САМАРСКИЙ УНИВЕРСИТЕТ
SAMARA UNIVERSITY

RAMAN SPECTROSCOPY METHOD FOR BONE ANALYSIS DURING OSTEORESORPTION

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1 Samarsky national research University named after academician S. P. Korolev (Samara University)

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Relevance of the study

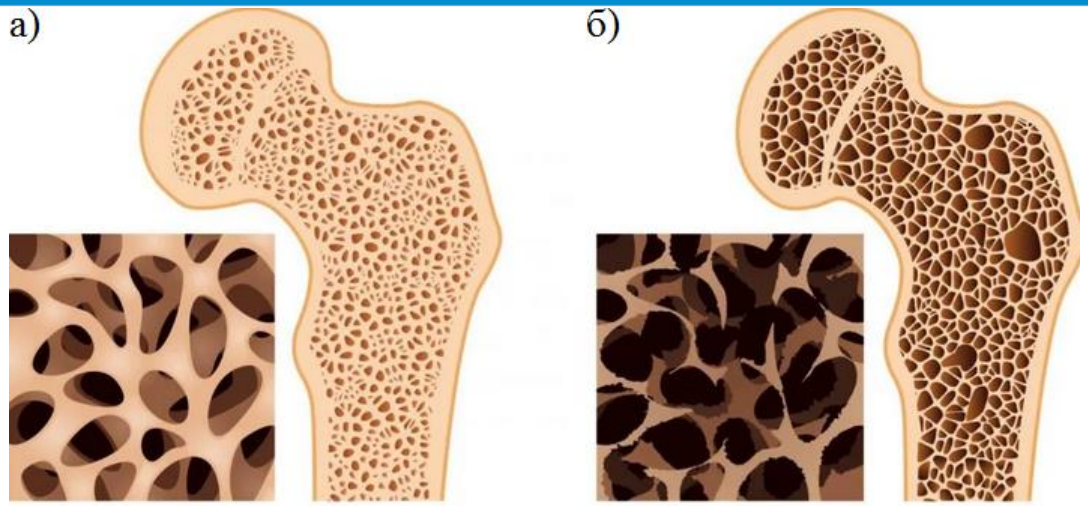


Figure 1 – Bone: a) healthy bone tissue b) bone affected by osteoporosis

Microgravity experienced by astronauts during space flights causes accelerated loss of bone mass, there is a violation of the mineral metabolism of bone tissue, leading to the development of osteoporosis.

The process of adaptation of bone tissue to microgravity conditions is expressed in its remodeling, accompanied by changes in the kinetics of calcium. Calcium dynamics can provide valuable information about the balance of bone resorption and neoplasm processes after space flight and during the recovery period.

Goals and objectives

The aim of this work is to apply the Raman spectroscopy method for spectral evaluation of bone tissue when modeling osteoresorption under anti-orthostatic hanging conditions.

Tasks:

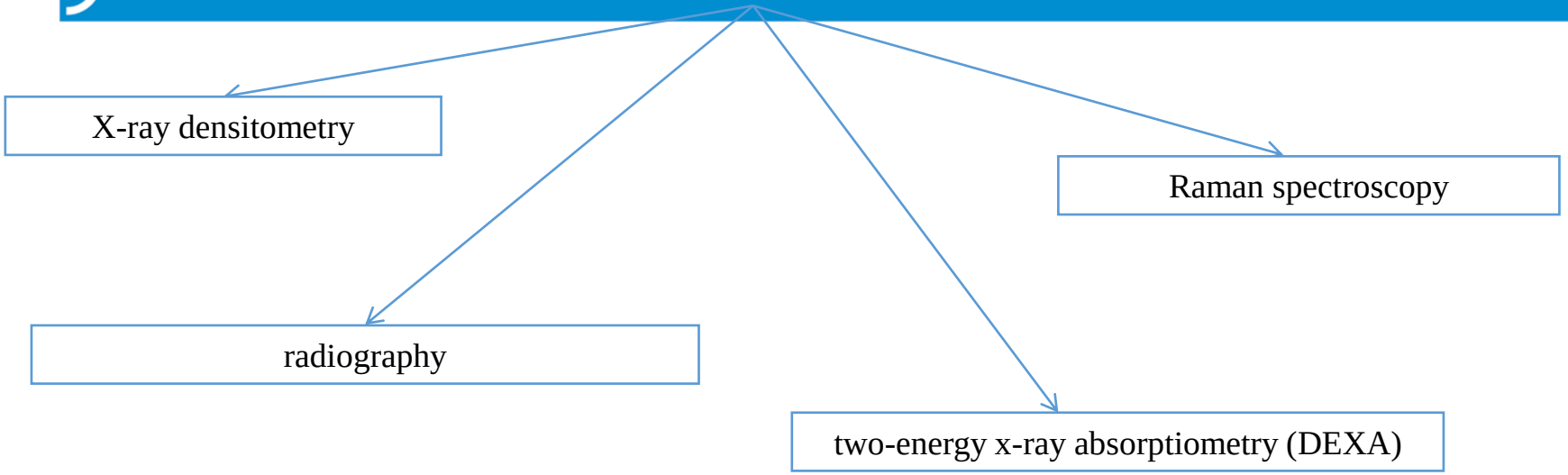
1) Study the structure and optical properties of bone tissue;

2) To conduct studies of cortical bone in the simulation of ostertibble in microgravity;

3) Perform spectrum analysis using the method of selecting the spectral contour and deconvolution of the Gaussian function in the MagicPlotPro software environment to increase the information content of the spectra;

4) To study the Raman spectra of rat bone tissue during osteoresorption using the linear discriminant analysis (LDA) method.

Research methods for detecting osteoresorption of bone tissue



Changes in simple radiographs can only be seen after approximately 30% of the bone is lost.

Raman spectroscopy quantifies such characteristics as the mineral-to-matrix ratio, carbonate substitutions, and crystallinity, which are uniquely different from the x-ray parameters that depend only on the presence of the mineral.

Experimental stand

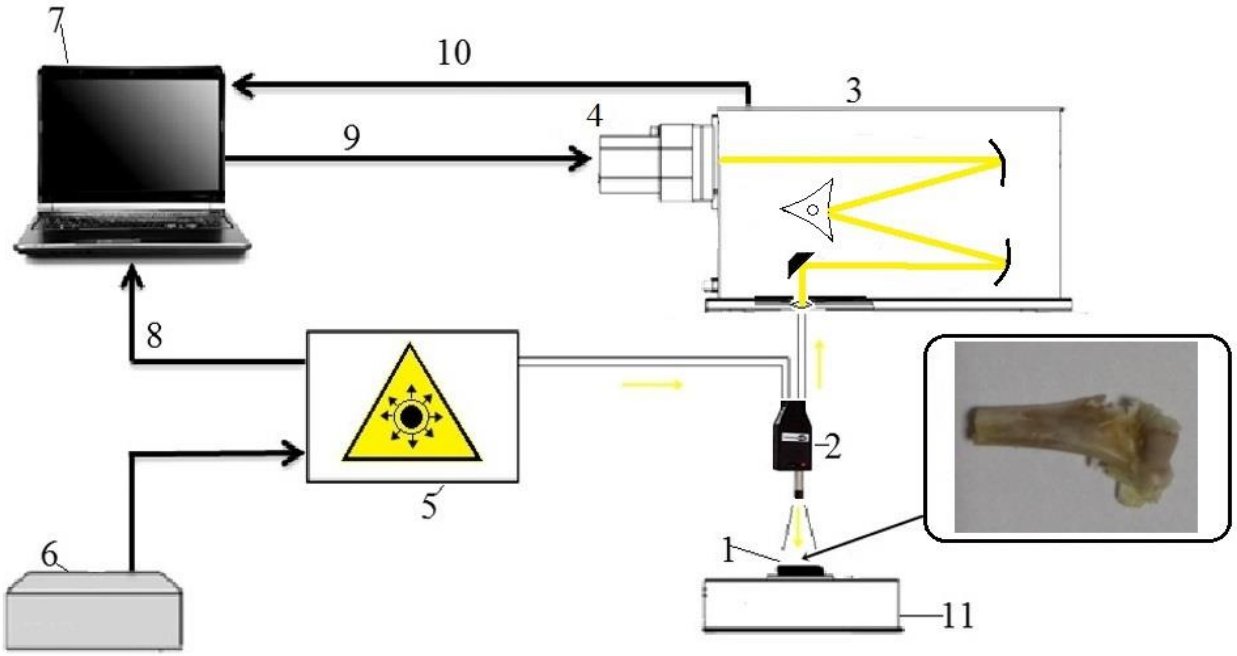


Figure 2 - experimental stand

Characteristics of the stand:

1 – spectral range: 200-1200 nm;
2 – exposure time from 0.01 to 10 s;
3 – spectral expansion of 0.15 nm;
4 – laser wavelength of 785 nm;
5 – laser power up to 500 mW.

Description of the installation:

1 – object under study;
2 – RPB785 RAMAN probe;
3 – Shamrock sr-303i spectrometer;
4 – built – in DV420AOE cooled camera;
5- LuxxMasterRamanBoxx laser module;
6 – laser module power supply;
7 – computer;
8, 9, 10 – electrical information cables;
11 – coordinate table with stepper motor (scanning step 0.2 mm)

Research materials

The experiment was performed on adult mongrel laboratory rats of both sexes, which were divided into groups according to the following study scheme:

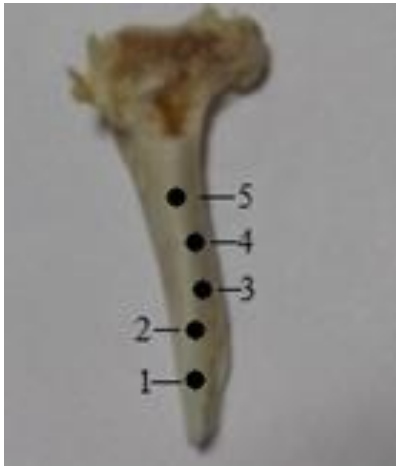


Figure 3 – test Sample

Animals of the **I** group were exposed to anti-orthostatic hanging for 28 days using the Novikov-Ilyin method.

II group included animals that were exposed to anti-orthostatic hanging and glucocorticoids for 28 days.

III and **IV** groups are similar to the first and second, with additional administration of hydroxyapatite at a dose of 100 mg/kg of body weight.

V group – intact animals (control).

Results of spectral analysis

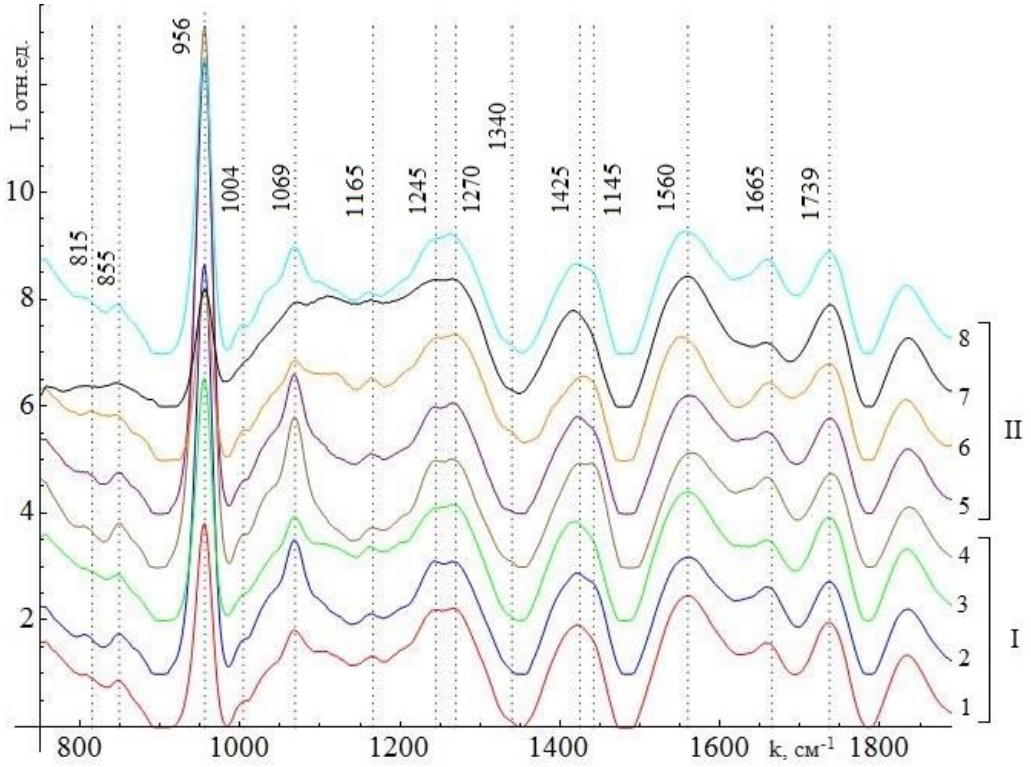


Figure 4 – Averaged spectra of rat cortical humerus bones:

I – Females: 1 – hanging for 20 days; 2 – hanging for 28 days + glucocorticoids at a dose of 40 mg/kg daily; 3 – hanging for 15 days + glucocorticoids at a dose of 40 mg/kg daily; 4 – hanging for 28 days + glucocorticoids at a dose of 40 mg/kg daily + hydroxyapatite at a dose of 100 mg/kg once on the first day;

II – Males: 5 – control; 6 – hanging for 45 days; 7 – hanging for 28 days + glucocorticoids at a dose of 40 mg/kg daily; 8 – hanging for 45 days + hydroxyapatite at a dose of 100 mg/kg once on the first day.

Table 1 - interpretation of the RAMAN spectrum

cm^{-1}	Substance, oscillation	cm^{-1}	Substance, oscillation
855	Hydroxyproline v(C–C) stretch	1270	Amide III, (α-helix) (C-N-H stretching)
956	v1 P–O symmetric stretch PO^{3-}_4	1560	Amide II (C-N bend)
1004	Phenlyalanine	1739	Phospholipids (C =O)
1069	C-O in plane stretch (CO_3^2 v1).		

Results

As can be seen from figure 3, the main spectral differences between the model of osteoporosis and the model of osteoporosis with HAP treatment are observed on the lines 855 cm-^{1} (Hydroxyproline ν(C–C) stretch), 956 cm^{-1} (PO^{3-}_{4} P–O symmetric stretch), 1069 cm^{-1} (Carbonate CO_3^{2} - ν1).

An increase in hydroxyproline in control samples and samples with HAP treatment corresponding to the 855 cm-1 line may indicate a decrease in bone resorptive processes.

The mineral component of the bone tissue corresponding to the lines 956 cm^{-1} and 1069 cm^{-1} is responsible for the quality and strength of the bone. The stiffness and modulus of bone bending depend significantly on the degree of mineralization, mineral crystallinity, and B-type carbonate substitution. Increased replacement of B-type carbonate in osteoporosis leads to increased bone fragility.

Amide III and amide I, represented by the main lines at 1245 cm^{-1}, 1270 cm^{-1}, and 1665 cm^{-1}, respectively, are classified as collagen structures and do not change significantly when comparing the control and samples with the female and male osteoporosis model.

For a more detailed analysis, the RAMAN lines were divided and normalized to the intensity of the line 1739 cm-1 (Phospholipids) using the linear discriminant analysis (LDA) method, the results of which are shown on the next slide.

Results of linear discriminant analysis (LDA)

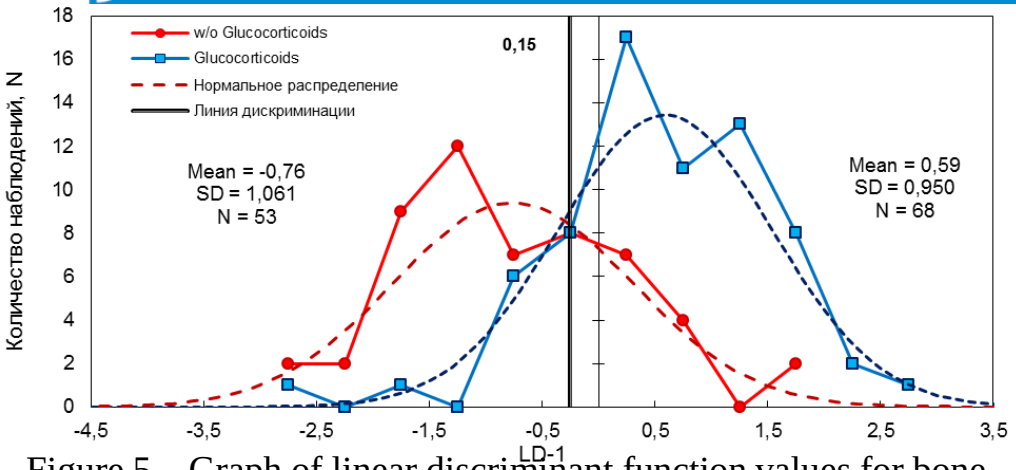


Figure 5 – Graph of linear discriminant function values for bone samples, divided by the effect of glucocorticoid dose.

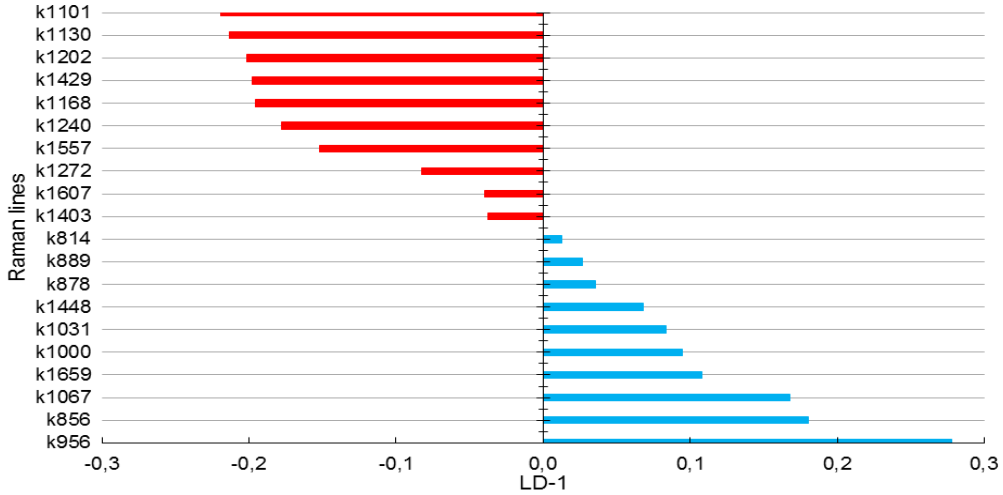


Figure 6 - Graph of the values of the structural matrix for differentiating samples based on the effect of the dose of glucocorticoids

An analysis of the relationship between groups of objects based on the influence of a daily dose of 40 mg/kg of glucocorticoids is shown in figure 3. it is Shown that the main differences between the two groups of samples are described by the discriminant function LD-1. The sample consists of 121 Raman spectra. Positive values of LD-1 are mainly characteristic of the RAMAN spectra obtained for samples under the influence of a dose of glucocorticoids of the group, and Vice versa negative values for the control group of samples.

The higher the value of LD-1 for a variable, the more it affects the observed difference in the component composition, which, for example, can be seen from the value of the coefficient k956, corresponding to the P-O symmetric stretch PO43-(V1) hydroxyapatite. Similarly, the relative intensities of the CR lines 1000 cm-1 proline and hydroxyproline, 1031 cm-1 phenylalanine, and the 1067 cm-1 line corresponding to the C-O in plane stretch of the hydroxyapatite carbonate ion CO32-(V1) predominate in bone tissue. The relative intensities of these lines are higher in the samples under the influence of glucocorticoids.

Conclusion

As a result, the spectra were deconvoluted using the method of selecting the spectral contour and deconvolution of the Gaussian function in the MagicPlotPro 2.7.2 software environment, which made it possible to conduct an extended composite qualitative and quantitative analysis of bone tissue in rats in microgravity. The main spectral differences of the studied objects (control samples, samples with the model of osteoporosis, samples with the model of osteoporosis in the treatment of hydroxyapatite) observed on the lines 855 cm-1, 956 cm-1, 1069 cm-1 were established.

The results obtained can later be used for the prevention and treatment of osteoporosis in astronauts, maintaining the health of crews during long-term interplanetary missions, as well as for correcting violations of mineral homeostasis and other diseases associated with impaired bone and cartilage metabolism and mineral homeostasis.

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THANK YOU FOR YOUR ATTENTION

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