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Functionalization of hydroxyapatite scaffolds with ZnO

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Abstract

This paper analyzes the effectiveness of a new method for treating the surface of bioceramics scaffolds intended for bone substitutes (SO). This employs hydroxyapatite ($Ca_5(PO_4)_3(OH)$, HA) obtained from the chemical treatment (weak acid) and pyrolysis (at 900°C) of fresh bovine bone. The inorganic matrix is formed by an interconnected pore structure and hydrophilic, very similar to the human cancellous bone, which is used in reconstructive surgery. The SO biologic response can be improved by surface modification with Zn, element known for its antibacterial action. The Zn fixing in HA is frequently performed by chemical synthesis processes, where Zn^{2+}, Ca^{2+} and PO_4^{3-} ions co-precipitation processes take place. The proposed method begins with the chemical treatment of the HA with an aqueous solution of phosphoric acid (30 wt. %). These scaffolds are submerged (under vacuum) in a dispersion of ZnO in an alcohol-glycerin solution, which allows relatively uniform distribution of oxide on the porous medium, when it is dried. The Zn chemical bonding on HA surface is accomplished by sintering (900 to 1100°C). This promotes a solid state reaction, which leads to the formation of Zn phosphates (in hydrated and non-hydrated forms), according to the analysis of X-ray diffraction, energy spectroscopy and electron microscopy observation. This process is of simple implementation and it would allow SO functionalization with other therapeutic agents of interest, represented by different ions.

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Keywords: Hydroxyapatite, Bioceramic functionalization, ZnO, Bone substitutes, Porous scaffolds.

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1. Introduction

Bone regeneration involves a series of complex and intricate biological events involving signaling molecules, growth factors and cells at the site of injury by Henkel et al. (2007). Tissue engineering uses for this purpose degradable scaffolds to promote bone regeneration. These scaffolds must be capable of: being replaced by host tissue, being porous to allow vascularization not producing an immunogenic response in a patient by Liu et al. (2007).

Hydroxyapatite (**HA**, $Ca_{10}(PO_4)_6(OH)_2$) is a widely used biomaterial for replacement of bone tissue due to its excellent biocompatibility by Liu et al. (2007). This calcium phosphate is the main constituent of bone inorganic phase; however, it is the most important reservoir of calcium and phosphorus in the body. A biodegradable matrix based on the biomaterial must have an inter-connected macro and microporosity, similar to the bone tissue, provide a suitable microenvironment for vascularization and regenerate further the tissue (Dorozhkin et al. (2010)).

Zinc (Zn) is known for its antibacterial properties and is a metallic element essential for cell proliferation and remodeling of the extracellular matrix (Rosen et al. (2012), Wang et al. (2007), Hie et al. (2011), and Yang et al. (2010)). Current evidence argues that Zn plays an important role in the mineralization (Peretz et al. (2001), Ito et al. (2005), Storrie et al. (2005), Wanga et al. (2010), Nagata et al. (2011), and Toledano et al. (2010)); catalyzes metalloproteinase (MMP) for remodeling; induces apoptosis and inhibits the mature osteoclast formation, and stimulates osteoblast proliferation (Hie et al. (2011) and Yang et al. (2010)). These properties would make Zn an element that would accelerate the repair process of bone tissue.

The aforementioned findings motivate this preliminary work, which proposes the synthesis of a three-dimensional hydroxyapatite structure by modifying the surface with zinc. The proposed technique involves the intrusion of an organic dispersion of ZnO in the array to achieve its anchor onto surface, and then promote chemical binding with the HA through a process of high temperature heat treatment (sintering).

2. Materials and Methods

2.1 Preparation of Hydroxyapatite scaffolds

The preparation of the scaffolds is made from cancellous fresh bovine bone of 5 femoral heads, which were cut into 50 pieces (15x10x10 mm^3). The samples were subjected to repeated sequences washed with aqueous solutions of acetic acid and hydrogen peroxide at 1% (by weight) in bi-distilled water (*Química Oeste*, Argentina) and rinsing in pure water, to degreasing and prevent hemoglobin fixation to bone. These pieces were oven dried and taken to an electric furnace (*Hornos Eléctricos ORL*, Argentina) for slow pyrolysis of the organic remains (10°C min^{-1}) under low airflow, up to the 900°C, where they remained for 2 hours. The obtained pieces are white and free from any color spot (Fig.1).

2.2 Preparation of the ZnO dispersion

Analytic grade ZnO particles (*Anedra*, Argentina) were dispersed in a 500 ml of organic solution at a concentration of 2% (by weight). This was formulated with 50% (by weight) of propanotriol ($C_3H_8O_3$) (*Saporiti*, Argentina) and double distilled water (*Química Oeste*, Argentina), so by having a viscous solution that limits particle sedimentation. Homogeneity and stability of the dispersion was kept in a beaker by means of magnetic stirring (*Decalab*, Argentina) at 100 r.p.m. and 90°C.

2.3 Preparation of functionalized scaffold

The HA scaffolds were immersed in a solution of phosphoric acid (*Química Oeste*, Argentina) to 30% (by weight) in distilled water for 20 seconds in order to generate a more reactive surface (with free chemical bonds), and rinsed in double distilled water in order to remove soluble phosphates. Then, the scaffolds were immersed in the ZnO particle

dispersion in a 500 cm^3 flask, which is maintained at 90°C under stirring for 20 seconds. The flask were placed in a vacuum (pressure less than 10^{-2} MPa) to ensure infiltration of the dispersion into scaffold pores for 10 min. Finally, the scaffolds were dried in an oven and subjected to slow heating (5 °C min^{-1}) using an electric furnace (*Hornos Eléctricos ORL*, Argentina) to the sintering temperatures (900, 1000 and 1100°C) in air, where they remained for three hours. The thermal cycle ended with a cooling at the same rate to room temperature.

2.4 Microstructure, local chemical composition and phase distribution

Microstructure and local chemical composition were analyzed by a scanning electron microscope, **SEM**, (*Philips 505,* Germany) and energy dispersive spectrometer, **EDS** (*EDAX 9100*, Germany), respectively. The crystalline phases of the scaffolds modified surfaces were determined with X-ray diffractometer, **XRD** (*Rigaku*, Japan), by using $Cu_{K\alpha}$ radiation (λ = 0.1542 nm) with Ni filter, and using a vertical goniometer in the 2θ range of 20-70°, with a step of 0.2° and at scan speed of 0.5° min^{-1}.

3. Results and Discussion

The SEM observations reveal the bone network of interconnected porosity, characterized by macropores up to 350-500 µm, integrating a three-dimensional structure (Fig. 1).

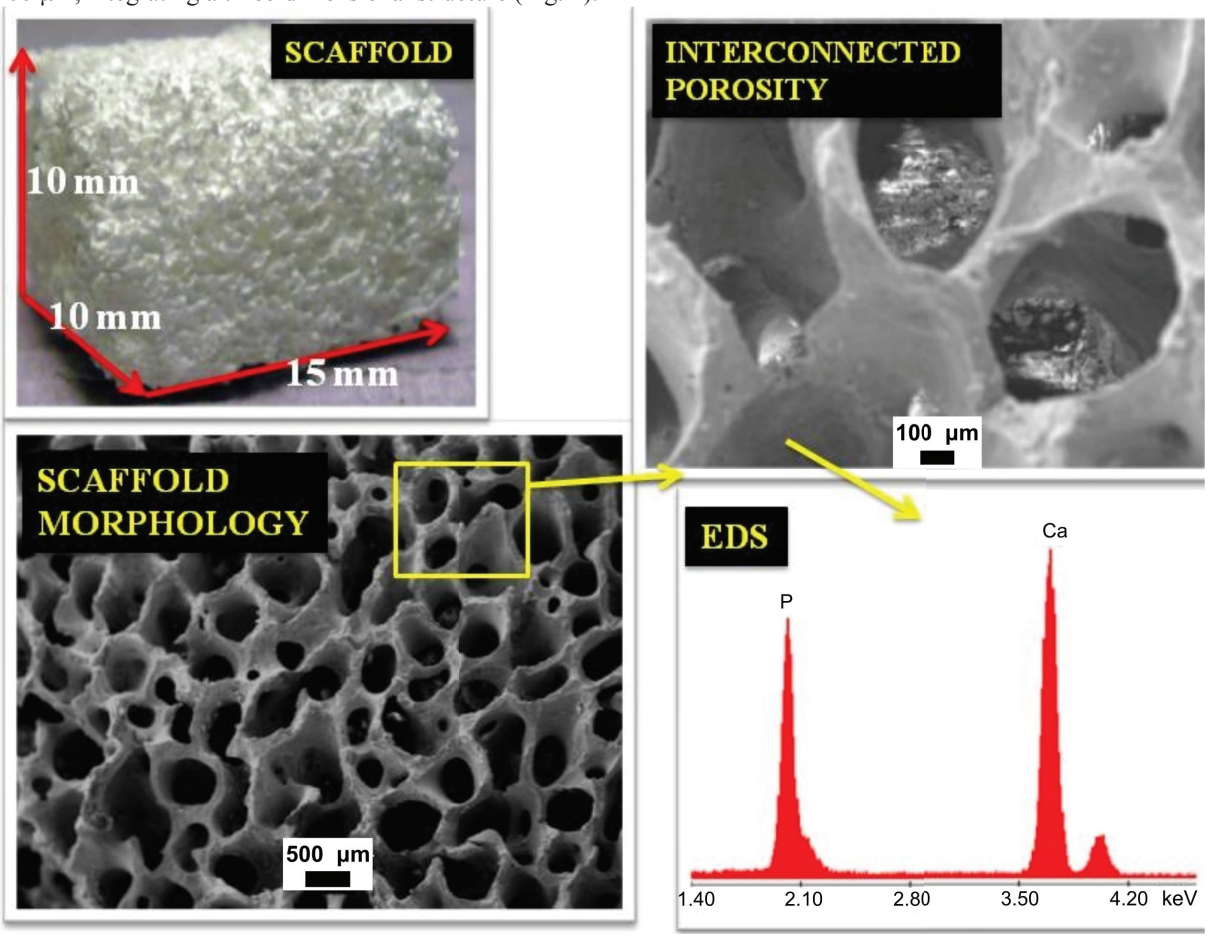

Fig. 1. HA scaffold surface sintered at 1000 °C, exhibiting white ZnO particles agglomerates.

The average concentration of zinc metal, measured with EDS on repeated determinations in different cuts of the scaffolds, and employing a 25 nm diameter electron probe scans over 100 x 100 μm^2 areas, is close to (1.01 ± 0.11) % (wt.) (Fig. 2). Furthermore, Zn is detected locally on HA crystals, indicating that a diffusion process of metal ions has taken place.

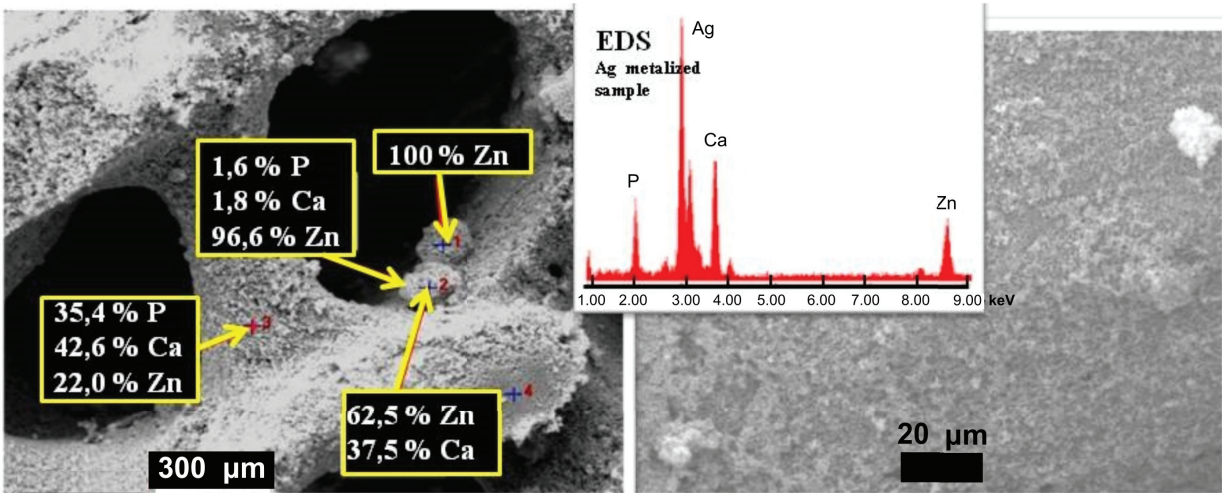

Fig. 2. SEM micrographs and local EDS (left) and global areas of about 100 x 100 μm^2 (right) analysis of HA scaffolds surfaces treated with ZnO particles and sintered at 1100°C.

These observations are consistent with the XRD analysis, where ZnO (36-1451 file in ICDD (2005)) is present in all oxide dispersion treated samples on the HA substrate. This substrate is mainly constituted by HA (34-0010 file in ICDD (2005)) and a small fraction of β-TCP (55-0898 file in ICDD (2005)) and tetracalcium phosphate, TTCP ($Ca_4O_9P_2$) (70-1379 file in ICDD (2005)), which are products of HA thermal decomposition (Fig. 3). However, an additional difference was revealed by the presence of peaks that may be associated with Zn phosphates in anhydrate and hydrated stages: (α-$Zn_3(PO_4)_2$, (29-1390 file in ICDD (2005)) and α-$Zn_3(PO_4)_2$.4 H_2O, (39-1352 file in ICDD (2005) and Chao et al. (1969)), known as *Hopeite* and *Parahopeite*, respectively. The techniques used do not allow to state with certainty the stoichiometry of the compounds due to its low global content. The heat treatment leads to a solid state reaction between HA surface and ZnO. The detailed comparison of the XRD peak position of HA with and without ZnO sintered at 1100°C shows no apparent displacement (Fig.3). This might also suggest that by changing HA crystalline parameters, which may be associated with ionic substitution of Ca by ions of Zn, in order to produce Zn substituted HA, it could result in a non-stoichiometric $Ca_xZn_xCa_5(PO_4)_3(OH)$ apatite type. Such phosphates are perfectly biocompatible and soluble in physiological medium (Anusavice (1996) and Mayer et al. (2000)). These compounds provide a source of Zn characterized by their bioavailability, which should be greater than that achieved with synthetic chemical processes, CS. These are based on the co-precipitation of precursors of Ca^{+2}, Zn^{+2} and PO_4^{-3} ions, dependent on pH, temperature and reaction time in aqueous solutions (Mayer et al. (2000) and Ito et al. (2000)). CS allows the Zn incorporation into HA crystal structure, in a process of ionic substitution of Ca^{2+} by Zn^{2+}.

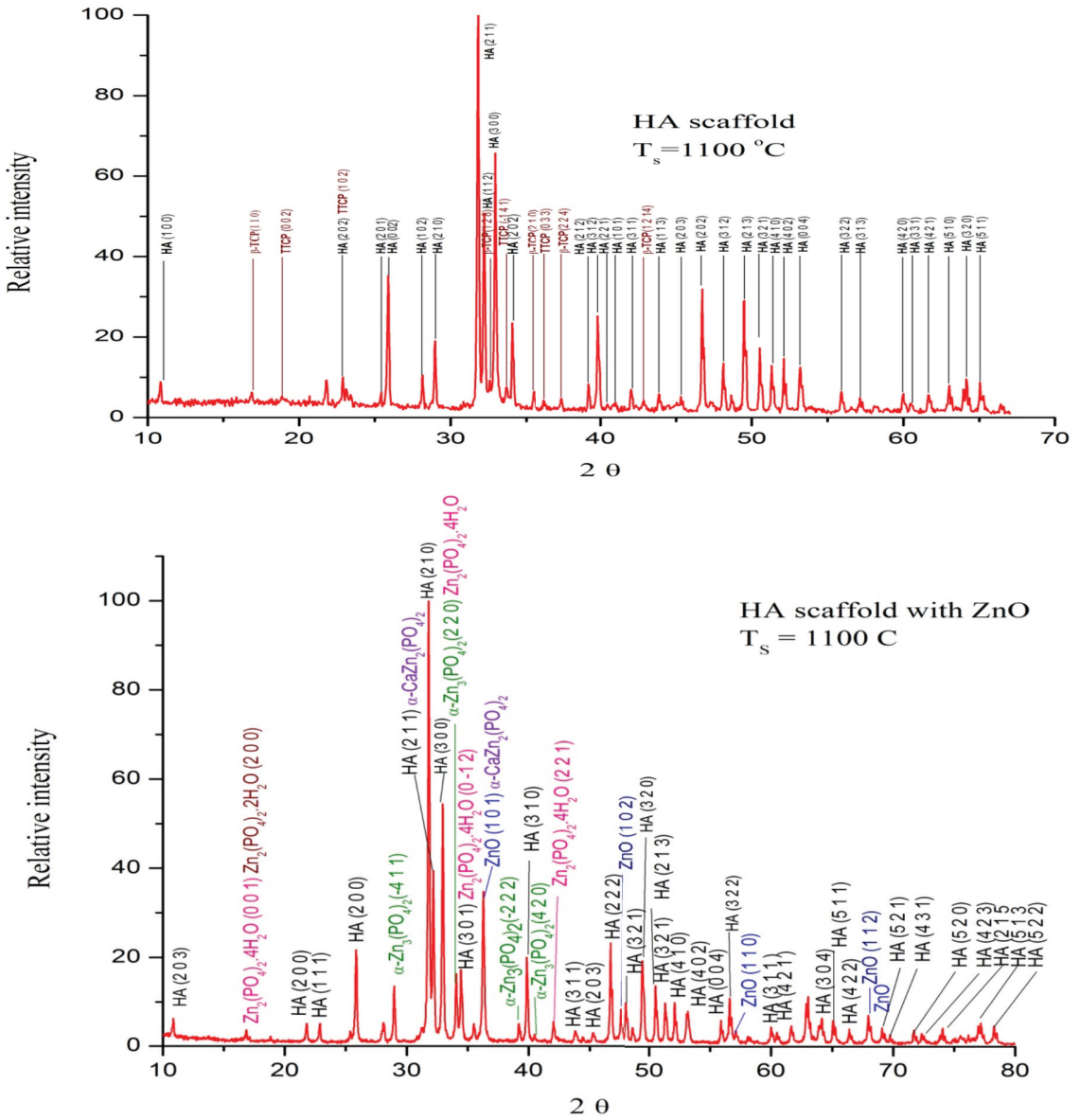

Fig. 3. HA scaffolds XRD spectra, (up) original HA, and (beneath) HA treated with ZnO, where Zn phosphates are formed on surface, when sintered at 1100°C.

The substitution of the Zn^{2+} cations is limited by the diffusion process to a value of 7.6 molar % of metal due to an energetically unfavourable condition (Mayer et al. (2000), Matsunaga et al. (2010), Faria et al. (2008), and Jin et al. (2010)). Finally, Zn from CS source has a bioavailability dependent on HA solubility, which is very low in the body, and its resorption can be extended for years. Furthermore, CS route only allows the synthesis of scaffolding granular products and it does not with 3-D porosity. However, the proposed process produces by sintering a solid state chemical reaction, which promotes chemical bonding between Zn^{+2} and PO_4^{-3} ions, atomic diffusion and partial thermal decomposition of the HA. These processes occur only at scaffold surface crystals, while preserving its three-dimensional porous structure.

Moreover, the concentration of Zn achieved by CS is limited by the stoichiometry of HA, characterized by a Ca/P molar ratio close to 1.67. Thus, the incorporation of Zn in molar ratio (Ca+Zn)/P leads to a lower Zn content, close to 11.65% (Mayer et al. (2000), Matsunaga et al. (2010) and Jin et al. (2010)), which represents a maximum of 2.5% by weight of HA. However, this Zn content produces cytotoxicity effect in the tissue, while values less than 1.2-1.5% allow osteoblast differentiation and growth (Pawlig al. (1999) and Mayer et al. (2000)), which is the range achieved with actual development. The biological validation by *in vivo* assays could quantify differences from untreated HA scaffolds.

4. Conclusions

This work demonstrated the feasibility of modified HA scaffolds surface with ZnO, preserving the three dimensional porosity. It provides a potentially osteoconductive capability, antimicrobial properties and a way to accelerate bone tissue reparative process. The developed process would reduce the risk of infection in bone reconstructive surgeries.

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