Bone Matrices of Different Origins Studied by FTIR Spectroscopy

R. Grecu, V. Coman, V. Avram, M. Băciuț, and G. Băciuț

Abstract — The bone matrix of deer antler, human bone and pig bone are compared in order to evidence the resemblance of their composition and structure at the level evidenced by FTIR spectroscopy. The variation of the organic/inorganic matrix content in bones of different origins was evaluated from the area of the characteristic absorption bands observed at ~1650 cm⁻¹ (collagen) and ~1030 cm⁻¹ (calcium phosphate). The higher content of organic matrix resulted for the deer antler and human skull. The deproteinization of the bone matrix by heat treatment is well evidenced by the FTIR spectra. The organic component is completely removed by the thermal treatment of bone samples at temperatures higher than 500°C. The spectra of these samples show also the removal of carbonate ion incorporated into the inorganic matrix and the modification of the crystallinity of hydroxyapatite. Based on the splitting of ν₄[PO₄²⁻] band from the 500-700 cm⁻¹ range of infrared spectrum a crystallinity index of hydroxyapatite was assessed. The evolution of this index is a monotone increasing for deer antler and human skull. The values of crystallinity index for the biogenic hydroxyapatite remain under that of the synthetic hydroxyapatite. The FTIR studies of the mentioned samples are useful for the characterization of deer antler, a potential new biomaterial for the bone reconstruction.

Keywords: bone matrices, deer antler, FTIR spectroscopy, thermal treatment, crystallinity index.

1. INTRODUCTION

The obtaining of a higher quality biomaterial for bone surgery as an alternative of the products on the market is the focus of many studies. Deer antler has notable regeneration properties (high growth speed and full regeneration every year) that recommend it as a promising biomaterial for the bone reconstruction.

The major constituents of bones are the organic matrix consisting in 85 to 95% of fibrous collagen and the bone mineral part represented by some varieties of calcium phosphate [1-3]. The poorly crystalline hydroxyapatite is predominant in the inorganic phase of the mature bones. The crystals are impure, ~5-6% by weight are carbonate substituted. The amount of water present in bone is an important determinant of its mechanical behaviour. Other constituents are non-collagenous proteins and polysaccharides and, in many types of bone, living cells and blood vessels. The bone cells are osteoblasts (responsible for the synthesis of bone matrix), osteocytes (involved in the maintenance of bone matrix) and osteoclasts (bone destroying cells). In the case of the antlers, all cells are dead by the time when they come to be used. The high porosity is a necessary feature for a faster resorption of a biomaterial with possible applications in the bone defect reconstruction by promoting osteoconduction (bone growth from the existing bone by stimulation of osteoblasts to form new bone). The SEM (scanning electron microscopy) study of deer antler [4] evidenced larger pores in comparison with those of pig bone.

Infrared spectroscopy evidences patterns both for the organic and inorganic components of the bone samples [5] and their evolution under the thermal treatment. In the perspective of using the mature antler of Romanian deer to obtain a new biomaterial with applications in the bone reconstruction, we studied the composition and the structure of deer antler, human and pig bones revealed by FTIR (Fourier transform infrared) spectral method and thermal analysis.

2. EXPERIMENTAL

The bone samples were prepared for FTIR investigation following some stages: the removal of the excess of soft tissue and marrow, the washing with physiological solution and the drying at 40°C. After the grinding into a fine powder the bones were degreased by washing with acetone till the disappearance of the 1740 cm⁻¹ band from the infrared spectrum. The investigated samples were antler tissue from the red deer (Cervus elaphus carpaticus), human skull and pig bone. In the case of deer antler, the studied samples consisted in a mixture of...
cancellous and compact parts of the bone tissue, excepting the case of a special mention. A commercial sample of hydroxyapatite was used as reference material.

FTIR spectra were recorded both on untreated and heat-treated powdered samples compressed in KBr disks using a Fourier transform infrared spectrometer JASCO 610. Thermal treatment of powdered bones was done in air, for 2 hours, in an electric furnace at 150, 600, 900 and 1200°C.

The thermal analysis (TG, DTG, DTA) was performed with a Derivatograph MOM OD 102 (Hungary). The sample quantity was 200 mg, the rate of increase of oven temperature 10°C min⁻¹, and the sensitivity 100 mg TG, 1/5 DTG, 1/5 DTA. Measurements were conducted in air in the temperature range of 30-1100°C.

3. RESULTS AND DISCUSSION

Vibrational spectroscopic methods (FTIR and Raman spectroscopy [6,7]) are powerful methods for the investigation of major components of bone samples, the protein (type I collagen) and hydroxyapatite. From infrared spectra of studied samples presented in Figure 1 one can note that deer antler, human skull and pig bone have the same absorption bands with small differences in their intensity.

The main absorption bands from infrared spectrum have been assigned [5] for deer antler. Thus, the strong band centered around 3420 cm⁻¹ is due to the water content of bone samples. The bands characteristic to ν(CH) vibrations of CH3 and CH2 groups from the 2800-3000 cm⁻¹ range have a medium intensity and a low analytical value. More intense are the bands assigned to amide groups of organic matrix: 1646, 1535, 1239 cm⁻¹. The inorganic bone phase has PO4 structural units characterized by the ν3 antisymmetric stretching (P-O) vibrational mode (resolved into well defined peaks at 601 and 561 cm⁻¹). The bands of medium intensity observed between 1400-1500 cm⁻¹ range as well as the band at 866 cm⁻¹ confirm the carbonation of all bone samples.

A quantitative evaluation of the relative content of the organic matrix in bones of different origins was done based on the ratio between area of the amide I band centered at 1646 cm⁻¹ and that of the complex band from 900-1200 cm⁻¹ range assigned to calcium phosphate.

The differences between the composition of cancellous and compact parts of the antler are well illustrated by the FTIR spectra (Figure 2) and also by the thermal analysis (Figures 3 and 4). In this case the ratio of organic/inorganic matrix evidenced by the FTIR spectra is clear greater for the sample prelevated from the cancellous part of antler tissue.

![Figure 1. FTIR spectra of bones of different origins: deer antler, human skull and pig bone.](image-url)

![Figure 2. FTIR spectra of two samples of deer antler (three years old).](image-url)

![Figure 3. TG, DTG and DTA curves of a sample of the compact part of deer antler (three years old).](image-url)
Figura 4. TG, DTG and DTA curves of a sample of the cancellous part of deer antler (three years old).

The literature data indicate a maximum exothermal peak for the type I collagen between 500-540°C depending on the extraction method [8,9]. For deer antler tissue we noticed three exothermal effects (See Figures 3, 4 and Table 1) determined by the presence of a collagen with different reticular degrees (maxima at 380°C and ~450°C). The third exothermal effect observed at ~560°C is assigned to the free collagen from deer antler that is unreticulated. The greater mass loss by these exothermal effects is for the sample prelevated from the cancellous part (44%) while for the compact one it is only 34%. The endothermal effect observed at 100°C is due to the water loss from the bone tissue.

Table 1. Thermal analysis results for the compact and cancellous parts of deer antler (three years old)

<table>
<thead>
<tr>
<th>Thermal Effect [°C] / Mass loss [%]</th>
<th>Total mass loss [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exo</td>
<td>I</td>
</tr>
<tr>
<td>Endo</td>
<td>10.0</td>
</tr>
<tr>
<td>Compact part</td>
<td>44.0</td>
</tr>
<tr>
<td>Cancellous part</td>
<td>54.0</td>
</tr>
</tbody>
</table>

*maximum and **range of exothermal effects respectively

The amount of organic component in samples of different origins decreases in the order human skull, deer antler, pig bone. The thermal treatment of powdered bones at 150°C produces the diminishing of the organic component. This process is illustrated for the deer antler, human skull and pig bone in Figures 5-7. One can notice also the reduction of the intensity of 1239 cm⁻¹ band (amide III) with the increase of temperature.

At 600°C the deproteination of bones is finished and the spectra of samples treated at higher temperatures put in evidence only the hydroxyapatite from the inorganic matrix. The absorption bands characteristic to carbonate ion are still present in spectra of samples treated at 900°C. From the infrared spectra we could not establish the nature of carbonate substitution site: hydroxy or...
phosphate. However, we can assume that the finally step of carbonate loss at temperatures higher than 900°C is from the hydroxy site if we have in view the modifications from the spectra of samples treated at 1200°C. At this temperature the bone samples consist of more crystalline carbonate free hydroxyapatite without any organic components.

In the spectra of samples treated at 1200°C the maxima at 3570 cm⁻¹ assigned to non-hydrogen bonded OH groups and 630 cm⁻¹ assigned to the OH libration are well evidenced. These two bands are also an indicative of a higher crystallinity of hydroxyapatite after the thermal treatment. In the less crystallized hydroxyapatite the band assigned to OH libration appears as a shoulder on the ν₄ phosphate band or just only as a broadening of this band in the spectrum of bone sample.

Termine and Posner [10,11] proposed a method that correlates the size change of hydroxyapatite crystals from bones with the splitting of the phosphate ν₄ band observed in the 500-700 cm⁻¹ region of the spectra. The evolution with temperature of the crystallinity index calculated using the Termine’s method is illustrated in Figure 8.

![Figure 8. The crystallinity index of studied samples.](image)

The effect of temperature on the crystallization of hydroxyapatite from the deer antler and human skull is more reduced comparatively to the pig bone. We mention the value of 5.71 for the crystallinity index calculated in the case of a commercial hydroxyapatite sample heated at 1200°C.

4. CONCLUSIONS

FTIR spectroscopy put in evidence the main components of studied bones, namely the organic matrix (collagen) and the mineral component (carbonate substituted hydroxyapatite).

The effect of thermal treatment is the complete deproteination of bones at temperatures around 600°C and the removal of carbonate ion from samples treated over 900°C. Another effect of this treatment at high temperature is the modification of hydroxyapatite crystallinity assessed by the index determined from the FTIR spectra.

These studies are useful to understand the properties of some new biomaterials with possible applications in bone reconstruction.

5. ACKNOWLEDGMENTS

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6. REFERENCES