

In vitro Bioactivity of Polycaprolactone/Bioglass Composites

Lachezar Radev^{1,*}, Dimitar Vladov¹, Irena Michailova², Ekaterina Cholakova¹,
Maria F. V. Fernandes³, Isabel M. M. Salvado³

¹Department of Fundamental Chemical Technology, University of Chemical Technology and Metallurgy, Sofia, 1756, Bulgaria

²Department of Silicate Technology, University of Chemical Technology and Metallurgy, Sofia, 1756, Bulgaria

³Materials Engineering and Ceramics Department, CICECO, University of Aveiro, 3810-193 AVEIRO, Portugal

Abstract A series of Polycaprolactone and Bioglass in the 85SiO₂-10CaO-5P₂O₅ (mol. %) systems were synthesized at different quantity of the organic/inorganic components. The 85S Bioglass was prepared via sol-gel method. It was added to the Polycaprolactone matrix at 20, 50 and 80 weight (wt. %) , respectively. *In vitro* bioactivity of the prepared composites was evaluated in 1.5 Simulated Body Fluid (1.5 SBF). The obtained composite materials before and after static *in vitro* test were characterized by FTIR and SEM. The obtained experimental data proved that the synthesized composites exhibit good *in vitro* bioactivity. After immersion in 1.5SBF, SEM depicts the presence of nano-needle-like hydroxyapatite on the immersed 20PCL/80BG and 50PCL/50BG samples.

Keywords 85SBioglass, Sol-Gel, Polycaprolactone, Composites, *In vitro* Bioactivity

1. Introduction

It is known that Polymer-Glass composites scaffolds enjoy the mechanical reliability of the polymer phase and the excellent bioactivity of the glassy phase[1]. In this context poly (ϵ -caprolactone) (PCL) is used to obtain different bioactive composites, which are already referred in the recent publications, focused on the production and application of PCL-based composites, loaded with different inorganic phases, such as Bioglasses (BG)[2-10], hydroxyapatite[7, 11-16], silica[17, 18], calcium silicate[19], tri calcium phosphate (TCP)[7, 20], ZrO₂[21], TiO₂[22], forsterite (Mg₂SiO₄)[23] and hardystonite (Ca₂ZnSi₂O₇)[24]. On the other hand, the promotion role of silicon in bone metabolism was first observed by[25, 26]. Some authors synthesized Si-substituted TCP[27] and hydroxyapatite (Si-HA) with different quantity of silicon[28]. They observed better bioactivity of these ceramics towards bone formation than those without silicon.

In the field of PCL/BG composites some authors find that the combinations of PCL/45S BG[2, 3] and PCL/Calcium Silicate BG[2] produce *in vitro* bioactivity, after soaking of the synthesized composites in SBF for different times. The obtained new precipitate promoted crystals with needle-like morphology. E. Tamij et al.[4] studied when

45SiO₂-24.5Na₂O-24.5CaO-6P₂O₅ (wt. %) BG was added to PCL, lower *in vitro* bioactivity acquired. M. Mohammadi et al.,[5] observed that two types of BG, described as 50P₂O₅-40CaO-10SiO₂ or 10Fe₂O₃ mixed with PCL promote Brushite formation after soaking in SBF solutions for 14 and 28 days. H-H. Lee and colleagues synthesized *in vitro* biodegradable composite in the presence of 70SiO₂-25CaO-5P₂O₅ (mol. %) nanofibrous BG and PCL[8]. They concluded that the synthesized composite membranes showed the rapid induction of apatite precipitates on their surface[8]. Furthermore, S. R oohani-Estefani at al., prepared 58SiO₂-38CaO-4P₂O₅ (mol. %) BG/PCL composites[9]. They demonstrated that the shape of the 58BG had significant importance for bone regeneration[9]. B. Lei et al., [10] investigated that the addition of 60SiO₂-36CaO-4P₂O₅ (mol. %) BG enhanced *in vitro* bioactivity of the investigated composites.

Briefly, in spite of numerous publications on PCL/BG composites information on the *in vitro* bioactivity of PCL/85S BG in 1.5 SBF solutions does not exist.

In this study we have synthesized sol-gel glass in the SiO₂-CaO-P₂O₅ system with high silicon content (85 mol. %) and then mixed it with PCL to prepare PCL/BG composites with different content of PCL and BG. PCL was selected, because it has FDA approval in several biomimetic devices, low cost, and most of all, it is slowly degradable. *In vitro* bioactivity of the prepared PCL/BG composites were evaluated in 1.5 SBF solution.

2. Experimental Part

* Corresponding author:

l_radev@abv.bg (Lachezar Radev)

Published online at <http://journal.sapub.org/ijmc>

Copyright © 2013 Scientific & Academic Publishing. All Rights Reserved

Preparation of BG and PCL/BG composites

BG in the SiO_2 -CaO- P_2O_5 system was used as filler. BG was synthesized via polystep sol-gel method. The composition was described as 85SiO_2 - 10CaO - $5\text{P}_2\text{O}_5$ (mol. %). The obtained BG was denoted as 85S Bioglass. Tetraethoxysilane (TEOS) and phosphoric acid (H_3PO_4) were used as sources. CaO was heated at 500°C for 2 hours before using as $\text{Ca}(\text{OH})_2$ in water medium. The synthesis of 85S Bioglass had already been described in [29].

Polycaprolactone (PCL) with $M_w=6000$ (Sigma-Aldrich) was dispersed in 10 ml chloroform then BG powder was added into PCL solution and continuously stirred for 24 hours to disperse 85S BG uniformly. After that the obtained mixture was heated at 50°C under vacuum to produce composites. The PCL/BG ratio were fixed as 50:50, 80:20 and 20:80 (wt. %). The synthesized composites were depicted as 50PCL/50BG, 80PCL/20BG and 20PCL/80 BG.

Biomimetic surface treatment

In the presented study, the treatments were conducted in 1.5 SBF solution, to stimulate the nuclei formation on the composite surface, in accordance by the method, described from Kokubo *et. al.*, [30].

The 1.5 SBF was prepared from different reagents as

follow: $\text{NaCl}=11.925\text{ g}$, $\text{NaHCO}_3=0.5295\text{ g}$, $\text{KCl}=0.3360\text{ g}$, $\text{K}_2\text{HPO}_4\cdot 3\text{H}_2\text{O}=0.3420\text{ g}$, $\text{MgCl}_2\cdot 6\text{H}_2\text{O}=0.4575\text{ g}$, $\text{CaCl}_2\cdot 2\text{H}_2\text{O}=0.5520\text{ g}$, $\text{Na}_2\text{SO}_4=0.106\text{ g}$, and buffering at $\text{pH}=7.4$ at 36.5°C with 9.0075 g of TRIS and 1M HCl in distilled water. A few drops of NaN_3 was added to the 1.5 SBF to inhibit bacterial growth [31]. After soaking, the PCL/BG specimens were removed from the SBF solution, gently rinsed with distilled water and then dried at 36.5°C for 12 hours.

Methods for analysis

FTIR spectroscopy was used to identify the functional groups of BG powder and PCL/BG composites with different weight ratio of the components. FTIR spectra were recorded using a Bruker Tensor 27 spectrometer equipped with MCT detector with a resolution of 4 cm^{-1} (64 scans). KBr pelletized discs containing 2 mg of sample and 200 mg KBr were made. SEM (Jeol, JSM-35CF, Japan) was used to ascertain the morphology and chemical constituents of the prepared composites before and after immersion in 1.5 SBF solution for 12 days at accelerating voltage of 15 kv.

3. Results and Discussion

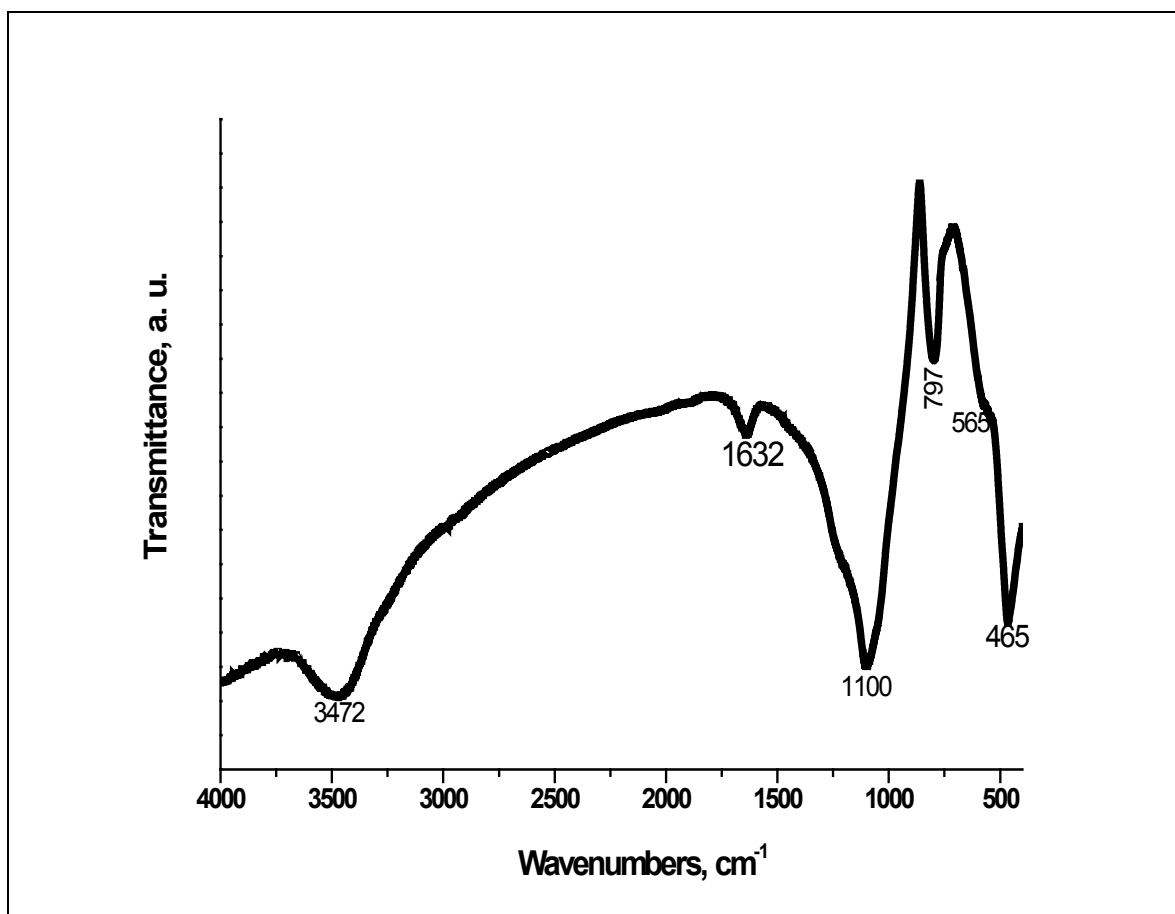


Figure 1. FTIR of 85S BG

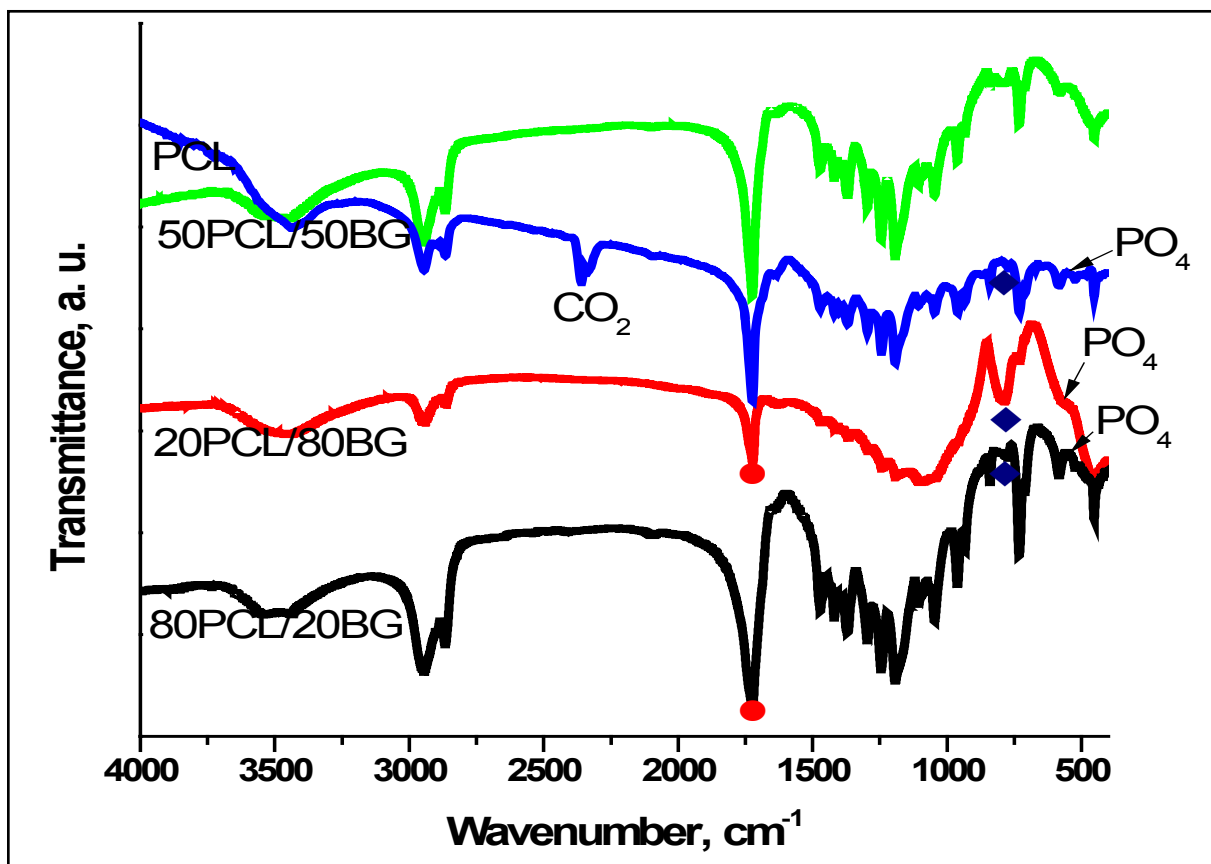


Figure 2. FTIR of pure PCL and PCL/BG composites at different weight ratio of the components before *in vitro* test

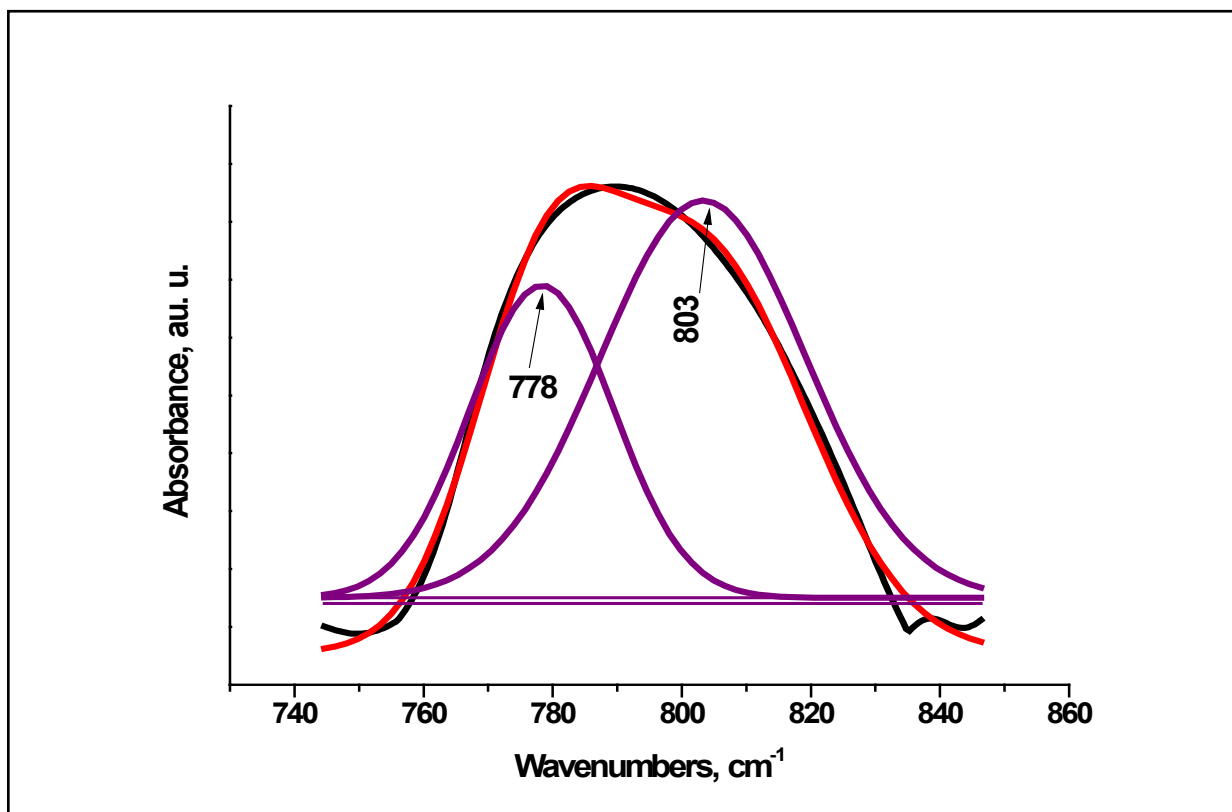


Figure 3. Curve-fitting spectra of the 50PCL/50BG composite before immersion in 1.5 SBF

FTIR spectrum of the 85S BG after its stabilization at 700°C for 3 hours is given in Fig. 1.

Fig. 1 presents the spectrum of the 85S BG, which shows very strong absorption peak at 1110 cm^{-1} assigned to Si-O-Si asymmetric stretching mode[32] and peaks centered at 797 and 465 cm^{-1} assigned to the symmetric stretching and rocking vibration of Si-O-Si band, respectively[32, 33]. The peak at 3472 cm^{-1} could be ascribed to the vibration of different OH groups[32]. The band with low intensity at 565 cm^{-1} corresponds to bending vibration of P-O bond and suggests that the phosphate enters in 85S BG as network former[33]. Moreover, the absorption peak at 1632 cm^{-1} is attributed to the water, absorbed on the surface Si-OH groups[32]. C. Vaid et al. concluded that the vibration bond, posited at $\sim 1630 \text{ cm}^{-1}$ and a broad band at 3000-3700 cm^{-1} was more prominent in the case of glasses, containing phosphorus due to the presence of more surface Si-OH groups[32].

Typical spectra of PCL and PCL/BG composites are given in Fig.2

The FTIR spectrum of pure PCL depicts the presence of carbonyl ($\text{C}=\text{O}$) stretching vibration at 1723 cm^{-1} , labeled as (●)[10-12, 23, 34]. Symmetric and asymmetric CH_2 stretching mode at 2934 and 2864 cm^{-1} are also visible[3, 11, 24, 34, 35]. On the other hand, the band at 1294 cm^{-1} could be ascribed to the backbone C-C and C-O stretching vibrations in the crystalline phase of PCL[23, 24, 34, 35]. The band at $\sim 1167 \text{ cm}^{-1}$ which is assigned to the amorphous phase of PCL[24, 35] is not visible in our case. It is noteworthy the presence of weak absorption peak at 1560 cm^{-1} , attributable to the antisymmetric stretching vibration of the COO^- in 50PCL/50BG and 80PCL/20BG composites [13]. Furthermore, the band, centered $\sim 700 \text{ cm}^{-1}$ could be ascribed to the presence of Si-O-Si bond in the sol-gel derived BG[32, 33]. B. Lei et al., proved that in the case of Nanoscale Bioactive Glass (NBG), reinforced PCL composites, the same band also been observed[10]. The presence of this band can be related to the presence of BG into the synthesized composites. As can be seen from the presented in Figure 2 FTIR spectra, the intensity of this band, labeled as (◆), is a function of weight quantity of BG into the composites, i. e. this band is quite visible for the 20PCL/80BG composite (Fig. 2, curve 3).

The more detailed information about the presence of PCL and BG in the 50PCL/50BG composite was analyzed by the curve-fitting in the region 745-845 cm^{-1} (Fig. 3).

From the presented Fig. 3 it can be seen that the band, centered at 778 cm^{-1} could be ascribed to the PCL while the band at 803 cm^{-1} could be related to Si-O-Si in accordance with FTIR data, presented in Fig. 1.

In addition, the well-defined, but not very intense, bands, posited at $\sim 570 \text{ cm}^{-1}$ could be defined as P-O stretching modes of PO_4^{3-} from 85S BG[36].

The surface morphology of the prepared PCL/BG composites before the *in vitro* test was studied by SEM as shown in Figures 4-6.

SEM image of the 50PCL/50BG (Fig. 4) shows that

85SBG has a good dispersion in the PCL matrix. On the other hand, SEM shows the presence of large cavities, due to the removal of chloroform which had previously been introduced during its preparation[11]. The sizes of these cavities are $\sim 40 \mu\text{m}$.

SEM of the 20PCL/80BG is given in Fig. 5

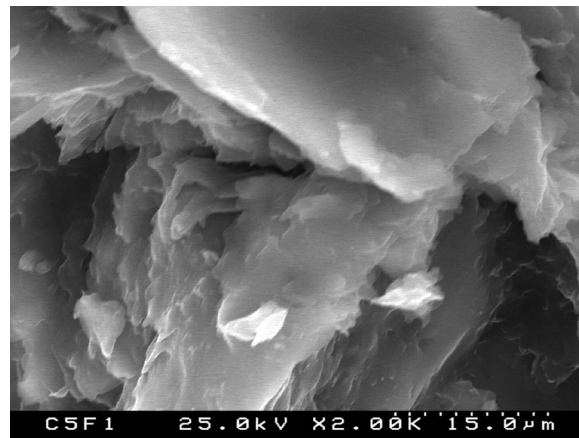


Figure 4. SEM for PCL50/50BG composite

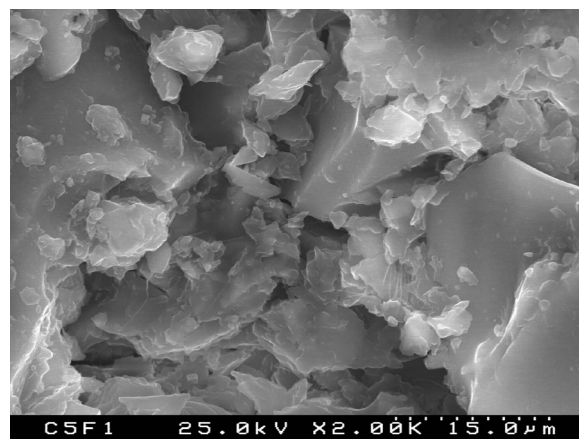


Figure 5. SEM for 20PCL/80BG

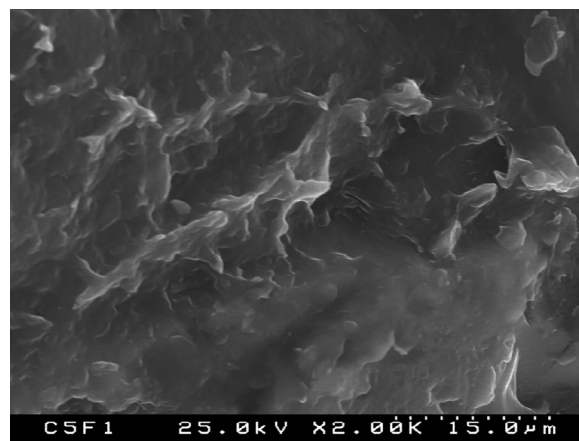


Figure 6. SEM of 80PCL/20BG

From the presented SEM image it is visible that when the quantity of BG increases, the quantity of particles attached on the PCL surface also increase. From the depicted in Fig. 5 SEM image we could also observe that the pore size decreased, compared with the 50PCL/50BG, presented in

Fig. 4. The SEM image also shows that the embedded BG particles have different sizes from 2 to 15 μm . The cavities observed in the sample have sizes smaller than those observed in the previous sample (fig. 4). This is due to the quantity of the two components from the prepared composite.

SEM of the composite with 80 wt.% PCL (80PCL/20BG) is presented in Fig. 6.

Fig. 6 shows the morphology of the sample before exposure in 1.5 SBF solution. The SEM image illustrates that the surface of the prepared composite is rough and porous. Almost no BG particles could be seen on the surface. In accordance with [4], this fact can be related to the inclusion of BG particles into the PCL matrix.

FTIR of the synthesized PCL/BG composites after *in vitro* test for 3 days are given in Fig. 7.

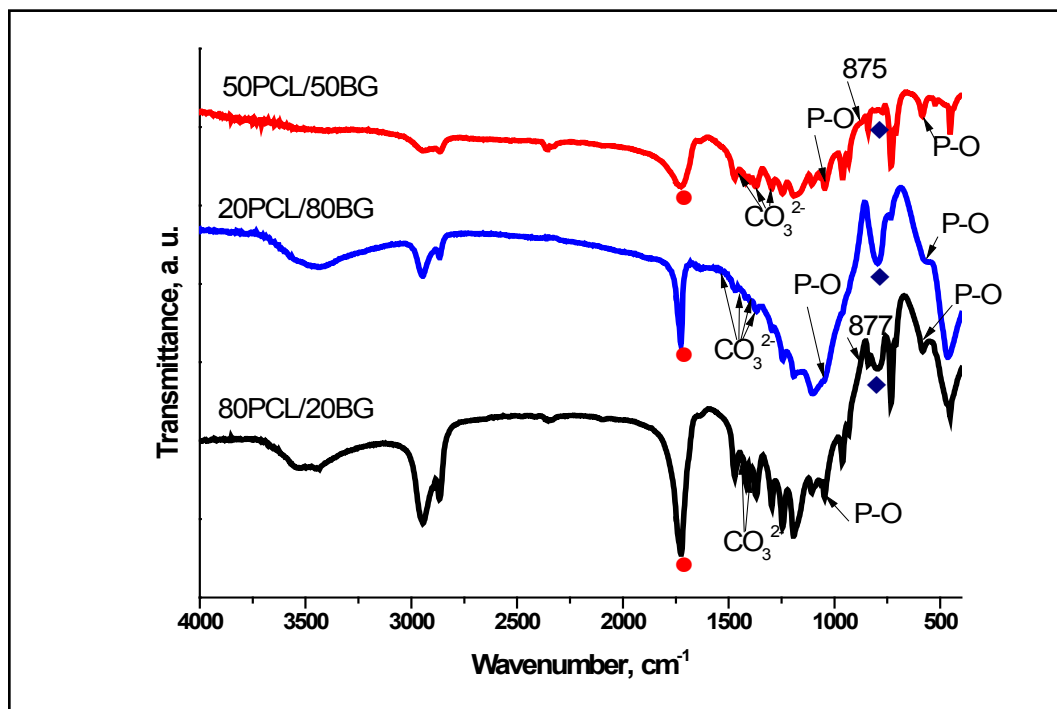


Figure 7. FTIR of the 50PCL/50BG, 20PCL/80BG and 80PCL/20BG composites, after *in vitro* test in 1.5 SBF for 3 days in static condition

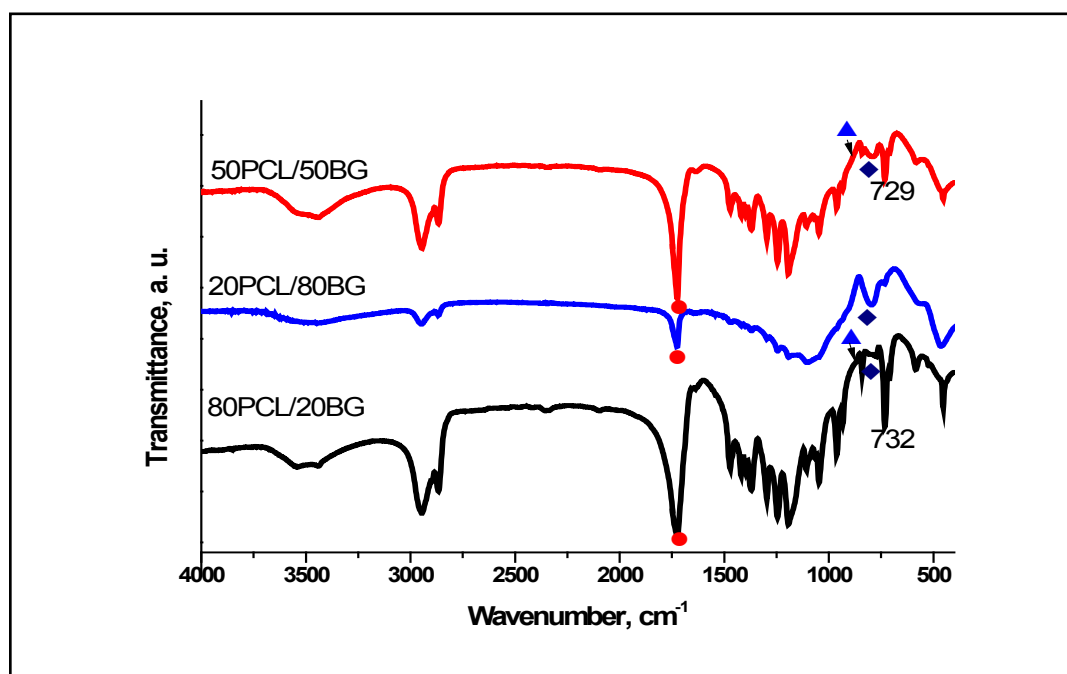


Figure 8. FTIR of the 50PCL/50BG, 20PCL/80BG and 80PCL/20BG composites after *in vitro* test in 1.5 SBF for 12 days in static conditions

The FTIR spectra of the three PCL/BG composites with different weight ratio of the PCL and BG after soaking in 1.5 SBF for 3 days, showed that the intensities of the bands significantly decreased with increasing of the immersion time up to 3 days of soaking, compared with the FTIR spectra of PCL/BG before immersion (Fig. 2). The decreasing of peaks intensity after the *in vitro* test was attributed to the partial hydrolysis of the organic part of the composites (PCL). This process is more visible for the C=O bond, presented from the absorption peak, posited at $\sim 1720\text{ cm}^{-1}$, and labeled as (●). On the other hand, in 80PCL/20BG composite (Fig. 6, curve 3), the intensity of the band at 797 cm^{-1} (labeled as ♦) became more visible. This peak could be ascribed to the Si-O-Si vibration mode of 85S BG (Fig. 1). This fact can be based also on the process of partial dissolution of PCL in 1.5 SBF solution. Furthermore, the P-O stretching modes of PO_4^{3-} at $\sim 570\text{ cm}^{-1}$ for the three composites are quite visible. These peaks are indicative for calcium phosphate crystalline phases on the surface of the soaked samples. Moreover, FTIR results presented at Fig. 7 also depict the presence of CO_3^{2-} bands at 1560 , 1462 and 1400 cm^{-1} (for the 50PCL/50BG), 1530 , 1460 , 1392 and 1370 cm^{-1} (for 20PCL/80BG) and at 1436 , 1420 and 1400 cm^{-1} (for 80PCL/20BG). The presence of these bands could be related to the CO_3HA formation on the surface of the soaked samples for 3 days in 1.5 SBF solution.

From the presented FTIR results we can summarize that:

- After *in vitro* test for 3 days, PCL partially dissolved in to 1.5 SBF solution.
- BG does not undergo any visible changes after immersion.
- Some CO_3^{2-} peaks could be observed for the three samples.

On the base of these conclusions we can conclude that the synthesized PCL/BG composites are *in vitro* bioactive. The observed results are in a good agreement with our preliminary studies[29].

FTIR spectra of the prepared PCL/BG composites with different weight ratio of the components after soaking in 1.5 SBF for 12 days are given in Fig. 8.

After comparison of the presented at Fig. 7 FTIR spectra with those depicted in Fig. 6 it is quite visible that after 12 days of soaking, the intensity of the characteristic bonds increased. Based on the obtained results, we can conclude that the synthesized composites are covered with a new phase of crystalline calcium phosphates. In this context, the peaks in the $1350\text{--}1550\text{ cm}^{-1}$ region are associated with CO_3^{2-} moieties (bending ν_3 and ν_4 modes)[5]. Moreover, the peaks centered at 875 cm^{-1} (labeled as ▲) and those at 1415 (1421) cm^{-1} , 1460 (1459) cm^{-1} could be ascribed to the presence of CO_3^{2-} into HA lattice, i.e. on the surface CO_3HA were formed[14-16]. Furthermore, peaks between $1000\text{--}1100\text{ cm}^{-1}$ are due to the triply generated asymmetric stretching mode of P-O bonds in PO_4^{3-} [5, 15, 16, 36, 37]. The peak, posited at 960 cm^{-1} could be ascribed to HPO_4^{2-} incorporated into the lattice. This peak could be ascribed to the dicalcium phosphate dihydrate (DCPD or Brushite) on the soaked

surface after 12 days of immersion.

Based on the presented FTIR results we can conclude that on the surface of the prepared PCL/BG composites, two types of products can be formed –DCPD and CO_3HA .

SEM of the immersed samples in 1.5 SBF solution after 12 days of soaking are given in Fig.9

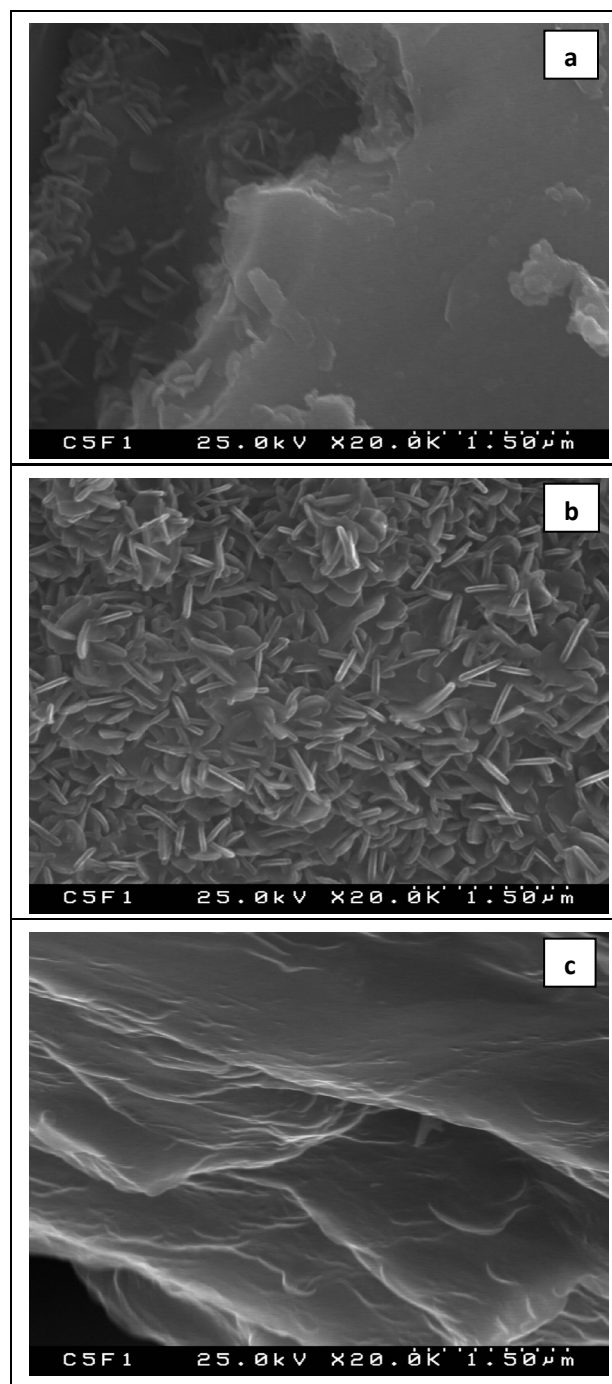


Figure 9. SEM of the 50PCL/50BG (a) 20PCL/80BG (b) and 80PCL/20BG (c) composites after *in vitro* test in 1.5 SBF for 12 days in static conditions

From the presented SEM images we can see that on the 50PCL/50BG (Fig. 9, a) and on the 20PCL/80BG (Fig. 9, b) needle-like hydroxyapatite can be observed[2, 3, 5, 7, 8, 10, 16, 19, 37, 38]. On the other hand, from the depicted in Fig. 9,

b SEM image, we can also see that the crystallized hydroxyapatite is nano-needle hydroxyapatite. In the 80PCL/20BG sample we could see that the surface morphology did not change, because BG was only 20 wt. %. On the base of these results we can conclude that BG was included in to the PCL matrix[3].

4. Conclusions

The PCL/BG composites have been prepared. BG was synthesized via sol-gel method using TEOS, CaO and H_3PO_4 as sources. The nominal composition of BG was $85SiO_2-10CaO-5P_2O_5$ (mol. %). PCL/BG composites were synthesized by mixing of PCL and BG at 20PCL/80BG, 50PCL/50BG and 80PCL/20BG weight ratio. *In vitro* bioactivities of the synthesized composites were examined in 1.5 SBF solutions for 3 and 12 days in static conditions. The obtained samples were characterized by FTIR and SEM. FTIR of the samples before *in vitro* test showed that BG was included in to PCL matrix. After 3 days of soaking in 1.5 SBF solution FTIR observed that the intensity of the characteristic bands of PCL significantly decreased. This result can be attributed to the process of partial dissolution of the organic part of the composites. On the other hand, some new carbonate bands could be observed. The presence of these new groups could be related to the carbonate containing hydroxyapatite, crystallized on the immersed sample. After 12 days of soaking, FTIR revealed the formation of DCPD and HA on the surfaces of the immersed samples. The obtained results depict that the synthesized PCL/BG composites are *in vitro* bioactive. SEM of these samples presented that needle-like HA can be observed. Based on the presented results we can conclude that the prepared PCL/BG samples exhibit good *in vitro* bioactivity.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Programme PEst-C/CTM/LA0011/2013 for finding this research.

REFERENCES

- [1] A. R. Boccaccini, M. Erol, W. J. Stark, D. Mohn, Zh. Hong, J. F. Mano. *Polymer/bioactive glass nanocomposites for biomedical applications: A review*. Composites Science and Technology, 70[13]:1764-76, 2010.
- [2] G. Chouzaouri, M. Xanthos. *In vitro bioactivity and degradation of polycaprolactone composites containing silicate fillers*, Acta Biomaterialia, 3[5], 745-56, 2007.
- [3] P. Fabbri, V. Cannillo, A. Sola, A. Dorigato, F. Chiellini, *Highly porous polycaprolactone-45S5 Bioglass® scaffolds for bone tissue engineering*, Composites Science and Technology, 70[13], 1869-78, 2010
- [4] E. Tamjid, R. Bagheri, M. Vossoughi, A. Simchi, *Effect of particle size on the in vitro bioactivity, hydrophilicity and mechanical properties of bioactive glass-reinforced polycaprolactone composites*, Materials Science and Engineering: C, 31[7], 1526-33, 2011.
- [5] M. S. Mohammadi, I. Ahmed, B. Marelli, Ch. Rudd, M.N. Bureau, Sh. N. Nazhat, *Modulation of polycaprolactone composite properties through incorporation of mixed phosphate glass formulations*, Acta Biomaterialia, 6[8], 3157-68, 2010
- [6] R L. Prabhakar, S. Brocchini, J. C. Knowles, *Effect of glass composition on the degradation properties and ion release characteristics of phosphate glass-polycaprolactone composites*, Biomaterials, 26[15], 2209-18, 2005.
- [7] S. I. Roohani-Estefani, S. Nouri-Khorasani, Z. F. Lu, R.C. Appleyard, H. Zreiqat, *Effects of bioactive glass nanoparticles on the mechanical and biological behavior of composite coated scaffolds*, Acta Biomaterialia, 7[3], 1307-18, 2011.
- [8] H.-H. Lee, H.-S. Yu, J.-H. Jang, H.-W. Kim, *Bioactivity improvement of poly(ϵ -caprolactone) membrane with the addition of nanofibrous bioactive glass*, Acta Biomaterialia, 4[3], 622-9, 2008.
- [9] S.I. Roohani-Esfahani, S. Nouri-Khorasani, Z.F. Lu, M.H. Fathi, M. Razavi, R.C. Appleyard, et al., *Modification of porous calcium phosphate surfaces with different geometries of bioactive glass nanoparticles*, Materials Science and Engineering: C, 32[4], 830-9, 2012
- [10] B. Lei, K.-H. Shin, D.-Y. Noh, I.-H. Jo, Y.-H. Koh, H.-E. Kim, et al., *Sol-gel derived nanoscale bioactive glass (NBG) particles reinforced poly(ϵ -caprolactone) composites for bone tissue engineering*, Materials Science and Engineering: C, 33[3]:1102-8, 2013.
- [11] M. G. Raucci, V. Guarino, L. Ambrosio. *Hybrid composite scaffolds prepared by sol-gel method for bone regeneration*, Composites Science and Technology, 70[13], 1861-8, 2010
- [12] M. Lebourg, J. Suay Antón, J.L. Gomez Ribelles, *Characterization of calcium phosphate layers grown on polycaprolactone for tissue engineering purposes*, Composites Science and Technology, 70[13], 1796-804, 2010
- [13] G. D. Guerra, P. Cerrai, M. Tricoli, A. Krajewski, A. Ravaglioli, M. Mazzocchi, et al., *Composites between hydroxyapatite and poly(ϵ -caprolactone) synthesized in open system at room temperature*, J Mater Sci: Mater Med, 17[1], 69-79, 2006
- [14] M. Lebourg, J. Suay Antón, J. L.G. Ribelles, *Hybrid structure in PCL-HAp scaffold resulting from biomimetic apatite growth*, J Mater Sci: Mater Med., 21[1], 33-44, 2010.
- [15] S. Joughehdoust, A. Behnamghader, M. Imani, M. Daliri, A. H. Doulabi, E. Jabbari. *A novel foam-like silane modified alumina scaffold coated with nano-hydroxyapatite -poly(ϵ -caprolactone fumarate) composite layer*, Ceramics International, 39[1], 209-18, 2013.
- [16] A. Rezaei, M.R. Mohammadi, *In vitro study of hydroxyapatite/polycaprolactone (HA/PCL) nanocomposite synthesized by an in situ sol-gel process*, Materials Science and Engineering: C, 33[1], 390-6, 2013.
- [17] M. Catauro, M. G. Raucci, F. De Gaetano, A. Marotta, *Sol-gel synthesis, characterization and bioactivity of*

- polycaprolactone /SiO₂ hybrid material*, J Mater Sci.,38[14], 3097-102, 2003
- [18] A. Schönbächler, O. Glaied, J. Huwyler, M. Frenz, U. Piele, *Indocyanine green loaded biocompatible nanoparticles: Stabilization of indocyanine green (ICG) using biocompatible silica-poly(ϵ -caprolactone) grafted nanocomposites*, Journal of Photochemistry and Photobiology A: Chemistry, 261[1], 12-9, 2013.
- [19] J. Wei, F. Chen, J-W. Shin, H. Hong, Ch. Dai, J. Su, et al., *Preparation and characterization of bioactive mesoporous wollastonite – Polycaprolactone composite scaffold*, Biomaterials, 30[6], 1080-8, 2009.
- [20] Y. Lei, B. Rai, K.H. Ho, S.H. Teoh, *In vitro degradation of novel bioactive polycaprolactone—20%tricalcium phosphate composite scaffolds for bone engineering*, Materials Science and Engineering: C, 27[2], 293-8, 2007.
- [21] M. Catauro, M. Raucci, G. Ausanio, , *Sol-gel processing of drug delivery zirconia/polycaprolactone hybrid materials*, J Mater Sci: Mater Med., 19[2], 531-40, 2008.
- [22] Santis RD, M. Catauro, L. D. Silvio, L. Manto, M. G. Raucci, L. Ambrosio, et al. *Effects of polymer amount and processing conditions on the in vitro behaviour of hybrid titanium dioxide/polycaprolactone composites*, Biomaterials, 28[18], 2801-9, 2007.
- [23] M. Kharaziha, M.H. Fathi, H. Edris. *Development of novel aligned nanofibrous composite membranes for guided bone regeneration*, Journal of the Mechanical Behavior of Biomedical Materials, 24, 9-20, 2013.
- [24] A. K. Jaiswal, H. Chhabra, S. S. Kadam, K. Londhe, V. P. Soni, J. R. Bellare. *Hardystonite improves biocompatibility and strength of electrospun polycaprolactone nanofibers over hydroxyapatite: A comparative study*, Materials Science and Engineering: C, 33[5], 2926-36, 2013.
- [25] E. M. Carlisle., *Silicon: A Possible Factor in Bone Calcification*, Science, 16[167], 279-80, 1970
- [26] K. Schwarz, D. Milne, *Growth-promoting Effects of Silicon in Rats*, Nature, 239, 333 - 4, 1972.
- [27] J. W. Reid, A. Pietak, M. Sayer, D. Dunfield, T. J. Smith, *Phase formation and evolution in the silicon substituted tricalcium phosphate/apatite system*, Biomaterials, 26[16], 2887-97, 2005.
- [28] N. Y. Mostafa, A. A. Shaltout, L. Radev, H. M. Hassan, *In vitro surface biocompatibility of high-content silicon - substituted calcium phosphate ceramics.*, Cent Eur J Chem., 11[2], 140-50, 2013.
- [29] L. Radev, D. Zheleva, I. Michailova, *In Vitro bioactivity of Polyurethane/85S Bioglass composite scaffolds*, Cent Eur J Chem. 11[9], 1439-46, 2013.
- [30] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura, et al. *Apatite Coating on Organic Polymers by a Biomimetic Process*, J Am Ceram Soc;77[11], 2805–8, 1994.
- [31] G. Falini, S. Fermani, B. Palazzo, N. Roveri, *Helical domain collagen substrates mineralization in simulated body fluid*, J Biomed Mater Res A., 87[2], 470-6, 2008.
- [32] Ch. Vaid, S. Murugavel, *Alkali oxide containing mesoporous bioactive glasses: Synthesis, characterization and in vitro bioactivity*, Materials Science and Engineering: C., 33[2], 959-68, 2013.
- [33] D. Arcos, M. Vila, A. López-Noriega, F. Rossignol, E. Champion, F.J. Oliveira, et al. *Mesoporous bioactive glasses: Mechanical reinforcement by means of a biomimetic process*, Acta Biomaterialia, 7[7], 2952–9, 2011.
- [34] V. Chiono, G. Vozzi, M. D'Acunzio, S. Brinzi, C. Domenici, F. Vozzi, et al. *Characterisation of blends between poly(ϵ -caprolactone) and polysaccharides for tissue engineering applications*, Materials Science and Engineering: C., 29[7], 2174-87, 2009.
- [35] J. Yu, P. Wu, *Crystallization process of poly(ϵ -caprolactone)-poly(ethylene oxide)-poly(ϵ -caprolactone) investigated by infrared and two-dimensional infrared correlation spectroscopy*, Polymer, 48[12], 3477-85, 2007.
- [36] M. Catauro, M. G. Raucci, F. de Gaetano, A. Buri, A. Marotta, L. Ambrosio. *Sol-gel synthesis, structure and bioactivity of Polycaprolactone/CaO • SiO₂ hybrid material*, Journal of Materials Science: Materials in Medicine, 15[9], 991-5, 2004.
- [37] F. Yang, J.G.C. Wolke, J.A. Jansen. *Biomimetic calcium phosphate coating on electrospun poly(ϵ -caprolactone) scaffolds for bone tissue engineering*, Chemical Engineering Journal, 137[1], 154-61, 2008.
- [38] M. Lebourg, J. S. Anton, J. L. Gomez-Ribelles, *Hybrid structure in PCL-HAp scaffold resulting from biomimetic apatite growth*, J Mater Sci: Mater Med., 21, 33-44, 2010.