

DIRECT MANUFACTURE OF HYDROXYAPATITE SCAFFOLDS USING BLUE LASER

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Abstract. The study deals with the direct manufacturing of hydroxyapatite scaffolds using selective polymerization of the slurry liquid phase. The bovine hydroxyapatite has great similarity with the human bone structure, making it able for a direct connection with the bone tissues. This study aims to obtain scaffolds using a new technique of rapid prototyping, obtained by polymerization of acrylic resin (liquid phase of slurry) by ultraviolet light present in a range of the band spectrum emitted by the blue laser light. Sub-micrometer hydroxyapatite was obtained by the calcination and grinding of bovine bone in a vibratory mill. Mixtures of hydroxyapatite and resin were prototyped in three-dimensional pieces and sintered afterward and subjected to blue laser emission path directed in a CNC equipment. Grounded particles obtained in the grinding vibratory mill, with equivalent diameter of 0.35 microns, were reactive enough to compensate the low green densification below 50 vol%. Polymerization tests realized indicated that the incidence of the laser with fluency of 170 mW.s/mm² promoted the curing of the 0.5 mm diameter pieces in depth about 0.5 mm, which allowed the prototyping of the scaffolds with sufficient mechanical strength for handling.

Introduction

Rapid prototyping is of great importance for the medical field, whether for planning surgery or for the direct manufacturing of implants. The prototyping of ceramic materials allows the obtaining of custom scaffolds, designed through a tomography scan and prototyped in an implant shape like the patient's bone defect, adding any desired porosity and complex geometries. This manufacturing flexibility arises in according to the type of construction of the parts which is made layer by layer, in other words, the part is obtained by adding successive layers of material to reach its final geometry.

The ceramic materials due to their high chemical stability, good biocompatibility and good tribological properties are potential candidates for use as scaffolds for bone disorders [1, 2]. The bioceramics may also be designed to deliver biologically active substances designed to repair, maintain, restore or improve the function of organs and tissues in the body. Several materials such as calcium phosphates, glasses and glass-ceramics are able to carry and then deliver drugs, hormones, growth factors, peptides or nucleic acids in a controlled way [3].

Hydroxyapatite is a bioactive bioceramic, biocompatible, osteoconductive, osteoinductive and very similar structurally, physically and chemically with bone mineral matrix, commonly used in bone replacements [4, 5]. However, the ceramic scaffolds typically do not have the exact shape and dimensions of the defect, being used more often in granules, blocks, and cylinders. According to Vallet-Regí and Ruiz-Hernández (2011) [3], scaffolds of bioceramics must meet requirements equivalent to bone porosity, and it is necessary to find methods that provide parts conformation with interconnected porosity, with porosity values in the range of microns, and that preserves the nanometric dimensions of precursor materials.

This study aims to obtain bovine hydroxyapatite scaffolds using a new rapid prototyping technique, obtained by polymerization of acrylic resin (liquid phase of the slurry) by ultraviolet light present in a range of band spectrum of light emitted by a blue laser.

Materials and Methods

For the manufacture of bovine hydroxyapatite by rapid prototyping by polymerizing the liquid phase of the slurry by ultraviolet light the raw material was prepared from bovine femurs. The hydroxyapatite powder was processed in a ball mill and vibrating mill to obtain submicron particles with sufficient reactivity to compensate the absence of pressure forming. Slurries of hydroxyapatite and acrylic photo reactive resin was underwent blue laser emission with directed path in a CNC equipment to obtain the three-dimensional prototypes.

Obtaining and characterization of hydroxyapatite. Hydroxyapatite was obtained from bovine femurs of animals screened by the *Sistema Brasileiro de Rastreabilidade Bovina e Bubalina-SISBOV (Brazilian System of Traceability Bovine and Buffalo)* to ensure that they were not contaminated by pathogens agents, bones were passed by autoclaving and chemical processing to remove organic material and was granulated in a mill until the grain size was below 750 μm and after was calcinated at 900 $^{\circ}\text{C}$. The process followed the ASTM F1185-03 specifications.

For the characterization of hydroxyapatite the X ray diffraction analysis (XRD), Fourier Transform Infrared Spectrometry (FTIR) and Wavelength Dispersive X-ray Fluorescence (WDXRF) were used. The X-ray diffraction tests were performed on X-ray diffractometer Multiflex, Rigaku, at the Laboratory of X-Ray Diffraction of the Center for Materials Science and Technology – CCTM/IPEN. Data were compared with the XRD and FTIR results made by Sigma-Aldrich hydroxyapatite patterns powder, that is allowed for clinical use, which has purity of 99.95%, molecular weight 502.31 g/mol, relative density of 3.140 g/cm³ and is insoluble in water.

The composition, according to ASTM F1185-03, which determines the maximum concentration of heavy metals allowed for hydroxyapatite obtained from natural sources, was held the analysis of WDXRF in spectrometer RIX3000, Rigaku, at the Laboratory of Fluorescence X-ray of the Center for Chemistry and Environment – CQMA/IPEN.

Milling. The hydroxyapatite was ground in a polyethylene jar with volume of 300 cm³ loaded with 40 vol% (500 g) of grinding elements (\varnothing 10 mm zirconia-3Y balls), giving a working volume of 100 mL. The jar was loaded at a concentration of 30 vol% solids and rolling at 104 rpm during 48 hours followed by vibratory mill during 48 hours. Milling was accomplished in an alcoholic medium using para-aminobenzoic acid as dispersant. The jar was discharged and the slurry was dried with hot air at approximately 80 $^{\circ}\text{C}$. Powders were granulated and classified into meshes (sieve) of stainless steel # 200 mesh \leq 74 micrometers.

Measurement of particle size. Tests were performed to measure particle size in the 5000ET Sedigraph equipment of the Micromeritics.

Rapid prototyping with blue laser. The sub micrometer hydroxyapatite powder was mixed with acrylic resin curing with ultraviolet light by Three Bond[®] and subjected to application of a laser light blue. An objective lens with an enlargement of 12.5x was applied to focus the laser beam.

Slurries in volume proportions of 1 HA: 3 resin and 1 HA: 1.5 resin were prepared and tested for resistance to handling and microstructure.

The laser used has 405 nm wavelength, 3 V voltage, 30 mW power and amperage below 250 mA. The commercial resin TB3003E by Three Bond[®] is liquid, with a viscosity of 2000 mPa.s, a density of 1.07 g/cm³, a Hardness of 75 Shore A and curing time of 100 seconds with an application of 30 mW/cm².

Sintering. The sintering of the prototypes were performed in an electric chamber box furnace Lindberg Blue/M in air atmosphere, from room temperature to 160 $^{\circ}\text{C}$ with a heating rate of 2.7 $^{\circ}\text{C}/\text{min}$, then to 600 $^{\circ}\text{C}$ at 4 $^{\circ}\text{C}/\text{min}$ from 600 $^{\circ}\text{C}$ to 1100 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$ and finally to the temperature of 1300 $^{\circ}\text{C}$ at 6 $^{\circ}\text{C}/\text{min}$, with a landing of 120 minutes followed by cooling inside the furnace to room temperature.

Results and Discussions

After the processing of bovine bone tissues, hydroxyapatite granules were obtained. The FTIR and XRD spectra obtained for the bovine hydroxyapatite granules and hydroxyapatite Sigma-Aldrich, used as a standard for comparison, are shown in Fig. 1a and Fig. 1b, respectively.

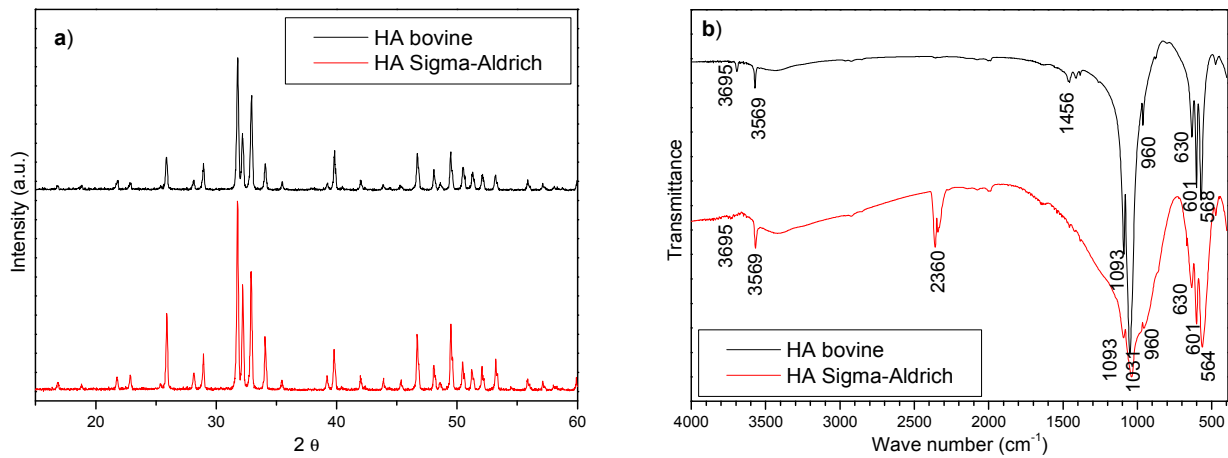


Fig. 1 – a) X-ray diffraction and b) FTIR spectra of hydroxyapatite.

As seen in Fig. 1a, bovine hydroxyapatite has the same crystallographic pattern of hydroxyapatite from Sigma-Aldrich. The XRD spectrum shows that the powder obtained from bovine bone is hydroxyapatite phase.

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) has a frequency of vibration for the phosphate and hydroxyl groups. In Fig. 1b the bands at 564, 568, 601, 960, 1031 and 1093 cm^{-1} are related to the vibrational mode PO_4^{3-} . The band at 1456 cm^{-1} in bovine hydroxyapatite refers to vibrations of CO_3^{2-} groups, indicating a carbonated hydroxyapatite. According to the authors Stoch et al. (2000 and 2003) and LeGeros and LeGeros (1993) [6, 7, 8] in biological apatites PO_4^{3-} ions are replaced by CO_3^{2-} ions. Kusrini and Sontang (2012) [9] also observed that bovine hydroxyapatite contains phosphate ions, hydroxyl and carbonate. The band at 2360 cm^{-1} in the Sigma-Aldrich hydroxyapatite is related to the vibrations of the CO_2 molecule.

The bands at 3569 and 630 cm^{-1} are related to the vibration of OH group characteristic of the structure of hydroxyapatite. The vibration of OH is only for hydroxyapatite and its intensity is considered low compared to the force vibration of P-O, due the stoichiometry of hydroxyapatite [7].

Hydroxyapatite showed no presence of heavy metals in their chemical composition as shown in Table 1, which is in accordance with the specifications of ASTM F1185-03, which determines that the maximum heavy metals in the chemical composition should be less than 43 ppm.

Table 1 – Chemical composition of bovine hydroxyapatite.

Elements	Percentage [%]
CaO	60.2 ± 0.5
P_2O_5	38.3 ± 0.5
MgO	1.2 ± 0.3
Na_2O	0.17 ± 0.03
SO_3	0.08 ± 0.03
SrO	0.03 ± 0.01
Fe_2O_3	0.03 ± 0.01
ZnO	0.02 ± 0.01

Millig. During the grinding performed in an alcoholic medium para-aminobenzoic acid (PABA) was added as deflocculant and able to reduce the average equivalent spherical diameter 0.35 μm after 48 hours in a ball mill and vibrating mill for 48 hours, as shown in Fig. 2. With the

use of this deflocculant all particles maintained the equivalent spherical diameter less than 1.0 micrometer.

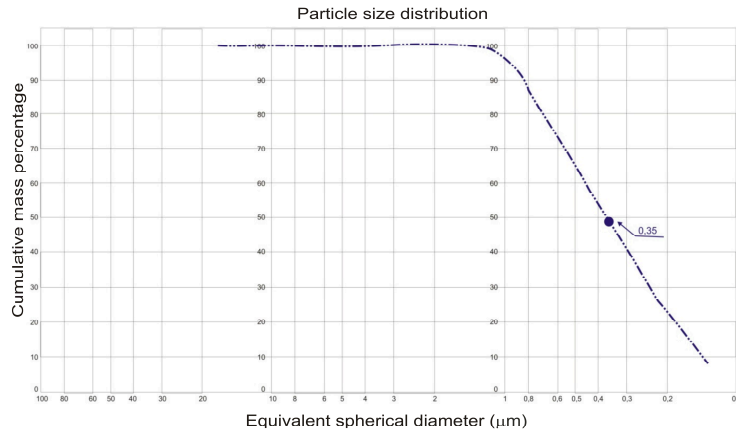


Fig. 2 – Performance of grinding in alcoholic slurry.

Rapid prototyping. Figure 3 shows the pictures of the acrylic resin polymerized after being subjected to the blue laser with emission path directed on CNC equipment. The incidence of the laser with fluence of 170 mW.s/mm^2 promoted the curing in the format specified in the project.

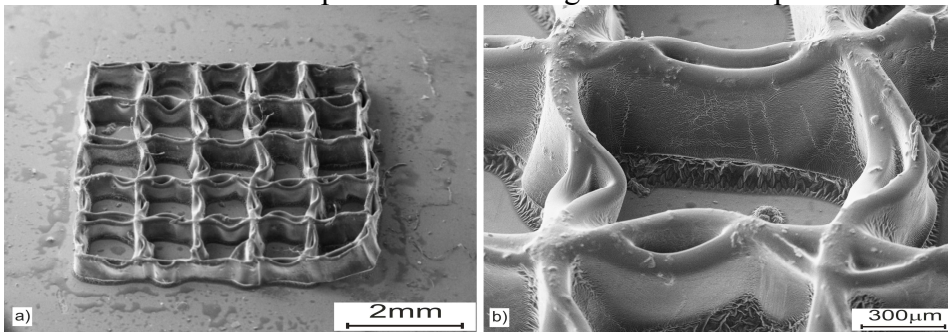


Fig. 3 – Images of the polymerized resin with ultraviolet light: in a) low magnification and b) higher magnification.

Figure 4 shows the microstructure obtained by prototyping the parts using blue laser and resin, using two proportions of resin and hydroxyapatite sintered at $1300 \text{ }^\circ\text{C}$. The first ratio was 1 HA: 3 resin in volume (Fig. 4a) and the second was 1 HA: 1.5 resin in volume (Fig. 4b). It can be observed that the ratio of 1 HA: 3 resin has high porosity and hence lower mechanical strength, allowing the part to be broken with handling. The ratio of 1 HA: 1.5 resin showed a good sintering, absence of porosity, and was used for the tests.

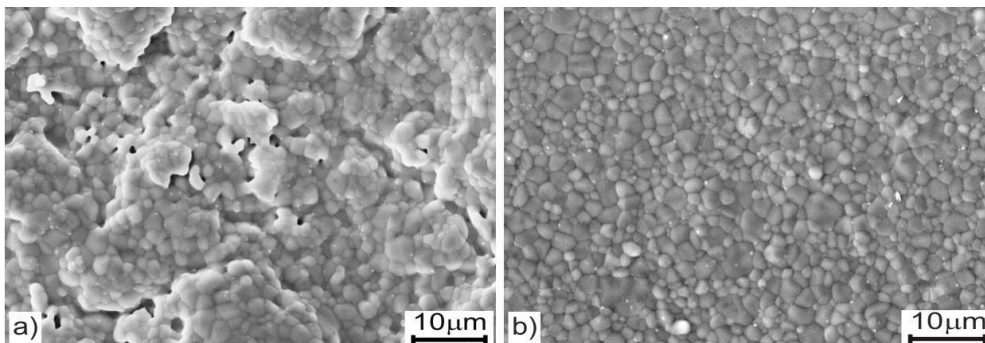


Fig. 4 – Images of the sample surface prototyped and sintered at $1300 \text{ }^\circ\text{C}$. In a) ratio of 1 HA:3 resin and in b) ratio of 1 HA:1.5 resin.

Figure 5 shows the three-dimensional design of the part. Three layers are designed with a thickness of 0.5 mm each and macropores of 2 mm. The designed size of part was 7x7x1.5 mm.

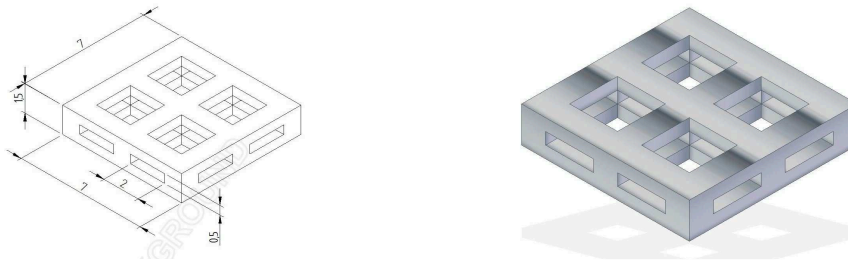


Fig. 5 – The three-dimensional design of the part.

Figure 6 shows the prototyping of a three-dimensional part and its microstructure. The prototyping was performed in three layers, but the delamination of the layers occurred. Regarding the microstructure is possible to observe a good sintering with very low porosity, which indicates the good reactivity of the powder.

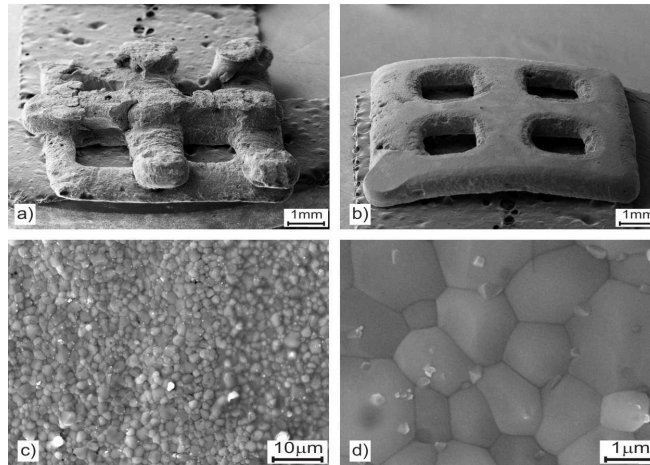


Fig. 6 – Three-dimensional parts prototyped and sintered at 1300 °C during 2 h, a) and b) prototyped three-dimensional parts, c) and d) microstructure on lower and higher magnification scale, respectively.

Conclusions

The milling process has proven quite suitable for obtaining fine hydroxyapatite powder. The use of para-aminobenzoic acid as deflocculant decreases the average equivalent spherical diameter to 0.35 μm and all particles are in equivalent spherical diameter less than 1 μm.

The incidence of laser with fluence of 170 mW.s/mm² proved to be sufficient for the polymerization of the resin and also the slurry of resin and hydroxyapatite mixture.

The slurry with ratio 1 HA: 1.5 resin (by volume) showed good sintering, mechanical strength to handling and low porosity which entitles it as an ideal relation for obtaining prototypes.

The powder exhibited good reactivity showing a microstructure with very low porosity.

To avoid the delamination between layers the prototyping just in bordering of solid part is being proposed.

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