SINTERABILITY STUDY OF BOVINE-DERIVED HYDROXYAPATITE AND SILVER MICROCOMPOSITES

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This article evaluates silver's potential as a sintering additive for hydroxyapatite. Bovine-derived hydroxyapatite powders with 0.1 – 2wt. % Ag were used for evaluation. The samples were consolidated by casting or pressing and sintered at 1000℃. The results of morpho-compositional analyses showed that silver has a positive effect in sintering, ensuring a better packing behaviour of the green body and locally contributing to wetting and relieving of ceramic particles. The proposed preparation method allows the obtaining of dense or porous materials, based on the requirements for final products.

Keywords: bovine bone, hydroxyapatite microcomposites, silver, sintering

1. Introduction

Hydroxyapatite is a biocompatible and osteoconductive calcium phosphate and is proposed as an alternative for bone reconstruction due to its similitude with the mineral bone component - a nonstoichiometric form of hydroxyapatite with multiple crystal lattice substitutions [1, 2]. One simple preparation method of such

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nonstoichiometric hydroxyapatite is the thermal processing of animal bones. The method is economic and risk-free (if the bones are processed at temperatures above 850°C) [3, 4]. Different sintering programmes may be applied to bovine bone-derived ceramics for improving their properties through microstructure control. Generally, the sintering of calcium phosphates aims for microstructure densification while limiting grain boundary diffusion [5]. Various substances are used in sintering for enhancing the favourable effect of thermal treatment upon the ceramic materials properties. Addition of powders with melting points below the sintering temperatures is used for stabilizing material structure and controlling grain dimensions during densification. Various fluorides, phosphates, carbonates or oxides were previously studied as sintering additives [6, 7]. If the sintered product is intended for implantation, the main requirement is that microstructure densification shall take place without toxic phase formation [6].

In this study, the silver is proposed as a sintering additive for bovine bone-derived hydroxyapatite. Silver is well-known for its antibacterial effect, which was tested for different forms of incorporation in hydroxyapatite: in both soluble and ionic form, silver induces an antibacterial effect in the ceramic material, which varies directly with the incorporated concentration [8, 9]. In the last years, studies on silver addition in hydroxyapatite tended to focus on nanoparticles and similar nanostructures [8, 10, 11, 12] for their enhanced antibacterial effect [13]. This approach is regarded restrainedly because the effect of silver nanoparticles on the human body and the environment is not fully clarified yet [14]. However, when material processing includes thermal treatments that induce melting of silver, the use of nanoparticles is not necessary.

A series of studies evaluated the effect of thermal treatment on hydroxyapatite and silver-based materials obtained by chemical synthesis [15, 16, 17]. The effects of sintering upon hydroxyapatite with silver were evaluated mostly for high silver concentrations (5-30 wt. %) [15, 18, 19]. Later, the cytotoxicity evaluation of high silver concentrations in hydroxyapatite dismissed the perspective of using these materials in implantable medical devices [20]. The first arguments for using silver particles as additive in the sintering of hydroxyapatite derived from bovine bone were experimentally confirmed during a previous study performed by the authors [21], in which silver's potential as wetting agent was evaluated together with its antibacterial effect for concentrations of maximum 2 wt.% (considered nontoxic [20, 22, 23]).

The scope of the present study is to refine the procedure for sintering hydroxyapatite after silver addition. The study compares two powder consolidation methods, namely casting and pressing. The study objectives are: a) to obtain casted and pressed ceramic samples using bovine-bone derived hydroxyapatite powder and commercial silver microparticles; b) to characterize materials morphology after each preparation stage and c) to evaluate silver's

influence upon the compositional characterization of hydroxyapatite-silver microcomposites.

2. Materials and Methods

The microcomposites were prepared with hydroxyapatite powders obtained in the laboratory and commercial silver powder. Hydroxyapatite was obtained by thermal processing of deproteinized bovine bone pieces. Thermal treatment was performed at 1000°C, according to a previously described and evaluated procedure [4, 24]. Hydroxyapatite powder was obtained by milling. The ceramic particles were granulometrically sorted in order to provide powders with 100-200μm particle size. The powdered mixture intended for sintering was obtained by mechanically mixing the hydroxyapatite powder with different quantities of silver microparticles (2-10 μm particle size). Five samples were obtained in this step and were labelled based on the silver concentration (expressed in weight concentrations, wt. %): HA-0.1%Ag, HA-0.2%Ag, HA-0.5%Ag, HA-1%Ag, HA-2%Ag. Samples evaluation was performed by comparison with hydroxyapatite without silver (labelled HA). In the consolidation stage, hydroxyapatite – silver mixtures were further blended with ultrapure water and the resulting paste was formed in cylindrical shapes by casting and by cold isostatic pressing at 25 MPa, respectively. The consolidated bodies were sintered at 1000°C for 6 hours [25].

The morphological features and microstructure of the samples were evaluated after each preparation stage by scanning electron microscopy (SEM) using a Phillips XL30 ESEM TMP equipment. The powders composition was evaluated after silver incorporation by energy dispersive spectroscopy (EDS) using the auxiliary microanalysis system coupled to the SEM microscope (EDAX Sapphire UTW). The sintered samples were also evaluated by Fourier-transform infrared spectroscopy (FT-IR) using a Bruker Tensor 27 equipment with ATR annex, in the 4000-600 cm^{-1} region, with 4 cm^{-1} spectral resolution and 32 scans/experiment.

3. Results and Discussion

3.1. Powder precursors

The morphological characteristics of the powdered samples are depicted in Fig.1 (before and after mixing). The SEM analysis performed on the as-received silver powder revealed a uniform particle size distribution (2-10μm). The metallic particles have a rounded shape and display a slight agglomeration tendency.

The analysis of the hydroxyapatite powder confirmed the particle dimension given by the granulometric sorting. The ceramic powder has a uniform particle size distribution (100 – 200μm) with few smaller ceramic particles

remained in the proximity of the main ones. The particles have an irregular shape with multiples sides and rounded edges.

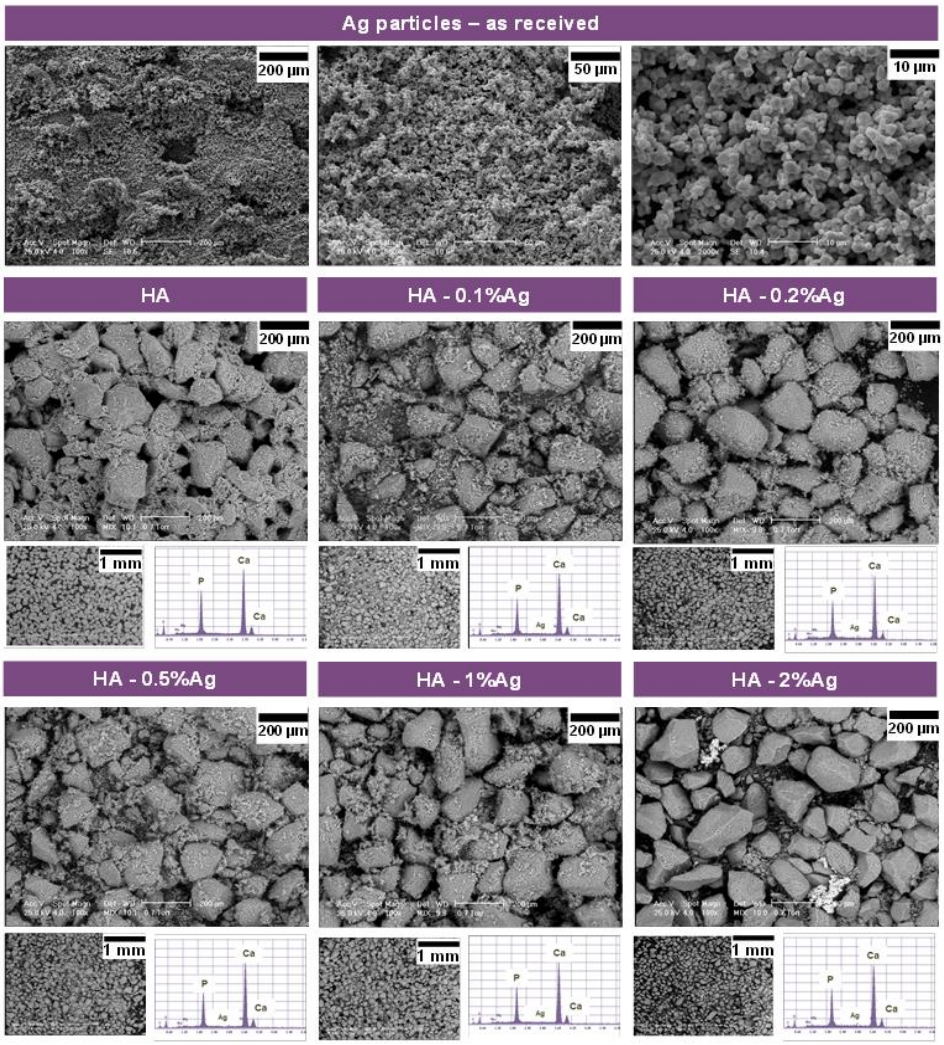

Fig 1. Morpho-compositional characteristics of precursors and powder mixtures with bovine-bone derived hydroxyapatite and different silver concentrations (wt. %)

The particle size distribution became bimodal after silver incorporation in hydroxyapatite, due to the differences between precursors particle size. The mechanical mixing provided a uniform dispersion of silver particles on the surface of hydroxyapatite particles. This observation is valid for all analysed samples, excepting the sample HA-2%Ag in which the silver particles exhibited a tendency for agglomeration between the ceramic particles. Similar agglomerations were

observed by other authors for concentrations of 1-5% silver after doping hydroxyapatite used in coatings [10] or porous scaffolds [11].

Fig. 1 also presents the characteristic EDS spectra of the powdered mixtures for sintering. Silver presence was identified in all analysed samples. The atomic Ca/P ratio of the samples varied between 1.73-1.91, confirming that the ceramic material is a nonstoichiometric hydroxyapatite [1]. No statistically significant correlation was identified between the atomic Ca/P ratio and Ag concentration. However, evaluation of this relationship was influenced by the local nature of the EDS analysis and by the agglomeration tendency observed for the samples with higher silver concentrations.

3.2. Green bodies

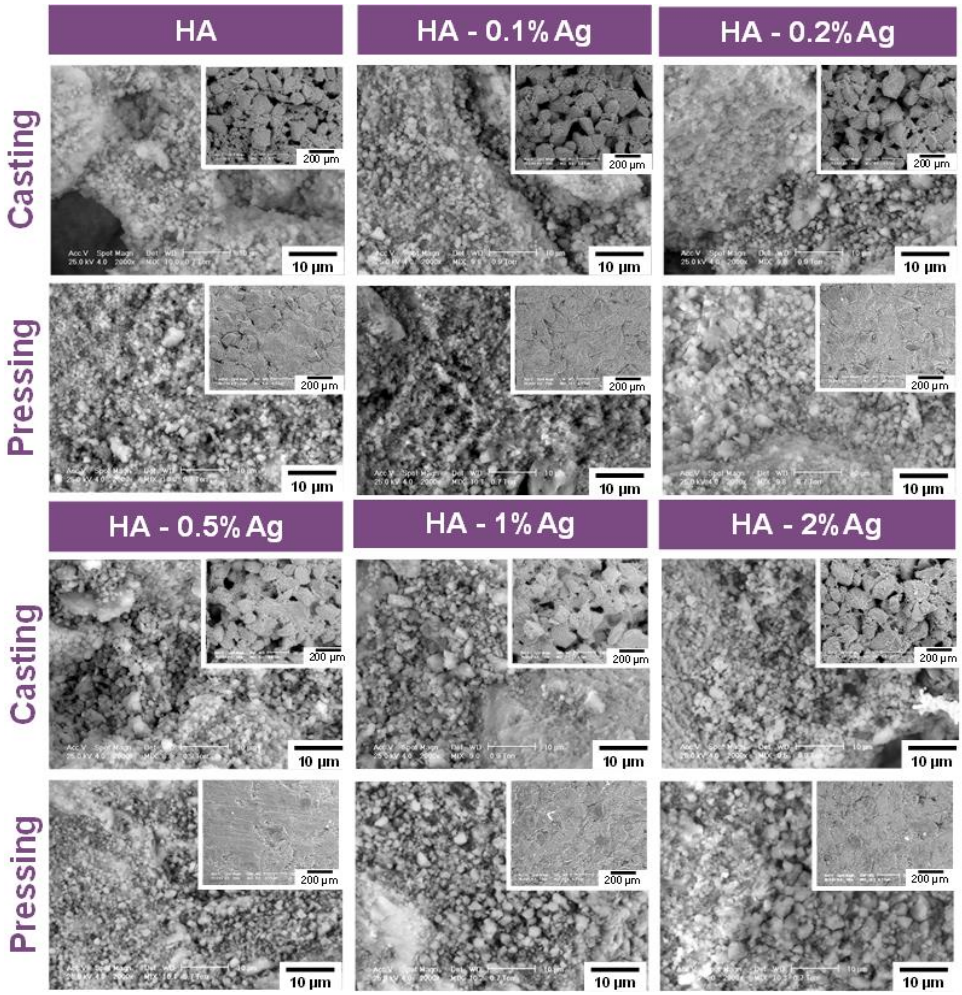

Fig 2. Comparison between the morphology of casted and pressed green bodies with bovine-bone derived hydroxyapatite and different silver concentrations (wt. %)

Fig. 2 displays the morphological features of the bodies obtained after the powder consolidation. Hydroxyapatite's granular morphology can be observed in the main SEM images in which the polyhedral grains are well-defined. This type of morphology is characteristic for similarly prepared hydroxyapatite [4, 25]. The casted samples are porous structures composed of consolidated ceramic particles. The particles shape is well-differentiated. The metallic particles are uniformly distributed on the surface of hydroxyapatite, locally contributing to a better particle connection. Agglomerations of silver particles were also observed during this stage for the samples with 1 – 2wt. % Ag (best depicted in the SEM results from insets in Fig. 2). Samples pressing significantly improved the packing behaviour of the consolidated body. The samples exhibited fewer pores and the shape of hydroxyapatite particles is less differentiated than the one of casted bodies. The increase of silver concentration slightly improved the packing behaviour with a compromise regarding the agglomerations of metallic particles.

3.3. Sintered bodies

The sintering of hydroxyapatite-silver particles was performed at 1000°C in order to avoid hydroxyapatite decomposition (which begins at approximately 1200°C for bovine bone-derived ceramics [4, 26]). This processing temperature, above silver's melting point (961.8°C), promoted the penetration of melted silver between the hydroxyapatite particles. This contributed locally to: a) maintaining a constant grain dimension, promoting densification without grain diffusion, and b) wetting the ceramic particles, facilitating the stress relieving of hydroxyapatite clusters and their reorganization into compact materials [21].

The positive effect of silver addition is better observed for the casted samples for which the porosity decreases with the increase of silver concentration (Fig. 3, inset). The results suggest an improved adhesion between the ceramic particles. This hypothesis was previously confirmed for pressed samples by the improved compression strength in direct correlation with silver concentration [21].

The FT-IR spectra presented in Fig.4 were obtained on the pressed samples. All spectra include the characteristic bands of hydroxyapatite: 970 cm^{-1} (ν_1 PO_4^{3-}) and 1040-1090 cm^{-1} ($\nu_3PO_4^{3-}$). The bands from 1400-1500 cm^{-1} indicate carbonate groups presence ($\nu_3CO_3^{2-}$) in material's structure. Hydroxyl (OH^-) characteristic bands were identified at 640 cm^{-1} (librational mode) and 3570 cm^{-1} (stretching mode) [27, 28, 29]. Silver addition in hydroxyapatite samples induced spectra modifications at: a) 1500 – 1800 cm^{-1}, corresponding to carbonate groups' $\nu_3CO_3^{2-}$ vibrations and bending mode of hydrogen-bonded H_2O molecules (Fig.4, detail A), and b) 3500 - 4000 cm^{-1}, corresponding to the stretching modes of hydrogen-bonded H_2O molecules (Fig.4, detail B). Slight intensity variations were also observed in the region corresponding to ν_4 PO_4^{3-} (600 cm^{-1}). These variations

manifests concomitantly with silver presence in the samples, without being affected by its concentration.

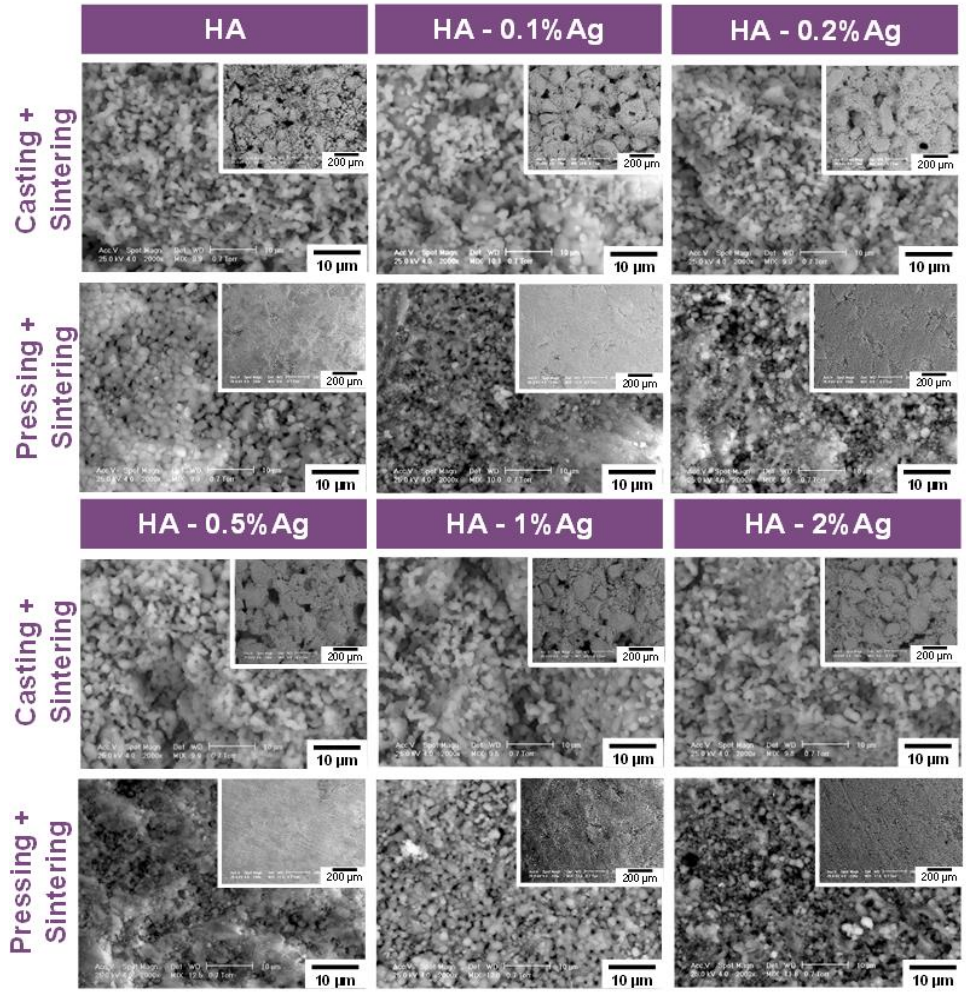

Fig 3. Comparison between the morphology of sintered bodies after casting and pressing bovine-bone derived hydroxyapatite and different silver concentrations (wt.%)

The aforementioned FT-IR results and previous insights regarding hydroxyapatite particle adhesion during sintering [21] outline the hypothesis of chemical adhesion between the hydroxyapatite particles through chemical compounds based on silver and molecules from the ceramic material. Although silver has an increased affinity for phosphorus, no significant variations of the phosphate bands were observed (excepting the minor variations at 600 cm^{-1}).

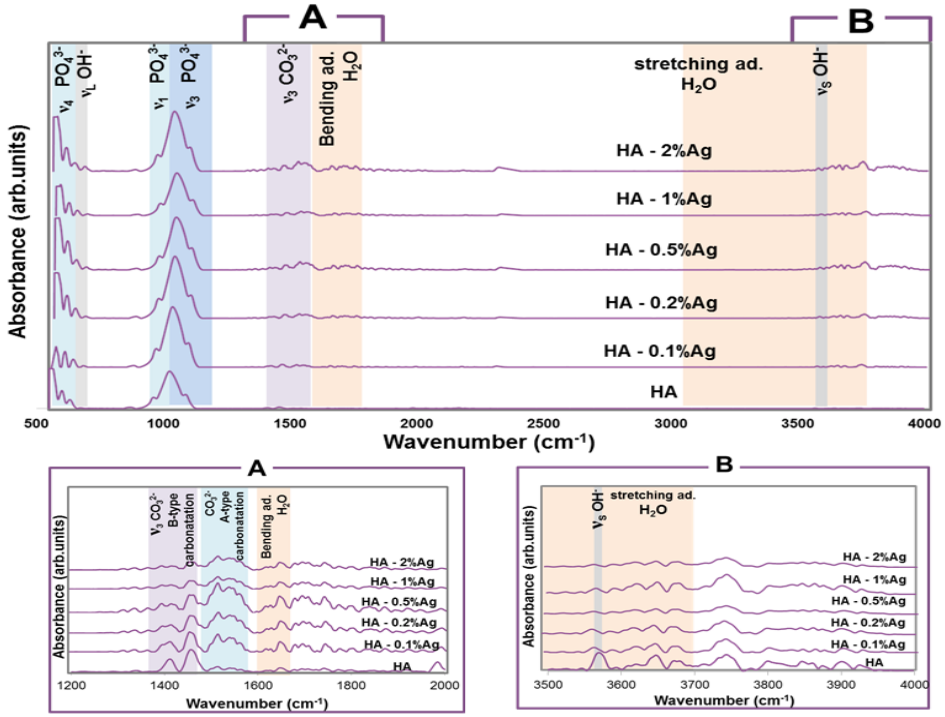

Fig 4. Representative FT-IR spectra of pressed sintered bodies with bovine-bone derived hydroxyapatite and different silver concentrations (wt. %)

5. Conclusions

Besides the well-known antibacterial effect, silver may be used as an additive in the sintering of bovine bone-derived hydroxyapatite, with positive effects in all processing stages. The silver particles used in this study ensured a bimodal distribution in the powdered mixture for sintering, contributing to a better packing behaviour in the green bodies. The mechanism of silver action as a sintering additive includes: formation of a metallic melt, local wetting, and film formation on the surface of ceramic particles with positive consequences in relieving particles clusters formed before sintering. These results require completion for an exhaustively characterization of the ceramic particle adhesion due to silver addition. Mechanical adhesion is argued by the morphology of bovine-bone-derived hydroxyapatite that facilitates the penetration and retention of the silver melted between the ceramic particles. Also, the hypothesis of chemical bonding, which derives from the FT-IR results (in which variations of carbonate and water band were observed), needs further confirmation. Silver's action was similar for both casted and pressed samples, with differences between these samples induced by the specific consolidation method. Sintering with silver led to the obtaining of dense ceramic materials in the pressed samples and porous materials in the casted ones. Powder consolidation may be further improved,

depending on the porosity required for the medical device, by adding supplementary porogen agents.

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