Bioactivity of PLGA-gel-derived bioglass composites

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Abstract

A series of PLGA (poly L-lactide-co-glycolide) based composites modified with gel-derived glasses in the form of foils were obtained by solvent casting procedure. As a modifier, glass particles with different chemical compositions from the $CaO–SiO_2$, $CaO–P_2O_5–SiO_2$, and $Al_2O_3–SiO_2$ systems were used. All glasses were synthesized by the sol-gel process. Evaluation of bioactive properties of obtained composites was made on the basis of surface changes occurring during contact with simulated body fluid. The changes of Ca, P and Si ions concentration in SBF after incubation of composites were also measured. The result showed that all composites with bioglasses ($CaO–SiO_2$, $CaO–P_2O_5–SiO_2$) exhibit formation of calcium phosphates layer after SBF test, however, kinetics of Ca, Si ion release and P uptake from SBF was dependent on bioglass chemical composition. The higher solubility, as well as faster consumption of phosphorus from SBF, was observed for materials from $CaO–SiO_2$ (T1/PLGA, S1/PLGA) compared to composites with respective bioglass particles from the $CaO–P_2O_5–SiO_2$(T_2/PLGA, S_2/PLGA). Our results showed that rate of Si and Ca release from the gel-derived glasses and P uptake from SBF are dependent on both: the concentration of respective ions in the materials and the presence of phosphates in their structure. For materials modified with gel-derived glasses from $Al_2O_3–SiO_2$ system no significant surface changes during contact with SBF were observed, and it seems that their behavior in physiological solution indicate that they are bio-inert materials.

Keywords: polymer-ceramic composites, bioactive properties

Introduction

Composite materials with the polymer matrix and bioactive glasses (melted and produced by sol-gel method) as modifiers are potential materials for regenerative medicine and tissue engineering. The use of sol-gel glasses as a composite modifier creates opportunities to produce "tailor-made" biomaterials with properties adapted to the functions it performs in a living organism. Especially, sol-gel derived bioactive glasses have a better ability to form surface apatite layers during contact with body fluid than melted ones. It may results in better material integration *in vivo* with bone tissue. Ability to form surface apatite layer during contact with body fluid is determined often as "material bioactivity" and a simulated body fluid (SBF) test is used to pre-evaluate it *in vitro*. These problems have been raised, among others, in our earlier works [1–3]. We have shown that the bioactive properties of sol-gel glasses are fully preserved in the case of polymer-based composites modified with these glasses. Moreover, it is assumed that the chemical composition of the sol-gel glasses is an important determinant of bioactivity [4–6].

The subject of current work was to determine the properties of 2D PLGA-based composites modified with $CaO–SiO_2$, $CaO–P_2O_5–SiO_2$, and $Al_2O_3–SiO_2$ sol-gel glasses. The behavior of produced composites in simulated body fluid was examined (SBF test). It is assumed that the SBF test result allows prediction of the bioactive properties of the material understood not only as the ability to directly connect to the bone but also as the ability to stimulate tissues for faster regeneration. So, the current research can form the basis for verifying the thesis about the usefulness of SBF test results to predict cellular behavior *in vitro* and *in vivo* indicating bioactive properties of the biomaterial.

Materials and Methods

Composites components

PLGA (poly L-lactide-co-glycolide) was synthesized via a ring opening process in the presence of low toxicity zirconium acetylacetonate as a copolymerization initiator [7]. The molar ratio of L-lactide to glycolide in the copolymer was 85:15 (as studied by 1 H NMR), and molecular masses of PLGA were: Mn = 80 kDa and Mw = 152 kDa. Chemical compositions of sol-gel glasses used as composites modifiers as well as refer-

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*Corresponding author: mlaczka@poczta.fm ence materials are given in Table 1. Tetraethoxysilane (TEOS; $Si(OC_2H_5)_4$), triethylphosphate (TEP; $OP(OC_2H_5)_3$), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) aluminium-tri-sec-butoxide (TBA; $Al(OCH(CH_3)C_2H_5)_3$), aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) were used as base components to start the sol-gel process. HCl solution was used as a catalyst for the hydrolysis and condensation reactions. The formed gel was dried at 40–120°C for 7 days and then subjected to thermal treatment at 700°C for 20 h [8]. Afterward, it was milled to obtain a bioactive glass powder with particle sizes ≤ 50μm. SiO_2 and Al_2O_3 were used as a reference materials.

Table 1. Chemical compositions of sol-gel glasses

Material	Chemical composition [mol%]			
	SiO_2	CaO	P_2O_5	Al_2O_3
T1	50	50		
S1	80	20		
T2	47	47	6	
S2	80	16	4	
AS1	80			20
AT1	50			50
SiO_2	100			
Al_2O_3				100

Composite Films Fabrication

The 2D bioglass (BG)/PLGA composites were fabricated by mixing glass particles with 10% w/v PLGA solution in methylene chloride (POCH, Gliwice, Poland) on a magnetic stirrer for 3 h, followed by partial evaporation of the solvent in air, slip casting of the viscous mixture on glass Petri dishes, then drying in air and vacuum to a constant weight. The volume fraction of bioactive glass in the composites was 50%. The composite sheets obtained were 0.11 mm thick. Further, obtained 2D composites are called as 50bioglass/PLGA films [2].

Evaluation of bioactivity

Bioactivity of the 2D composites was assessed by an *in vitro* simulated body fluid (SBF) test according to the method proposed by Kokubo *et al.*[8]. In brief, SBF was prepared by dissolving the following chemicals (POCH, Gliwice, Poland) in UHQ-water: 141 mM NaCl, 4 mM KCl, 0.5 mM $MgSO_4$, 1 mM $MgCl_2$, 4.2 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 1.0 mM KH_2PO_4. The resulting SBF was buffered to pH 7.40 with Tris(hydroxymethyl)aminomethane/HCl. The samples were immersed in SBF solution with the mass/volume ratio 1:1000 and incubated at 37°C for 3, 7 and 14 days. Subsequently, the samples were carefully washed in UHQ water and air-dried for further research.

Microstructure and chemical composition of PLGA and composite films before and after soaking in SBF during 7 and 14 days were examined by SEM/EDS analysis (Nova NanoSEM, FEI, USA). The EDS spectra were averaged for the whole analyzed surface.

Other tests

Moreover, the ATR-FTIR spectroscopy (Bruker VERTEX 70 V spectrometer, USA) was applied to obtain information about structural changes of fabricated materials after 14 days incubation in SBF. The spectra were registered in the 550–4000 cm^{-1} wavenumbers range, with the use of a platinum single-crystal diamond ATR unit. 128 scans were accumulated at 4 cm^{-1} resolution.

The samples of SBF solution, remaining after 3, 7 and 14 days incubation of produced 2D composites, were analyzed for the content of silicon Si, calcium Ca and phosphorus P in it to determine the ions release from the examined samples. For assessment of ion concentrations in the incubation fluid inductively coupled plasma atomic emission spectrometry (ICP-OES; Plasma 40, Perkin Elmer, USA) was used. The test was performed in triplicate, and results were presented as mean ± standard deviation (SD).

Results and Discussion

SBF test results

SEM observations and EDS analysis

The SEM/EDS analysis was aimed at assessing the surface morphology of composite films after contact with SBF and to determine the changes in the chemical composition of the surface after 7 and 14 days incubation. The study was based on SEM microphotographs and average EDS analyses (Fig. 1, 2).

50CaO–SiO_2/PLGA films

All samples after 7-day incubation in SBF have an altered surface morphology (Fig. 1). Numerous “cauliflower” precipitates were formed, forming a thick, sometimes cracked layer. Created, as a result of contact with the SBF, forms are characteristic of the apatite crystallizing on the surface. This is also demonstrated by the EDS analysis (Fig. 1), which indicates elevated levels of calcium and phosphorus after contact with SBF. Extension of incubation time in SBF up to 14 days resulted in an intensification of surface changes (Fig.1). The surface layer has a clearly “cauliflower” form and appears to be thicker and more tight in comparison to the samples incubated for 7 days.

50CaO–P_2O_5–SiO_2/PLGA films

Films modified with three-component CaO–P_2O_5–SiO_2bioglasses showed similar surface changes to those described earlier for 50CaO–SiO_2/PLGA samples (Fig. 1). However, these changes for 50S2/PLGA films were less intense, as evidenced by the presence in the EDS spectrum a little bit amount of Si. The EDS surface analysis in the region of cauliflower forms showed an

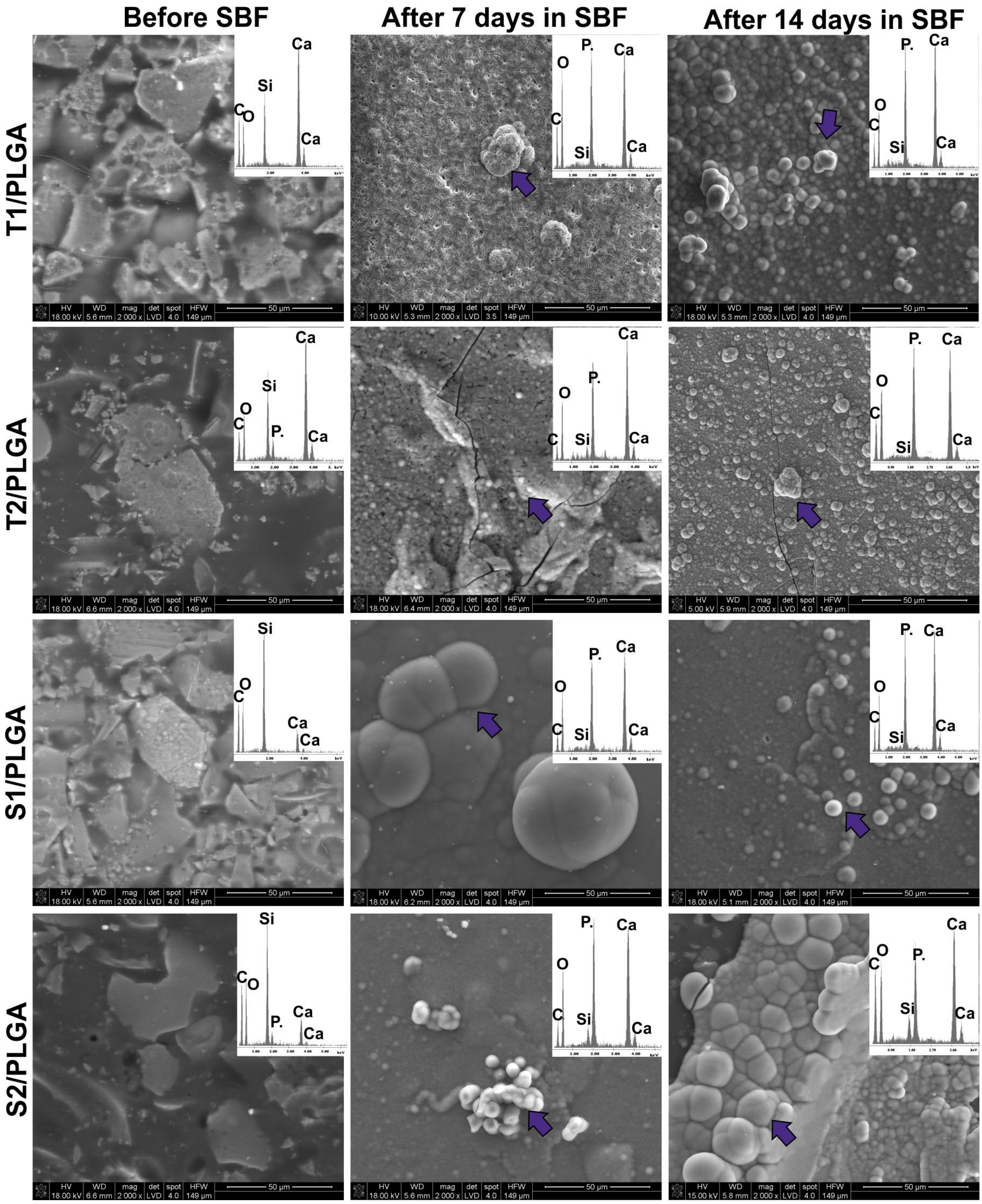

Figure 1. SEM microphotographs and EDS analyses of 50CaO–SiO_2/PLGA films, 50CaO–P_2O_5–SiO_2/PLGA films before, and after immersion in SBF. Arrows indicate representative apatite precipitations.

increased amount of calcium and phosphorus indicating calcium phosphates crystallization [10–12].

50Al_2O_3–SiO_2/PLGA and reference materials: 50SiO_2/PLGA, 50Al_2O_3/PLGA films

After 7 and 14-days incubation in SBF, no significant surface changes were observed for films containing Al_2O_3. These materials have similar surface morphologies before and after incubation. Only slight changes in the chemical composition of the surface layer that are recorded by the EDS analysis are noticeable (Fig. 2). Surface morphology of reference materials after incubation in SBF has not changed. The films containing an inorganic modifier had a corrugated surface which resulted from the presence of SiO_2 and Al_2O_3 grains below the surface layer. EDS analysis of samples after incubation in SBF showed in the surface layer only the elements that are components of the film similar to results for samples before incubation. Surface morphologies of the reference materials SiO_2 and Al_2O_3 after incubation in SBF were unchanged.

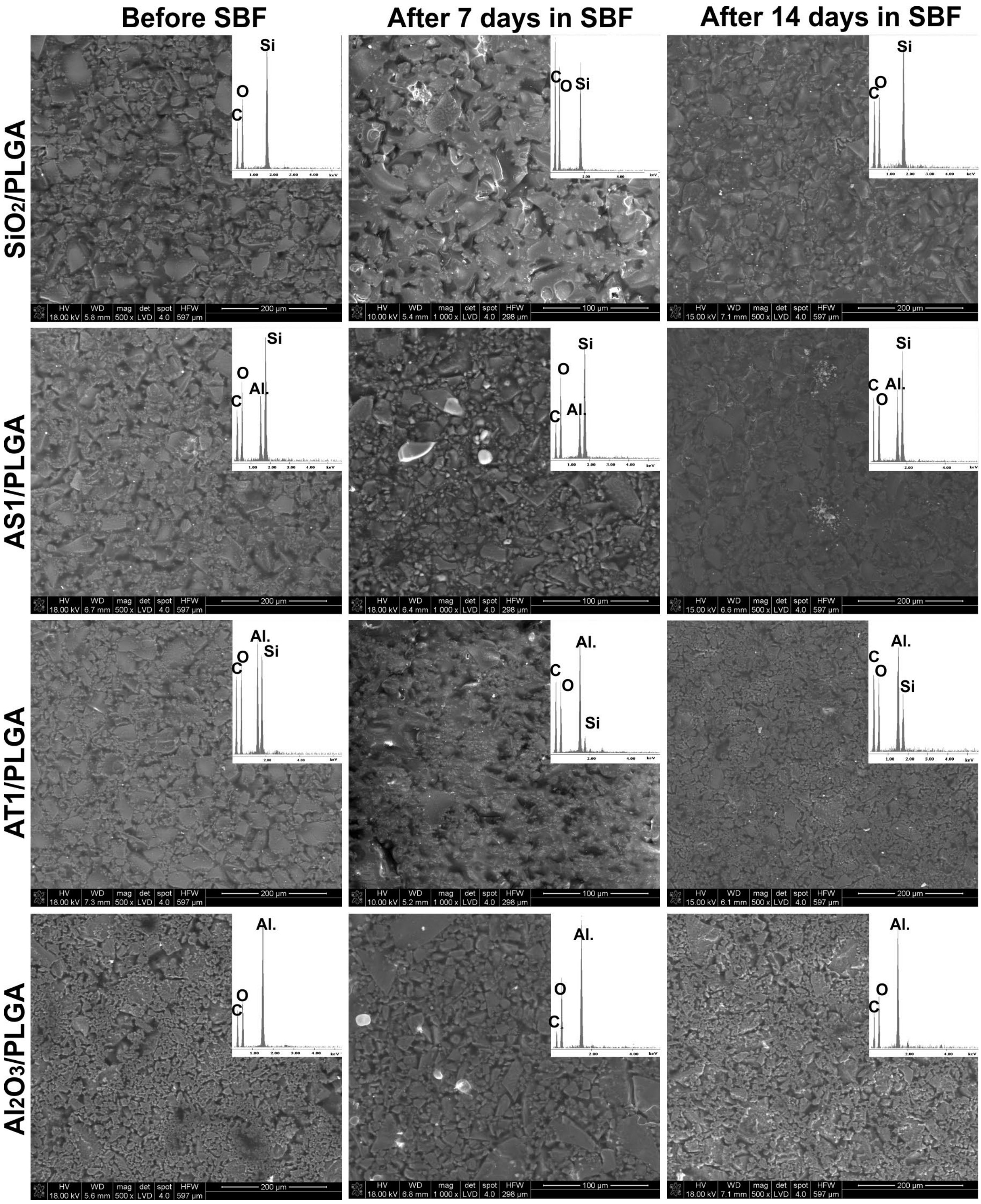

Figure 2. SEM microphotographs and EDS analyses of 50Al_2O_3–SiO_2/PLGA and reference materials: 50SiO_2/PLGA, 50Al_2O_3/PLGA films before and after immersion in SBF.

ATR–FTIR results

The ATR-FTIR spectroscopy was used to determine the structure of the composite surface after incubation in SBF (Fig. 3). EDS analysis showed formation on 50CaO–SiO_2/PLGA and 50CaO–P_2O_5–SiO_2/PLGA films the surface layers enriched in calcium and phosphorus. This could indicate surface crystallization of apatite demonstrating the bioactive properties of the materials. Using a spectroscopic method, phosphate structures on the surface were expected to be identified. Their presence would be evidenced by the appearance of the double band about 564–604 cm^{-1} occurring in the ATR–FTIR spectra of apatite [13–15].

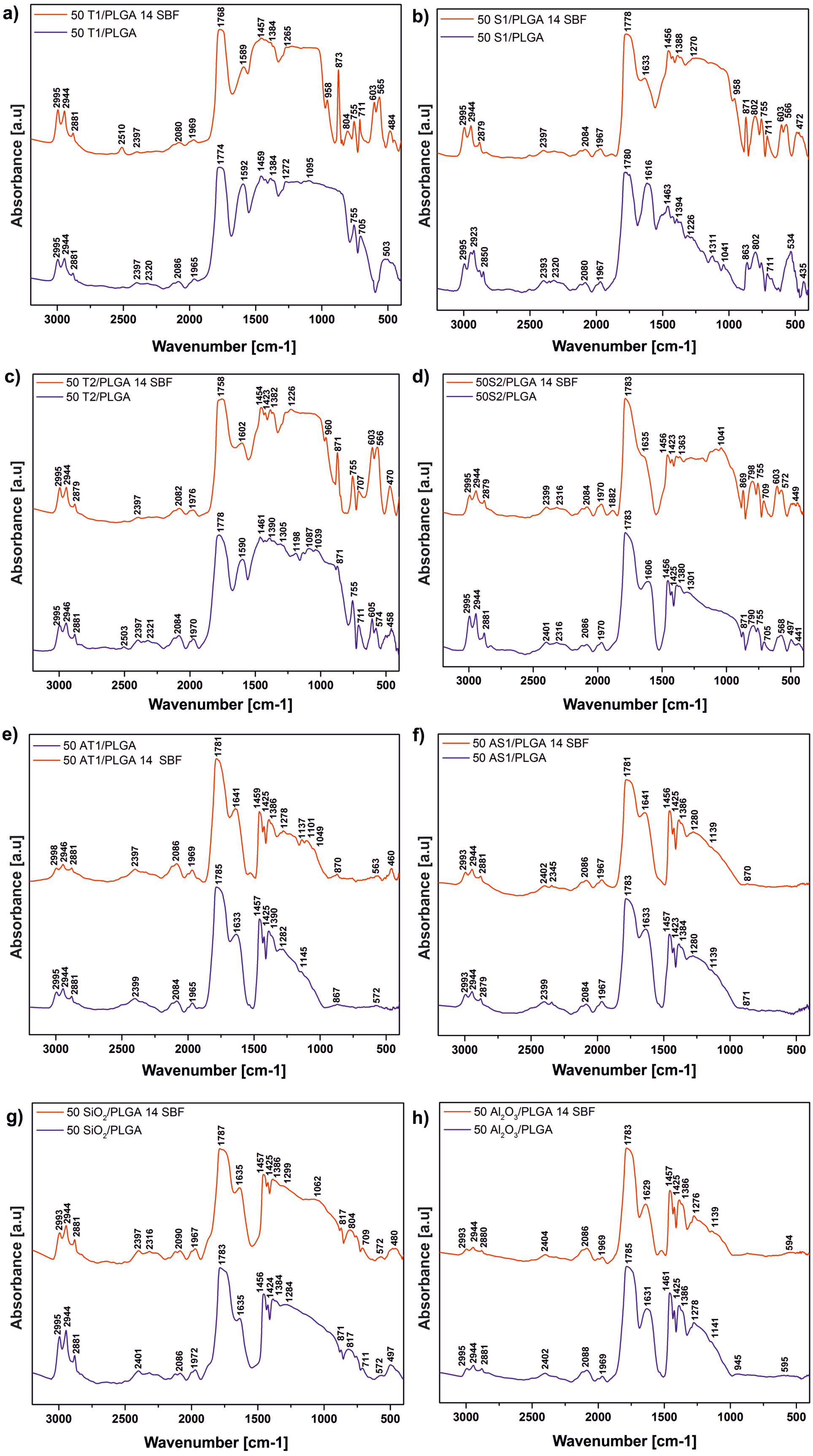

Figure 3. ATR-FTIR spectra of 50 CaO-SiO$_2$/PLGA, 50 CaO-P$_2$O$_5$-SiO$_2$/PLGA, and 50Al$_2$O$_3$–SiO$_2$/PLGA and reference materials: 50SiO$_2$/PLGA, 50Al$_2$O$_3$/PLGA films before and after immersion in SBF.

50CaO–SiO_2/PLGA films

In the spectra of 50CaO–SiO_2/PLGA films before incubation in SBF, there are no bands characteristic for phosphates. After 14-days incubation in SBF, a distinct band at 563–603 cm^{-1} appears indicating the apatite crystallization (Fig. 3 a,b).

50CaO–P_2O_5–SiO_2/PLGA films

The 50CaO–P_2O_5–SiO_2/PLGA films before incubation in SBF showed the IR absorption in the range 560-609 cm^{-1} related to the presence of PO_4^{3-} groups in bioglasses that form a phosphate structure (Fig. 3c, d). After SBF incubation these bands (560–609 cm^{-1}) have clearly a doublet shape and become more intense compared to the spectra of materials before incubation. That indicates the surface crystallization of calcium phosphates (apatite) during contact with SBF. ATR–FTIR analysis is not a basis for the quantitative assessment of the phosphate layer being formed, however, taking into account the highest intensity of the 560–609 cm^{-1} band for 50T2/PLGA film, it can be assumed that in this case, the phosphate structures are best shaped [14,15].

50Al_2O_3–SiO_2/PLGA films and reference materials: 50SiO_2/PLGA and 50Al_2O_3/PLGA films

ATR-FTIR spectra of 50Al_2O_3–SiO_2/PLGA and reference materials films after SBF incubation do not show any significant changes indicating surface crystallization of apatite (Fig. 3 e–h). So their behavior in SBF indicates that they are bio-inert materials [16].

Ions release from materials to SBF

It is currently assumed that the key impact on the osteogenesis process is a controlled release of ions, especially silicon Si and calcium Ca, from biomaterials [17]. These experiments are usually conducted *in vitro* using animal or human tissues containing osteoprogenitor cells that must receive appropriate chemical and mechanical stimulation from their local environment instructing them to differentiate into osteoblasts and form new bone [18].

Our research is to be the basis for such *in vitro* experiments for composite films 50CaOSiO$_2$/PLGA and 50CaO–P_2O_5–SiO_2/PLGA, and reference materials (Fig. 4). For these materials results of SBF test indicated their bioactive properties. The solubility study of the reference materials was used as a control.

Si ions release

Figure 4c. presents the changes in Si release in a function of time, during 14 days of incubation in SBF for composites modified with materials from the binary and ternary systems CaO–SiO_2 (T1/PLGA, S1/PLGA), CaO–P_2O_5–SiO_2 (T2/PLGA, S2/PLGA).

After 3 days the highest level of Si was detected for T1 material, whereas composite with T2 bioglass shows the lowest Si solubility. After 7 days the sequence according to the decreasing amount of Si was as follows: T1>S1>T2>S2. Longer incubation time (14 days) results in increased solubility of Si from T2/PLGA material. It is clearly visible, that bioglass from the ternary system are less soluble than respective materials from the binary system. Moreover, the rate of Si ions releases from the S system glasses (S1,S2) decrease in the 14th day of incubation, despite the higher silica concentration in comparison with T system glasses (T1,T2). In Si ions release from the BG/PLGA composite films, it's direct proportional dependence on the sil-

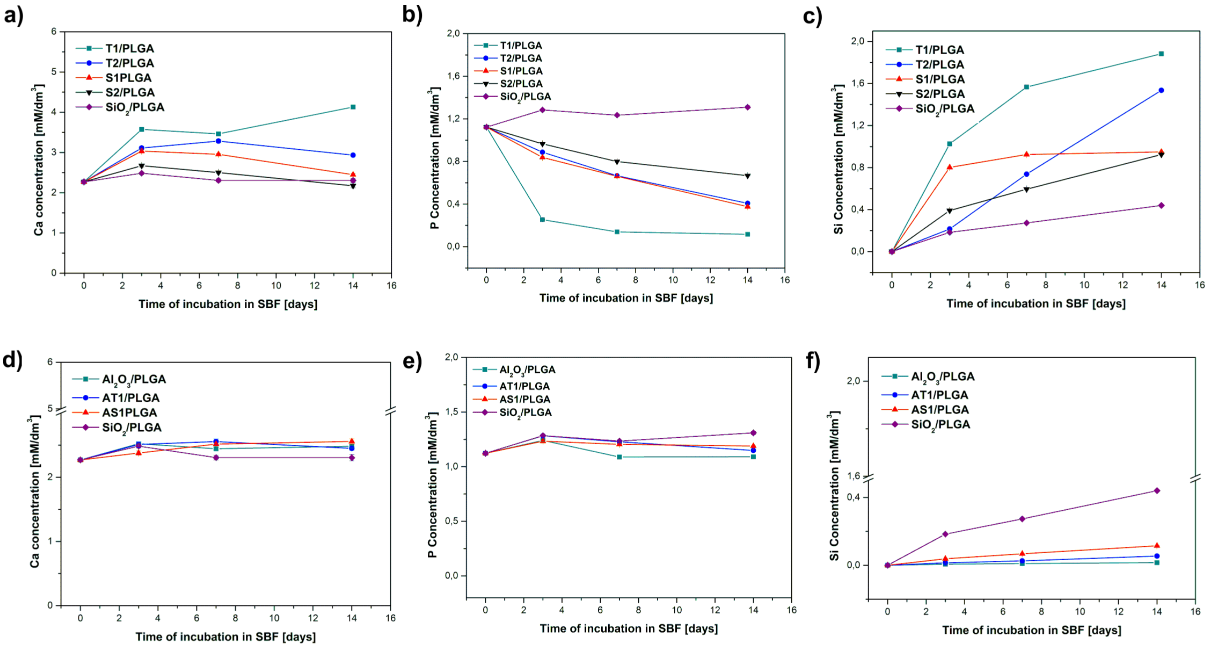

Figure 4. Changes in Ca, P and Si concentration of 50CaO–SiO_2/PLGA, 50CaO–P_2O_5–SiO_2/PLGA, 50Al_2O_3–SiO_2/PLGA and reference materials: 50SiO_2/PLGA, 50Al_2O_3/PLGA films during 14 days immersion in SBF.

ica content in bioglasses used as composite modifiers has not been observed.

Using 50SiO_2/PLGA and 50Al_2O_3–SiO_2/PLGA (Fig. 4f) composite films as reference materials it is visible that Si release is in this case at the lowest level compared to 50CaO–SiO_2/PLGA and 50CaO–P_2O_5–SiO_2/PLGA composites.

Ca and P ions release

Figure 4a presents the Ca concentration changes in the incubation medium during 14 days for the 50CaO–SiO_2/PLGA, and the 50CaO–P_2O_5–SiO_2/PLGA composites films. Ca concentration in incubation fluid grows fastest until the 3rd day of SBF incubation and remains in direct correlation with its quantity in composite films. This relationship was observed for the materials from the same system (binary or ternary). After longer SBF incubation time (7 and 14 days) downwards trend in the Ca release is observed for S type and T2 bioglasses used as composite modifiers, while a slightly increase in Ca amount occurred for the T1 bioglass. A decrease in Ca concentration results from ion uptake caused by calcium phosphate formation on the composite surface. The decrease of P concentration in SBF (Fig. 4b) confirmed CaP layer deposition on composite films. Probably due to the high release rate of Ca from T1 material and almost all consumption of phosphorus the slightly increase in Ca amount after 14 days of incubation is observed. Ca and P concentration in SBF during contact with reference materials remain almost constant due to its inert behavior (Fig. 4d,e) [19,20].

Conclusions

In the present study, a series of bioglass/PLGA composites in the form of films were characterized in the SBF test with respect to bioactivity evaluated as CaP formation on their surfaces. As polymer matrix modifier there were used gel-derived bioglasses (T1, T2, S1, S2) and almost inert glasses from the Al_2O_3–SiO_2 system. Obtained composite films with bioglass addition reveal bioactive properties confirmed by surface changes after incubation in simulated body fluid (SBF). These composites were covered by calcium phosphate with the "cauliflower" shapes characteristic for apatite, while no surface changes were observed for reference materials. Although that all proposed here bioglasses exhibit bioactive properties, their dissolution kinetics and rate of CaP formation was depend on chemical composition. It was shown that phosphorus consuming from SBF occurs much faster for materials from the CaO–SiO_2 system compared to adequate bioglasses from CaO–P_2O_5–SiO_2 system [20]. According to the obtained results it can be observed that rate of Si and Ca release from the gel-derived glasses is probably influenced by two factors: the initial concentration of respective ions in the materials and the presence of phosphates in their structure.

Acknowledgments

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