameter petri dishes and cultured with DMEM medium (complemented with 10% fetal bovine serum (FBS), 100 Unit/ml penicillin and 100 μg/ml streptomycin). Cells were maintained at 37 °C in a fully-humidified atmosphere at 5% CO_2 in air. The media was first changed 7 days after culturing and after this initial change, every 3 - 4 days until cells reached confluence.

The different materials to be tested were treated with 70% ethanol to remove the superficial grease, then were washed extensively with deionized water and finally were sterilized with UV for a period of 20-30 minutes. Cells were seeded in 12-well plates (1×10^4 cells/cm^2) for the proliferation test. The medium was changed every 3-4 days and viability and proliferation of cells was observed by inverted optic microscope for 14 days. This observation was conducted directly under a glass surface or in a well surface rounded opaque ceramic. The image was recorded using a Canon inverted microscope camera. The adhesion of cells on different materials was tested seeding 3×10^5 cells/cm^2 on 24 well-plates and incubated 24 h in contact with the materials. After that, cultures were washed with PBS to remove the culture medium and non-adhered cells. Adhered cells were detached with trypsin treatment, and quantified employing a hemocytometer. A treatment to induce biomineralization was started 14 days after cells were grown with the different glass or glass-ceramic tested. DMEM supplemented with 1×10^{-7} M dexametasone (DXM), 50 μg/ml ascorbic acid, 10 mM β-glycerophosphate (β-GP) and 10% fetal bovine serum (FBS) was used as the medium. The medium was changed every 7 days and the treatment was maintained for a total period of 14 days. The pH of the culture medium was quantified using a digital pHmeter.

Calcium deposition was determined using the alizarin red S method [18]. The medium was removed and cultures were rinsed with PBS, fixed in ice-cold 70% ethanol for 1 h. After this, samples were washed three times with PBS, rinsed with deionized water and then stained for 10 minutes with 4 mM alizarin red S dye pH 4.2, at room temperature for brightfield images. Cultures were rinsed five times with deionized water, followed by rinsing in PBS (15 minutes), to reduce non-specific alizarin red S stain. Mineralized nodules were recorded by digital photography and samples were treated as describe above for SEM analysis.

Results and Discussion

For some years the bioactivity of different special ceramic materials (glass, glass-ceramics) has been studied as well as the capacity of bonding with soft and hard tissues, in order to determine possible uses for repairing and reconstructing sick and damaged tissues, especially in bones [19].

According to the theory, the multivalent B_2O_3 ion should have a negative influence on bioactivity. However, in a previous study it was found to enhance this property [14]. The compositions with B_2O_3 additions, except compositions C2 and C3 both with second and third cycle of treatment, exhibited bioactivity. An explanation for this non-bioactive behavior could be that these samples were submitted to the longest nucleation cycle promoting the formation of more crystalline phase, which made the interaction between SBF and the vitreous material phase more difficult.

The glasses of different compositions were submitted to three (3) different heat treatments (Table 2), in order to induce devitrification, to achieve glass-ceramic formation. The samples showed a white opacity surface to the naked eye, characteristic of glass to glass-ceramic transformation occurrence. Fig. 1 gives SEM images of cross-sections of glass-ceramics from compositions 1-5, heat treated under cycles I-III, where the development of dendritic crystals and a residual intercrystalline vitreous phase in all materials except that of composition 1 where the crystalline phase showed a nodular morphology are seen.

Table 3 shows the EDX analysis of the crystalline phase obtained in all compositions after heat treatment cycle 1. The B_2O_3 and Al_2O_3 additions do not crystallize, remaining in the vitreous phase.

X-ray diffraction spectra obtained are similar for all compositions studied except that of composition 4, where the maximum intensity signals appeared inverted in reference with all others, see arrows in Fig. 2.

According to the Journal of Crystalline Diffraction Pattern (JCPDF) data, it was found that the phase present in all compositions is $Na_4Ca_4(Si_6O_{18})$ known as combeite, and the difference between the signal intensities in both spectra (arrows in Fig. 2), is due to the different crystal structures of combeite. In all the glass-ceramics hexagonal combeite was found(JCPDF 75-1687), with the exception of composition 4 (Fig. 2(b)), where it was identified as rhombohedral combeite (JCPDF 75-1686) [20].

The glass and glass-ceramics were immersed *in vitro* (SBF) and apatite deposition was analyzed by SEM-EDX. Results are shown in Fig. 3 column (a) corresponding to different glass compositions. It is observed that both compositions 1 and 5 gave no CaP deposition after 4 weeks SBF incubation, this effect is due to the presence of high Al_2O_3 additions1% and 1.5%, respectively. They react to only form a silica surface layer, although in the latter case the Al_2O_3 is counteracted by the 1% B_2O_3 addition when the B_2O_3/ Al_2O_3 equals to 0.66. Otherwise, glass compositions 2, 3 and 4 [Fig. 3, column (a)] have besides the Si, an evident apatite (CaP) layer deposition.

The relationship between B_2O_3 and Al_2O_3 could be used to modify the rates of surface dissolution, Al_2O_3 is especially important in controlling glass surface durability, its melting

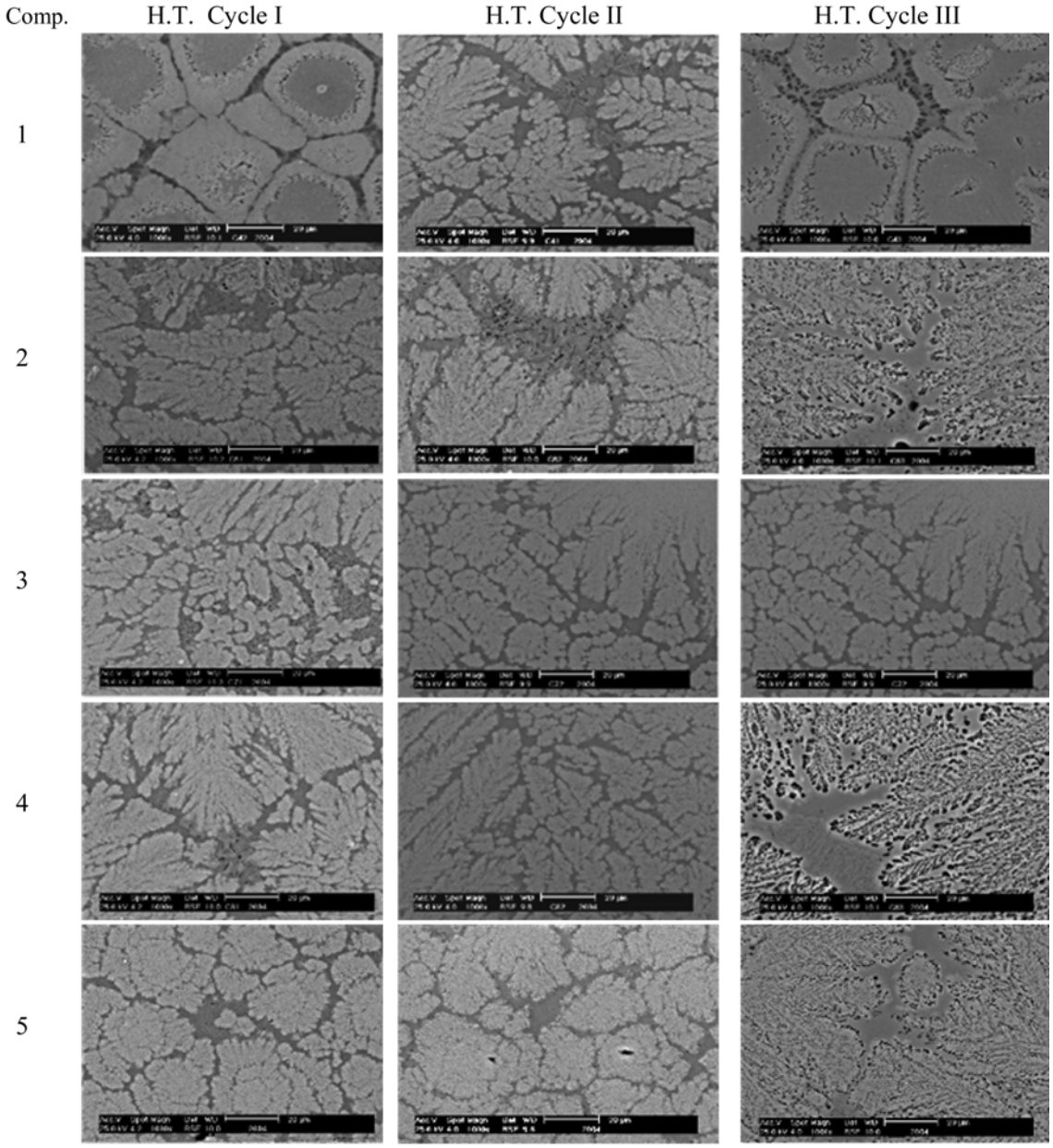

Fig. 1. SEM images of cross-sections of glass-ceramics from compositions 1-5. H.T. = Heat treated under cycles I-III. Notice the microstructural changes.

Table 3. EDX analysis of the crystalline phase in all samples for heat treatment cycle 1

Element	O	Na	Mg	Si	K	Ca
Weight %	27.1	14.2	1.7	33.8	2.1	21.1
Atomic %	40.6	14.9	1.7	28.9	1.3	12.7

and forming characteristics. However, it is well established that Al_2O_3 in contrast to B_2O_3, can inhibit bone bonding, also its reactivity leading to formation of apatite at the surface.

The glasses of different compositions were converted by heat treatment into glass-crystal composites, these behave differently in the SBF, depending on the degree of crystallization due to the heat treatment cycle (see Table 2), from cycle I, as is seen in Fig. 3, column (b), only the composition 3 shows the formation of a Ca layer after 4 weeks. This effect should be due to the presence of the 1% B_2O_3 addition, which enhances the hydroxyapatite (HCA) deposition, [14]. The others only show a Si-rich layer formation. Although composition 4 has a larger B_2O_3 addition, it has less K_2O and MgO. The glass-ceramics corresponding to cycle II [Fig 3, compositions 1 to 5, column (c)], submitted to less nucleation and longer crystal growing time, shows that composition 4 allows the CaP layer to form on top of the Si layer. The same effect was observed for the last treatment with a longer nucleation and a shorter crystal growing time (cycle III) [Fig. 3,

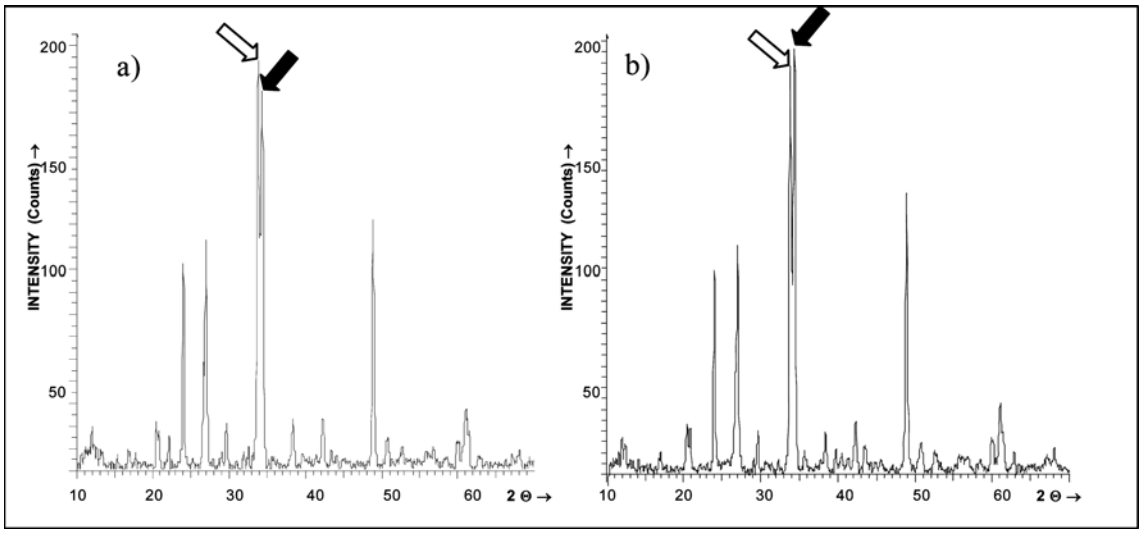

Fig. 2. X rays diffraction spectra for: a) compositions 1-3, 5 and b) composition 4.

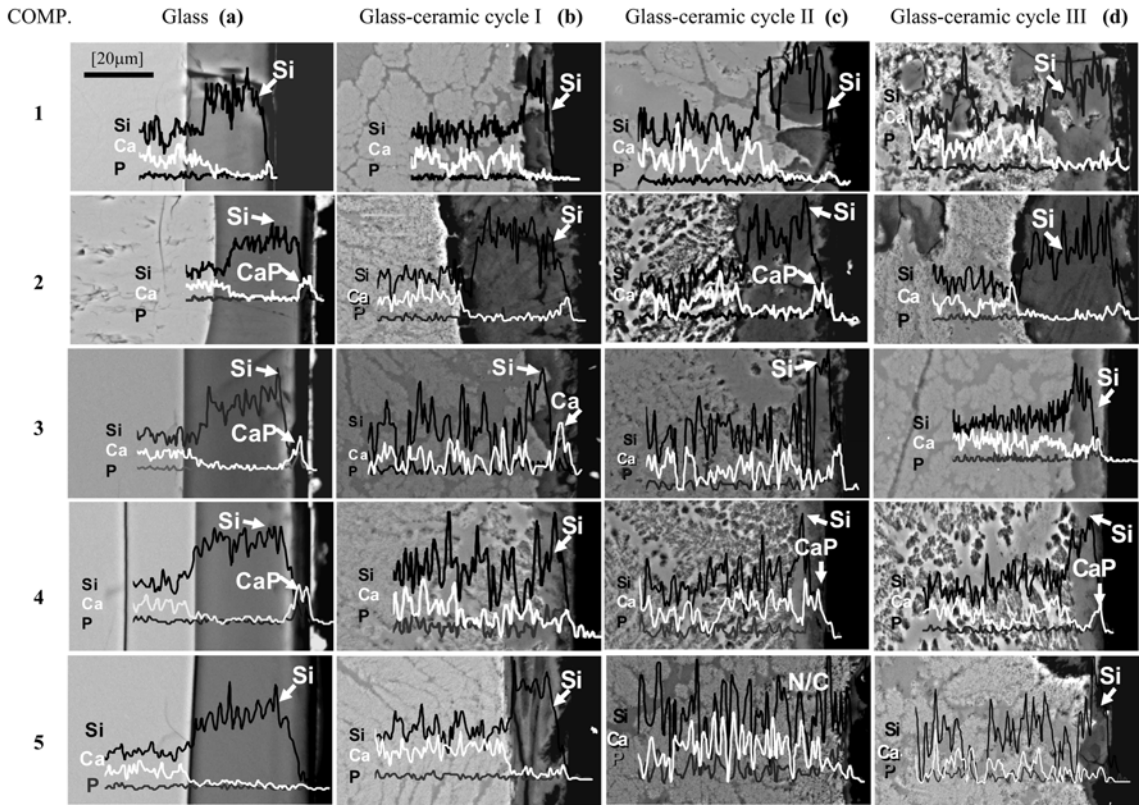

Fig. 3. Scanning electron microscopy images of cross sections of glasses 1-5 (a); glass-ceramics cycle I: 1-5 (b); glass-ceramics cycle II: 1-5 (c); glass-ceramics cycle III: 1-5 (d), soaked in simulated body fluid (SBF) for 4 weeks, with their line scan profiles superimposed. Si: Silicon, Ca: calcium, P: phosphorus, CaP: apatite, N/C: no-change.

column (d), composition 4], while for composition 5 with less MgO and a higher Al_2O_3 addition shows no change, the others allows only the Si layer formation.

Cell adhesions on glass surfaces of different compositions were low when compared to the controls (values ranged from 4% for the adhesion of glass composition 1 to 20% for composition 5, see Table 1 for different compositions). Contrary to this, cells adhered more on glass-ceramics, showing values up to 40%, in the case of composition 5, when compared to the controls (for glass-ceramics with cycles I to III). During the entire cell culture period (28 days) cells showed viability for all compositions tested, except for composition 1 (1% Al_2O_3) where all the cells died after 3 days of culture. This was tested using trypan-blue staining of the cells in culture.

In this study, calvaria bone cells were treated with

dexametasone (DXM) for 14 days (as previously described), to induce osteogenesis in these cell cultures [21, 22]. The osteogenic response on bone cells was evaluated using SEM-EDX and alizarin red staining. The last method is used to reveal calcium deposition on the surface, based on the calcium binding properties of the alizarin compound. Red colorations are visible when calcium is present in such an area.

When glass and glass-ceramics were in contact with cells and the culture media, DMEM, changes of pH of the media were observed when compared to the controls. The pH was measured at different time intervals. Table 4 reports the pH values of the culture media 3 days before starting the treatment of cultured of cells with DXM and at the 14th day of treatment (total period of study was 28 days). Variations up to 0.5 units were observed for compositions 1 to 4. In contrast, for composition 5, both, glass and glass-ceramics (for all cycles) tend to show a slightly alkaline pH, however not as elevated as the rest of the compositions. A decrement in pH was observed in the media at the end of the experimental period, similar to that observed in the controls. It is important to point out that the pH of the culture media for composition 5 (both, glass and glass-ceramic) remained constant through the whole experiment (see Table 4).

Table 4. Culture medium pH

Glasses		
Composition	Initial pH	Final pH
1	8.30	7.90
2	8.27	7.87
3	8.24	7.95
4	8.07	7.89
5	8.21	7.96
Glass-ceramic cycle I		
Composition	Initial pH	Final pH
1	8.30	7.84
2	8.40	8.01
3	8.36	7.95
4	8.07	7.89
5	8.02	8.01
Glass-ceramic cycle II		
Composition	Initial pH	Final pH
1	8.30	7.90
2	8.29	7.75
3	8.23	7.88
4	8.10	8.00
5	8.02	8.01
Glass-ceramic cycle III		
Composition	Initial pH	Final pH
1	8.30	7.93
2	8.33	7.96
3	8.19	8.02
4	8.19	8.06
5	8.04	8.04
Control	7.78 ± 0.06	7.80 ± 0.06

Initial pH corresponds to day 3 of the cell culture. The final pH corresponds to day 28 of cell culture (day 14 of DXM treatment)

Previous authors [5, 19, 24, 25], report that when glasses are in contact with physiological fluids they release silica into solution in the form of silicic acid [$Si(OH)_4$] (due to cation exchange with H^+ or H_3O^+ ions) causing external alkalinization. On the other hand, changes in [H^+] could markedly affect the metabolism as well as the cellular function [23], producing moderate changes in the pH, harmful in some cases and potentially beneficial in others [24]. The present study shows that glasses as well as glass-ceramics of composition 5, have higher compatibility with tissue cultures. For the same composition, as was mentioned before, a smaller change in the pH media was found, during the entire experimental period, as reported in previous studies, were the strong alkalinization in presence of bioactive glasses which ended affecting negatively the cellular function [23].

In order to assess if osteoblast-like cells could mineralize on the surfaces of the different materials under study, treatment of cultures with DXM (1.10^{-7} M), ascorbic acid (50 μg/ml) and β-GP (10 mM) were started at the 14th day after cells seeded, and maintained for an additional 14 day period, changing the media each third day.

Previous to the treatment of the culture, we could observe growth in the different compositions with the exception of glass composition 1, as was mentioned. Once the DXM treatment was started, we could observe a significant decrease in cell viability in general. For glass compositions 2 and 3 (0.5 and 1.0% B_2O_3, respectively) we observed few cells in the culture with the appearance of osteocyte and abundant granular material, probably due to glass and glass-ceramics degradation, because in both compositions, no alumina was present.

In contrast, for glasses of composition 4 and 5, we observed cells attached to the glass surfaces in all cultures with DXM treatment. Cells appeared to have osteocyte morphology at the end of the experiment, as we can see in Fig. 4-B (glass, composition 5). In the case of glass of composition 4, cells remained alive and granular material was present in the media.

We did not observe mineralized nodules (alizarin red positive) on any surface of the glass tested, including composition 5 (Fig. 4-B, inset).

By contrast, we observed mineralized nodules on the glass-ceramics, especially for compositions 4 and 5 (Fig. 4-A and 4-C, inset). For composition 4 (1.5 % B_2O_3), it was possible to find a particulate substance around the material

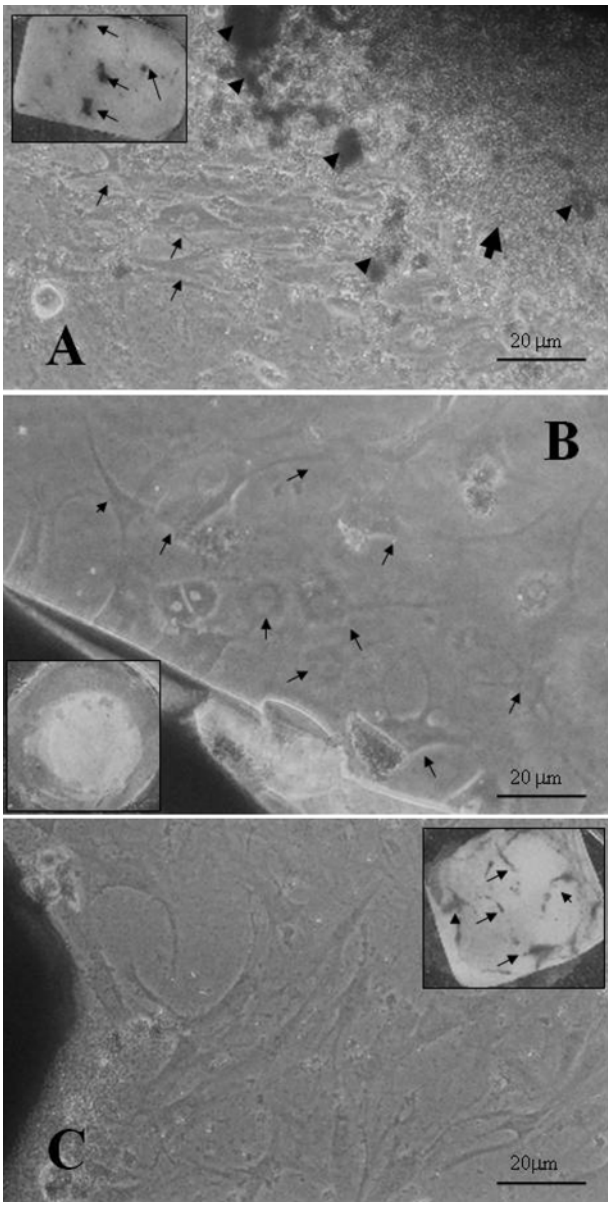

Fig. 4. Cell culture micrographies. A.-Glass-ceramic composition 4 (1% B_2O_3). Arrows show bone cells growing around glass-ceramic; arrowhead show granular deposition material in the culture medium Inset. alizarin-red staining (al-red) of glass-ceramic composition 4. Hydroxyapatite nodule mineralization are indicated by arrow B-Glass composition 5 ($B_2O_3/Al_2O_3 = 0.66$). Arrows show bone cells growing on glass surface Inset. Glass composition 5 al-red results, no staining or hydroxyapatite nodule formation is evident. C-Glass-ceramic composition 5. Bone cells growing around the glass-ceramic are observed. Inset. Glass-ceramic composition 5 al-red staining. Several hydroxyapatite-mineralized nodules can be observed on the surface of the material (arrows).

in both the glass and glass-ceramics (Fig. 4-A). The same effect was observed for the cell cultures with glass and glass ceramics for compositions 2 and 3. For composition 5 we did not find this effect on the material (Fig. 4-C), probably due to the alumina present in this composition.

The SEM-EDX spectra of the different materials submitted to osteoblast-like cells mineralization show differences when compared to spectra corresponding to SBF (Fig. 3). Glasses for composition 1, 4 and 5 reveal a small layer of Si while compositions 2 and 3 show no-change [Fig. 5, column (a)]. Otherwise, glass-ceramics submitted to cell culture conditions resulted in less bioactivity as compared to spectra from SBF [Fig. 5, columns (b), (c) and (d)], especially in the case of composition 4, that only showed a Si layer for all glass-ceramic cycles. In the case of glass-ceramics of composition 5 a film of hydroxyapatite was observed, only for the samples with the 3rd cycle. This result contrasts with that of the composition 4 where no film of apatite was found, probably because of the different testing media in each case. However with SBF, the presence of salts with similar concentrations to those of blood fluid [6, 17], the samples incubated with the culture medium, were probably exposed to proteins presents in the fetal bovine serum (FBS) used to complement the medium, also the proteins from bone cells, once these were introduced into the materials. It might be good to point out the possibly inhibiting effect of the proteins on the exchange between the substrate and the medium, thus hampering the formation of apatite. Several authors have pointed out the property of certain materials to rapidly interact in non-specific way with the proteins present in the biologic fluids [5, 26-29].

However, in the inset of Fig. 4-A and 4-C, mineralized nodules can be observed as a product of bone cell activity on some localized regions on the glass-ceramics surface of compositions 4 and 5. Probably the localization of the hydroxyapatite nodules produced regions without hydroxyapatite content for composition 4. In both cases (glass-ceramics of compositions 4 and 5), one can assume that the materials are biocompatible and allow a biomineralization process. However composition 5 is advantageous because the presence of 1.5% of alumina hampers the biodegradation of the material.

Summary

The $Na_2O.K_2O.MgO.CaO.SiO_2.P_2O_5$ based glass and glass-ceramic, proved to be reactive with the SBF, depending on composition, B_2O_3/Al_2O_3 additions and the glass-ceramic conversion heat treatment, allowing the development of a SiO_2-rich layer, and in some cases, the presence of a layer rich in calcium and phosphorous that according to other studies corresponds to the apatite layer previously identified. This behavior suggests a biocompatibility in the former group and a bioactivity behavior in the last one, with the exception of the glass-ceramic containing $B_2O_3/Al_2O_3 = 0.66$ with a second heat treatment cycle.

Even though the bioactivity results in SBF, did not match exactly with the cell culture test, probably due to the inhibiting effect of proteins adsorbed on the surface. It shows that the bioactivity determination must be completed by submitting the material to different tests as the media has an effect in controlling the CaP formation and cell attachment, as proteins present in live media, may have interfered in previous results.

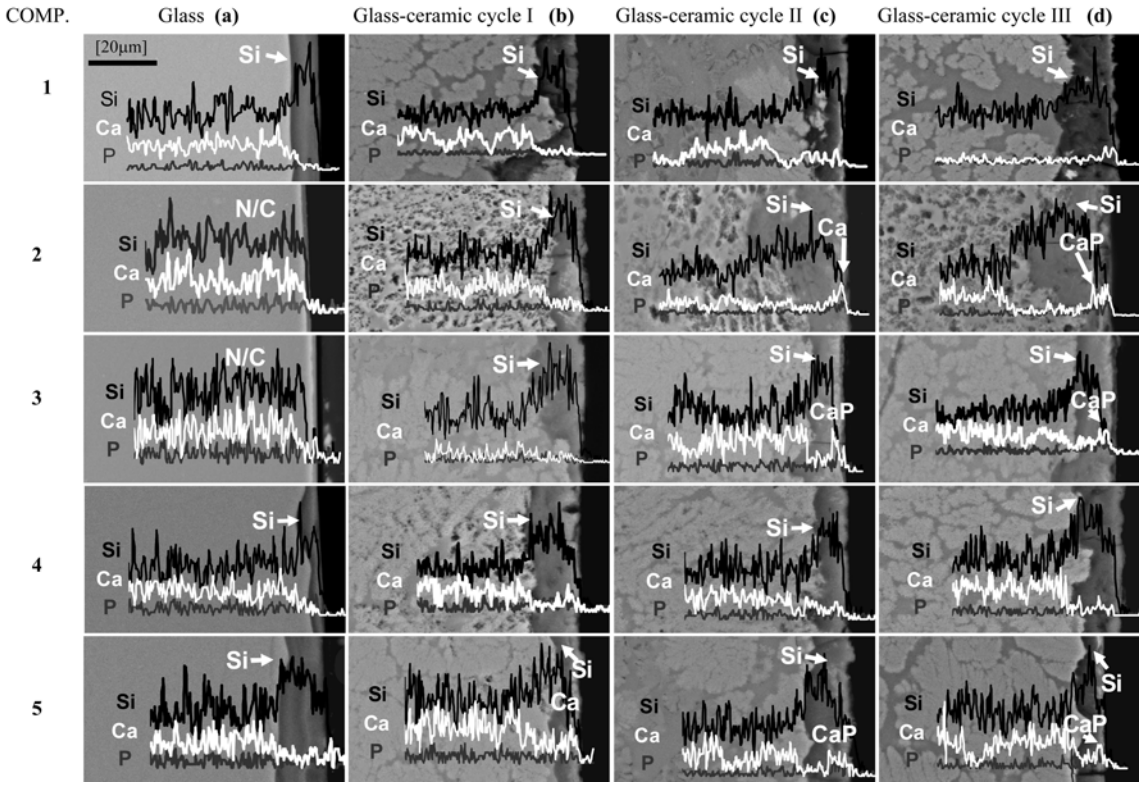

Fig. 5. Scanning electron microscopy images of cross sections of glasses 1-5 (a); glass-ceramics cycle I: 1-5 (b); glass-ceramics cycle II: 1-5 (c); glass-ceramics cycle III: 1-5 (d) submitted to osteoblasts-like cells mineralization, for 14 days treatment with DXM, analyzing the compositional changes with line scan compositional profiles superimposed.

We can assume then, that the materials analyzed in the present study, having B_2O_3 and Al_20_3, posses a good osteogenic potential and could be used to coat dental implant surfaces as well as general bone surfaces. Other posterior, *in vivo* assays must be carried out to ensure this.

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