COMPLEX ANALYSIS ON HEAT TREATED HUMAN COMPACT BONES

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Studiul oaselor umane sau animale constituie un obiectiv major al ingineriei biomaterialelor şi medicinei reconstructive, acest tip de biomaterial natural-biologic oferind rezultate excelente atunci când este utilizat în condiţii optime, în timpul interventiilor chirurgicale complexe. Ne-am propus caracterizarea osului compact uman folosind analiza termică (DSC-TGA şi dilatarea termică), imagistica (SEM) şi compoziţională (EDS), în scopul de a evidenţia caracteristicile de utilizare a acestora în grefarea osoasă. În acelaşi timp, folosind metodele amintite, vom evidenţia modificările şi interacţiunile dintre minerale şi faza organică care apar după tratamentul termic, rezultatele fiind utilizabile pentru obţinerea unor produse de tip grefă osoasă.

The study of the human and animal bones represents a major objective of the biomaterials engineering and reconstructive medicine domain, as this type of natural-biological biomaterial offers excellent results when it is used in the optimal conditions, during complex surgical operations. We proposed the characterization of human compact bone using thermal analysis (TGA-DSC and thermal expansion), imagistic (SEM) and compositional (EDS), in order to highlight the best characteristics for their use as bone graft. In the same time, using the named methods allow also to highlight the changes and interactions among mineral and organic phase, that occur after heat treatment, applicable to obtain products for the bone grafts.

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1. Introduction

The study of the human and animal bones represents a major objective of the biomaterials engineering and reconstructive medicine domain, as this type of natural-biological biomaterial (only if it has suffered some treatments for the initial state in the sampling moment) offers excellent results when it is used in the optimum conditions, during complex surgical operations, using or not membrane materials for support, depending on the surgical situation [1-6].

At the structural level, the bones are composed of organic and inorganic compounds and water, being actually a composite phase, each phase contributing to the remarkable properties of bone. Organic portion consists mainly of collagen and protein, while the inorganic component is mainly hydroxyapatite (HA) and tricalcium phosphate (TCP), and a small percentage of other elements incorporated into the structure, such as magnesium carbonate, sodium, etc [7-9].

From the microscopic point of view, the bones are characterized by composition, crystalline structure, morphology, particle size and orientation. The carbonates content of biological apatites ranges from 4 to 8% and increases with age. The size of crystals is very important when the biological and mineral apatites are compared in terms of solubility. [1, 2, 10-12].

We have proposed the characterization of human compact bone samples (taken following a coxofemoral prosthetic surgery involving resection of femoral head and part of the upper femur in most cases) which has been heat treated in controlled atmosphere furnace at 200ºC, 600ºC, 1200ºC respectively, using thermal analysis (TGA-DSC and thermal expansion) imagistic (SEM) and compositional (EDS), in order to highlight the best characteristics for their use in reconstruction operations. The upper limit temperature was 1200ºC, whereas above this value is converted from β-TCP to α-TCP [2, 13]. In the same time, using the named methods allow also to highlight the structural changes and interactions among mineral and organic phase, that occur after heat treatment.

2. Methods

The bones used to perform the experiments (part of the femoral head and femoral compact bone) were collected from local hospitals (Bucharest - Romania), following certain surgical coxofemural prosthesis operations (according to agreed procedures on patient privacy and medical ethics), and were frozen immediately after sampling. All femoral bones were placed in individual containers. Preparation of a biomaterial-biological origin for possible applications in bone reconstruction involves, first of all, removal of organic components to avoid immunological and antigenic contamination. As a first step to remove

tissue, blood and protein, macroscopic impurities and adhered substances, including salts, ligaments and tissues stuck to the bone, samples were cleaned with blade surgery and forceps, treated with jet hot water, steam (100ºC and 1atm) and solvents. Cortical bone samples were dried, by placing in a desiccator. The samples were cut into wedges quadratic form (5mm x 5mm x 5mm) using a jig saw with diamond blade. Heat treatments were carried out in furnaces with controlled atmosphere (Ar) at 200ºC, 600ºC and 1200ºC, with 12 hours maintaining time.

All samples preparation and analysis were performed in the laboratories of the University *Politehnica* from Bucharest, Materials Science and Engineering Faculty. The TGA-DSC analysis was performed with a SDT Q600 instrument, in a temperature range between 25 and 1250ºC, using a 10C/min heating rate. The instrument chamber has been purged with 20ml/min Ar flow rate. The thermal expansion analysis was performed on a Unitherm 1161V Anter dilatometer using the same thermal parameters as in TGA-DSC analysis.

For SEM microscopy and EDS microanalysis the samples were examined in a scanning electron microscope (Philips XL 30 ESEM TMP) equipped with secondary electron detector in low-vacuum and solid state BSE detector with two diodes, plus an auxiliary microanalysis EDS system (EDAX Sapphire, UTW, 128eV resolution). Due to special performance of the microscope, in neither case was necessary to cover the samples with conductive material.

3. Results and Discussion

Choosing the temperature for the treatment in this study is consistent with other publications [5, 9, 14-17] in which thermal analysis was used and that have revealed two thermal events, the first one happened at the occurrence of an endothermic peak with maximum in the range 70-80°C, associated with a mass decrease in the curves of the sample obtained (the range of 50-200ºC), secondary exothermic event of high intensity in the temperature range 350-600ºC, also accompanied by a considerable mass loss. The first event could correspond to the distortion triple helix structure of collagen in bone matrix, event that is dependent on the degree of hydration of the material and eliminate free water hydroxyapatite matrix attached. The second event corresponds to the decomposition and combustion of organic phase of bone accompanied by the volatilization of gaseous compounds from this process. Weight loss accompanying the first event are in the 5-10% and those for the second event is 20-30% of the mass of material, percentages that are consistent with the percentage of water, organic matter of total bone mass. Weight loss reported in the literature represents an indicator of water quantity, respectively the organic phase of the mineral matrix of each

material. Such analysis reveals that up to 600ºC there is not complete elimination of volatile components; instead this continues right up to 1200ºC [13-15].

Since with the application of heat treatment addition bone samples can be obtained (extensively used for surgical correction of bone defects). It is a proven fact that the heat treated bones crystalline phase composition is similar to natural bones. The temperature effect on bone morphology and composition was previously studied [3, 5, 11, 15] through TGA-DSC, SEM/EDS combined methods, the results confirmed by XRD methods being similar.

In order to obtain sustainable and reproducible results, we performed complementally thermal analysis (TGA-DSC - thermal expansion), spectral analysis (EDS), and imagistic determinations (SEM). The SEM images shown are representative figures of the characteristics observed in several areas collected from living humans, male and female, middle age. EDS presented spectra are average for five analyses (on 200x200μm^2) on each sample.

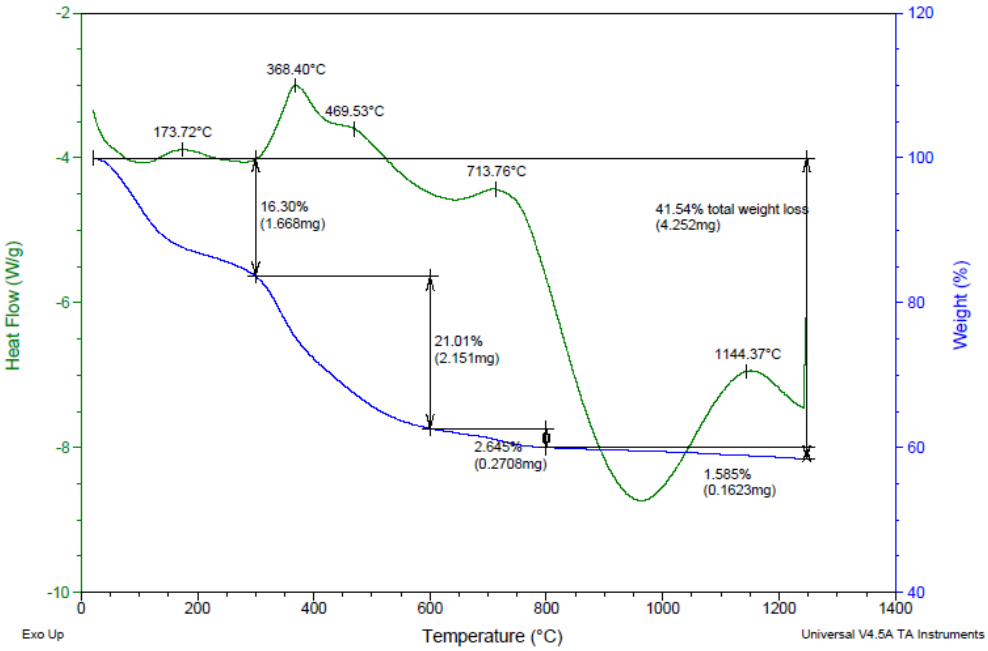


Fig. 1. Comparisons of the TGA spectra (right axis) and DSC analysis (left axis) obtained after the investigation of the heat treated human bone samples

TGA analysis shows a decrease of weight during heating to 1250ºC from 10.236mg at 25ºC to 4.252mg at final temperature, which corresponds to 41.54% weight loss. First significant weight loss is recorded at 250ºC (1,668mg) representing 16.3%, and being associated to the massive dehydration of sample. Following this interval the sample reduced its weight with 21.01% (2.1541mg) at

600ºC, being associated to the bone structure collagen elimination. This reaction continues up to 800ºC, with a lowered rate, the difference between 600 and 800ºC being of 2.645% (0.27mg). Above this temperature, a fine TGA curve descending slope is observed, at maximum analysed temperature the weight loss being of 0.1623mg (1.585%), this being associated with the collagen remains removal and the incipient transformation of HA in β-TCP.

DSC curve is complementary to the TGA results, bringing additional information on the domains in which structural changes occur and the maximum temperatures at which they occurred. The first detectable change has a maximum at 174°C and it occurred between120-140°C. After this temperature at 310ºC a transformation began with a maximum at 370ºC that was completed at 415°C. At this temperature a new transformation started that was completed at 480ºC, its maximum being located at 470ºC. The next transformation had the maximum at 655ºC being completed at 715°C. After this interval there was a surge of decreasing slope up to 925ºC, temperature at which a transformation completed at 1000°C began, with a minimum at 960ºC. After the last temperature of this interval a major transformation began, being completed at 1245ºC, with a maximum at 1145°C, that can be ascribed to the partial transformation of HA into β-TCP.

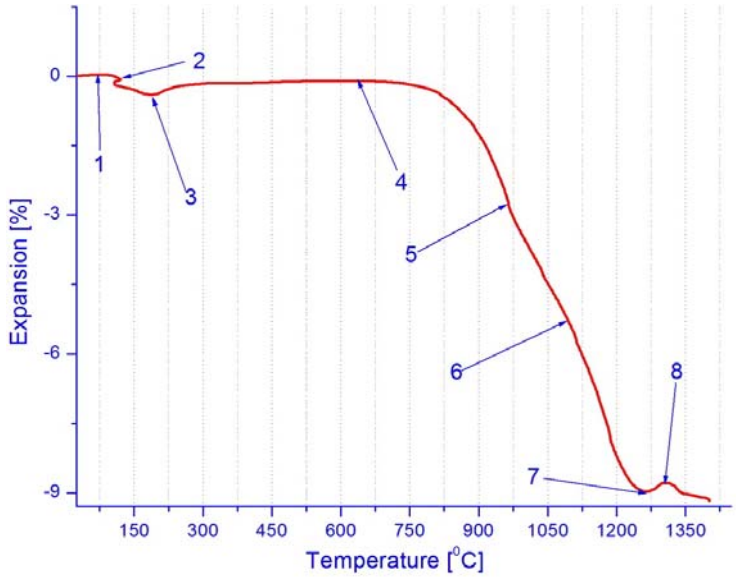


Fig. 2. Human bone expansion test curve

Thermal expansion test resulting curve (see Fig. 2) shows the transformation beginning points and the temperature intervals of transformation. The first transformation was found at 73ºC followed by a transformation at 120ºC

where an interesting endotherm transformation appears whose ending point can be found at 185ºC. This transformation causes an instantaneous sample temperature decrease by 15ºC. The transformation that took place between points 1 and 3 caused a volume and mass loss. From this point an expansion process begins and continues up to 614ºC (point 4), from which a new transformation begins. At 964ºC (point 5) an inflexion point appears which indicates a new transformation start, that takes place between 964ºC and 1040ºC (point 6). Between point 4 (618ºC) and point 7 (1260ºC) a massive contraction can be observed. Than at 1260ºC a new expansion interval starts that ends at 1360ºC where the β-TCP – α-TCP transformation takes place.

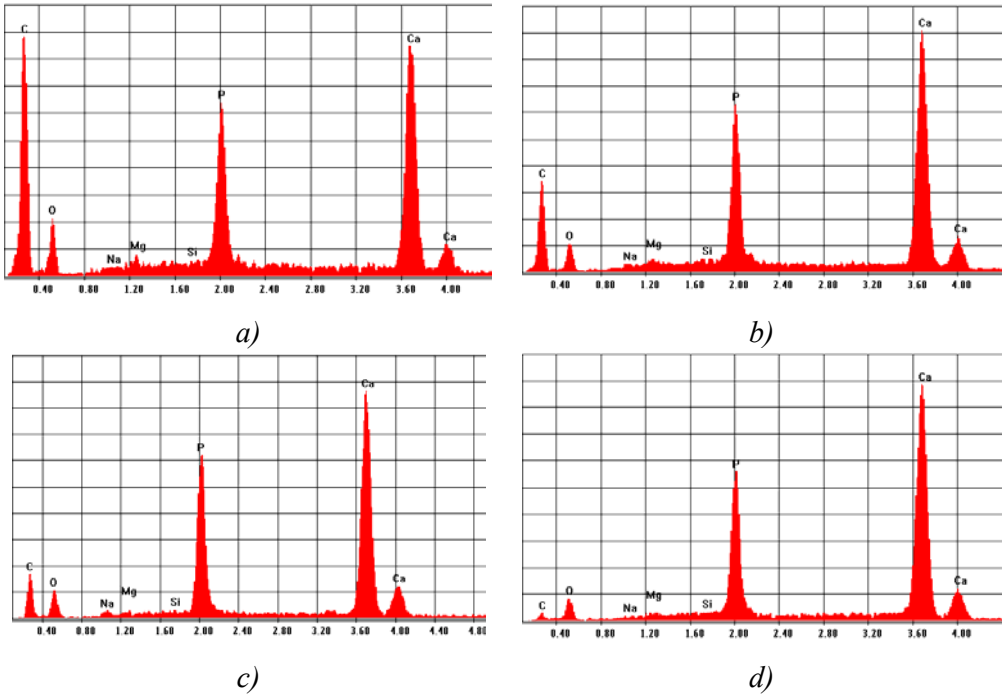


Fig. 3. EDS spectra comparison obtained after the investigation of the untreated samples (a), heat treated at 200ºC (b), 600ºC (c), and 1200ºC (d) respectively

Spectral results, presented in Fig. 3, confirm the stoichiometric composition of the standard samples and point to a ratio of the Ca/P of the studied bone sample close to the ideal value, with the presence of other chemical elements characteristic for a normal bone chemical composition (Na, Mg, Si, O, C). Mg is one of the most abundant trace elements present in biological hard tissues. In the nonstoichiometric HA, it partly changes into TCP at high temperatures. Usually, the presence of Mg in TCP stabilizes and increases the ratio value TCP/HA by

increasing the temperature more than 800ºC. It is noted that the heat treatment temperature does not influence significantly the qualitative composition of bone samples. However it is remarkable the decreasing of the C% content with the increasing of the temperature. The data presented in Table 1 confirm the fact that the apatite of the biological hard tissues is nonstoichiometric, with structural imperfections due to the incorporation in the crystalline network of the said chemical elements present in small quantities.

Table 1

Values of the Ca/P according to EDS analysis

Ca/P ratio over temperature			
Untreated bone	200℃	600℃	1200℃
1.69	1.66	1.54	1.48

The results of qualitative and quantitative EDS compositional analysis performed on samples subjected to heat treatment at 600ºC intended to remove organic components and water show a slight modification of the Ca/P ratio in terms of reducing its value below 1.6.

All presented images where obtained in MIX signal, which is a combination of the secondary (SE) and backscattered electrons (BSE) signals, the results being in fact a morpho-compositional analysis.

Macroscopically, there have been observed colour changes, delaminating, fracturing and distortion processes. Significant changes have occurred at structural and morphological level. At the presented magnification, collagen microfilaments can be distinguished. Similar organic filaments have been previously reported by other authors, this type of formations being discovered in some microcracks of the cortical bone [3, 5]. Normal bone, untreated, contains small pores, but overall gross bone microstructure is very dense due to the presence of organic matter associated with inorganic mineral-impregnated bone. After the bone was heated to 200°C and 400°C for 12 hours, the microstructure has changed as a result of water disposal and organic matter content as collagen, present in small amounts being probably proteins, polysaccharides and lipids [2].

With increasing the temperatures above 600ºC, pores formation is different than for untreated bone. Surfaces are not preponderantly smooth, but rough and branched. High temperatures heat treatment implies the appearance of apatite crystals [3].

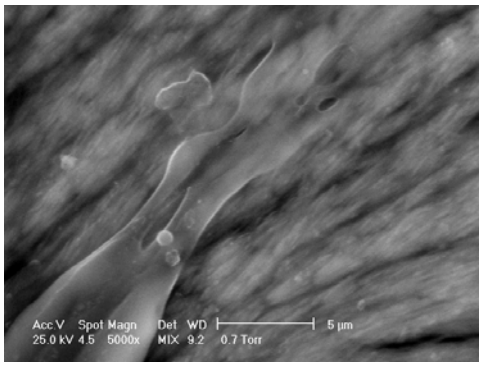


Fig. 3. Bone sample after surgical removal of the major organic components.

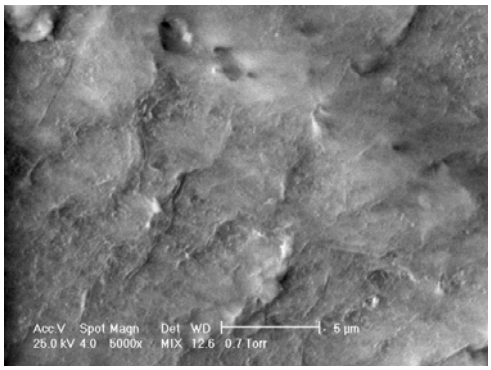
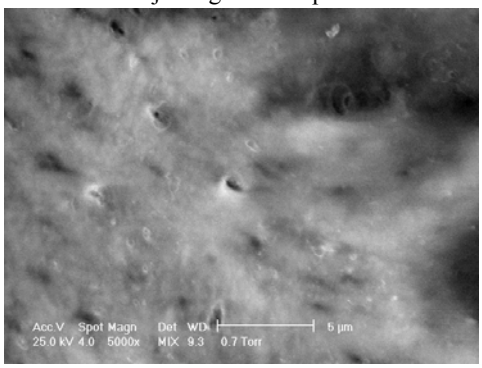


Fig. 4. Morpho-compositional analysis of the bone sample heated at 200ºC.



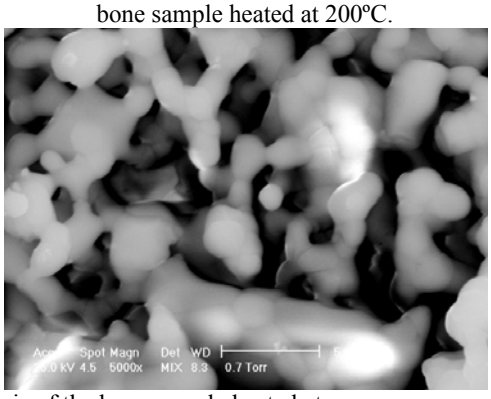


Fig. 5. Morfo-compositional analysis of the bone sample heated at 600ºC (left), respectively 1200ºC (right)

Degeneration of the organic matrix under the influence of temperature has a great impact on the mechanical properties of bone [13-17]. Electron microscopy images revealed a significant structural difference in bone architecture of the femoral bone according to treatment. Mesostructure is responsible for the differences in bone mineral density. SEM microscopy shows the bone structure, but offers only a two-dimensional image bone structure, which only leads to a qualitative analysis of the mesostructure.

4. Conclusions

There are important structural, morphological and compositional differences between the bone in the natural state and the dehydrated one or heat treated at different temperatures. The mechanisms of degradation under heat treatment must be an objective for obtaining a good quality bone substitute material and finally the proper bone regeneration.

Correlation of the analysis methods used in this study allowed a comprehensive characterization of the changes produced on the bone heat treated up to 1200ºC. The temperature has a significant effect on the emphasis of compact bone morphology. The variability of composition, structure and morphology is essential for understanding the contribution of these factors on bone mass and constitution.

TGA-DSC and thermal expansion measurements revealed the weight loss and structural modifications during heat treatment of the human bones subjected to heat treatment in order to obtain bone grafts with optimal composition.

Using scanning electron microscopy techniques could help to characterize the morphological changes that occur during processing of bone samples. SEM technique serves not only to distinguish inorganic characteristics, but also offers the possibility to identify structural components of cells and micro-morphological details. Morphological analysis and thermal behaviour of studied materials (along with the advantage of totally removal of risks of transmitting any type of disease) highlights the capabilities of studied materials to be used as biomaterials for bone reconstruction.

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