

SYNTHESIS AND *IN VITRO* CHARACTERIZATION OF MELT DERIVED 47S CaO–P₂O₅–SiO₂–Na₂O BIOACTIVE GLASS

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*In our previous study, glass in weight ratio of 47% SiO₂ - 26% CaO - 21% Na₂O and 6% P₂O₅ have been prepared by the conventional melt quenching process. The referred glass have been characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS) confirming the composition and its amorphous state well. The *in vitro* bioactivity study of all pellets prepared were carried out by soaking in a simulated body fluid (SBF) for 1, 3, 7, 15, 20 and 30 days at 37°C. The analysis of SBF after each immersion time was carried out by inductively coupled plasma optical emission spectrometry (ICP/OES). The XRD, FTIR, SEM and EDS analysis of the glass surfaces after the *in vitro* essays, reveal the formation of an amorphous CaO–P₂O₅ - rich layer on the surface of the specimens after 20 days in the solution. After 30 days immersion time, a crystalline hydroxycarbonate apatite layer was formed that is similar in composition and crystallinity to the biological apatite. Osteoblast cell culture experiments were performed to assess the biocompatibility activity. Cell counts of the osteoblasts cultured on the bioactive glass samples were studied and compared with the polystyrene plates and Bioglass. The cells cultured on the bioactive 47S consistently highlighted the biocompatibility of this glass compared to cells cultured on polystyrene plates used as reference compounds.*

INTRODUCTION

Bioactive glasses and glass-ceramics have been extensively investigated as bone grafts or fillers owing to their ability to form a direct bond to living bone through the development of surface layer of carbonated hydroxyapatite [1-4]. Recent years have witnessed the use of several types of bioceramics to repair damaged and diseased bones. These bioceramics are generally classified as bioinert (e.g. Al₂O₃ and ZrO₂), bioactive (e.g. Bioglass[®] and hydroxyapatite (HAP)) or biodegradable ceramics (e.g. tricalcium phosphate and bone cement). Bioglasses and glass-ceramics have received special interest due to their unique characteristics, including (i) a rapid rate of surface reaction that leads to their direct attachment to bone via a chemical bond [5]; (ii) the use of these compositional for particular clinical applications; and (iii) their excellent controllability over a wide range of chemical properties and rate of bonding with tissues [6, 7].

It is well documented that a series of reactions with their kinetics that take place on the surface of these mate-

rials after immersion in tissue or experimental fluids, is responsible for the onset of the apatite formation [8-10]. Hench et al, synthesized the first bioactive material (Bioglass[®]) [11] with composition SiO₂ 45 wt.%, CaO 24.5 wt.%, Na₂O 24.5 wt.%, P₂O₅ 6 wt.%. This material has obtained by melting and rapid quenching. It was able to form a chemical bond directly with natural bone [12, 13]. This is the case of certain compositions of glasses, glass ceramics [14, 15] and hydroxy-apatite with different ratio of Ca/P [16, 17,18]. It is probable to obtain either amorphous calcium phosphate phase with possibly variable composition or crystalline apatite.

The apatite-like layer formation is responsible for direct chemical bond with bone. It avoids the formation of a fibrous capsule and, therefore, decreases the failure possibilities of the prostheses [19]. Therefore, the formation of the mentioned layer of biologically active bonelike carbonate-containing hydroxyapatite on its surface in the body seems to be necessary for glass and glass ceramics to bond living bone. The specific criteria for ideal material used in bone tissue engineering are summarized as follows [20] (1) ability to deliver cells,

(2) excellent osteoconductivity, (3) good biodegradability, (4) appropriate mechanical properties, (5) highly porous structure: porosity > 90% and pore size > 400-500 μm, (6) irregular shape fabrication ability, and (7) commercialization potential.

Bioactive glasses meet the first criteria. It has been shown that a silica hydrogel layer is formed, when these materials are in contact with simulated body fluid. This layer allows the subsequent crystallization of the apatite-like phase [21].

The mechanism for the nucleation and growth of these calcium based phases on bioactive glass is still not quite clear, but a number of hypotheses have been proposed in many publications. Meanwhile, some work [22] concluded that the combination of a large number of hydroxyl groups and negative charges on the bioactive gel surface were necessary for the induction of carbonate hydroxyapatite (HCAp). They demonstrated that the negative charge would accumulate cations such as Ca²⁺ on the surface and would ultimately lead to precipitation of calcium phosphate.

In other publications on bioactive glasses, it has been shown that the high surface area provides a high concentration of surface silanol groups (Si-OH) that serve as nucleation sites for the crystallization of HCAp. Karlson has proposed that the silanol groups on the gel layer are flexible enough to supply the correct atomic distances required by the crystal structure of HCAp [23].

The aim of this study was to describe the kinetics of HCAp layer formation "in vitro" in favor of glass with 47 wt% of SiO₂ and to check the toxicity of glass when it's in contact with osseous cells.

In our research group, we view to study many compositions of bioactive glasses in the goal to determine qualitatively and quantitatively the real limits between different areas (groups A, B and C) in the Hench's ternary diagram. The 47S6 compound is one of these compositions. However, changes of SiO₂, CaO and Na₂O amounts in the bioactive glasses induce modifications in the physico chemical properties like the temperature of vitreous transition (*T_g*) and other parameters necessary for the bioactive glasses synthesis and consequently modifications in their general behaviour.

EXPERIMENTAL

Elaboration and physic- chemical characterization

Sodium Silicate (Na₂SiO₃), Calcium Silicate (CaSiO₃) and Sodium Phosphate (NaPO₃) were used to obtain glass composition 47S formed by: (47 % SiO₂, 26 % CaO, 21 % Na₂O and 6 % P₂O₅ in weight ratio). The weight percentages of all compounds used in glass fabrication are presented in Table 1.

Raw materials were weighed and mixed for one hour in a polyethylene bottle. Premixed products were melted in a covered Pt crucible at the temperature range 1300-1350°C for two hours. Samples were cast into a Layton mold to form 8×13 mm cylinders. Thermogravimetric analysis (TGA) (Labsys TGA-DTA/DSC, Setaram) was performed on the powders calcined to determine the temperature of vitreous transition (*T_g*). After annealing at *T_g* for 8 hours, the cylinders were cut into disks. Then, those materials were coated in resin in order to detain one surface on air. All glass disks coated in resin were prepared by wet grinding with 600, 1200 grit Silicon carbide paper followed by wet grinding with 2400 grit paper. Polished samples were cleaned in an acetone bath and air-dried [24].

The characterization of the bioactive glass samples was carried out using X-Ray diffraction (XRD) with a BRUKER AXS D8 ADVANCE diffractometer ($\lambda = 1.54059$ Å, Bragg-Brentano geometry, θ - θ) and using scanning electron microscopy (SEM) in Jeol JSM 6301F. Semi quantitative chemical analysis on glass surfaces, covered by gold-palladium layer to allow surface conduction, was performed by energy dispersive spectroscopy (EDS) in Jeol JSM 6400.

The newly formed layer was characterized by infrared absorption analysis in a Fourier transform infrared (FTIR) Bruker Equinox 55 spectrometer.

The XRD, SEM and EDS studies were carried out directly on the surface of the glass samples; The FTIR analyses were performed on ~ 1 mg of material scraped from the glass surface with metallic blade. Spectra were obtained between 4000 and 400 cm⁻¹ wave number, with a resolution of 2 cm⁻¹.

Table 1. Glass composition.

Components	(wt.%)
CaSiO ₃	50
NaSiO ₃	41.4
NaPO ₃	8.6

In vitro analysis

Immersion in SBF

The assessment of "in vitro" bioactivity was carried out in simulated body fluid (SBF), which has a composition and ionic concentration similar to human plasma as shown in Table 2.

The SBF-K9 solution [25] was synthesised in our research group by dissolving the following reagent chemicals in deionizer water: NaCl, NaHCO₃, (CH₂OH)₃CNH₂, KCl, CaCl₂·2H₂O, KH₂PO₄, MgCl₂·6H₂O and 6NHCl to regulate the pH at 7.4 [24]. Reagent amounts were added, one by one in the order given in Table 3. "In vitro" test samples were performed under static conditions soaking in sealed polyethylene bottles with 8 ml of SBF-K9 solution at 37°C. The solutions

were kept at 37°C in an incubator at static condition for the following time intervals: 1, 4 hours, 1, 3, 7, 15, 20 and 30 days.

The samples were removed from the incubator, rinsed gently, first with pure ethanol and then using deionized water, and left to dry at ambient temperature. ICP-OES was used to analyze the ionic concentration of the SBF after immersion of the glass samples.

Cell culture studies

Human osteoblast cell line hFob (immortalized with SV40 virus) is maintained in Dulbecco's Modified Eagle's Medium (BioWhittaker™, Cambrex) supplemented with 10% heat-inactivated fetal bovine serum (BioWhittaker™, Cambrex), 2mM L-glutamine, 100 U ml⁻¹ penicillin, and 100 µg ml⁻¹ streptomycin. Cells were cultivated in a humid 5% CO₂ atmosphere at 37°C.

The bioactive glass-conditioned medium containing the dissolution products of 45S5 or 47S were prepared by incubating 1% w/v biomaterials particulate (100-600 µm in diameter). Particulates were removed by filtration through a 0.20 µm filter (Sartorius, UK). And the collected medium supplemented as described above for the complete medium.

hFob cells were seeded at 15000 cells per well in a 24 wells plates. After 24 h, medium was removed, and replaced by fresh complete medium (control) or Glass-Conditioned Medium respectively for 24 h of contact time.

Cell proliferation evaluation

The viability of cells was determined by the standard colorimetric 3-(4,5-dimethyl-2thiazoly)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cells grown in 24 well multiplates were incubated for 2 h at

37°C in cells incubator with the MTT solution (1 mg/ml) (Sigma). The yellow tetrazolium salt was metabolized by viable cells to purple crystals of formazan. The crystals were solubilized in Dimethyl Sulfoxide solution (Sigma).

The product was quantified spectrophotometrically by measuring absorbance at 570 nm wavelength using a Dynatech MR5000 microplate reader.

Statistical Analysis

The quantitative results from the MTT tests were analyzed using Statview V, one-way ANOVA followed by PLSD Fisher test to determine where significant differences lay (P 0.05).

RESULTS

SBF Solution analysis

The solutions of the bioactivity tests were analyzed using the induced coupled plasma (ICP-OES) spectroscopy in order to determine the evaluation of the elemental concentration of Ca, Si and P ions as function of immersion time. These results are very useful to understand the phenomenon of ions transfer which will take place between the glass surface and the synthetic physiological liquid.

Changes in SBF composition

Figures 1, 2 and 3 correlate the elemental concentrations of Si, Ca and P, before the immersion in SBF solution and after different immersion times for glass disk. It shows the profiles of dissolution of melt-derived 47S disks in SBF.

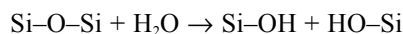
Table 2. Ion concentration (mM) in SBF - K₉ and in human blood plasma [24].

Ion	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	HCO ₃ ⁻	HPO ₄ ²⁻	SO ₄ ²⁻
SBF - K ₉	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5
Human Plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

Table 3. FTIR band assignment.

Wave number (cm ⁻¹)		Vibration mode
This work	Published data [23-25]	
1460-1415	1465-1415	C–O stretching
1250	1250-1150	P–O stretching
1070	1095	Si–O–Si stretching
1043-1024	1045, 1025	P–O stretching
960	960	P–O stretching
920	940-860	Si–O–Si stretching of non-bridging oxygen atoms
870	878	C–O stretching
727	840-720	Si–O–Si symmetric stretch of bridging oxygen atoms between tetrahedra
600	600-560	P–O bending (amorphous)
560	560-500	P–O bending (crystal)
461	470-455	Si–O–Si bending

In SBF, the concentration of silicon released into solution increases rapidly during the time of immersion. The silicon concentration in the SBF increases from a value of 0 ppm to approximately 35 ppm. This release of silicon ions indicates the first stage of dissolution by breaking up of the outer silica layers of the network. The solid silica dissolves in the form of monosilicic acid $\text{Si}(\text{OH})_4$ to the solution resulting from breakage of Si-O-Si bonds and formation of Si-OH (silanols) at the glass solution interface.



The curve which presents the evolution of the concentration of calcium shows 3 stages. A transitional stage which the concentration of calcium increases until 145 ppm during the first 120 h. The calcium concentration did not change significantly between the 5th day and the 20th day. Finally, a decrease is observed for time between 20 and 30 days, reaching value of 137 ppm.

Concurrent with the increase in silicon, the concentration of phosphorus decreases rapidly over the first few hours of dissolution. This behavior typically is attributed to the incorporation of phosphorus ions together with calcium ions from the dissolution medium within the Ca-P rich reaction layer forming on the glass surface.

Formation of apatite-like layer

Formation of an apatite-like layer on glass surfaces after soaking in SBF solution is evidenced from the following analyses: XRD, FTIR and SEM-EDS.

XRD patterns of glass before and after soaking in SBF for several days are shown in Figure 4. On the latter, we superimposed the diagrams corresponding to the various times of immersion in the SBF. The layer formed on the surface of glass is not crystallized same after 20 days immersion. As can be observed, after 20 days of immersion in SBF, a slight reflection (211) of an apatite-like phase starts to appear. Further sharpening of the peak and an important shifting corresponding to the (002) reflection. Then, a slight shifting of the maxima corresponding to (004) apatite reflection were observed after 30 days of soaking in SBF.

Figure 5 shows the IR spectra on the glass surface before immersion in SBF and the detached precipitates formed on the glass surface after 1, 3, 7, 15, 20 and 30 days in SBF. The FTIR spectra of a commercially synthesized HA are presented, for comparison reasons. Bands were assigned on the basis of published data according to Table 3 [26-28]. Roughly, all FTIR measurements yielded similar spectra although with different band intensities. From the tabulated assignments it is clear that a silica-rich layer is present, together with a carbonate- containing hydroxyapatite layer.

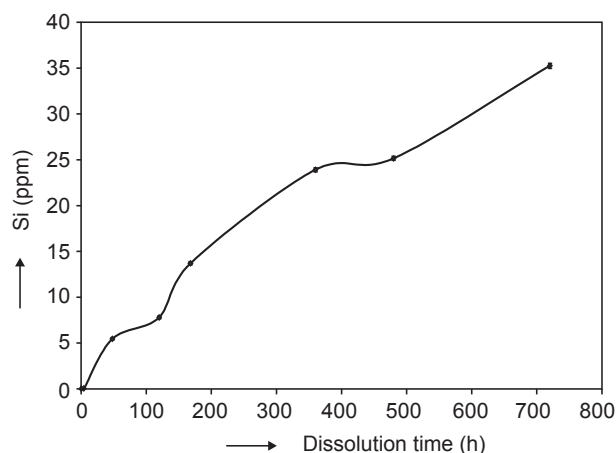


Figure 1. Evolution of silicon concentration in SBF versus soaking time for sample 47S.

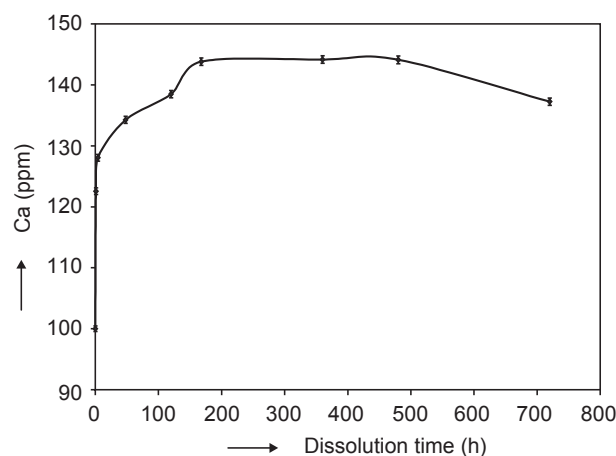


Figure 2. Evolution of calcium concentration in SBF versus soaking time for sample 47S.

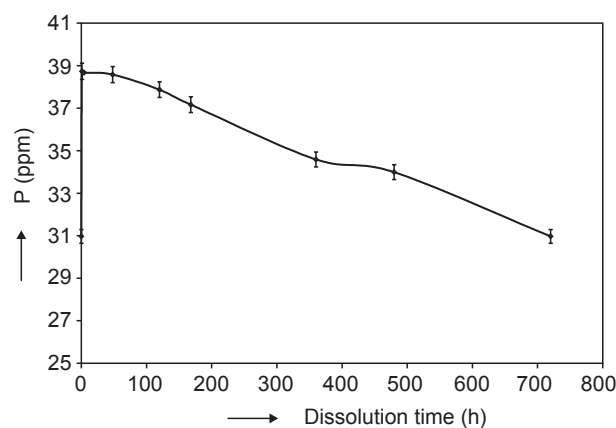


Figure 3. Evolution of phosphorus concentration in SBF versus soaking time for sample 47S.

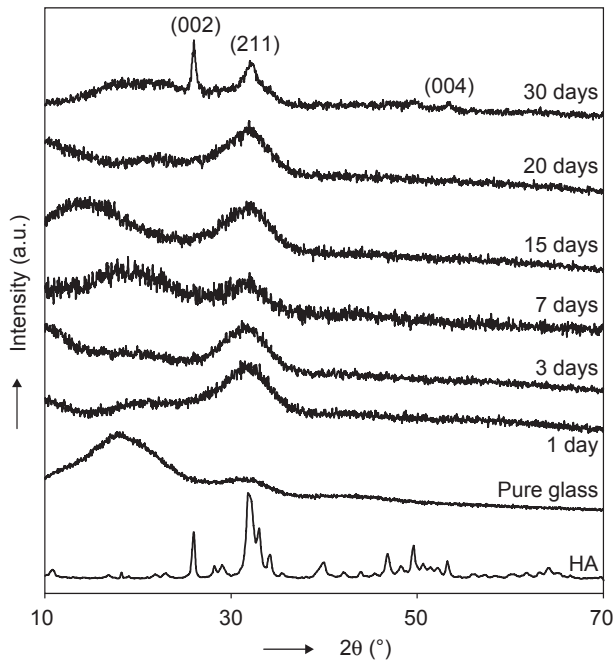


Figure 4. XRD patterns of the glass before and after soaking in SBF for different periods of time. XRD pattern of hydroxyapatite is included for reference purpose.

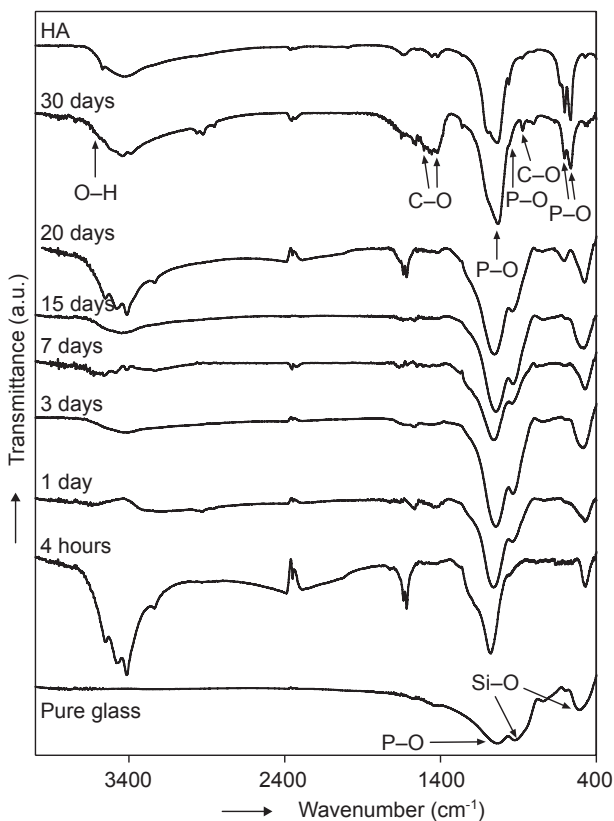


Figure 5. FTIR spectra obtained for glass before and after soaking in SBF. FTIR spectrum of hydroxyapatite is included for reference purpose.

This analysis shows changes in surface composition before and after soaking in the SBF solution. The spectrum before the immersion reveals silicate absorption bands at about 492, 727, 920 and phosphate absorption at 1025 cm⁻¹. The following changes were observed after various reaction times:

- After 4 hours: the band of silicate at 492 was shifted to 466 cm⁻¹ and the band of phosphate at 1025 cm⁻¹ was disappeared taking place to band of silicate at 1070 cm⁻¹. Additionally, the hydroxyl band at about 3550 cm⁻¹.
- After 1day: the band at 1070 cm⁻¹ disappeared and appearance of a phosphate band at 1045 cm⁻¹.
- After 3 days: the band at 1045 cm⁻¹ shifted to 1030 cm⁻¹.
- After 7 and 15 days: there were no significant changes.
- After 20 days: appearance of a phosphate band at 600 cm⁻¹.
- After 30 days: appearance of phosphate absorption bands at about 560, 600, 960, 1250 cm⁻¹, carbonate absorption bands at about 870, 1411 and 1457 cm⁻¹ and hydroxyl band at about 3550 cm⁻¹. The presence of two peaks at 600 and 566 cm⁻¹ means that this layer is crystalline after 30 days.

Figure 6 shows the SEM micrographs of 47S before and after 2 day, 20 days and 30 days immersion in SBF solution. The starting material (Figure 6a) shows the surface of glass before immersion in physiological liquid synthetic SBF. It presents a smooth and amorphous aspect.

Than after immersion, the morphology of surface changed and we can observe the formation of a silica gel layer. After 2 days immersion in SBF (Figure 6b), this layer has approximately a height of 0.5 μm. The thickness of silica gel layer increases during 20 days of immersion (Figure 6c), it reaches 4 μm. After soaking in SBF for 30 days (Figure 6d), the glass surface was fully covered by a layer of spherical particles less than 0.2 μm in diameter. This phase is formed on top of silica gel layer. It was identified as hydroxycarbonate apatite like layer.

EDS profiles of the glass surfaces before and after soaking in SBF are shown in Figure 7. After 20 days, the silicon content decreased from 20.26% in weight to 11.51% while the calcium and phosphate contents increased. After 30 days, the remaining quantity of silicon presents only 3% in mass and Ca/P ratio increase.

Cell culture studies

Background signal (wells with no cells) was deducted from the absorbance values. To compare the results obtained, the absorbance values were expressed

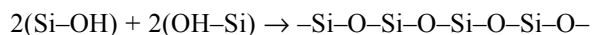
as a function of the control (without materials). The absorbance value obtained with the control was considered as indicating 100% viability. The relative percentages of viability were expressed in terms of the control. After 48 h in conditioned medium, a decrease of cell proliferation was detected for 47S compared with the control ($p < 0.05$) (Figure 8). After 72 h and 96 h, there is not difference between the two materials and the control (Figures 9 and 10).

DISCUSSION

The 47S glass develops a dual silica-calcium phosphate layer in SBF. The following discussion will be based on the mechanism proposed by Le Geros [28] to explain the glass surface activity, namely the existence of a silica gel layer as a supplier of calcium phosphate sites.

The ICP-OES analysis shows that the silicone increases in the solution of SBF. This result is attributed to a loss of soluble silica in the form of $\text{Si}(\text{OH})_4$ to the

solution resulting from breakage of Si-O-Si bonds and formation of Si-OH (silanols) at the glass solution interface. Then a condensation and repolymerization of SiO_2 rich layer on the surface that is depleted in alkalis and alkaline earth cations.



This result is confirmed by SEM (Figure 4b, 4c). It shows the formation of a silica gel layer which increases in thickness by increasing the immersion time: after 20 days the X-ray measurements indicate the formation of a peak at 32° characteristic to an amorphous calcium phosphate phase. This result show that Ca^{2+} and PO_4^{3-} migration groups to the surface through the SiO_2 rich layer forming a $\text{CaO-P}_2\text{O}_5$ rich film on top of the SiO_2 rich layer, followed by growth of an amorphous $\text{CaO-P}_2\text{O}_5$ rich film by incorporation of soluble calcium and phosphate from solution.

After 30 days immersion in SBF, the SEM micrographs and the X-ray measurements indicate the forma-

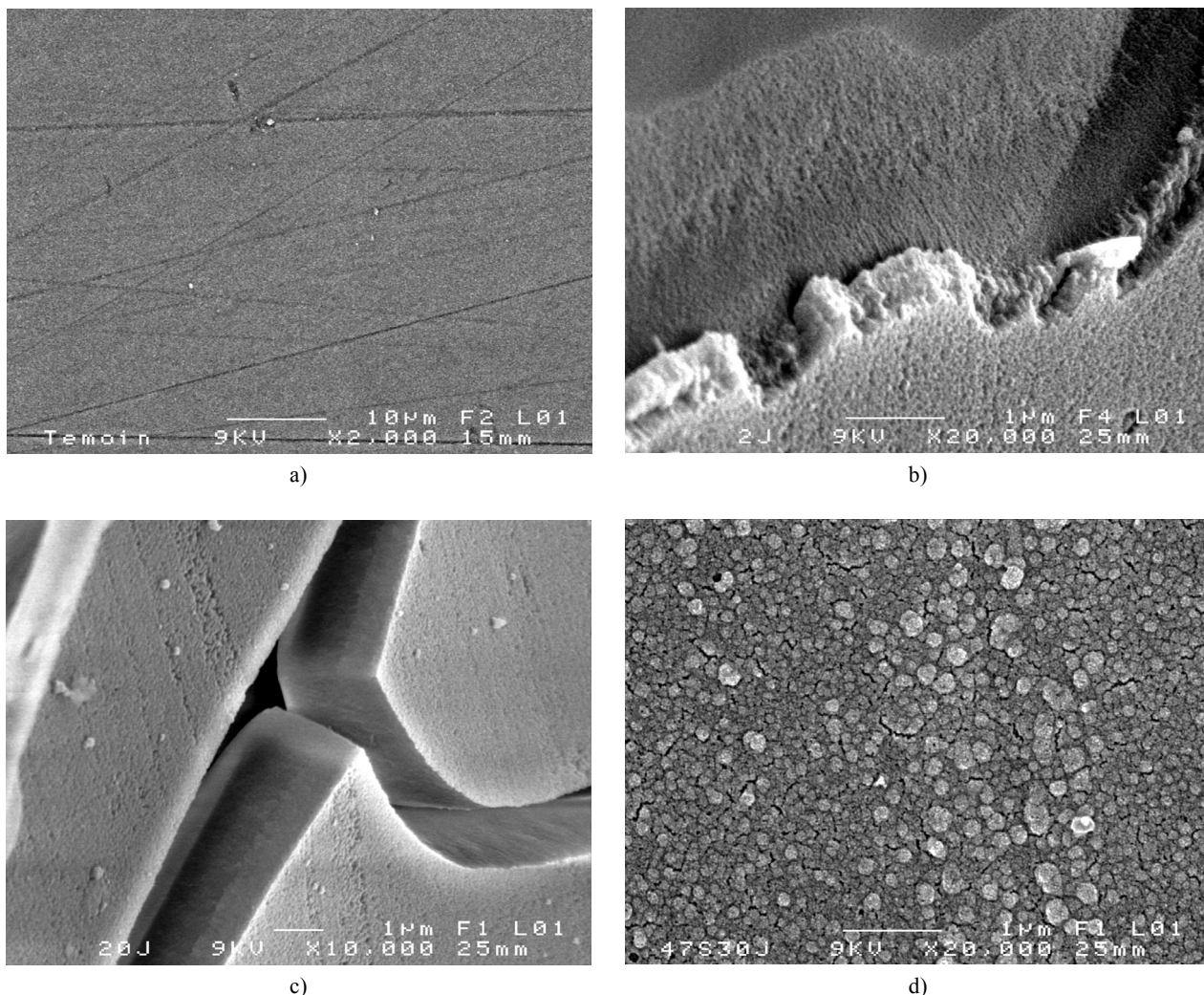


Figure 6. Scanning Electron Micrograph of the cross-sections of the specimens before immersion a) and after immersion in SBF for b) 2 days, c) 20 days and d) 30 days.

tion of micro-crystalline hydroxyapatite phase. This result is at the origin of crystallization of the amorphous CaO–P₂O₅ film by incorporation of OH⁻ and CO₃²⁻ anions from solution to form hydroxyl carbonate apatite (HCA) layer.

The width of XRD diffraction peak reflects crystal size. The shifting of the maxima observed between the XRD pattern of the glass before soaking and after soaking at 30 days, concomitant with the appearance of much sharper apatite diffraction peaks (Figure 2), indicates two things: (1) Difference in composition between the original glass (before soaking) and the forming apatite-like layer; and (2) growth of the apatite crystals

relative to the period of soaking. The formation of the new layer on the surface of glass starts about the 20th days. Indeed, the peak at 32° was visualized and represents an amorphous calcium phosphate layer.

The sharp (002) reflection appeared at 30 days immersion in SBF. It indicates the crystallization of the phase hydroxyapatite. This reflection presents the preferred orientation of the apatite crystallite.

After immersion in SBF, the FTIR spectra indicate the formation of new bands. The main characteristics of the spectrum of the unreacted bioglass pellet are attributed to the amorphous silica glass, e.g. the bands at 920 cm⁻¹ assigned to Si–O–Si stretching vibration and a band at 492 cm⁻¹ assigned to Si–O–Si bending one [26,29,30]. The spectra from the reacted pellets for 4 hours in SBF reveal new band at 1070 cm⁻¹ that can be assigned to the amorphous silica gel rich layer formed on the surface of bioglass® pellets. After one day of immersion, the band silicate at 1070 cm⁻¹ disappeared taking place to a band at 1040 cm⁻¹ assigned to P–O stretching vibration. There were no significant changes between 3 and 15 days. After 20 days, the FTIR spectra showed the formation of a new band at 600 cm⁻¹. It can be assigned to the amorphous CaO–P₂O₅ phase layer. The appearance, at the spectra from immersed 30 days in SBF, of the band at 560 cm⁻¹ proves that after 30 days in SBF a crystalline phase of HCAP layer is developed [23, 30, 31-33]. Peak at 920 (Si–O bonds) was not detected at 30 days immersion, indicating that the silica-rich layer is polymerized for the immersion time used. Additional characteristics from the HCAP layer are two bands at 1411 and 1457 cm⁻¹ assigned to C–O stretching vibration and one at 870 cm⁻¹ assigned to

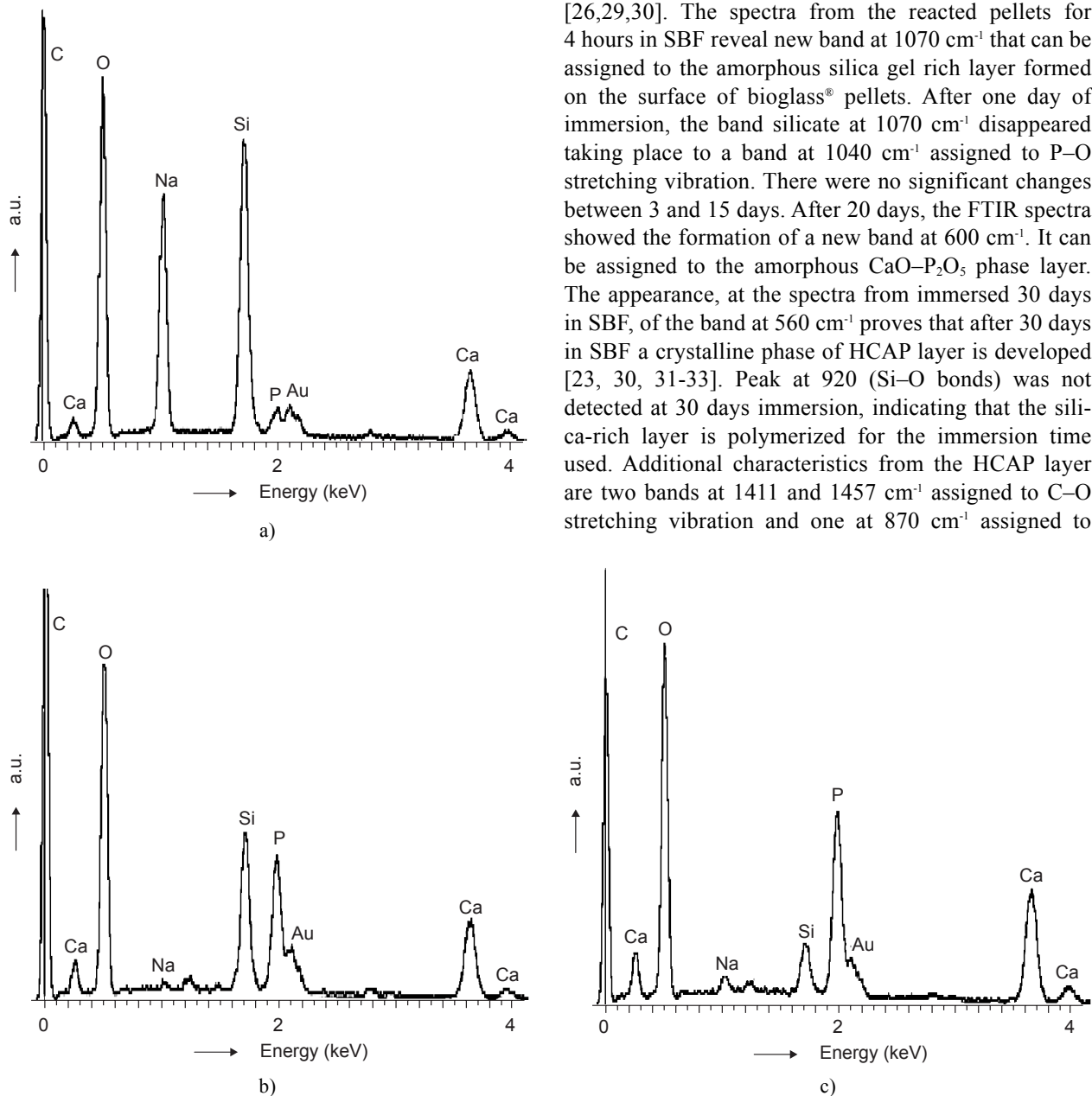


Figure 7. EDS patterns of the 47S glass surface: a) before soaking in SBF, b) after 20 days and c) after 30 days.

C–O out-of-plane bending vibration of the carbonate group [27, 32]. The phosphate peaks became more intense and sharp with the increase in immersion time, indicating the growth of crystalline apatite "*in vitro*". The appearance of the phosphate and carbonate absorption bands in the spectra of the materials formed on glass surfaces after soaking in SBF not only confirms the formation of an apatite-like layer (as determined by XRD and EDS analysis but also determines that the apatite-like material is a carbonate hydroxyapatite (HCAP) similar in composition and structure to bone apatite and also found on bioactive glass [1-4]. Formation of HCAP *in vivo* on the surfaces of calcium phosphate materials (hydroxyapatite and biphasic calcium phosphates), reported previously, has been associated with the reactivity of the materials [33]. From the spectra in Figure 5, it is clearly concluded that an amorphous layer is formed after 20 days immersion in SBF. While after 30 days the layer is converted to HCAp crystalline phase. The presence of the carbonate group in the 30 days immersed in SBF samples is documented from the two weak broad bands at 1400-1490 cm^{-1} and the one band at 870 cm^{-1} .

Hydroxyl stretch is observed at about 3550 cm^{-1} in the spectra of the 47S glass after 4 hours immersion in SBF. It indicates the formation of Si–OH (silanols) at the glass solution interface. This band persists and it is seen after 20 and 30 days. It indicates the formation of hydroxyl-carbonate-apatite (HCA) layer.

From the analysis by scanning Electron Microscopy, it is demonstrated that morphologically no apparent changes appeared on the surface until the 20th days of the specimens. Only, the thickness of silica gel layer increases by 0.5 μm to 4 μm and it is covered by a few aggregates of the amorphous apatite phase with diameter less than 100 nm.

After 30 days immersion in SBF, the surface of the specimens is still fully covered by a layer of apatite with aggregates of approximately 250 nm in size.

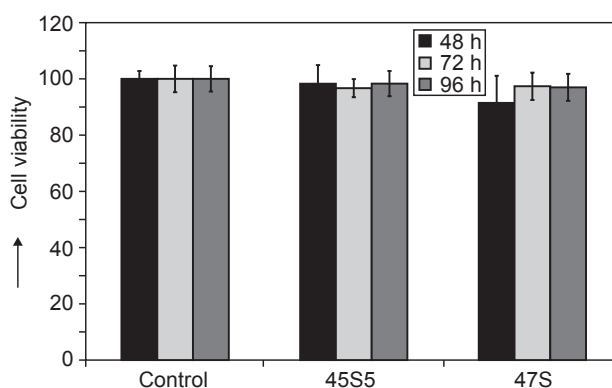


Figure 8. Osteoblasts proliferation after 48 h, 72 h and 96 h of culture in contact with particulate glass 47S (bioglass®) (45S5) is given for comparison purposes).

The EDS studies reveal the inclusion of phosphorus in the composition of the newly formed layer since the after 20 days of immersion (Figure 7 b). The phosphorus present on the newly formed layer proceeds from the SBF solution. It also shows an increase in the calcium content and a decrease in the silicon content as a function of the soaking time. The decrease in Si with increasing Ca and P concentrations with time on newly formed layers also indicates the formation of an apatite-like layer material.

Cytotoxicity of ionic products of 45S5 and 47S Bioactive glass dissolution has been controlled with Human immortalized osteoblast cell hFob. In this work and after 48 h of treatment, cell proliferation decreased only with 47S, this toxicity was light (8.5%) compared with control cells. The difference between composition 45S5 and 47S explained this result with pH medium variation. After this time, cell proliferation increased to be the same as 45S5 and 47S. This experiment demonstrated that there is no difference between the two materials and that there is not real cytotoxicity. Xynos et al showed that ionic product of 45S5 dissolution increased osteoblast proliferation with longer incubation time [34]. Hench observes cells cycle variation after 6 days incubation [35].

The cell culture experiments showed good viability of hFob cell line after 48h incubation in conditioned medium. In addition, the observations [34] showed that bioactive materials seemed to provide a favourable template for bone deposition. Indeed, bioactive glasses possess a composition and morphology that supports bone cell phenotype and can be used as templates for "*in vitro*" bone growth. The number of cell adhesion to the bioglass samples increases at 72 h culture time, which indicates the good "*in vitro*" biocompatibility of the 47S bioactive glass compared to that of the references compounds.

CONCLUSION

This "*in vitro*" study combined different techniques to determine the composition and properties of materials that are formed on glass surfaces after soaking in SBF solution. The combined application of, ICP-OES, SEM-EDS, FTIR and XRD techniques allowed the monitoring of the formation of hydroxyl carbonate apatite layer. Amorphous CaO–P₂O₅–rich layer is formed on the top of silica gel layer. As well as the crystalline carbonate-containing apatite, develops in particulate glass, as shown from this study. The formation of an amorphous CaO–P₂O₅–rich layer was recognized on the surface of the specimens after 20 days in SBF. After 30 days, the amorphous phase becomes crystalline and the surface of glass is covered completely by the HCAP layer. This phase as consisting of crystallites is similar to biological apatite in bone.

"*In vitro*" biocompatibility assessments indicate that our bioactive glass 47S stimulates no toxicity and promotes proliferation.

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SYNTÉZA A IN VITRO CHARAKTERISTIKA TAVENINY ODVOZENÉHO 47S CaO–P₂O₅–SiO₂–Na₂O BIOAKTIVNÍHO SKLA

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V naší předchozí studii bylo připraveno sklo ve hmotnostním poměru 47 % SiO₂ - 26 % CaO - 21 % Na₂O a 6 % P₂O₅ a to konvenčním procesem ochlazování taveniny. Příslušné sklo bylo popsáno rentgenovou difrakcí (XRD), Fourierovou transformační infračervenou spektroskopií (FTIR), rastrovací elektronovou mikroskopií (SEM) a energeticky disperzní spektroskopií (EDS), které potvrdily složení a také amorfní stav materiálu. Studie in vitro bioaktivity všech připravených pelet byly připraveny namáčením do simulované tělní tekutiny (SBF) po dobu 1, 3, 7, 15, 20 a 30 dní při teplotě 37°C. Analýza SBF po každé době ponoření byla provedena indukčně sřaženou plazmovou optickou emisní spektrometrií (ICP/OES). Analýza XRD, FTIR, SEM a EDS povrchu skla po zkouškách in vitro prokázala vytváření vrstvy bohaté na amorfni CaO–P₂O₅ na povrchu vzorků po 20 dnech v roztoku. Po 30 dnech doby ponoření se vytvořila vrstva krystalického hydroxyuhlíčanového apatitu, která se podobá svým složením a krystalicitou biologickému apatitu. Byly provedeny pokusy s osteoblastickou buněčnou kulturou s cílem vyhodnotit aktivitu s ohledem na biologickou kompatibilitu. Byly studovány počty buněk osteoblastů kultivovaných na vzorcích bioaktivního skla a porovnány s polystyrenovými destičkami a materiálem Bioglass®. Buňky kultivované na bioaktivním vzorku 47S trvale vykazovaly biologickou kompatibilitu tohoto skla ve srovnání s buňkami kultivovanými na polystyrenových destičkách použitých jako referenční směsi.