Strategies for production of naturally-derived calcium phosphates particles

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ABSTRACT

Besides their economical and biological advantages, naturally-derived ceramics manufacturability allows them to become candidates for solid freeform fabrication. In this study, controlled variations of heat treatment parameters were used for fine tuning of the structural and morpho-compositional properties of bovine bone-derived ceramics. A preliminary DSC-TGA study performed in air and argon atmosphere established the temperature and working environment influences upon material's thermal stability and phase composition. After thermal processing, SEM-EDS and FT-IR results showed that cooling conditions (air vs. frozen water) may be used for controlling the microstructural densification of the naturally-derived materials. An opportunity for controlling the material's structure was suggested by FT-IR results, but extended studies are needed for clarification. Similarly to chemically synthetised calcium phosphates, naturally-derived ones may be used for SFF if further processing stages are clarified.

Keywords: hydroxyapatite, bovine bone, heat treatment, naturally-derived ceramics

1 INTRODUCTION

Current biomaterial solutions for assisting bone regeneration meet only partially the design and manufacturing requirements for bone substitution materials. Many upgrade opportunities aim to develop smart biomaterials and devices, with adaptable features which allow their use in various biological environments,

Among the present materials that are used for bone substitution, the calcium orthophosphates (namely hydroxyapatite and various forms of tricalcium phosphate) have adequate properties for replacing bone tissue and may be obtained through several preparation methods. Besides classical chemical synthesis, a series of methods use animal bones as precursors for calcium orthophosphates. All these methods aim, through various processing stages, to eliminate bone's organic component and to improve the remained mineral component: a nonstoichiometric hydroxyapatite with multiple lattice substitutions (the most important ones are carbonate groups CO_3^{2-}, magnesium Mg^{2+} and sodium Na^- ions). The immediate advantages of these methods are their simplicity (no further doping procedures are required) and the availability of raw material [1, 2].

Various naturally-derived calcium phosphates are commercially available for assisting bone substitution [2]. Their use in manufacturing tridimensional scaffolds through solid freeform fabrication techniques (SFF) has multiple development opportunities, both related to method and material optimization.

In this paper, thermal processing method was used to prepare naturally-derived calcium phosphates. In addition to its simplicity, thermal processing is economical and provides microbiological-safe products, if high temperatures are used [1].

Various optimization strategies are proposed in this article through controlled variation of heat treatment parameters. The proposed research objectives are to evaluate the modifications induced in bovine bone tissue by thermal processing in different environments and to compare the structural and morpho-compositional features induced in the ceramic bulk materials by alternative heat treatment programmes.

2 MATERIALS AND METHODS

The choice of heat treatment parameters was based in the results of a combined thermal study, consisting of differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) in the range 25-1250°C, with 10°C/min heating rate, in air and argon atmosphere.

The ceramic bulk pieces were prepared through thermal processing of deproteinised bovine bone [3,4], at temperatures ranging between 1000-1200°C, with a heating rate of 10°C/min and a mantaining time of 6 hours. Different batches of materials were obtained by varying the cooling environment (slow cooling in air at room temperature versus rapid cooling – quenching – in frozen water).

The bulk products were evaluated through Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy coupled with energy dispersive spectroscopy (SEM-EDS).

3 RESULTS AND DISCUSSION

3.1 Thermal behaviour of bone precursor

The thermal analyses performed on bovine bone in the 25-1250°C temperature range revealed some thermal events that are typical for bone degradation and confirmed the influence of working environment upon the outcome of degradation reactions.

The bovine bone samples that were tested in air atmosphere (fig.1) showed three main thermal events, each corresponding to a critical stage in bone's thermal degradation. The first thermal event (exothermal) took place between 25-500°C and was accompanied by significant mass loss (almost 10% until 300°C and 50% until 500°C). This event is associated with the removal of adsorbed water from bovine bone's surface and with the degradation, combustion and removal of bone's organic component (exothermal peak at 400-500°C in fig.1). During a thermal treatment performed up to 500°C, temperature's influence was manifested only upon the organic component [1]. At temperatures near 500°C most of the organic component was already removed and some thermally induced modifications of the mineral component were revealed.

The second thermal event, an endothermal one, took place between 500 - 600°C without significant mass loss. This is a transitional event in bone thermal degradation, which corresponds to the end of organic component's removal and to the beginning of a mineral component recrystallization.

At 600-1200°C the results revealed a slight endothermal event, which was observed as a plateau in the DSC curve. The event is associated with minor mass loss (approximately 3%) at 900°C. In this temperature range, while the recrystallization of biological hydroxyapatite advances, the material preserves its thermal stability. The process was previously observed in morphology studies [3, 7] from which three stages of recrystallization were distinguished: lattice diffusion (up to 750°C), surface diffusion (up to 900°C) and grain boundary diffusion (up to 1000°C). The process is associated with crystal lattice rearrangements which lead to the removal of carbonate groups and water molecule embedded in the initial hydroxyapatite crystalline structure [7]. These phenomena are represented by the mass loss identified in TGA analysis [1, 4, 7]. The beginning of a fourth thermal event was observed starting with 1200°C, which suggests the hydroxyapatite's transformation in beta-tricalcium phosphate (β-TCP).

The thermal analysis performed in inert (argon) atmosphere promoted the decomposition of biological hydroxyapatite as suggested by the visible features of the DSC curve between 900°C and 1200°C. Although mass loss was not significantly altered in argon versus air atmosphere, an acceleration tendency was observed starting with 900°C.

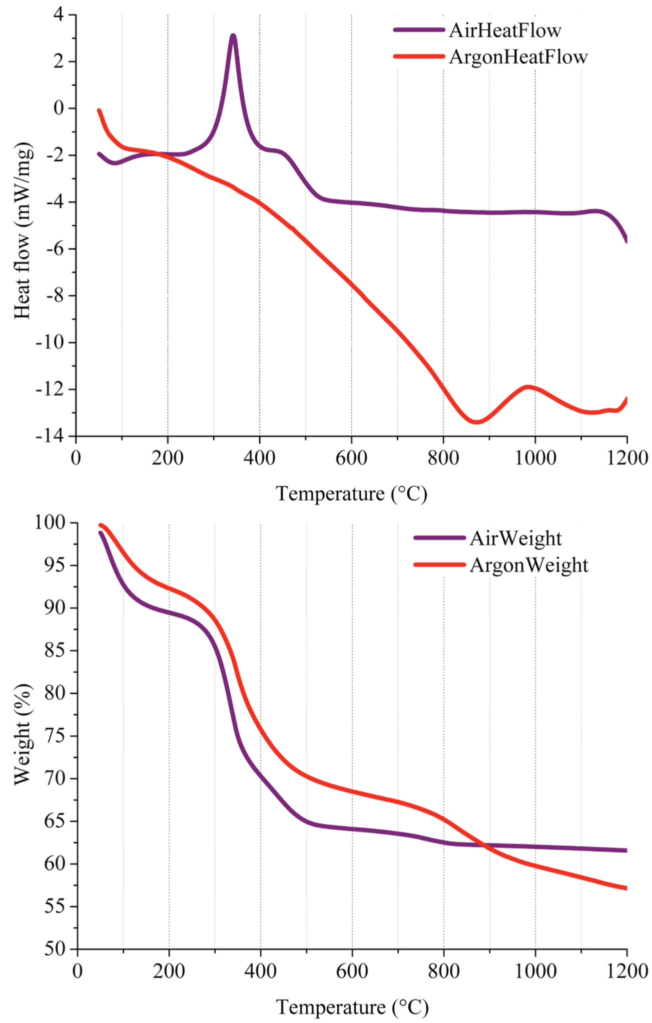

Figure 1: DSC (top) and TGA (bottom) curves for bovine bone reveal its thermal degradation between 25 - 1200°C in air and argon atmosphere.

These results confirm both temperature's influence upon the preparation of naturally-derived calcium phosphates and the effects induced by the heat treatment environment. The analysis suggests that thermal processing of bovine bone tissue may be performed in air atmosphere, up to 1200°C, if production of hydroxyapatite with various degrees of crystallinity is wanted, or may be performed in argon atmosphere if biphasic calcium phosphates are preferred. The second case (thermal analysis in argon) is relatively rarely tested [3, 8] and further extensions above 1200°C are needed in order to define the proper strategies for controlling the phase composition of these naturally-derived compounds.

3.2 Tunable bulk products

If the thermal processing of bovine bone exceeds 650°C, the resulted naturally-derived material is a porous ceramic and its porosity is a consequence of organic component's degradation. The further increase in heat treatment temperature stimulates the recrystallization of the remained material, with significant influences upon the morphology of the final products.

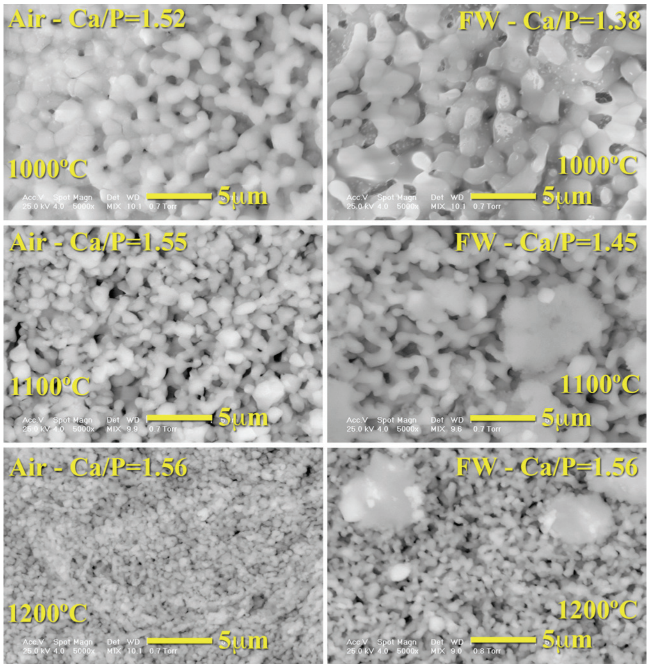

Figure 2: Representative SEM morphologies for bovine samples heat treated at different temperatures, and cooled in air atmosphere (left column) versus frozen water (FW – right column), as noted on each figure. Ca/P ratio is also mentioned for each morphology.

Thermal processing at 1000℃ with slow cooling in air atmosphere, at room temperature, led to the obtaining of a ceramic material with granular aspect and particles embedded in a porous structure, with uniformly distributed small-sized pores (fig.2). A further increase of the temperature at 1100℃ promoted particles growth and improved their attachment. Also, due to the solid material reorganization, the pores combined themselves in irregular shapes ones, increasing their dimensions. At 1200℃, the material exhibited a densified microstructure, with strongly attached particles. This evolution attests that the aformentioned grain boundary diffusion continues at temperatures above 1000℃ and ends with significant densification of hydroxyapatite's microstructure.

For quenching (rapid cooling in water with ice), the densification effect began from heat treating bovine bones at 1000℃. The ceramic microstructure exhibited attached particles embedded in a compact structure with few localised pores. Similarly with slow cooling, the heat treatment performed at 1100℃ led to grain boundary diffusion and an increase in pores size. Thermal treatment at 1200℃ followed by quenching led to a dense morphology with fewpores, in which particles shape is hardly distinguishable at microscopic level.

A series of EDS analyses were performed simultaneously with the SEM study and Ca/P ratio (fig.2) was chosen as an indicator for the compositional evaluation of heat treated bovine bone samples. The EDS results revealed that Ca/P ratio varies directly with heat treatment temperature, the higher values being obtained for samples treated at 1200°C (Ca/P = 1.56, corresponding to a calcium deficient hydroxyapatite [9]).

The comparison between Ca/P ratios of differently cooled samples confirmed the influence of cooling conditions upon the composition of naturally-derived calcium phosphates with decreased Ca/P ratios (but with an abrupt increase at higher temperatures) for the quenched samples.

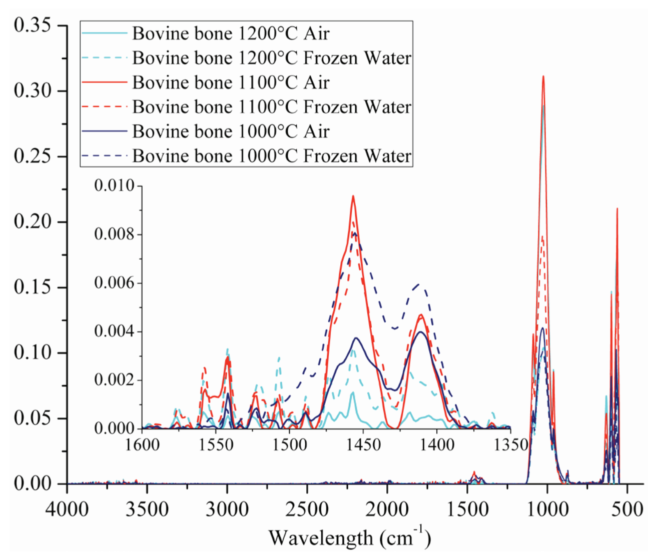

Figure 3: FT-IR spectra for bovine bone processed at 1000 - 1200°C. Comparation between slow cooled samples (continous lines) and quenched samples (dashed lines)

The FT-IR spectra (fig.3) of the bulk components encloses peaks characteristic for hydroxyapatite [10-13]. The peaks identified between 1500 - 1400 cm^{-1}, corresponding to ν_2 and ν_3 stretching vibration of CO_3^{2-} groups, show that the heat treated material is a B-type carbonated hydroxyapatite (in which the CO_3^{2-} groups substitute phosphate PO_4^{3-} from the hydroxyapatitic structure).

Thermal processing at 1100°C did not induce significant changes in FT-IR spectra outcome, except for a minor diminishment of CO_3^{2-} peaks intensities. The material has a high degree of crystallinity, as suggested by the sharp and well-defined aspect of CO_3^{2-} peaks (1500-1400 cm^{-1}) and PO_4^{3-} peaks (1150 – 900 cm^{-1} and 600-550 cm^{-1}).

The increase of heat treatment temperature at 1200°C led, as suggested by the DSC-TGA analysis (fig.1), to structural modifications confirmed by FT-IR study (fig.3) through a decrease in PO_4^{3-} peaks intensity (ν_3 stretching band at 1150 – 900 cm^{-1}) and a modification of the general aspect of CO_3^{2-} peaks.

The cooling conditions influence (slow cooling in air versus quenching in frozen water) is also revealed in the CO_3^{2-} groups characteristic region (1500-1400 cm^{-1}) with broader bands and increased intensities for the quenched samples.

The careful management of cooling conditions leads to reproducible morphologies, similar microstructures being obtained by the authors in preliminary studies [13, 14]. Also, the modifications induced in the general outcome of the CO_3^{2-} bands in FT-IR spectra suggests that quenching encourages the movement of these groups from their initial location within the crystalline structure du to thermal stresses induced by rapid cooling. Extended studies may reveal the possibilities for controlling the structural organisation of the naturally-derived calcium phosphates and their correlation with material properties.

4 CONCLUSIONS

The complementary analyses performed in this study revealed the influences induced by temperature and working environment and cooling conditions upon the morphological, structural and compositional characteristics of naturally-derived calcium phosphates. Similarly to heat treatment temperature and working environment, the cooling conditions may be controlled for inducing the desired characteristics in bovine bone-derived ceramics. Although intensively studied for metallic materials due to its associated durification, quenching is less studied for ceramic materials. Its implementation in heat treatment programmes performed at high temperatures may contribute both to a better control of mechanical properties (through microstructure densification) and to fine tuning of degradation properties (through stabilization of α-tricalcium phosphate, a highly biocompatible and biodegradable phase).

Currently, this field is limited by the compositional variability of bovine bone precursors, due to differences in cattle age, breed, diet and living region, which may affect the level of control achieved for thermally-induced properties.

Similarly with calcium phosphates prepared through chemical synthesis, the perspectives for using naturally-derived calcium phosphates in additive manufacturing for bone substitution are optimistic. The next stages in adapting these materials for solid freeform fabrication techniques concern the adequate powder processing, evaluation of specific SFF factors upon the material (like thermal effect induced by selective laser sintering), and identification of biocompatible binders which assure mechanical resistance during manufacturing and subsequent utilization.

5 ACKNOWLEDGEMENT

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