Bone and Tissue Regeneration Insights

SHORT REPORT

Properties of Hydroxyapatite from Bovine Teeth

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Abstract: The objective of this work was to study the production of hydroxyapatite (HA) from bovine teeth. Hydroxyapatite (HA) was produced from bovine teeth powder after calcination at 1150 °C. It was discovered that the sample preparation process influences its properties, so, crystal structure and thermal stability of HA were investigated. The X-Ray diffraction analysis (XRD) results confirmed that HA has been successfully produced. Fourier transform infrared spectroscopic (FT-IR) study confirmed the presence of hydroxyl (OH^-) and phosphate (PO_4^{-3}) functional groups. The scanning electronic microscope (SEM) was employed to identify the surface morphology of HA, and showed the nanoporous structure throughout the matrix. The sample constituents such as Ca, P, K ... etc., and their values were determined by Energy dispersive X-Ray (EDX).

Keywords: bovine teeth, bovine hydroxyapatite (BHA), hydroxyapatite properties, XRD, SEM, calcium and phosphate, FT-IR, EDX

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Introduction

For more than a century a research has been carried out trying to find a suitable material to repair or replace bone segments, either by "Autograft" which is clearly osteogenic, and is considered as a limited supply especially when it is used in children as a bone graft, or by "Allograft" which is demonstrated lower osteogenic capacity and it is slower than the new bone formation.[1] To reduce or eliminate the need for bone grafting substantial effort that has been invested in finding a suitable artificial bone. A variety of biomaterials have been investigated as bone graft substitutes, such as the hydroxyapatite which is frequently used as a calcium phosphate based compound, it is classified as bioactive material.[2,3] The HA as a ceramic material has a poor mechanical properties, that why it is used in non-load bearing applications such as orthopedic dental and maxillofacial applications. The HA chemical constituents and structure is similar to the human hard tissues. It is generally accepted that the human bone contains large amount of calcium and phosphate, calcium (about 85%) causes bone hardness.[4] Moreover human and animals teeth have the same complex structure, which is consisting of three layers, the first outer layer is enamel which contains of 92%–96%wt HA, 1%–2%wt organics and 3%–4% H_2O, while the second layer is the tooth bulk which consists of dentin 70% HA, 18% organics and 12% H_2O, and the third layer is the outer surface consist of HA cement.[5]

There are two types of HA either synthetic or from natural origin; synthetic HA is the most frequently used, although it is expansive and does not completely match with the human teeth chemical composition, but it is more reliable.[4] On the other hand, the natural HA has an economic advantage, but there are significant concerns about the use of natural HA, due to the potential transmission of dangerous diseases when it is not well prepared,[4] these infections include transfer of viruses such as Creutzfeldt Jakob, encyphalopathy, BSE, HIV, Hepatitis B and C and AIDS etc.[6]

Due to the attractive properties of HA, various techniques have been developed to produce synthetic hydroxyapatite such as wet precipitation, hydrothermal and electrochemical … etc. On the other hand natural HA can be produced from bovine bones, human teeth, goats, swine bone, sheep bone, sheep teeth and egg shell.[4]

The aim of the present work is to prepare HA derived from bovine teeth and evaluate the material chemical properties. We focus on the bovine teeth which are readily available in Egypt, and it has been proven to be a good source of HA.[3]

Materials and Experimental Procedure

The research methodology included two stages, the first is the HA preparation and calcination, and the second was identifying the properties by using XRD, IR, EDX, Brunauer, Emmett and Teller (BET) for surface area determination, thermal stability and SEM.[3]

Bovine hydroxyapatite preparation

The process of preparing HA from bovine teeth was originally developed for its better results and lower cost; the bovine teeth were collected from the local slaughter houses. The teeth samples were cleaned by boiling to remove organic substances and collagen. This was done to avoid soot formation in the material during the calcinations process.[6] Raw teeth impurities that were sticking on the teeth were shaved and removed, and then irrigated with a brush in running water, followed by boiling in distilled water for 30 minutes. This process was repeated three times till it yielded white and clean teeth, and then the teeth samples were dried in the sun for 3 days.

The teeth were calcined in the muffle at 735 °C (7 °C/min), and then hold for 1 hr, then left to cool, it was observed that at 450 °C huge amount of vapors evolved from the sample. The calcinations was in humid atmosphere to avoid any dehydration in the furnace, the sample was sintered again to 1150 °C (7 °C/min) for 1 hr.[4] This sintering process took place to ensure that the organics are completely removed and that the material is safe and to avoid any microbial contamination.[2,7,8]

For ensuring the HA purification an initial pH was determined for the distilled water, then the pH was determined for the annealed teeth water, which was prepared after the teeth were milled and stirred for several minutes in distilled water, the pH results were compared. This experiment was repeated till the pH of the teeth washed water is equal to the water pH, which ensure the bovine hydroxyapatite (BHA) purity.[9]

Characterization

The following tests were performed on sintered samples of size less than 45 micrometer.

The teeth powder was analyzed by using XRD, the measurement was carried out by a monochromatic narrow beam and nickel filtered; the scan speed was equal to 2 deg/min, and sampling pitch = 0.018°, in case of samples containing iron that can be analyzed by using the standard cu X-Ray tube.

The FT-IR was used to determine the chemical function by the wavelength range was up to 4000 cm^{-1} versus the transmittance, the KBr is used as a reference to prevent fluctuations in the output.

The EDX used by the HA powder sample was coated by carbon to improve the resolution, and then the X-Rays were obtained to determine the chemical elementary composition of the sample. The inorganic elements were determined in presented percentages.[2]

The BET model Coulter SA 3100 Beckman was used to measure surface area based on the nitrogen gas adsorption. The sample was pretreated in a test-tube and under vacuum, then degasified for 24 hr at 400 °C. The surface area was performed by relative pressure which was obtained, and after the nitrogen adsorption, it is calculated by Langmuir equation then integrated to all layers to measure specific surface area (m^2/g).

The HA thermal stability was studied by using one apparatus named SDT Q600 test the differential thermal analysis (DTA) and Thermo gravimetric analysis (TGA), which were used for more precise tests. It is used to determine any transitions or reactions by increasing the temperature, the thermal tests carried out up to 1200 °C with constant rate 10 °C/min.

The SEM model Jeol JSM 6360-SEM was used for pore structure determination, the bovine teeth was placed on an aluminum holder, adhered with a carbon conductive tape, and covered with a golden cap and of thickness approximately 200 °A, used for high resolution. All the images were taken with a backscattered electrons detector, this allows visual inspection for better understanding of the material structure.[7]

Results and Discussions

In this research the results testing properties of the produced BHA which is produced, imply that all possible diseases existing in the bovine teeth had been completely eliminated.[6]

The results of this study of high temperature of calcinations during BHA production 1150 °C implies that any possible disease existing in the bovine teeth that has been completely eliminated furthermore, earlier reports have suggested that interesting bioceramics could be produced from high-temperature calcined. This has also been confirmed by cell culture and animal studies.[9]

The XRD pattern as (See Fig. 1) shows the HA characteristics, the pattern sharpness peaks indicate the good sintering process of the HA and it is free from organic components. The pattern shows the bovine teeth sample was calcined after defatting at different 2θ. The obtained result was compared with the HA previous studies (See Fig. 2), and it was observed that the result of BHA prepared is similar to the XRD HA pattern.[2,7,9–11]

The results of the earlier studies for the FT-IR detectable transmittance peaks were identical to the HA pattern in this study (See Fig. 3); contains PO_4^{+3} and OH^- without expressing any impurities (See Table 1 and Fig. 4).[9,11,12]

The EDX result (See Fig. 5) revealed the presence of inorganic elements which were identified as calcium and phosphate, in addition of traces of magnesium and potassium, these elements values were

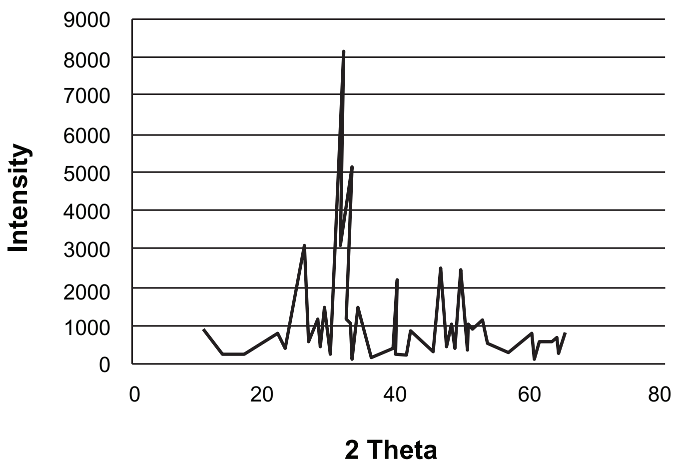

Figure 1. X-ray diffraction pattern of the calcined bovine teeth.

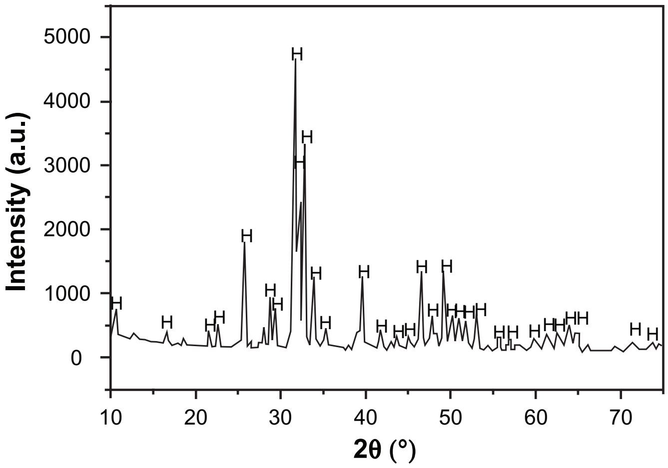

Figure 2. X-ray diffraction pattern result of previous study hydroxyapatite.[7]

Table 1. BHA FT-IR peaks transmittance.

Absorbance frequency Vibration cm^{-1}	Assignments
569.4	O-P-O bending
603	P-O symmetric stretching bands
1048.3	PO asymmetric stretching
2924.7	PO asymmetric stretching
3445.5, 3572 and 3572	O-H in plane bending

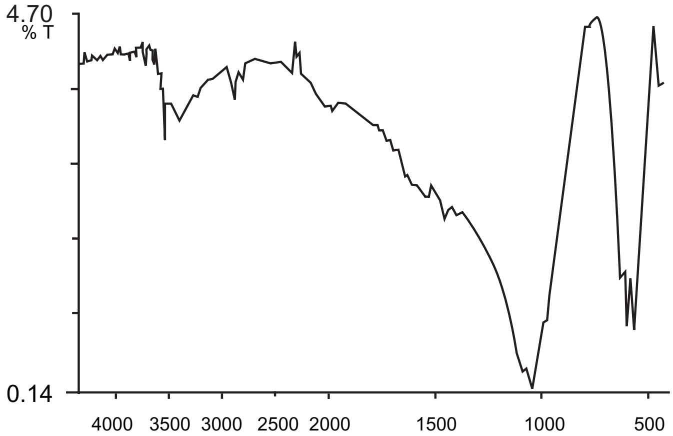

Figure 4. Infrared spectrum transmittance line of hydroxyapatite powder sintered at 1150 °C.

permitted to be used in medical applications with reference to USA 1990 Pharmacopeia.[11]

The nourishments, origin, sex and age, sintering temperature are factors that may have impact on the sample constituent such as Ca, P, K etc.

The BET results (See Figs. 7, 8) showed that the specific surface area is equal to 1.666 m^2/g and the specific total pore volume = 0.0178 ml/g, and the observed isotherm graph (See Fig. 6).

As regards the HA TGA and DTA results (See Figs. 9, 11), there is no significant weight loss or endothermic/exothermic reactions observed from the thermogram, which ensure the HA thermal stability compared to previous studies (See Fig. 10), however other studies results contradict the present research results. But, if any weight loss from 50–200 °C it can be due to the water evaporation. Also, weight loss

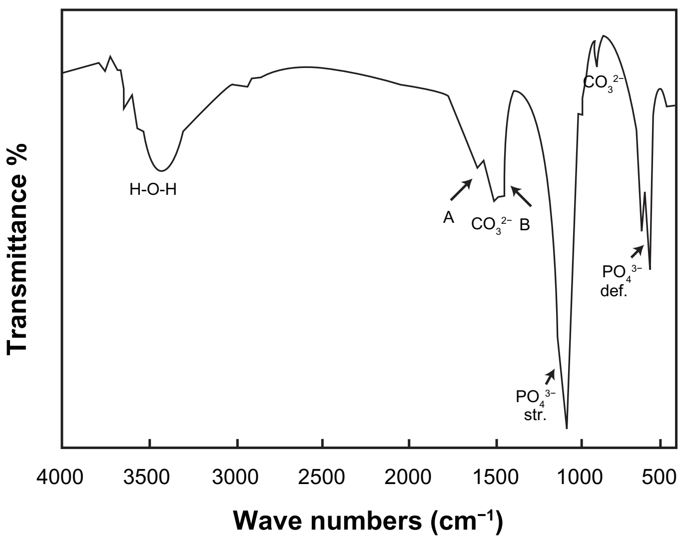

Figure 3. Infrared spectrum transmittance line result of previous study hydroxyapatite.[10]

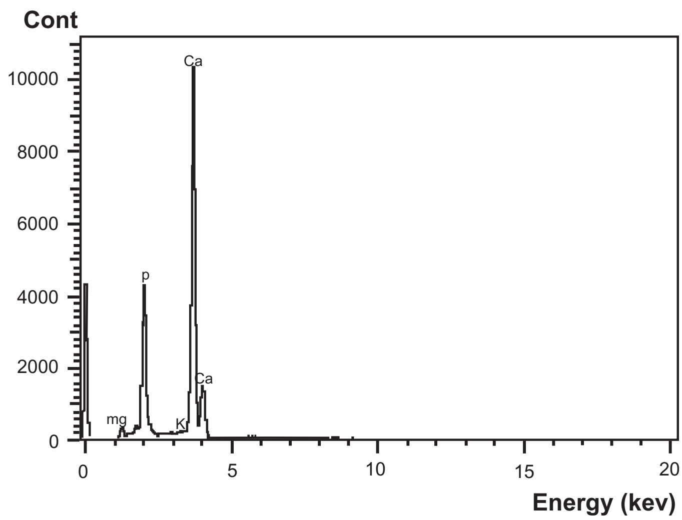

Figure 5. EDX pattern.

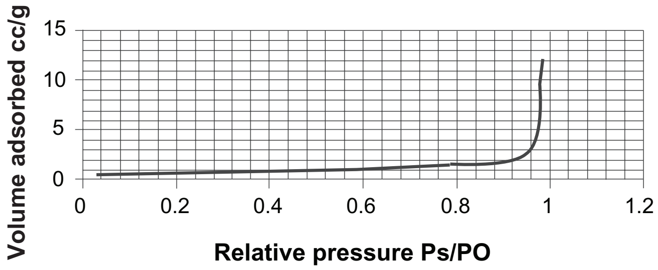

Figure 6. Nitrogen adsorption isotherm obtained at liquid nitrogen temperature for HA powder sintered at 1150 °C.

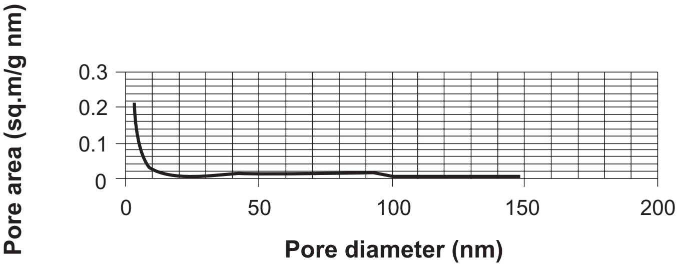

Figure 7. Pore is versus diameter.

occurred between 580–620 °C indicate decomposition of organic traces, and so the prepared BHA is free from any organics materials.[9]

The SEM result indicated that the HA has a porous architecture throughout the matrix (See Fig. 12). As it can be seen from the gross morphology, all the pores are in order of nanometer size; the mean pore diameter is equal to 135 nm, these pores were created due to the removal of organic constituents associated with the native bone, and therefore it can be expected that the HA have the capability to support the bone tissue in-growth upon implantation and provide mechanical interlocking and firm fixation.[7,9]

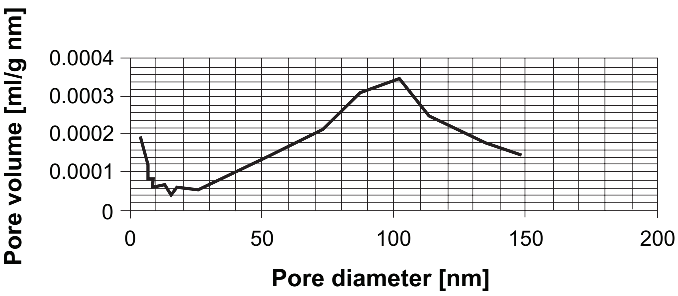

Figure 8. Pore volume versus diameter.

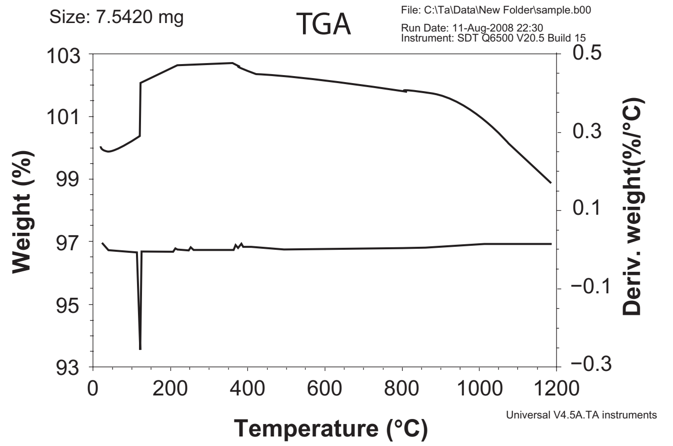

Figure 9. Thermo gravimetric analysis of the hydroxyapatite powder sintered at 1150 °C.

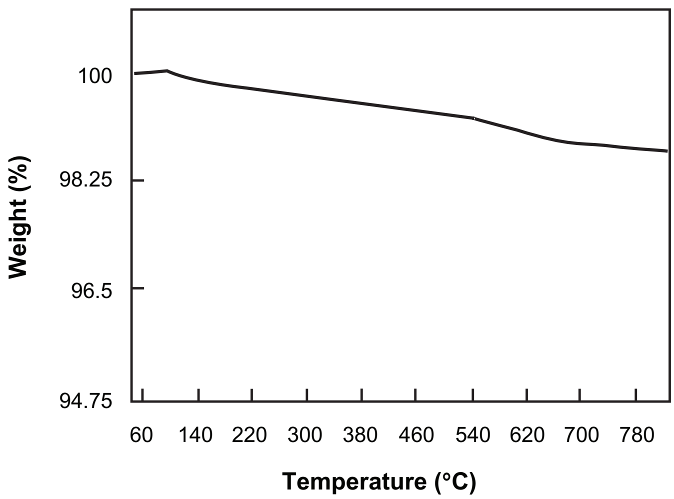

Figure 10. Thermo gravimetric analysis result of previous study hydroxyapatite.[10]

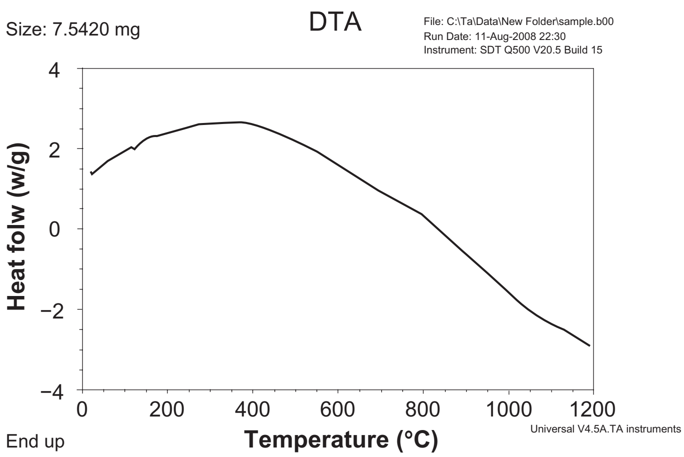

Figure 11. Differential Scanning Calorimetric of the hydroxyapatite powder sintered at 1150 °C.

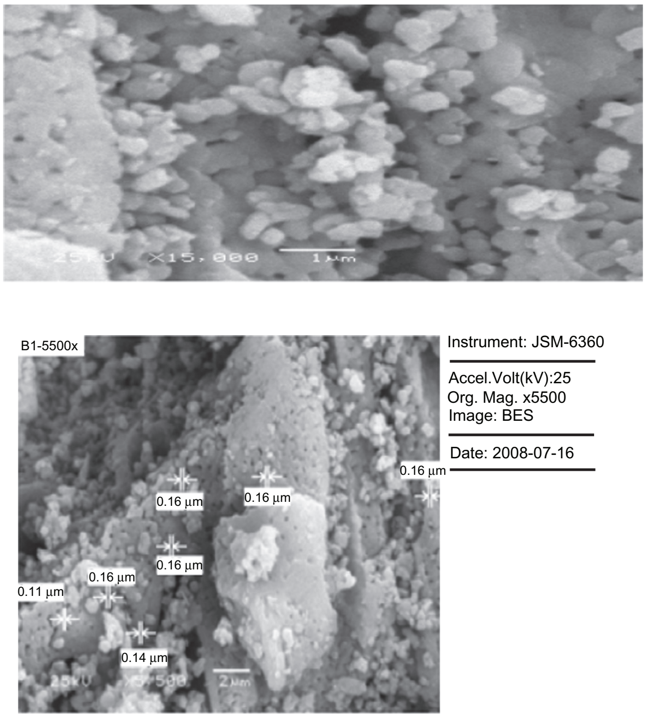

Figure 12. SEM micrographs of BHA sintered at 1150 °C.

Conclusions

The present investigation is demonstrating an easy, cost effective and reproducible process of HA from the bovine teeth. The overall results indicated that the HA is purely prepared,[4] for HA nonporous present they would be beneficial for bone tissue engineering, the BHA can be simply prepared followed by calcinations at 1150 °C as proposed in this study. This sintering process avoids any transmission of diseases. Also establishing standardized parameters for collecting animal teeth which would present similar and better results.

We conclude that the prepared HA from bovine teeth is considered as an economic process. However, due to the HA poor mechanical properties, its application is limited to non-load bearing applications, therefore we must find out ways to overcome this limitation.

Disclosures

The authors report no conflicts of interest.

References

1. Oktar FN. Hydroxyapatite-TiO_2 composites. *Materials Letters*. 2006;60:2207–10.
2. Jay Arre Toque MK, Herliansyah, Hamdi M, et al. The effect of sample preparation and calcination temperature on the production of hydroxyapatite from bovine bone powders. *Biomed 06 IFMBE Proceedings*. 2007;15:152–5.
3. Kim YG, Dong Seok Seob, Jong Kook Lee. Comparison of dissolution resistance in artificial hydroxyapatite and biologically derived hydroxyapatite ceramics. *Journal of Physics and Chemistry of Solids*. 2008;69:1556–9.
4. Oktar FN, Karacayli U, Gunduz O, et al. Effect of Sintering Temperature on Mechanical Properties and Microstructure of Sheep-bone Derived Hydroxyapatite (SHA). *IFMBE Proceedings, 13th International Conference on Biomedical Engineering*. 2008;23:1271–4.
5. Lei Dang, Hukins DW, Wardlaw D. Mechanical properties of calcium sulphate/hydroxyapatite cement. *Bio-Medical Materials and Engineering*. 2006;16(6):423–31.
6. Deepak K. Pattanayak, Divya P, Sujal Upadhyay RC, Parasod, et al. Synthesis and evaluation of hydroxyapatite ceramics. *Trends biomater Artif Organs*. 2005;18:2.
7. Tang YJ, ChunTang Lv, YueFeng Tang, et al. Preparation of uniform porous hydroxyapatite biomaterials by a new method. *Applied Surface Science*. 2008;254:5359–62.
8. Guzman Vazquez C, Pina Barba, Munguia N. Stoichiometric hydroxyapatite obtained by precipitation and sol gel processes. *Revista Mexicana de Fisica*. 2005;51(3):284–93.
9. Goller G, Oktar FN, Agathopoulos S, et al. Effect of sintering temperature on mechanical and microstructural properties of bovine hydroxyapatite (BHA). *J Sol Gel Sci Techn*. 2006;37:111–5.
10. Murugan R, Ramakrishna S, Panduranga Rao K, et al. Nanoporous hydroxyl-carbonate apatite scaffold made of natural bone. *Materials Letters*. 2006;60:2844–7.
11. Oleg Prokopiev, Igor Sevostianov, Joseph Genin, et al. Microstructure and elastic properties of sintered hydroxyapatite. *International Journal of Fracture*. 2004;130:183–90.
12. Nilsson M, Wielanek L, Wang JS, et al. Factors influencing the compressive strength of an injectable calcium sulfate-hydroxyapatite cement. *Journal of Materials Science: Materials in Medicine*. 2003;14:399–404.