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A new calcium releasing nano-composite biomaterial for bone tissue engineering scaffolds

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Abstract

A biomaterial with bioactive glass nanoparticles (nBG) and Ca^{2+} incorporated into alginate matrix was developed. Films characterization was carried out by SEM, IR, tensile strength measurements, bioactivity assay, degradation and swelling studies. Ca^{2+} release from films was analyzed. Freeze-dried-scaffolds were also fabricated. Films showed the development of a homogeneous matrix and the mechanical properties were improved when nBG were incorporated. The bioactive nature of nBG containing films was confirmed by studies in simulated body fluid. Degradation was negligible and a good swelling capacity was observed. Moreover Ca^{2+} was released in a controlled manner. In scaffolds fabricated by freeze-drying, pores were seen to be uniform and well distributed. According to the characterization results, these composite biomaterials are attractive candidates for the fabrication of bone tissue engineering scaffolds.

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Keywords: Composites; Biomaterials; Scaffolds; Films; Calcium.

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1. Introduction

The new generation of biomaterials to develop scaffolds for bone tissue engineering (BTE) includes the elaboration of nano-composites (n-c) in order to resemble de bone extracellular matrix, with the possibility of using the scaffold itself as a drug delivery system [1]. In this context, inorganic biomaterials such as ceramics and glasses, as well as organic systems such as biodegradable polymers, are being used to fabricate composite scaffolds [2]. Among inorganic systems, bioactive glasses are a special class of highly bioactive materials exhibiting interesting properties such osteoconductive and osteocompatible characteristics, as well as the promotion of osteogenesis and angiogenesis [3]. Further, studies confirmed that the addition of ceramic nanoparticles into a polymer matrix usually improves the mechanical properties of the matrix [4]. The possibility to deliver different biological factors to the surrounding tissue to stimulate new bone formation, as well as to release different drugs intended for the treatment of bone diseases is a strategy that makes the scaffold a multifunctional matrix for BTE [5,6]. Lately, metallic ions as therapeutics agents (MITA) have been considered for new biomaterials development [7,8]. In fact, a large amount of biomaterials for BTE contain calcium ions, Ca^{2+} [9], which stimulate the differentiation of bone cells, osteoblasts proliferation, bone metabolism and bone mineralization [7]. According to the above considerations, this work presents the development of a new n-c biomaterial which combines alginate with nBG and incorporates Ca^{2+} for BTE applications.

2. Materials and Methods

A mixture of sodium alginate with nBG (nominal composition of *Bioglass® 45S5*, size: 35-40 nm), loaded with Ca^{2+} ions (by using $CaCl_2.2H_2O$, which was purchased from MERCK, Germany), was casted into molds to obtain n-c films. The morphology and homogeneity of n-c films were evaluated by scanning electron microscopy (SEM). n-c films were also analyzed by Infrared (IR) spectroscopy. Mechanical properties of n-c films in compare with films without nBG were assessed by tensile strength measurements, where ten samples of each type of film were used. The bioactive nature of n-c films was tested in Simulated Body Fluid (SBF) at 37°C for 7 days and the growth of hydroxyapatite (HA) crystals on the films surface was determined by SEM and confirmed by X-ray diffraction (XRD). Degradation and swelling studies of n-c films were made at 37 °C in phosphate buffer at pH 7.4. Ca^{2+} release from n-c films was studied at 37 °C in phosphate buffer at pH 7.4 during 60 days and the quantification was made by a UV-direct capillary electrophoresis method previously developed [10].

Scaffolds of the same composition as n-c films were fabricated by freeze-drying. Morphology and porosity of n-c scaffolds were studied by SEM.

3. Results and Discussion

3.1. Characterization of films

Figure 1 shows the surface of n-c films; a uniform and homogeneous matrix with nBG dispersed in it is observed.

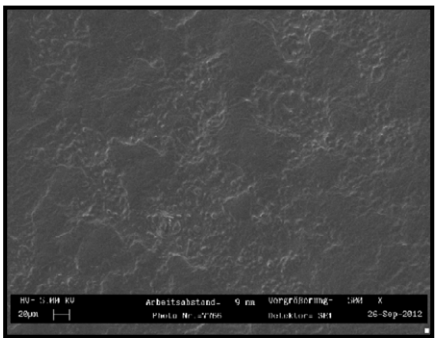

Figure 1. SEM image of the surface of a n-c film before bioactivity studies.

The interaction between Ca^{2+} and the films matrix, either for films with or without nBG, is shown by IR spectra, Figure 2.

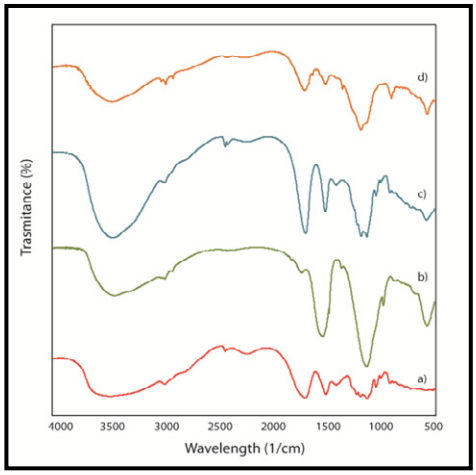

Figure 2. IR spectra of a) nBG, b) alginate, c) films without nBG, d) n-c films.

The addition of nBG to the alginate matrix increased significantly ($p < 0.05$) the tensile strength in comparison with films not containing nBG, as shown Figure 3.

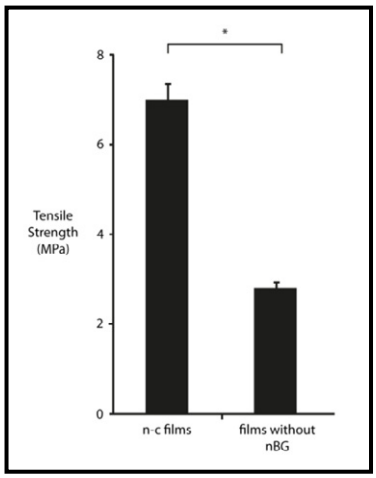

Figure 3. Results of tensile strength measurements on films with and without incorporation of nBG.

SEM images of films immersed in SBF are shown in Figure 4a,b. The *in vitro* bioactivity was confirmed by the deposition of HA crystals on the surface of the n-c films after 3 and 7 days of immersion in SBF, as indicated in the XRD spectrum in Figure 5.

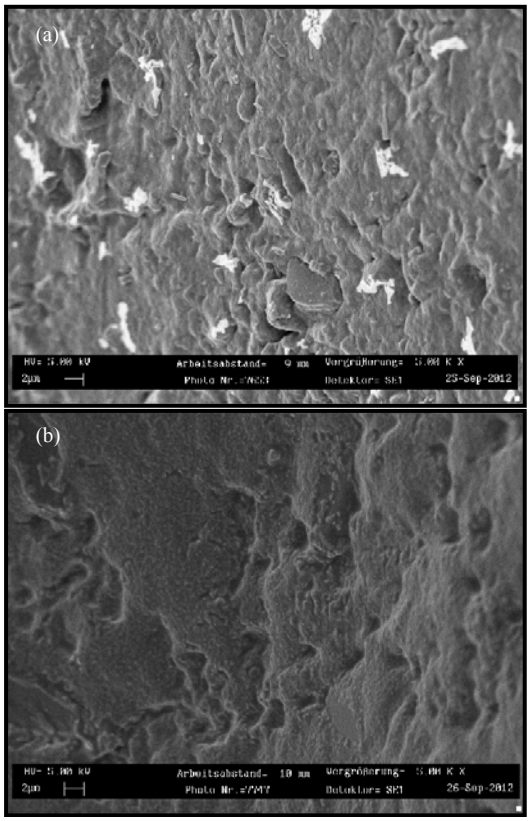

Figure 4. SEM images of (a) n-c films after 3 days in SBF showing the growth of crystals on the surface, and (b) n-c films after 7 days in SBF.

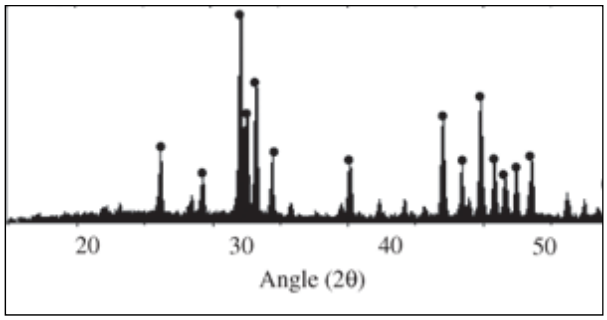

Figure 5. XRD pattern of a composite film after 7 days of incubation in SBF showing characteristics peaks of hydroxyapatite (•) and the presence of amorphous material.

3.2. Degradation and Swelling studies of films

No significant changes in weight loss were observed indicating that n-c films still remained shaped until day 60 (Figure 6a). The swelling study indicated a good swelling capacity of the films with a weight gain percentage of about 100% (Figure 6b).

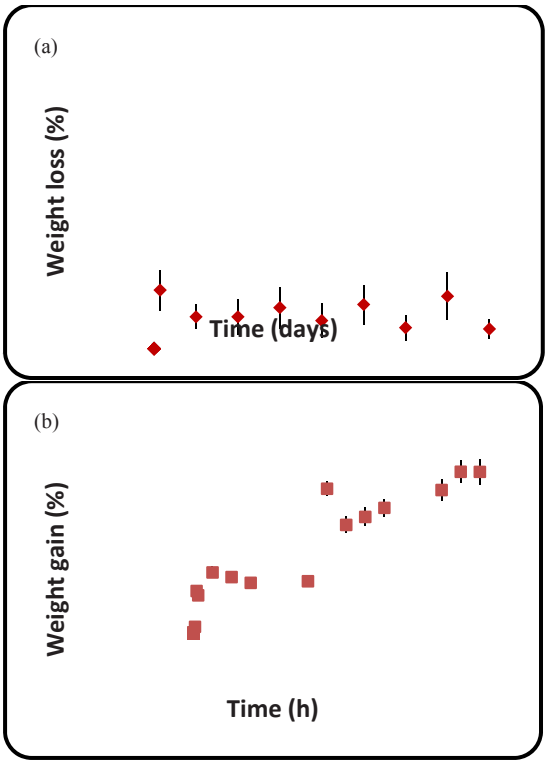

Figure 6. Results of (a) weight loss as function of time, showing that there is no significant weight loss until the end of the study, and (b) swelling study showing a weight gain of 100% after 15 days, where films double their weight.

3.3. Release study of films

The amount of Ca^{2+} released increases until day 30. After that and until day 60, the release of Ca^{2+} tends to remain stable. About 30-35 µg/ml of Ca^{2+} were released from day 30 until the end of the study. Figure 7 shows the release profile of Ca^{2+}.

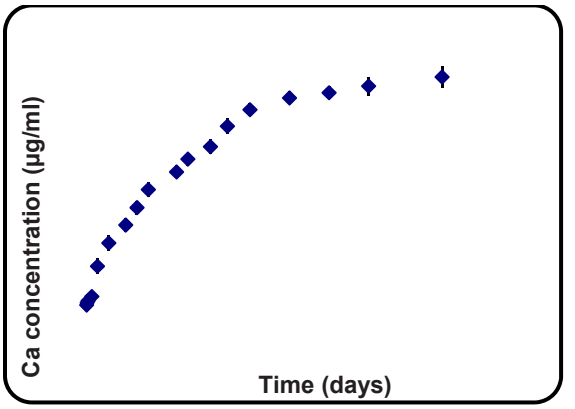

Figure 7. Ca^{2+} release profile in phosphate buffer at pH 7.4 during 60 days.

3.4. Characterization of scaffolds

Figure 8 shows the morphology and porosity of n-c scaffolds. Pores are uniform and well distributed (Figure 8a), and the presence of bioactive glass nanoparticles can be observed (Figure 8b).

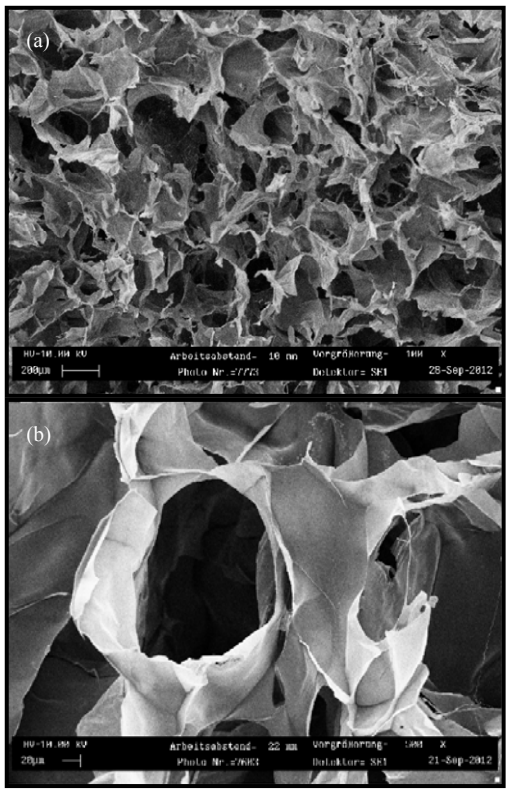

Figure 8. SEM images of nBG containing composite scaffolds at (a) low and (b) high magnification.

4. Conclusions

Nanocomposite, biodegradable and bioactive films and scaffolds with Ca^{2+} releasing ability as multifunctional substrates for BTE were developed. The incorporation of nBG into alginate films significantly improved the tensile strength. Biomineralization studies indicated the deposition of apatite on the films surface, suggesting their bioactive behavior which is a consequence of the high bioreactivity of the added nBG. Films do not completely degrade after 60 days, which indicates their stability over time. The release of Ca^{2+} is seen to be controlled and without an initial burst effect, which might be an advantage when cellular *in vitro* studies, such as citocompatibility assays, are envisaged. Future assays will be focused on a full characterization of the scaffolds, the study of the Ca^{2+} release in relevant biological conditions and the investigation of the capability of these scaffolds to promote the proliferation of bone cells.

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