Optical analysis of the osteoporotic bone tissue and evaluation of its prevention using allogenic hydroxyapatite

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Abstract. The results of the experimental research of the osteoporotic bone tissue and the Raman spectroscopy evaluation of its prevention using allogenic hydroxyapatite during resorption are presented in this work. The Raman spectra were obtained and their deconvolution and comparative analysis was made in this work. The coefficients allowing evaluating the osteoporosis model treatment using hydroxyapatite were implied. The treatment of more intensive osteoporosis model did not show any changes, which indicates that the treatment of this osteoporosis model was not effective in this case.

1. Introduction

Osteoporosis is one of the most important issues of health care, but the frequency of osteoporosis cases has grown steadily in recent decades. Osteoporosis is a systemic disorder of the bone tissues with the simultaneous loss of organic matrix and minerals caused by low osteoblast activity [1]. The bone mass per its volume decreases. That leads to the bone weakness and fractures [2].

Glucocorticoid- or steroid-induced osteoporosis is a form of secondary osteoporosis and results from pathological increase of endogenous glucocorticoid production by adrenal glands or is caused by corticosteroid synthetic analogue injections for treating different diseases [3]. Glucocorticoids are often used in kidney, liver, heart transplantation, etc. The steroid osteoporosis causes decrease in weight of all bones, but the process is more active in trabecular than in cortical bones [4].

The effect of the glucocorticoid effect on the bone lies in its influence on the osteoblasts. The collagen synthesis is oppressed, that in the end results in the decline of bone mineral density [5]. In this regard it is necessary to develop new methods of osteoporosis evaluation during steroid-induced osteoresorption.

The optical methods are rather popular among the methods [6]. The Raman spectroscopy method is widely used in biomedical researches. In particular, it can be applied for diagnostics of diseases and evaluation of treatment [7].

The aim of this work is optical analysis of the osteoporotic bones and evaluation of the conducted treatment using allogenic hydroxyapatite.

2. Materials and methods of research
The mature female rats at the age of 6-9 months with a mass of 180-230 grams were used as the samples in the research. The animals were divided into three groups. The first group (control) is a group of healthy animals. The second group (cortisone) is a group of rats, in which the model of osteoporosis was created by cortisone (steroid hormone medication with anti-inflammatory, anti-exudate (anti-edema), desensitizing (antiallergic), immunosuppressive, antishock and antitoxic effect) injections. The third group (cortisone + HA) is a group of animals in which the model of osteoporosis was made by injection of cortisone medication with the subsequent course of treatment using the powder of hydroxyapatite (HA). The amount of the injected drugs per rat mass estimated 10 mg/kg and 40 mg/kg (the second and third groups were divided into two subgroups).

More over the bones were analyzed for mechanical breaking and bending strength.

The bone spectral characteristics were studied using the experimental stand. The Raman probe focuses laser radiation on the subject (in the distance of 7.5 mm from the window with focal point diameter of ~0.15 mm) and collects the backscattered radiation which is then directed through the optic fiber to the spectrometer (spectral resolution of 0.15 nm (~1 cm\(^{-1}\))) with the built in chamber [8].

The chamber is cooled down to -60 °C for noise level decrease. The transporter with a stepper motor allowed the sample spatial scanning [9].

The spectrum processing was conducted using the software package Wolfram Mathematica 10. During the processing the researched spectrum was cleared up from noises using the smoothing median filter (5 points), the approximation line (a fifth order polynomial) of autofluorescent component was determined on the chosen interval of 400-2200 cm\(^{-1}\) using an iteration algorithm, and then this component was subtracted and as a result the selected Raman spectrum was received [10].

3. Results

Some lines in the spectra overlap the nearby lines. In order to analyze them, spectrum decomposition was conducted (the Gaussian function was used). The analysis was made using MagicPlotPro 2.7.2 software.

The received averaged Raman spectra of the groups of rats with the models of osteoporosis are shown in figure 1. The Raman lines correspond to the oscillation modes of the substances and connections, presented in table 1.

The main spectral differences were found on the wavenumbers 857 cm\(^{-1}\), 956 cm\(^{-1}\), 1430 cm\(^{-1}\) and 1660 cm\(^{-1}\).

During the study of osteoporosis, above all, the attention is payed to the change on the wavenumbers 956 cm\(^{-1}\) and 1067 cm\(^{-1}\), as the defects of the constructions of trabecula ossea, their thinning and increase of the distance between them, that takes place at osteoporosis, is due to the phosphate ion \(\text{PO}_4^{3-}\) replacing by carbonates \(\text{CO}_3^{2-}\) in the formula of hydroxyapatite [17]. In the received Raman spectra it is illustrated by the decrease of the intensity peaks on the wavenumber 956 cm\(^{-1}\) (phosphate ion \(\text{PO}_4^{3-}\)) replacing by carbonates \(\text{CO}_3^{2-}\)) in groups "cortisone (40 mg/kg)", "cortisone + HA (40 mg/kg)", and "cortisone (10 mg/kg)".

The wavenumber 1033 cm\(^{-1}\), corresponds to the "breading" mode of phenylalanine benzene ring. This amino acid is important for osteoporosis study, as it participates in the process of collagen synthesis and defines such collagen fibril properties as rigidity and elasticity. The reduction of this peak prevails in the groups of samples "cortisone (40 mg/kg)" and "cortisone + HA (40 mg/kg)" as compared to the other samples.

The water loss from collagen causes the significant change in the collagen fibers (fibrils) that become less elastic leading to the increase of the fibril breakage possibility under tension, which causes the bone elasticity and rigidity decrease in general [18].

The type I collagen is the basic protein component of bones, providing their strength. The amino acid sequence of collagen is rich in proline about half of which hydroxylates with formation of hydroxyproline in the process of collagen breakdown [19]. Therefore it is possible to use information about change of amide I and hydroxyproline on the wavenumbers 1660 cm\(^{-1}\) and 857 cm\(^{-1}\) accordingly for the analysis of change in a bone matrix.
Figure 1. Averaged Raman spectra of the bone samples, where a – control group, b - cortisone + HA (10), c - cortisone (10), d - cortisone + HA (40), e - cortisone (40)

The wavenumber 1389 cm\(^{-1}\) corresponds to the vibrations of glycosaminoglycans that are part of proteoglycans.

On the basis of the lines described above, the coefficients characterizing the component composition of the bone samples were implied. The intensity on the wavenumber 1067 cm\(^{-1}\) was chosen as a denominator:
\[ K = \frac{I_j}{I_{1067}} \]

where \( I_j \) – intensity on the wavenumbers \( j \) of the described above components.

The two-dimensional diagrams considering the implied coefficients are shown in figure 2.

Table 1. Interpretation of the Raman lines of bone tissue.

| Wavenumber \( k \), cm\(^{-1}\) | Substance, molecular vibration | Reference |
|-------------------------------|--------------------------------|-----------|
| 857                           | hydroxyproline \( \nu \) (C-C) | [11]      |
| 924                           | Proline, \( \nu \)(C–N)        | [12]      |
| 956                           | \( \nu 1 \) (PO\(_4\))^\(_3^-\) phosphate | [12]      |
| 1001                          | \( \nu \)(C–C) Phenylalanine   | [13]      |
| 1033                          | Phenylalanine                  | [14]      |
| 1067                          | \( \nu 1 \) \( \text{CO}_2\) - B-type carbonate | [11], [13] |
| 1165                          | Pyranose ring                  | [14]      |
| 1262                          | Amide III                      | [11]      |
| 1389                          | CH\(_3\), glycosaminoglycans    | [14]      |
| 1430                          | C–H bending                    | [15]      |
| 1551                          | Amide II                       | [14]      |
| 1587                          | Amide I                        | [11]      |
| 1660                          | Amide I                        | [11]      |
| 1737                          | (phospholipids (C=O valent))   | [16]      |

The diagrams show that all coefficients in the groups "cortisone 40" and "cortisone + HA 40" are characterized by more sharp deviation from control group as compared to the groups "cortisone 10" and "cortisone + HA 10", which is caused by development of osteoporosis. All the coefficients also indicate that the group "cortisone + HA 10" shifts to control group as compared to the group "cortisone 10", while the group "cortisone + HA 40" does not show these results in relation to the group "cortisone 40" in case of coefficients \( I_{1430}/I_{1067} \), \( I_{1033}/I_{1067} \) and \( I_{1389}/I_{1067} \). This suggests that the treatment of this osteoporosis model using the HA powder is ineffective.

Figure 2 shows the increase of the both coefficients in the groups of osteoporosis models of 10 mg/kg and 40 mg/kg from 0.3-0.42 to 0.42-0.7 and 0.42-0.69 for \( I_{857}/I_{1067} \) and from 0.43-0.67 to 0.57-0.73 and 0.5-0.88 for \( I_{1033}/I_{1067} \) accordingly as compared to control group. The development of osteoporosis is followed by the destruction of collagen and freeing of the amino acids that are part of it. Thus, the amount of hydroxyproline and phenylalanine rises as compared to the substituted carbonates; it can serve as markers of osteoporosis development [20, 21].

The similar increase of values can be seen in figure 2-c. It can be explained by the increase of non-regenerative collagen connections in relation to carbonates; as a result elasticity of collagen fibers [22], as well as of glycosaminoglycans, that can serve as the markers of bone resorption [23] decreases.
Figure 2. The two-dimensional ratio of the implied coefficients
4. Conclusion
Spectral changes were detected on the wavenumbers 857 cm\(^{-1}\), 956 cm\(^{-1}\), 1033 cm\(^{-1}\), 1389 cm\(^{-1}\), 1430 cm\(^{-1}\) and 1660 cm\(^{-1}\) for the groups of samples (control group, the group with the model of osteoporosis and the group with the model of osteoporosis after the HA treatment) as a result of this work. The coefficients allowing estimating the efficiency of treatment of the cortisone osteoporosis model (10 mg/kg) using HA were introduced. There were no changes during the HA treatment of the model with cortisone of 40 mg/kg, which in this case indicates that treatment of this osteoporosis development model was ineffective.

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