Method of creation and same mechanical properties of hydroxyapatite envisaged for bone tissue replacement

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Introduction. The creation of artificial organs and substitutes for biological tissue and systems is one of the most vital problems of biomechanics. Various biomaterials, such as hydroxyapatite, titanium, Ti-Al-V alloy, polyethylene, composites based on porous nano-hydroxyapatite/collagen, biocomposites with different hydroxyapatite-collagen ratios as well as many others, play an important role in the creation of artificial materials for replacing the bone tissue [1-4]. In the present study, a procedure is described for obtaining natural hydroxyapatite (NHAp) from bone tissue of cattle. The structure of bone tissue before and after deproteinization was investigated by the method of optical microscopy. Some characteristics of the cattle bone tissue and NHAp were determined. The bone tissue of cattle and NHAp have been examined because of their potential use as bone substitutes [3].

Methods. The NHAp was obtained from the bone tissue of cattle. Before deproteinization, the bone was freed from the soft tissue and fat and cut into 2-4 mm-thick layers. Then, the bone specimens were placed in a furnace and heat-treated in a suspended state in a stream of air at a temperature gradually increasing from room temperature to 400-415 °C for 1.5 h, after which it was kept at constant temperature for 5.0-5.5 h.

The results of experiments, performed by absorbtion method of infrared spectroscopy, wave interval 400-3500 cm^{-1}, using spectroscope SCIMITAR 800 MIR (USA), show that the protein was removed from the heat-treated specimens of bone tissue practically completely. Fig. 1 show that the stripes of absorption which characterize protein (1240, 1540 and 1660 $см^{-1}$) are absent. Opposite, the stripes of absorption which characterize the mineral part of bone tissue remained unchanging.

The structure of bone tissue before and after deproteinization was investigated by the method of optical microscopy (see Fig. 2). Samples were investigated in reflected light with differential interference contrast microscopy, using Leica DMLP microscope (Germany).

Some characteristics of mechanical properties of the cattle bone tissue before and after deproteinization were determined. Specimens of cattle bone tissue before deproteinization were loaded in tension at strain rates of 0.2 mm/min, 2.0 mm/min, 20.0 mm/min and 200.0 mm/min. These specimens were 70 mm long, 5 ± 0.3 mm wide and 1.3 ± 0.1 mm thick.

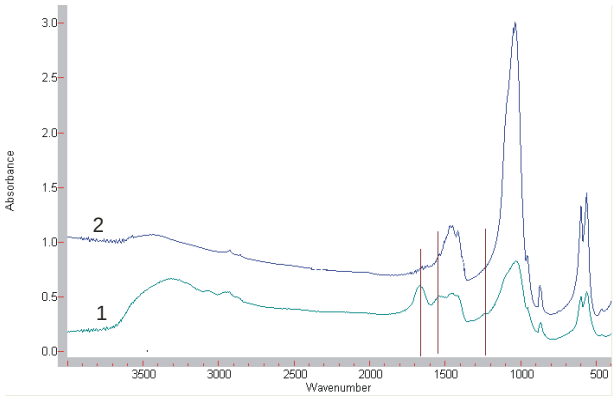

Fig. 1. Infrared absorption spectrum of the initial bone tissue (1) and those deproteinized at t=400-415 ^{o}C (2)

Thirty-two specimens were tested. Eight specimens from each group were tested to obtain an average value. The experiments in uniaxial tension were carried out with an IMP 0.5 automatic testing machine controlled by MTS testing system (USA). The tests were continued up to failure of the specimens. On the basis of experimental results the Young's modulus (initial modulus of elasticity), ultimate tensile stress and ultimate tensile strain were determined. Relationships of $\sigma - \varepsilon$ (see Fig. 3), $\sigma^* - V$, $\varepsilon^* - V$, and E - V (see Fig. 4) were obtained.

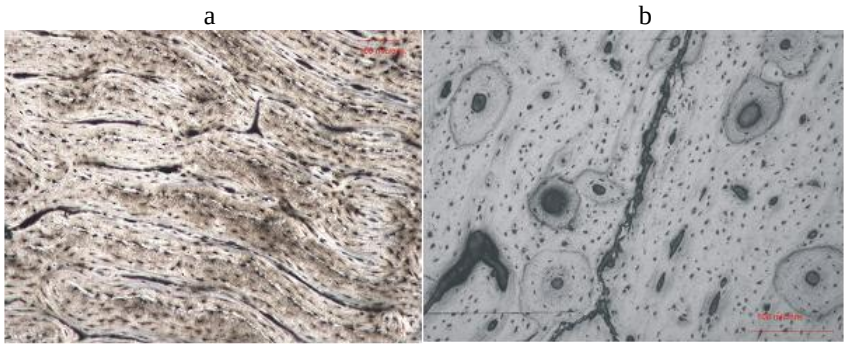

Fig. 2. Micrographs of cattle bone tissue before (a) and after (b) deproteinization. Magnification 100x

Also specimens of catle bone tissue before and after deproteinization were loaded in compression at strain rate of 0.5 mm/min. The specimens had the form of a parallelepiped 10 ± 0.1 mm long, 4.2 ± 0.2 mm wide and 4.2 ± 0.2 mm thick for catle bone tissue before deproteinization, and 20 ± 0.1 mm long, 6.5 ± 0.5 mm wide and 6.5 ± 0.5 mm thick after deproteinization. Twenty

specimens were tested. Ten specimens from each group were tested to obtain an average value. The compression tests were carried out with an INSTRON-4301 testing machine (GB). The tests also were continued up to failure of the specimens. On the basis of experimental results the Young's modulus, ultimate compression stress and ultimate compression strain were determined, and $\sigma - \varepsilon$ relationships were obtained.

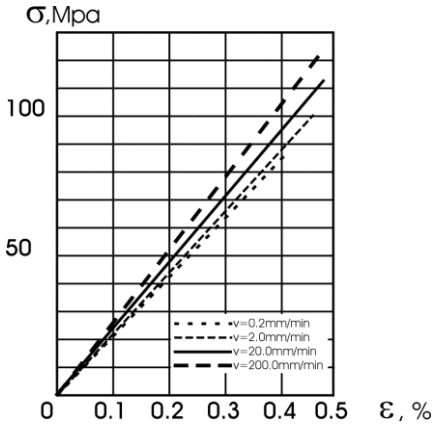

Fig. 3. Relationship $\sigma - \varepsilon$ for cattle bone tissue before deproteinization in tension at different strain rates

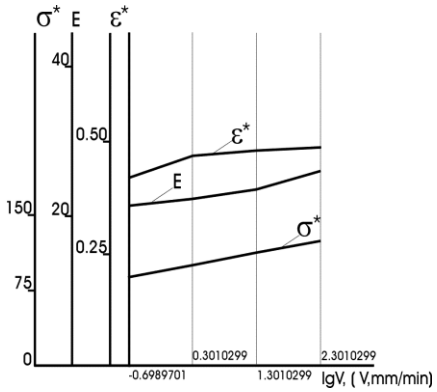

Fig. 4. Relationship $\sigma^* - V$, $E - V$ and $\varepsilon^* - V$ for cattle bone tissue before deproteinization in tension

Also four-point bending tests were carried out using an INSTRON-4301 testing machine. Six samples of catle bone tissue before and six samples after deproteinization were tested to obtain an average value. The test was carriet out using rectangular bars with (45.0 ± 1.0) × (4.5 ± 0.5) × (4.5 ± 0.5) mm. The samples were tested with a crosshead speed of 0.5 mm/min and the span was 20 × 40 mm. The four-poin bend strengths (σ) were calculated using the equation (1):

$$\sigma = 3F\frac{(L-L_1)}{2bd^2} \qquad (1)$$

where F is applied force (N); L, the span of the support loades (mm); L_1, the separation of the loading span (mm); b, sample width (mm); d, sample thickness (mm).

The density (ρ) and porosity (μ) of cattle bone tissue before and after deproteinization were determined by water displacement. The specimens had the form of a parallelepiped 25.0 ± 1.0 mm long, 8.0 ± 1 mm wide, and 3.0 ± 0.5 mm thick. The ρ and μ of these materials were determined on analytical scales XT 220 A (Switzerland). The ρ and μ of the cattle bone tissue before and after deproteinization are expressed as follows (equations 2 and 3):

$$\rho = \frac{m_1}{m_3 - m_2} \times \rho_{H_2O} \quad (2)$$

$$\eta = \frac{m_3 - m_1}{m_3 - m_2} \times 100\% \quad (3)$$

where m_1 is mass of dry specimen (g); m_2, the mass of moist specimen in water (g); m_2, the mass of moist specimen in air (g); $\rho_{H_2O} = 0.988$, the density of water (g/cm^3). The results of experiments are shown in Table 4.

Results. All the results for cattle bone tissue before and after deproteinization (NHAp) were analysed statistically using the standard test. The determined characteristics of mechanical properties are shown in Table 1, Table 2 and Table 3.

Table 1. Characteristics of the mechanical properties of cattle bone tissue in tension at different strain rate

Strain rates, mm/min	**Ultimate stress, MPa, mean ± SD**	**Initial modulus of elasticity, GPa, mean ± SD**	**Ultimate strain, %, mean ± SD**
0.2	88.12 ± 17.62	21.30 ± 2.71	0.417 ± 0.05
2.0	100.0 ± 19.50	22.24 ± 1.38	0.463 ± 0.08
20.0	112.75 ± 20.78	23.47 ± 3.12	0.479 ± 0.07
200.0	124.85 ± 16.23	25.84 ± 1.77	0.486 ± 0.05

Table 2. Characteristics of the mechanical properties of cattle bone tissue before and after deproteinization in compression

Materials	**Ultimate stress, MPa, mean ± SD**	**Initial modulus of elasticity, GPa, mean ± SD**	**Ultimate strain, %, mean ± SD**
Cattle bone tissue before deproteinization	107.5 ± 34.03	2.963 ± 0.818	2.857 ± 0.318
Cattle bone tissue after deproteinization	38.92 ± 13.22	2.754 ± 0.532	1.234 ± 0.035

Table 3. Characteristics of the mechanical properties of cattle bone tissue before and after deproteinization by four-point banding

Materials	**Ultimate stress, MPa, mean ± SD**	**Initial modulus of elasticity, GPa, mean ± SD**
Cattle bone tissue before deproteinization	195.80 ± 42.33	22.510 ± 1.870
Cattle bone tissue after deproteinization	103.73 ± 27.12	18.345 ± 2.117

Table 4. Densities and porosities of cattle bone tissue

Materials	**Density, g/cm^3**	**Porosity, %**
Cattle bone tissue before deproteinization	1.980	5.83
Cattle bone tissue after deproteinization	1.463	49.8

Discussion. Experimental data provide a necessary basis for biomechanical analysis, although overall knowledge is certainly not exhaustive. Several facts have emerged clearly from these experiments.

On the basis of the results of experiments, performed by the method of infrared spectroscopy we can do a following conclusion – a new method for removing protein from cattle bone tissues by heat treatment at a temperature under 415 ^{o}C allows one to completely preserve the mineral structure of bones with the purpose of its further use as a filler for biocomposite materials.

From results of optical microscopy we can assert that, on the microphotographs, of bone samples before thermal treatment (see Fig. 2a), clearly we can see in the literature [4] described bone lamellas which forms multi-layer cylindrical structural elements – osteons. Moreover, in the bone samples which were exposed to thermal treatment (see Fig. 2b), these structures are expressed more intensive. Taking into account that all bone arhitectonic structures of lamellas and bone fibrillas are preserved after thermal treatment and it is possible to see them in microscope we can assume that after elimination of protein bone holds it's initial bone architectonic structure.

It is necessary to note, that in bone samples before thermal treatment we can see luminating microsectors, especially good on the photograph (see Fig. 2a), it is possible to assume that this effect is caused due to interaction of light beam with protein substance – collagen in these regions. However, in the bone samples after thermal treatment (see Fig. 2b) we are not able to see such phenomenon.

The mechanical tests showed following results: increasing strain rate of cattle bone tissue before deproteinization increases ultimate tensile stress, modulus of elasticity and ultimate tensile strain. The experimental results of present paper showed that by increase of strain rate of specimens from 0.2 mm/min to 200 mm/min, ultimate stress, initial modulus of elasticity and

ultimate strain increased on 29,42%, 17,57% and 14,2%, respectively. Thus from present results we can assert that ultimate tensile stress more depends from a strain rate that both modulus of elasticity and ultimate strain.

From the experimental results of cattle bone tissue in compression we can see that ultimate stress decreases considerably after deproteinization (see Table 2). Opposite, initial modulus of elasticity changes insignificantly.

From experimental results by four-point bending also we can see that both ultimate stress and initial modulus of elasticity higher than in compression tests (see Tables 2 and 3).

Also from the results of experiments we can see that the protein has considerably lower density than mineral part of bone tissue. Thus we can report, that porosity of bone tissue after thermal treatment increases considerably (see Table 4).

References

1. Silva Marques J. M., Gomes P. S., Silva M. A., Silverio Cabrita A. M., Santos J. D., Fernandes M. H. Growth and phenotypic expression of human endothelial cells cultured on glass-reinforced hydroxyapatite. J. Mater. Sci. Mater. Med, 2009. V 20, No 3, – P. 725–731.

2. Zhang S. M., Cui F. Z., Liao S. S., Zhu Y., Han L. Synthesis and biocompatibility of porous nano-hydroxyapatite/collagen/alginate composite. J. Mater. Sci. Mater. Med, 2003.V 14, No 7, – P. 641–645.

3. Sena L. A., Caraballo M. M., Rossi A. M., Soares G. A. J. Mater. Sci. Mater. Med, 2009.V 20, No 12, – P. 2395–2400.

4. Young B., Lowe J. S., Stevens A., Heath J. W. WHEATER'S Functional Histology. A Text and Colour Atlas. CHURCHILL LIVINGSTONE ELSEVIER, 2006. – P. 457.

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