Thermal processing temperature influence on hard tissue chemical composition

F. MICULESCU*, L. T. CIOCAN[a], M. MICULESCU, M. BRÂNZEI, N. GHIBAN
University „POLITEHNICA", Bucharest, Romania
[a]*„Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

In terms of chemical composition, some elements are essential to the human body functions and others are toxic. Due to the latter, the need to control their concentrations in human tissues appears. In Romania, there are few data on exposure to heavy metals, but in advanced countries there is a growing demand to assess this issue. In this study, the changes in the concentration of Pb, Cd, Sr, Zn, Cu, Fe, and Al in cortical bone as function of heat treatment temperature was analyzed, whereas the preparation of samples for compositional analysis and processing can involve heating at different temperatures in order to remove organic component. Elemental concentrations determination is also important, as hard tissues can be used to produce hydroxyapatite used for bone reconstruction operations. The analyzed samples were taken from subjects from the same areal. In addition to the above-mentioned elements, the concentrations of the major elements of the bone (Ca, P, Na, Mg, K, Si) were determined. To obtain a comprehensive traceability tissue samples from four individuals, of both sexes were analyzed. Heat treatment structural transformations were determined by thermal expansion. Comparing the chemical composition (determined comparatively by energy dispersive spectrometry - EDS and X-ray fluorescence spectrometry - XRF) of the natural state bone and the corresponding thermal treated ones from the same specimen (at 400, 800 and 1200°C), a statistically significant decrease in Pb, Hg and Cd quantity was observed in the heat treated material. Fewer changes in the other chemical elements concentration were observed. The heavy elements concentration decrease was found to be proportional to the temperature level.

(Received March 25, 2013; accepted July 11, 2013)

Keywords: Bone thermal treatment, Thermal expansion, Chemical composition by EDS, XRF

1. Introduction

Elemental chemical composition of human hard tissues is quite diverse, meaning that some of the chemical elements are essential to the human body functions and others are toxic. Due to the latter, there is a need to control their concentrations organs and human tissues.

In this study, the changes in the concentration of Pb, Cd, Sr, Zn, Cu, Fe, and Al in compact bone as function of heat treatment temperature was analyzed, whereas the preparation of samples for compositional analysis and processing can involve heating at different temperatures in order to remove organic component and to avoid disease transmission. Elemental concentrations determination is also important, as hard tissues can be used to produce hydroxyapatite used for bone reconstruction operations. [1-2]. By conducting this study we analyse the possibility of heavy elements concentrations growth with heat treatment temperature increase, which would make more difficult the use of compact bone for bony reconstruction products. The analyzed samples were taken from subjects from the same areal.

Throughout childhood and a great part of the adult life, exposure to heavy chemical elements due to environmental and occupational sources lead to increasing concentrations in calcified tissues, reflecting them as integrated or cumulative exposure.[3]

In addition to the above-mentioned elements, the concentrations of the major elements of the bone (Ca, P, Na, Mg, K, Si) were determined.

To determine the quantitative composition changes depending on the heat treatment temperature, tissue samples from 4 individuals of both sexes were analyzed. Quantities of hard tissues - compact bone samples (taken following the coxofemoral prosthetic surgery involving resection of femoral head and upper part of the femur in most cases) were initially analyzed without thermal processing, and then after heat treatment at 400, 800, respectively 1200°C. The thermal treatment has been made in controlled atmosphere furnace. Structural changes induced by the heat treatment temperature were determined by thermal expansion.

Comparing the chemical composition (determined comparatively by energy dispersive spectrometry - EDS and X-ray fluorescence spectrometry - XRF) of the natural state bone and the corresponding thermal treated ones from the same specimen (at 400, 800 and 1200°C), a statistically significant decrease in Pb, Hg and Cd quantity was observed in the heat treated material. Fewer changes in the other chemical elements concentration were observed. The heavy elements concentration decrease was found to be proportional to the temperature level.

2. Methods

The temperature effect on compact bone structure was previously studied. [4-9] Due to the significant amounts of water and collagen, the heat treatment applied for their removal, in order to conduct the composition tests, may change the chemical composition too. The hard tissues 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 implied, first of all, removal of organic components. As a first step to remove including salts, ligaments and tissues stuck to the bone, samples were cleaned with blade surgery and forceps, treated with jet water and solvents. Cortical bone samples were dried, by placing in a desiccator. For thermal dilatometry analysis, the samples were cut into wedges rectangular form (50mm x 5mm x 5mm) using a jig saw with diamond blade. Heat treatments were carried out in furnaces with controlled atmosphere (Ar) at 200ºC, 800ºC and 1200ºC, with 4 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 thermal expansion analysis was performed on a Unitherm 1161V Anter dilatometer in a temperature range between 25 and 1250ºC using a 10ºC/min heating rate. The instrument chamber has been purged with 20ml/min Ar flow rate.

In order to achieve the proposed objectives in terms of chemical composition, two methods based on energy dispersive spectrometry (EDS) were used. The first method was based on X-ray microanalysis, identifying elemental concentrations from B to U, and the second method was energy dispersive spectrometry with polarized radiation fluorescence (XRF) used for the determination of trace elements from Na to U. The complementarity of the two selected methods ensured a complete elemental compositional analysis of hard tissues collected for this purpose. For EDS microanalysis a EDAX Sapphire system, UTW, with 128eV resolution was used. The micro-analytic operating conditions were as follows: 0° tilt angle, 35° TOA, 25 kV accelerating voltage, and 10mm working distance. The XRF instrumentation used for bone elemental analysis was a SPECTRO equipped with a 50W Rh and a Si-drifted detector with a resolution of 148eV (1000cps Mn Kα). The obtained spectra were evaluated with TURBOQUANT software package matrix effects that will occur are taken into account.

3. Results and discussion

The temperature for the treatment in this study is consistent with other publications [10-14] in which thermal expansion was used and that have revealed the thermal events. Since with the application of heat treatment addition bone samples can be obtained (extensively used for surgical correction of bone defects), the elemental chemical composition modifications become very important. The major interest consists in the possibility of heavy elements concentration increases with the thermal treatment temperature.

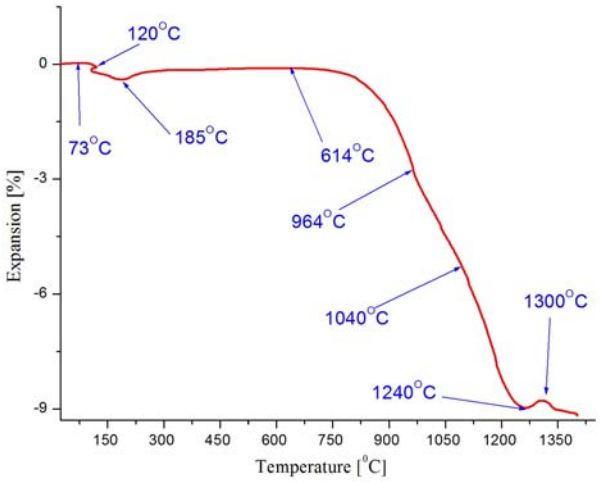

Fig. 1. Bone thermal expansion curve.

Thermal expansion test resulting curve (see Fig. 1) 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 73ºC and 185ºC caused a volume and mass loss. From this point an expansion process begins and continues up to 614ºC, from which a new transformation begins. At 964ºC an inflexion point appears which indicates a new transformation start, taking place between 964ºC and 1040ºC. Between 618ºC and 1260ºC, a massive contraction can be observed. Than at 1260ºC a new expansion interval starts that ends at 1300ºC where the β-TCP – α-TCP transformation takes place. [15-17]

For chemical composition analysis, each sample was analyzed three times in repetitive condition to assess the precision of the analytical results. Each analytical data for an analyzed sample is provided on the base of the three spectra that are normalized before estimating the elemental concentrations.

Apart from the major and essential chemical elements (Na, Mg, K, Si, O, C) the presence of other elements has not been identified, which leads to the conclusion that they are found in concentrations below the limits of detection of the EDS analyzer.

The quantitative analysis for Ca and P by EDS, presented in Table 1, confirm the almost stoichiometric composition of the untreated samples close to the ideal value. Increasing the heat treatment temperature increased

the concentration of Ca, but the P concentration remaining approximately constant. This led to the change of Ca/P ratio from the stoichiometric value. The results of qualitative and quantitative EDS compositional analysis performed on samples subjected to heat treatment at 400, 800 and 1200ºC intended to remove organic components and water show a slight modification of the Ca/P ratio in terms of increasing its value at 2.09 at 800°C. Above this temperature (1200°C), the Ca/P ratio slightly decreased. These data lead to the conclusion, supported by the literature results, [6-7, 18] that increasing the heat treatment temperature the Ca concentration increases due to the hard tissues organic component mass decrease.

Table 1. Ca and P, EDS quantitative analysis function of heat treatment temperature.

Element	No TT	TT 400°C	TT 800°C	TT 1200°C
P [%]	13.13	13.53	13.23	14.12
Ca [%]	22.33	26.27	27.78	28.89

It is noted that the heat treatment temperature slightly influences the qualitative composition of bone samples. It is remarkable the decreasing of the C% content with the increase of the temperature. EDS analysis induce the fact that with the temperature increasing 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 2. EDS vs. XRF Ca/P ratio value variation.

Ca/P	No TT	TT 400°C	TT 800°C	TT 1200°C
XRF	1.67	1.96	2.06	2.04
EDS	1.7	1.94	2.09	2.04

Comparative analysis by EDS methods continued using XRF method. The Turboqunt program takes into account the fundamental parameters of fluorescence excitation and the coincidence of the characteristics lines in all the above spectra for a higher exactness assessing of elemental concentrations.

From the obtained spectra, it was confirmed that hard tissue sample contains the well known major elements as Ca, K, Si, Cl, S, Na, Mg, but minor ones as Fe, Cu, Zn, Al (essential) and Sr, Pb, Cd (toxic). The quantitative results obtained from the data analysis (see Table 3) are expressed in wt% for major and essential chemical elements, respectively ppm for trace elements.

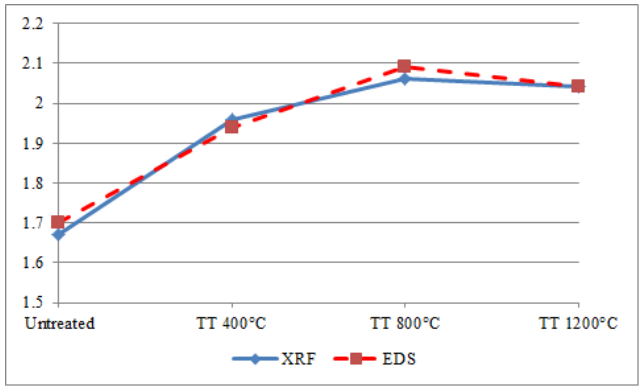

Fig. 1. Comparison of Ca and P average concentrations obtained by EDS (red), and XRF (blue).

Although the detection limits of the two compared methods are very different for light elements like Ca and P analysis, the average analysis results for each group are very close, on the order of a few tenths of a percent (see Figure 1). The analysis of the concentrations of the main elements shows less variability as compared to the analysis by EDS method. In this respect, the Ca / P ratio is stoichiometric (1.67) for untreated hard tissue analysis, is 1.96 by treatment at 400°C, 2.06 and 2.04 respectively by raising the temperature to 800 and 1200°C. Given the small variation of this report, it can be concluded that the data obtained by both methods are comparable. Analyzed compositions differences by the two methods may appear due to the fact that EDS analysis coupled to the scanning electron microscopy involves only the analysis of matter micro-volumes.

Regarding the average concentration of other elements present in higher concentrations, the analysis made only by XRF method, revealed a greater variation of the Si. We determined that the concentration increased from 1.3% in untreated tissue case to 1.47 in the case of heat treatment at 1200°C. Variation of Mg concentration showed a clear downward trend, from 0.58% to 0.19% at 1200°C, relationship related to the element elimination due to its low melting temperature. Na and K concentrations showed small variations with the heat treatment temperature.

The average elemental concentrations of the samples give a significant picture of the heavy metal burden function of samples heat treatment temperature, but more important, suggests differences among heavy elements concentration variation in hard tissues.

Fe concentration decreased rapidly to 400°C, the total reduction being around 16% at 1200°C, from 769 to 642ppm. It is noted that concentration reduction was limited at temperatures above 400°C.The average concentration of iron in the untreated samples was similar to that assayed by other authors [3]

Table 3. XRF average chemical quantitative analysis results.

Element [%]	No TT	TT 400°C	TT 800°C	TT 1200°C
Ca	23.735	27.17	28.535	30.418
P	14.157	13.832	13.835	14.855
Na	0.513	0.442	0.429	0.432
Mg	0.585	0.475	0.337	0.091
K	0.218	0.189	0.197	0.194
Si	1.301	1.247	1.259	1.474

Element [ppm]	No TT	TT 400°C	TT 800°C	TT 1200°C
Al	370.5	292.0	240.4	240.53
Fe	769.7	689.0	640.6	642
Zn	154.1	138.6	124.5	133.0
Cu	16.32	14.8	13.6	12.9
Sr	246.4	191.4	170.5	142.6
Pb	9.413	7.76	6.887	3.532
Cd	2.605	<2	<2	<2

The concentration of copper in the hard tissues was much higher than the values assayed by other authors of 3.6ppm. [19] Concentration decreased by 11% from 16.32 ppm (samples without heat treatment) at 12.9ppm for samples processed at 1200°C. The same trend we found in the concentration of Zn, with a minimum average composition at 1200°C of 133ppm reduced from 154ppm in untreated samples. From the essential elements, the most important change was recorded for Al, its concentration decreased by 35%, from 370 to 240ppm at 1200°C.

Variation of the average concentrations of chemical elements showing the highest toxicity is shown in Fig. 2d). The highest concentration was that of Sr, 246ppm for the untreated tissues. The concentration variation slope is significantly decreasing; the minimum concentration of 142ppm was recorded at 1200°C, 42% lower than the initial one. Even these concentrations are greater than those reported in the literature for populations living in polluted areas.

Cadmium is one of the most popular elements assayed in bones.

Untreated samples showed a mean concentration of 2.6ppm. Increasing the heat treatment temperature over 400°C led to obtaining Cd concentrations lower than the detection limit of the XRF method (2ppm).

Lead is one of the elements that is very frequently assayed in bones. The lead concentration in the hard tissues assayed in our investigations varies very much as function of thermal treatment temperature (9.4ppm to 3.5ppm at 1200°C).

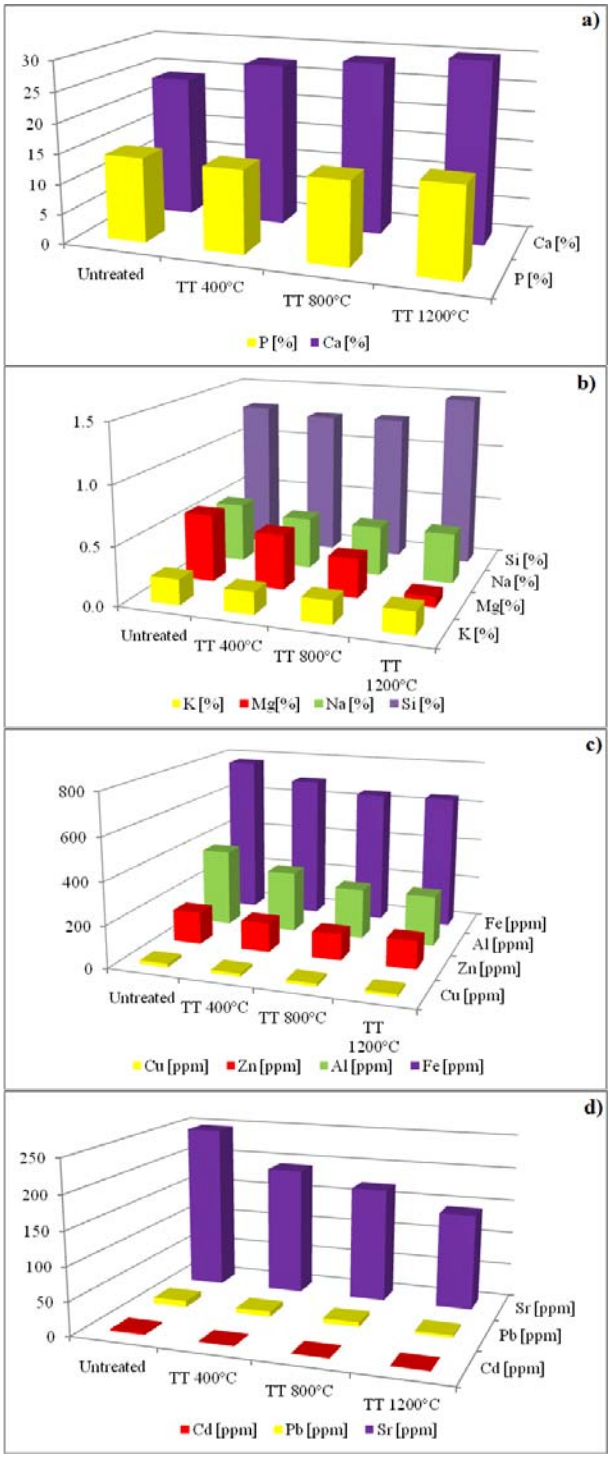

Fig. 2. Variation of the average concentrations of chemical elements.

This was the most drastic change of all analyzed elements; the decrease from 25°C to 1200°C was of 62%. However, even the maximum concentrations are within those reported in the literature. For example, the average lead concentrations presented in other paper varies from 5 to 65ppm [19 - 20].

XRF results showed differences in concentrations of all analyzed chemical elements in untreated and heat treated at 400, 800 and 1200°C compact bone samples. The major elements showed significant increases in concentrations. Si it presents the same behavior, but the concentration slope increase with temperature is lower. All the other chemicals indicated concentrations decrease with increasing of heat treatment temperature.

Comparative analysis of the presented data indicates that one of the XRF method limitations is because hard tissues consist mainly of light elements. The phenomenon of radiation scattering from matrices with low Z signal produces a wide and deep background signal. Since the element of interest concentrations are usually small, of ppm order, it is important to obtain a high signal / background ratio.

However, the use of EDS and XRF complementary methods allows a complete analysis of the hard tissues elemental chemical composition. Limitations occur when elemental concentrations are of ppm level, [21] as shown in the analysis of Cd.

The study clearly indicated that function of the hard tissue samples thermal processing temperature the elemental concentrations vary significantly. Major chemical elements increase in concentration with increasing heat treatment temperature, while the minor chemical elements or present as elemental traces concentrations are becoming smaller at elevated temperatures.

4. Conclusions

XRF technique can be used to determine the concentration of heavy metals in human hard tissue at ppm level, while EDS microanalysis coupled to electron scanning microscopy is well suited to determine the concentration of major elements such as Ca, P, C and O, which completes the results obtained by XRF.

When using tissues for processing and use as biomaterials, the bones heavy metals concentration determination is very important and XRF technique gives an idea of their remaining amount in the hard tissues, indicating future exposures endogenous risk. In the cadmium and mercury case the XRF technique still has some limitations due to detection limits of 2ppm level.

The sample preparation method is extremely important for trace elemental analysis. When samples are thermally treated at high temperatures, low concentrations of elements present in hard tissues decreases with increasing temperature. These results indicate that, when possible, chemical composition analysis should be performed on samples without thermal treatments.

At the same time, an additional finding is that of indications for use of products from hard tissues treated at temperatures above 1000°C for bone reconstruction operations due to lower concentrations of toxic elements than for the thermally unprocessed ones.

Acknowledgments

This work was supported by CNCSIS-UEFISCSU (UEFISCDI), project number PN II-RU 104/2010.

References

[1] E. M. Raif, M. F. Harmand, Biomaterials **14**, 978 (1993).
[2] A. Fischer, J. Kwapuliński, D. Wiechuła, T. Fischer, M. Loska, Sci. Total. Environ **389**, 315 (2008).
[3] K. Kaczanowski, H. Gła¸ K. Szostek, Variability and Evolution **5**, 95 (1996).
[4] J. O. Nriagu, Science **272**, 223 (1996).
[5] R. Lobinski, C. Moulin, R. Ortega, Biochimie **88**, 1591 (2006).
[6] V. Benezra, L. W. Hobbs, M. Spector, Biomaterials **23**, 921 (2002).
[7] T. R. Helliwell, S. A. Kelly, H. P. L. Walsh, L. Klenerman, J. Haines, R. Clark, N.B. Roberts, Bone **18**, 151 (1996).
[8] S. I. Voicu, A. C. Nechifor, B. Serban, G. Nechifor, M. Miculescu, J. Optoelectron. Adv. Mater. **9**(11), 3423 (2007).
[9] V. Benezra, L. W. Hobbs, M. Spector, Biomaterials **23**, 921 (2002).
[10] G. E, Stan, S. Pina, D. U. Tulyaganov, J. M. Ferreira, I. Pasuk, C. O. Morosanu, J. Mat. Sci.-Mat. Med. **21**, 1047 (2010).
[11] S. Joschek, B. Nies, R. Krotz, A. Gofpferich, Biomaterials **21**, 1645 (2000).
[12] S. I. Voicu, A. C. Nechifor, B. Serban, G. Nechifor, M. Miculescu, J. Optoelectron. Adv. Mater. **9**, 3423 (2007).
[13] D. Tadic, M. Epple, Biomaterials **25**, 987 (2004).
[14] E. Rusen, C. Zaharia, T. Zecheru, B. Mărculescu, R. Filmon, D. Chappard, R. Bădulescu, C. Cincu, J. Biomech **40**, 3349 (2007).
[15] G. E. Stan, C. O. Morosanu, D. A. Marcov, I. Pasuk, F. Miculescu, G. Reumont, Appl. Surf. Sci. **256**, 1617 (2009).
[16] C. Ooi, M. Hamdi, S. Ramesh, Ceram. Int. **33**, 1171 (2007).
[17] F. Miculescu, I. Antoniac, L.T. Ciocan, M. Miculescu, M. Brânzei, A. Ernuteanu, D. Batalu, A. Berbecaru, U.P.B. Sci. Bull., Series B **73**, (2011).
[18] H. W. Kuo, S. M. Kuo, C.H. Chou, T. C. Lee, Sci. Total Environ. **255**, 45 (2000).
[19] V. A. Granadillo, J. A. Navarro, S. Campos, A. Avila Major, J. Cardozo, R. A. Romero, Trace Elem. Med. **9**(3), 139 (1992).
[20] F. Miculescu, L. T. Ciocan, M. Miculescu, A. Ernuteanu, Dig. J. Nanomater. Bios. **6**(1), 225 (2011).
[21] J. I. Catanese, J. D. B. Featherstone, T. M. Keaveny, J. Biomed. Mater. Res. **45**, 327 (1999).

*Corresponding author: f_miculescu@yahoo.com