

# Production of polyhydroxybutyrate and hydroxyapatite (PHB/HA) composites for use as biomaterial

## *(Produção de compósito de poli-hidroxibutirato e hidroxiapatita (PHB/HA) para utilização como biomaterial)*

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### Abstract

The composite materials using polyhydroxybutyrate as matrix and hydroxyapatite as reinforcement (PHB/HA) were produced by molding, using pressing at 100 MPa and 165 °C for 30 min. The amount of filler was varied from 0 to 20 wt% to evaluate the effect of the HA particles on mechanical properties and the bioactivity of the composites. The increase of the HA amount reduced the compressive strength of the composites nearly 20% while bending strength reduced nearly 30%. However, the bioactivity after 14 days immersion in SBF was improved with the increase of HA amount, with formation of apatite layer on the surface of the PHB/HA composite.

**Keywords:** polyhydroxybutyrate, hydroxyapatite, composite, biomaterials.

### Resumo

Materiais compósitos com matriz de poli-hidroxibutirato e material de reforço de hidroxiapatita (PHB/HA) foram produzidos por moldagem a partir de prensagem em 100 MPa e 165 °C durante 30 min. A quantidade de reforço variou de 0 a 20% em massa para avaliar o efeito das partículas de HA nas propriedades mecânicas e na bioatividade dos compósitos. O aumento da quantidade de HA reduziu a resistência à compressão dos compósitos em aproximadamente 20%, enquanto a resistência à flexão diminuiu 30%. No entanto, a bioatividade após 14 dias de imersão em SBF foi melhorada com o aumento da quantidade de HA, sendo observada camada de apatita na superfície do compósito PHB/HA.

**Palavras-chave:** poli-hidroxibutirato, hidroxiapatita, compósito, biomateriais.

## INTRODUCTION

Many biofunctional materials have been studied to improve their use as bone substitute. These materials exhibit properties which enhance the healing process [1]. Polyhydroxybutyrate (PHB) is an aliphatic polyester produced by microorganisms under unbalanced growth conditions [2, 3]. It is generally biodegradable (via hydrolysis) and thermoprocessable, making them attractive as biomaterials for applications in medical devices and tissue engineering. PHB decomposes to 3-hydroxybutyric acid (3HB), a normal constituent of human blood that evidences the high biocompatibility and non-toxicity of this polymer [4]. Recent studies also showed that 3HB molecules increase calcium influx in the cultured cells, suppress the death of cells, and prevent apoptosis induced by serum withdrawal [5, 6]. PHB is of particular interest for bone tissue application as it was demonstrated to produce a consistent favorable bone tissue adaptation response with no evidence of an undesirable chronic inflammatory response after implantation periods of

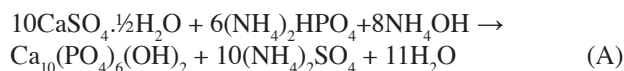
up to 12 months. Bone is formed close to the material and subsequently becomes highly organized, with up to 80% of the implant surface lying in direct apposition to new bone [7]. PHB-based biomaterials have also been related to piezoelectric and promote bone growth *in vivo* [8, 9].

Around 60 wt% of bone is made of hydroxyapatite [HA, Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>] and therefore HA has been intensively investigated as the major component for scaffold materials in bone tissue engineering [10]. HA has a close chemical resemblance to mineral part of the bone and consequently presents excellent biocompatibility [11]. However, its low fracture toughness limits the application at load-bearing sites. The high tensile strength and fracture toughness of cortical bone are attributed to the tough and flexible collagen fibers reinforced by HA crystals. Hence, calcium phosphates alone cannot be used for load-bearing scaffolds. However, its use as reinforcement of composite materials becomes very interesting to imitate the natural bone properties. The rheological and thermal properties of PHB can be tailored by incorporating appropriate amounts of

HA. The HA particles can modify the surface chemically and physically, so that samples with high content of HA tend to exhibit pronounced differentiation resulting from high surface roughness and large amount of exposed HA [12]. The mechanical properties of PHB/HA scaffolds have been reported to be higher than those for pure PHB depending on the HA amount, the distribution of the particles in the matrix, the method used to conform the scaffold and the kind of mechanical property evaluated [13-16]. The goal of this work was to produce composite materials using PHB as matrix and HA as filler and evaluate the influence of the HA amount on the mechanical properties and bioactivity of the composites.

## MATERIALS AND METHODS

*Production of the composites:* an aqueous 0.2 M hemihydrated gypsum suspension [ $\text{Ca}(\text{SO}_4) \cdot \frac{1}{2}\text{H}_2\text{O}$ ] was reacted with 0.12 M  $(\text{NH}_4)_2\text{HPO}_4$  solution to precipitate hydroxyapatite (HA) powders with molar Ca/P ratio of 1.667 at pH 9.5 according to Equation A. A 3M  $\text{NH}_4\text{OH}$  solution was used to control de pH of the reaction. The precipitated powders were filtered and dried for 24 h and the dried powders were calcined for 2 h at 900 °C in air atmosphere. The PHB, produced from the *Wautersia eutropha* bacteria, was provided by PHB Industrial. The PHB was purified using the extraction Soxhlet, using ethanol as solvent according to [17]. The HA and PHB powders were dry-mixed in varying amount of HA particles: PHB (0% HA), PHB/HA-10 wt% and PHB/HA-20 wt%. Subsequently the mixtures were warm pressed at 165 °C, 5 °C below the melting temperature, and 100 MPa for 30 min.



*Composite characterization:* mechanical strength testing was carried out in a standard test device (Emic, DL 10000) with a constant cross-head speed of 1 mm/min. For the compressive tests, a 5 kN load cell and specimens with a diameter of 10 mm and height of 20 mm were used. The bending tests were performed according to DIN 843-1 using a 0.5 kN load cell and specimens of 60 mm x 10 mm x 10 mm. Micrographs were obtained using a scanning electron microscope (SEM, Hitachi, TM-1000, Tabletop Microscope), using at least five micrographs for the measurement of the particle size. Fourier-transform infrared spectroscopy (FTIR) measurements were performed in a Bruker, FS66 spectroscope. The samples were prepared using KBr pellet, and the spectra were obtained in the range from 4000 to 500  $\text{cm}^{-1}$ . X-ray diffraction analysis (XRD, Siemens, Diffract ACT 1000) was operated with monochromated  $\text{CuK}\alpha$  radiation ( $\lambda=1.54056 \text{ \AA}$ ).

*In vitro analysis:* simulated body fluid (SBF) was prepared according to literature [18]. *In vitro* analysis was performed by immersion of the samples in SBF solution at controlled temperature of  $36.5 \pm 0.5 \text{ }^\circ\text{C}$  and  $\text{pH}=7.4$  for

14 days. The solution was changed every 48 h and the dimension of the samples was 5 mm x 5 mm x 50 mm. After the immersion time, the samples were washed in deionized water and dried at 50 °C.

## RESULTS AND DISCUSSION

*Characterization of the raw materials:* Fig. 1A shows the micrograph of the HA powder after calcination at 900 °C, where big plate-like agglomerates can be observed. These agglomerates were formed by small HA particles. The X-ray pattern is shown in Fig. 1B. HA peaks and  $\beta$ -tricalcium phosphate [ $\beta$ -TCP,  $\text{Ca}_3(\text{PO}_4)_2$ ] peaks were observed; however, no gypsum peak was detected. The formation of  $\beta$ -TCP can be associated to the reduction of the pH during the reaction, due to formation of sulfuric acid during the reaction. However,  $\beta$ -TCP is a resorbable biomaterial and enhance the biological properties of monolithic HA

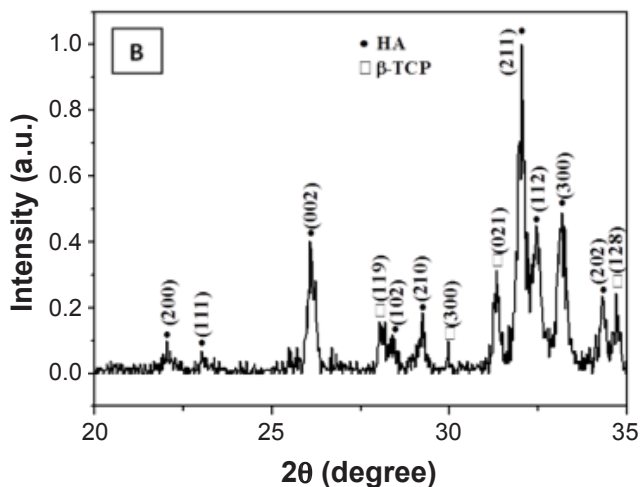
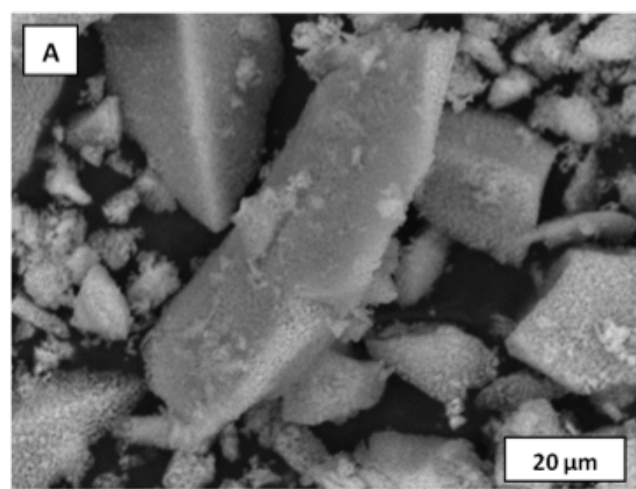


Figure 1: SEM micrograph showing the morphology of the particles (A), and X-ray diffraction pattern (B) of the HA powder after calcination at 900 °C.

[Figura 1: Micrografia obtida por microscopia eletrônica de varredura mostrando morfologia das partículas (A) e difratograma de raios X (B) do pó de HA após a calcinação a 900 °C.]

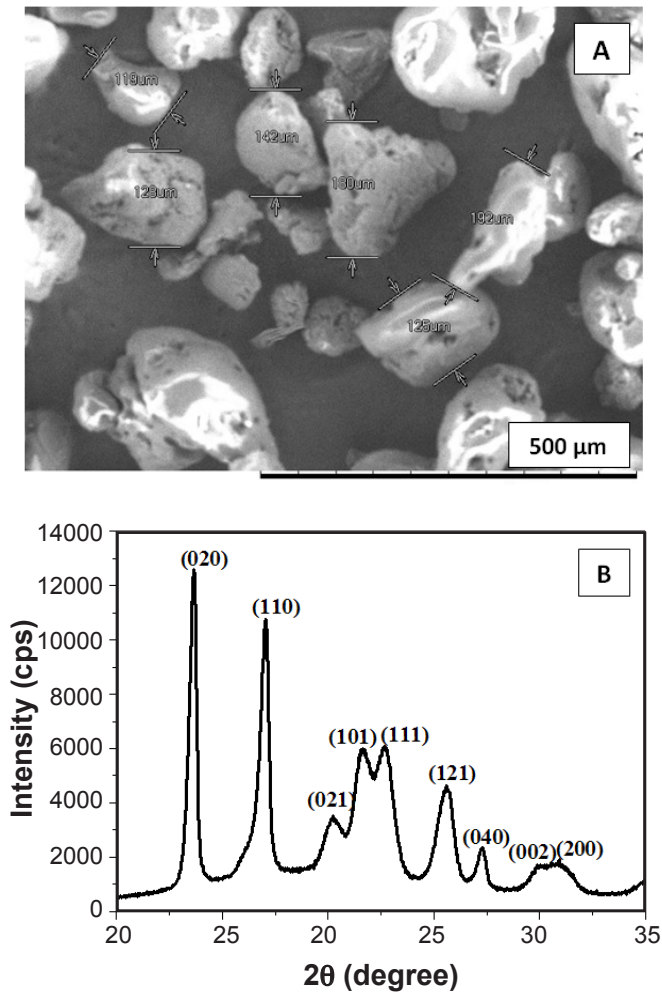


Figure 2: SEM micrograph showing the morphology of the particles (A), and X-ray diffraction pattern (B) of the PHB powder.  
 [Figura 2: Micrografia obtida por microscopia eletrônica de varredura mostrando morfologia das partículas (A) e difratograma de raios X (B) do pó de PHB.]

[18, 19]. Micrograph of PHB particles is shown in Fig. 2A. An average particle size of 196  $\mu\text{m}$  was measured and the PHB particles showed an irregular morphology. PHB is a crystalline polymer, the XRD pattern is shown in Fig. 2B, where intense peaks relating to PHB can be observed. The more intense peaks were observed at  $2\theta = 13.6^\circ$  and  $17.1^\circ$ , related to the PHB lattice planes (020) e (110), respectively. PHB showed crystallinity of 51%, in accordance to others works that reported PHB crystallinity between 50 and 80% [20].

*Mechanical characterization of the composites:* Fig. 3 shows the results of mechanical properties of the composite samples with different HA amounts. The samples without HA reached the highest compressive strength,  $\sigma_c$  of  $23.7 \pm 2.1$  MPa, and the highest bending strength,  $\sigma_f$  of  $1.05 \pm 0.12$  MPa. The increase of the HA amount lead to decrease of the mechanical properties, such that the samples containing 10% HA reached a compressive strength of  $16.4 \pm 2.1$  MPa and a bending strength of  $0.88 \pm 0.55$  MPa. Increase of the HA amount to 20% lead to further decrease of the mechanical properties. The addition of

HA promoted a decrease of the mechanical strength probably due to a deficient load transmission between the ceramic and polymeric phase. Hayati *et al.* [16] described an increase of the mechanical resistance related to the addition of HA on a PHB matrix. Samples with 5 and 10 wt% HA reached increases of 20 and 50% on the compressive strength of porous PHB/HA scaffolds, respectively. They related the increase on the mechanical properties to a homogenous distribution of the HA nanoparticles on the matrix. The increased interface area works like a reinforcement that improves the load transmission between the matrix and the ceramic nanoparticles. The final porosity was similar for all samples. Our results however showed a decrease on the mechanical properties, related with the increased size of the particles of approximately 5  $\mu\text{m}$ , since a good distribution of the HA particles on the PHB matrix was achieved. Galego *et al.* [21] reported a compressive strength of 67 MPa for

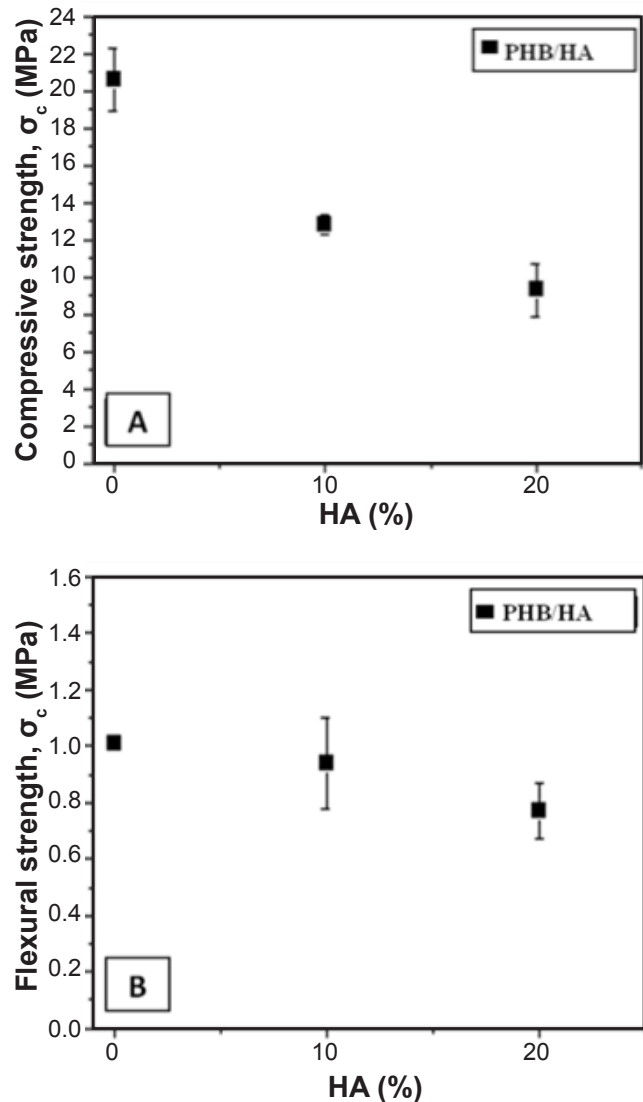


Figure 3: Results of mechanical tests of the PHB/HA composites: (A) compressive strength; and (B) flexural strength.  
 [Figura 3: Resultados de ensaios mecânicos dos compósitos PHB/HA: (A) resistência à compressão; e (B) resistência à flexão.]

PHB/HA composite with 30 wt% HA. This sample was produced by pressing at temperature 5 °C above the melting point of the polymer. The compressive strength was measured on cylindrical samples of 8 mm diameter and 10 mm height, that tends to promote increased strength values; no porosity value was reported in the work.

Zhuo *et al.* [13] reported that HA could enhance the bending strength of HA/PHB composites. The HA content increased the bending strength until a HA content of 10 wt% and the maximum bending strength reached was 32.74 MPa, an increase of about 41% comparing with pure PHB. They also described that a further increase of the mechanical properties can be reached by addition of a silane coupling agent that improves the interface between the HA particle surface and the PHB matrix. Other works also presented increase of approximately 30% of the mechanical properties by addition of HA particles [14, 15]; in both cases the enhancement of the mechanical properties was related to the utilization of nanosized particles and the good distribution in the matrix. Doyle *et al.* [7] reported a reduction of the mechanical strength of PHB/HA composites by addition of HA particles. The composites were formed via injection molding of PHB and PHB/HA mixtures at 170 °C. The *in*

*vitro* and *in vivo* properties demonstrated a slow rate of degradation that may be controlled by reinforcement with HA particles. The tissue adaptation response to the PHB and PHB filled with 40 vol% HA showed favorable to improve the bone healing. Under stresses, the dissipation of energy promotes a friction or damping in the polymeric material. In a polymer matrix composite, the energy dissipation may also come from the filler-matrix interface where friction between the two phases can occur. A high damping capability is accompanied by a decrease in dimensional stability. At the temperature of 165 °C, the addition of HA particles limited the mobility of the polymeric matrix and hence reduced the damping of the composites, the degree of which depended upon bioceramic volume percentage. An increase on the melting temperature of the PHB by addition of HA particles has been reported by Sadat-Shojai *et al.* [12] by use of DSC analysis to evaluate the melting of PHB/HA composites with different HA contents. They related a shift of 10 °C on the melting temperature after addition of 20 wt% of HA.

*In vitro analysis of the PHB/HA composites:* SBF analysis confirmed that the addition of 10 wt% HA promoted an increase on the bioactivity of the samples in comparison with pure PHB. Fig. 4 shows the morphology of pure PHB

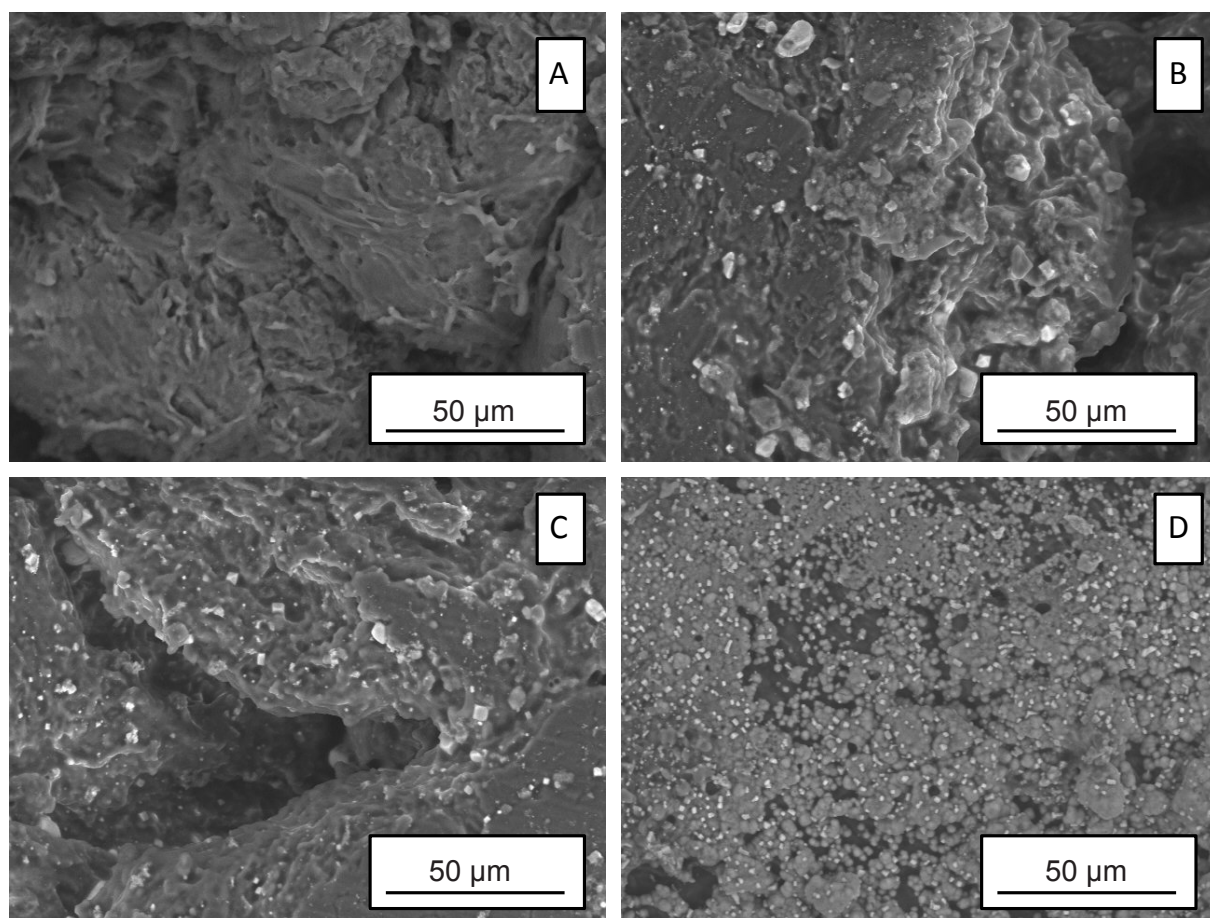


Figure 4: Surface morphology of the PHB sample before (a) and after immersion in SBF for 7 days (b), and of the PHB/HA sample with 10 wt% HA before (c) and after immersion in SBF for 7 days (d).

[Figura 4: Morfologia superficial das amostras de PHB antes (a) e após imersão em SBF por 7 dias (b), e de amostra de PHB/HA-10% antes (c) e após imersão em SBF por 7 dias (d).]

and PHB/HA-10 wt% samples before and after immersion in SBF for 7 days. Only a small number of biomimetic HA crystals could be observed on the surface of the PHB sample after the immersion in SBF. The PHB/HA sample showed however more formation of biomimetic HA on their surface after immersion in SBF for 7 days. Studies have described formation of apatite crystals on the surface of PHB/HA composites after immersion in SBF. Ni and Wang [22] evaluated the effect of HA on the bioactivity of PHB using HA amounts of 0, 10, 20 and 30 vol% HA. It was observed formation of small crystals after 1 day immersion and more near-spherical crystals were observed on the specimen after 7 days immersion. The formation of a complete layer of apatite crystals was observed only after 7 days on all HA/PHB composites. Mineral crystals were also found on unfilled PHB after its immersion in SBF for 14 days, but the growth of these crystals was very slow. After 28 days immersion, the mineral layer formed was still very thin and rough and only after 56 days immersion was a uniform mineral layer observed. Szuberta *et al.* [23] studied the apatite layer formation on the surface of HA samples modified by incorporation of PHB. It was reported the formation of a layer with the near-spherical grains on the surface pellet of HA grafted by PHB after 28 days immersion in SBF. They reported that the mixture of PHB and HA can be more bioactive than pure HA, however no mechanism to explain this hypothesis was presented.

## CONCLUSIONS

The addition of HA in the polymeric matrix of PHB promoted decrease of the mechanical strength of the PHB/HA composites, due to formation of discontinuities on the matrix promoted by poor interface between the matrix and the ceramic particles. However, the increase of the HA amount enhanced the bone-like apatite formation on the composite surface after immersion in SBF, which indicated an increase of the bioactivity.

## REFERENCES

- [1] H.K. Uthoff, P. Poitras, D.S. Backman, J. Orthop. Sci. **11**, 2 (2006) 118.
- [2] Y. Doi, S. Kitamura, H. Abe, Macromol. **28**, 14 (1995) 4822.
- [3] H.Y. Li, R.L. Du, J. Chang, J. Biomater. Appl. **20**, 2 (2005) 137.
- [4] Y. Zhao, B. Zou, Z. Shi, Q. Wu, G.Q. Chen, Biomaterials **28** (2007) 3063.
- [5] S. Cheng, Q. Wu, F. Yang, M. Xu, M. Leski, G.Q. Chen, Biomacromolecules **6** (2005) 593.
- [6] S. Cheng, G.Q. Chen, M. Leski, B. Zou, Y. Wang, Q. Wu, Biomaterials **27** (2006) 3758.
- [7] C. Doyle, E.T. Tanner, W. Bonfield, Biomaterials **12** (1991) 841.
- [8] Y. Ando, E. Fukada, Polymer Phys. **22** (1984) 1821.
- [9] S.K. Misra, S.N. Nazhat, S.P. Valappil, M. Moshrefi-Torbati, R.J.K. Wood, I. Roy, A.R. Boccaccini, Biomacromolecules **8** (2007) 2112.
- [10] R.Z. LeGeros, J.P. LeGeros, Bioceram. **15** (2002) 3.
- [11] M. Jarcho, J.F. Kay, K.I. Gumar, R.H. Doremus, H.P. Drobeck, J. Biosci. Bioeng. **1**, 2 (1977) 79.
- [12] M. Sadat-Shojai, M.-T. Khorasani, A. Jamshidi, S. Irani, Mater. Sci. Eng. C **33** (2013) 2776.
- [13] G.Z.Q. Zhuo, L.M. Dong, C. Wang, Q.F. Zan, J.M. Tian, Adv. Mater. Res. **105-106** (2010) 104.
- [14] H.T. Jiang, P. Hu, L. Liu, Y. Li, Isinghua Sci. Technol. **7** (2002) 463.
- [15] I.Y.-W. Wang, Q. Wu, J. Chen, G.-Q. Chen, Biomaterials **26** (2005) 899.
- [16] J.A.N. Hayati, H.R. Rezaie, S.M. Hosseinalipour, Mater. Lett. **65** (2011) 736.
- [17] H.C. Birnboim, J. Doly, Nucl. Acids Res. **7**, 6 (1979) 1513.
- [18] T. Kokubo, H. Takadama, Biomaterials **27** (2006) 2907.
- [19] M. Descamps, T. Duhoo, F. Monchau, J. Lu, P. Hardouin, J.C. Hornez, A. Leriche, J. Eur. Ceram. Soc. **28**, 1 (2008) 149.
- [20] M. Vallet-Regí, J.M. Gonzalez-Calbet, Prog. Solid State Chem. **32**, 1 (2004) 1.
- [21] N. Galego, C. Rozsa, R. Sánchez, J. Fung, A. Vázquez, J.S. Tomás, Polym. Test. **19** (2000) 485.
- [22] J. Ni, M. Wang, Mater. Sci. Eng. C **20** (2002) 101.
- [23] M. Szuberta, K. Adamskaa, M. Szybowicz, T. Jesionowskia, T. Buchwaldb, A. Voelkela, Mater. Sci. Eng. C **34** (2014) 236.

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