Mechanical Properties of Cattle Bone Tissue and Natural Hydroxyapatite

Viktors Filipenkovs[1], Lauris Rupeks[2], Ivars Knets[3], Natalija Borodajenko[4], Zilgma Irbe[5], Liga Mezmale[6], Ineta Rozenstrauha[7], Visvaldis Vitins[8], [1-8]*Riga Technical University*

Abstract. **Creation of artificial organs and substitutes for biological tissue is one of the most important problems of biomechanics. In the present study, a procedure is described for obtaining natural hydroxyapatite (NHAp) from bone tissue of cattle. The results of experiments, performed by absorption method of infrared spectroscopy, show that the protein was removed from the heat-treated specimens of bone tissue practically completely. The structure of bone tissue before and after deproteinization has been investigated by the method of optical microscopy. The characteristics of mechanical properties such as Young's modulus (initial modulus of elasticity), ultimate tensile stress, ultimate tensile strain, ultimate compression stress have been determined. The density and porosity of cattle bone tissue before and after deproteinization have been determined by water uptake.**

Key words: **bone tissue, mechanical properties, hydroxyapatite, bone substitutes.**

I. 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, porous nano-hydroxyapatite/collagen/alginate composite, biocomposites with different hydroxyapatite-collagen ratios, hydroxyapatite/polycaprolactone–chitosan composites, nanocrystalline hydroxyapatite, as well as many others play an important role in the creation of artificial materials for replacing the bone tissue [1-5].

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 to be used as bone substitutes.

II. METHODS

The NHAp was obtained from the bone tissue of cattle. Before deproteinization, the soft tissue and fat were removed from the bone and it was cut into 2-4 mm thick layers. Then, the bone specimens were placed in the furnace and heat-treated in a suspended state in a stream of air at a temperature gradually increasing from room temperature to 400 – 415 ^{0}C for 1.5 h, after which it was kept at a constant temperature for 5.0 – 5.5 h. Air was supplied into the furnace by a compressor through a pipe.

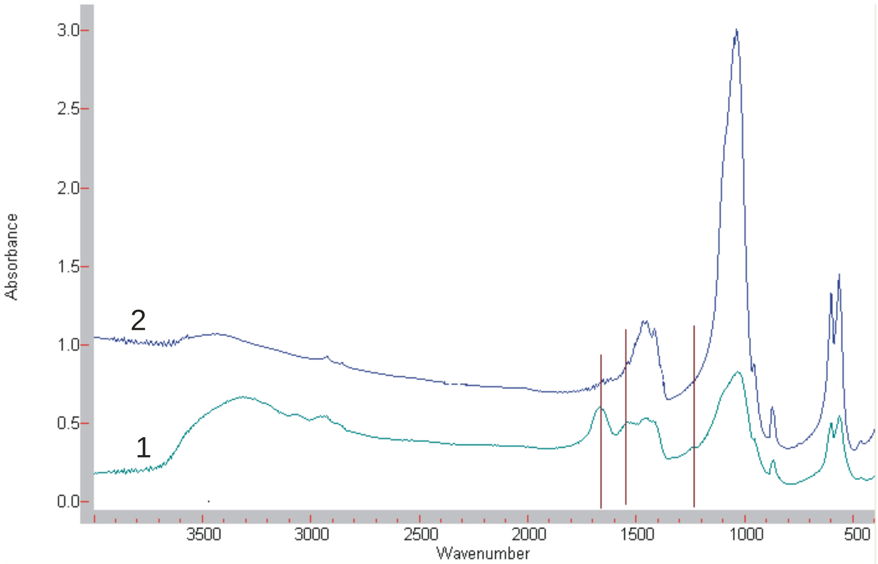
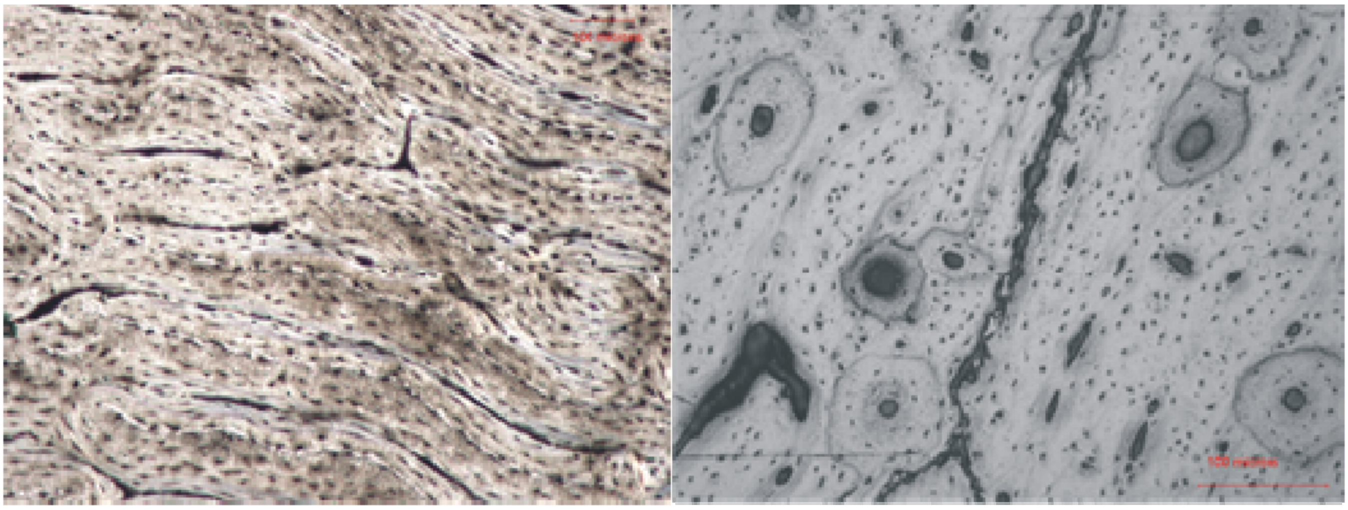


Fig. 1. Infrared absorption spectrum of the initial bone tissue (1) and those deproteinized at t = 400 – 415^{o}C (2)



a b

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

The results of experiments, performed by absorption 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 shows that the stripes of absorption, which characterize protein (1240, 1540 and 1660 $см^{-1}$), are absent [6]. On the contrary, 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 (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. Thirty-two specimens were tested. Eight specimens from each group were tested to obtain an average value. The experiments under 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 σ - ε (Fig. 3), σ^* - V, ε - V and E – V (Fig.4) were obtained.

Also specimens of cattle 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 cattle bone tissue before deproteinization, and 20±0.1 mm long, 6.5±0.5 mm wide and 6.5±0.5 mm thick for cattle bone tissue 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 were also 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. Orientation of samples was always the same.

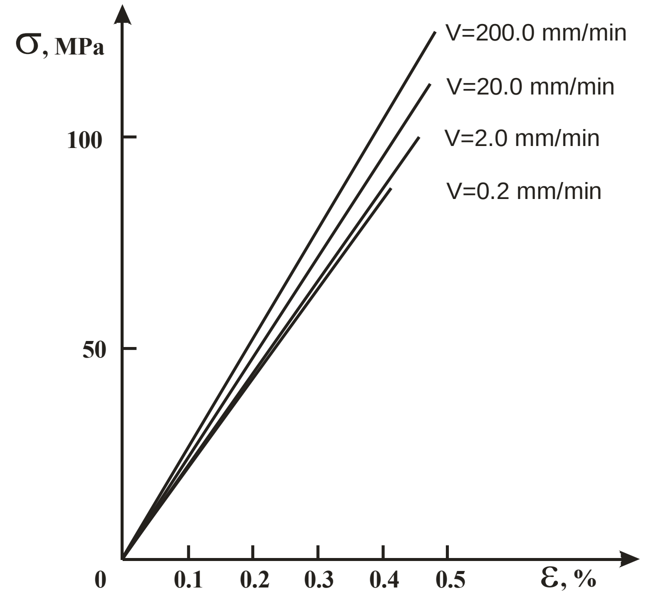


Fig. 3. Relationship σ-ε for cattle bone tissue before deproteinization in tension at different strain rates

Also four-point bending tests were carried out using an INSTRON-4301 testing machine [7]. Six samples of cattle bone tissue before and six samples after deproteinization were tested to obtain an average value. The tests were carried out using rectangular bars with dimensions of (45.0±1.0)×(4.5±0.5)×(4.5±0.5) mm. The samples were tested at a crosshead speed of 0.5 mm/min and the span was 20×40 mm. The four-point bend strength (σ) was calculated using the equation (1) [8]:

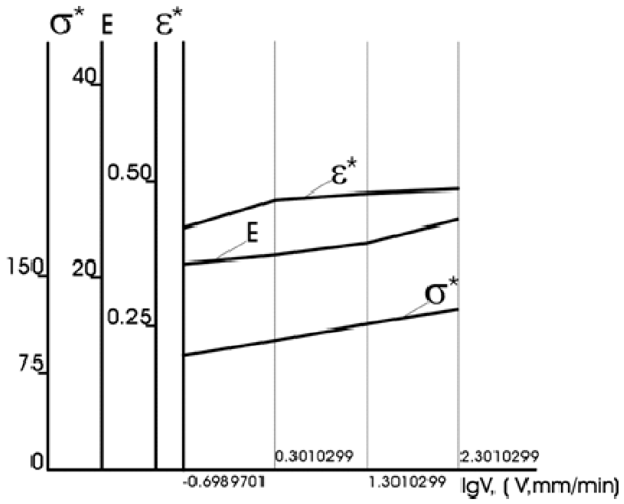


Fig. 4. Relationship $\sigma^* - V$, $E - V$ and $\varepsilon - V$ for cattle bone tissue before deproteinization in tension

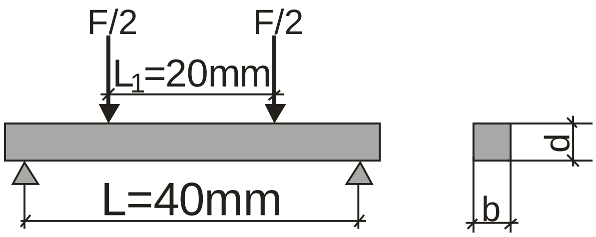


Fig. 5. Schematic illustration of a specimen in the four-point bend test

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

where F is the applied force (N); L, the span of the support loads (mm); L_1, the separation of the loading span (mm); b, sample width (mm); d, sample thickness (mm) (Fig. 5).

The density (ρ) and porosity (η) of cattle bone tissue before and after deproteinization were determined by water uptake [9]. The specimens had the form of a parallelepiped 25.0±1.0 mm long, 8.0±1.0 mm wide, and 3.0±0.5mm thick. The ρ and η of these materials were determined on analytical scales XT 220 A (Switzerland). The ρ and η of the cattle bone tissue before and after deproteinization are calculated by the equations 2 and 3:

$$\rho = \frac{m_1}{m_3 - m_2} \times \rho_{H_2O} \tag{2}$$

$$\eta = \frac{m_3 - m_1}{m_3 - m_2} \times 100\% \tag{3}$$

where m_1 is mass of dry specimen (g); m_2, the mass of moist specimen in water (g); m_3, 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.

III RESULTS

All results for cattle bone tissue before and after deproteinization (NHAp) were analysed statistically using a standard test. Mechanical characteristics 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 BENDING

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	9.078 ± 1.641	7.032 ± 1.436

TABLE 4

DENSITY AND POROSITY 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

IV. 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 experimental results, performed by the method of infrared spectroscopy, the following conclusion can be made – a new method for removing protein from cattle bone tissue 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.

Infrared absorption method and optical investigation using a light microscope have showed that mineral part of bone tissue remains unchanged after protein removing.

The experimental results of present paper showed that by increasing the strain rate of bone tissue specimens from 0.2 mm/min to 200 mm/min, ultimate stress, initial modulus of elasticity and ultimate strain increased by 41.68%, 21.31% and 16.55%, respectively. Thus from present results it can be asserted that ultimate tensile stress more depends on a strain rate that both modulus of elasticity and ultimate strain.

From experimental results of cattle bone tissue in compression, it can be concluded that ultimate stress decreases considerably after deproteinization (Table 2). However, initial modulus of elasticity changes insignificantly.

From experimental results of cattle bone tissue by four-point bending, it is also possible to observe the same relationship but in a more distinctive way (Tables 2 and 3). As seen from the data in Table 2, Table 3 and Table 4, mechanical characteristics of bone tissue, such as ultimate stress and initial modulus of elasticity, depends on the density and porosity of the materials. Bone tissue as a natural composite consisting of hydroxyapatite and protein has characteristics of mechanical properties on much higher level than hydroxyapatite and protein individually. It confirms studies conducted in [10-11].

Also from the results of experiments it is possible to conclude that protein has considerably lower density than mineral part of bone tissue. Thus, it can be reported that porosity of bone tissue after thermal treatment increases considerably (Table 4).

REFERENCES

1. J. M. Silva Marques, P. S. Gomes, M. A. Silva, A. M. Silverio Cabrita, J. D. Santos, M. H. Fernandes. Growth and phenotypic expression of human endothelial cells cultured on glass-reinforced hydroxyapatite.J. Mater. Sci. Mater. Med. V 20, No 3, pp. 725-731 (2009).
1. S. M. Zhang, F. Z. Cui, S. S. Liao, Y. Zhu, L. Han. Synthesis and biocompatibility of porous nano-hydroxyapatite/collagen/alginate composite. J. Mater. Sci. Mater. Med. V 14, No 7, pp. 641-645 (2003).
2. Lidia A. Sena, Mirta M. Caraballo, Alexandre M. Rossi, Gloria A. Soares. Synthesis and characterization of biocomposites with different hydroxyapatite-collagen ratios. J. Mater. Sci. Mater. Med. V 20, No 12, pp. 2395-2400 (2009).
3. Xiufeng Xiao, Rongfang Liu, Qiongyu Huang, Xiaohong Ding. Preparation and characterization of hydroxyapatite/polycaprolactone-chitosan composites. J. Mater. Sci. Mater. Med. V 20, No 12, pp. 2375-2383 (2009)
4. Jiwen Wang, Leon L. Shaw. Synthesis of high purity hydroxyapatite nanopowder via sol-gel combustion process. J. Mater. Sci. Mater. Med. V 20, No 6, pp. 1223-1227 (2009)
5. V. V. Filipenkov and I. V. Knets. Technology and some mechanical properties of biocomposites based on mineral components. J. Mechanics of Composite Materials. V. 41, No 2, pp.187-194 (2005)
6. V. A. Gibson, S. M. Stover, J. C. Gibeling, S. J. Hazelwood, R. B. Martin. Osteonal effects on elastic modulus and fatigue life in equine bone. J. Biomechanics. V.39, No 2, pp. 217-225 (2006)
7. Y. H. Hsu, I. G. Turner, A. W. Miles. Mechanical properties of three different compositions of calcium phosphate bioceramic following immersion in Ringer'ssolution and distelled water. J. Materials in Medecine. V. 20, No 12, pp. 2367-2374 (2009)
8. Cheng Yu Lin, Noboru Kikuchi, Scott J. Hollister. A novel method for biomaterial scaffold internal architecture design to match bone elastic properties with desired porosity. J. Biomechanics. V. 37, No 5, pp. 623-636 (2004)
9. J. Brandt, S. Henning, G. Michler. Nanocrystalline hydroxyapatite for bone repair: an animal study. J. Materials in Medicine. V. 21, No 1, pp. 283-294 (2010).
10. I. V. Knets, G. O. Pfafrod, J. Z. Saulgozis. Deformation and fracture of hard biological tissue [in Russian], Zinatne, Riga (1980)

Viktors Filipenkovs, Dr.sc.ing. (2001), Senior Researcher at Riga Technical University, Institute of Biomaterials and Biomechanics. Research interests include biomaterials, mechanics of composites, mechanics of deformable solids, biomechanics, mechanics of biological tissue. He is the author of more than 45 publications in scientific periodicals.
Address: 14/24 Azenes Street, Riga, LV- 1048, Latvia
Phone: +371-67089202; E-mail: v_filipenkov@hotmail.com

Lauris Rupeks, Medical Doctor (1989), MBA at Riga Technical University (2002), Researcher at Riga Technical University, Institute of Biomaterials and Biomechanics. Research interests include biomaterials, mechanics of composites, mechanics of deformable solids, biomechanics, mechanics of biological tissue, mechanics of implant – biological tissue interface. He is the author of more than 10 publications in scientific periodicals.
Address: 14/24 Azenes Street, Riga, LV 1048, Latvia
Phone: +371-67089202; E-mail: laru@hotmail.com

Ivars Knets, Dr.habil.sc.ing. (1977), Professor at Riga Technical University, Institute of Biomaterials and Biomechanics. Full Member of the Latvian Academy of Sciences (1992). Research interests include biomaterials, mechanics of composites, mechanics of deformable solids, biomechanics, mechanics of biological tissue. He is the author of more than 500 publications in scientific periodicals.
Address: 1 Kalku Street, Riga, LV- 1658, Latvia
Phone: +371-67089340; E-mail: knets@acad.latnet.lv

Natalija Borodajenko, Mg.sc.ing., Researcher at Riga Technical University, Institute of General Chemical Engineering. Research interests include biomaterials, organic chemistry. She is the author of more than 10 publications in scientific periodicals.
Address: 3/3 Pulka Street, Riga, LV-1007, Latvia
Phone: +371-67089605; E-mail: rbiac@rtu.lv

Zilgma Irbe, Mg.sc.ing. (2008), Researcher at Riga Technical University, Institute of General Chemical Engineering. Research interests include biomaterials, organic chemistry. She is the author of more than 10 publications in scientific periodicals.
Address: 3/3 Pulka Street, Riga, LV-1007, Latvia
Phone: +371-67089605; E-mail: rbiac@rtu.lv

Liga Mezmale, Mg.sc.ing, (2011), Scientific Assistant at Riga Technical University, Institute of General Chemical Engineering. Research interests include biomaterials, organic chemistry. She is the author of more than 5 publications in scientific periodicals.
Address: 3/3 Pulka Street, Riga, LV-1007, Latvia
Phone: +371-67089605; E-mail: rbiac@rtu.lv

Ineta Rozenstrauha, Dr. sc. ing. (1999), Associate Professor at the Institute of Applied Chemistry, Riga Technical University. Research interests include industrial waste investigation, chemical, mineralogical and environmental assessment of properties, recycling of various types of waste: metallurgical wastes, ash from incineration, fly-ash, sewage sludge, etc. She is the author of more than 60 publications in scientific periodicals.
Address: 14/24 Azenes Street, Riga, LV-1048, Latvia
Phone: +371-67089277; E-mail: Ineta@ktf.rtu.lv

Visvaldis Vitins, Dr.sc.ing. (1989), Senior Researcher at Riga Technical University, Institute of Biomaterials and Biomechanics. Research interests include biomaterials, mechanics of composites, mechanics of deformable solids, biomechanics, mechanics of biological tissue. He is the author of more than 70 publications in scientific periodicals.
Address: 14/24 Azenes Street, Riga, LV- 1048, Latvia
Phone: +371-67089126; E-mail: Visvaldis.Vitins@rtu.lv

Viktors Fiļipenkovs, Lauris Rupeks, Ivars Knēts, Natālija Borodajenko, Zilgma Irbe, Līga Mežmale, Ineta Rozenštrauha, Visvaldis Vītiņš. Naturālā hidroksiapatīta un liellopa kaulaudu mehānisko īpašību raksturojums.

Mākslīgo cilvēka orgānu izveidošana un dabīgo bioloģisko audu aizstājēju radīšana tiek uzskatīta par mūsdienu biomehānikas vienu no galvenajiem un svarīgākajiem uzdevumiem. Dažāda veida biomateriāli, tādi kā alumīnija oksīdu saturoši savienojumi, hidroksiapatīts, titāns, Co-Cr-Mo un Ti-Al-V sakausējumi, metilakrilāts, polietilēns, uz poraina nanohidroksiapatīta bāzaes veidoti materiāli, kolagēnu un alginātu saturoši savienojumi kā arī daudzi citi ir ļoti nozīmīgi mākslīgo biomateriālu radīšanai, kuri savukārt tiek izmantoti kaulaudu defektu aizvietošanai. Šajā darbā ir aprakstīta termiskās apstrādes metodikas izstrādāšana naturālā hidroksiapatīta iegūšanai no dabīgajiem liellopa kaulaudiem. Mūsu eksperimentu rezultāti, kuri tika veikti ar infrasarkanās spektroskopijas metodi, parādīja, ka olbaltums no termiski apstrādātajiem kaulaudiem ir izdalīts praktiski pilnībā. Kaulaudu struktūra tika analizēta ar optiskās mikroskopijas palīdzību gan pirms, gan pēc termiskās apstrādes un olbaltuma eliminācijas. Tāpat noteiktas dabīgo kaulaudu mehāniskās īpašības gan pirms, gan pēc deproteinizācijas, kā arī šo materiālu blīvums un porozitāte. Pamatojoties uz iegūtajiem datiem iespējams izdarīt secinājumu, ka jaunā olbaltumvielu eleiminācijas metode – termiski apstrādājot dabīgos kaulaudus temperatūrā zem 415 ^{O}C ļauj pilnībā saglabāt kaulaudu struktūru, kas savukārt vērtējams kā pozitīvs moments lai šo materiālu tālāk varētu izmantot biokompozītu veidošanā. Eksperimentālie rezultāti tāpat parāda, ka kaulaudu graujošais spriegums deproteinizācijas rezultātā ievērojami samazinās, turpretī sākotnējais elastības modulis mainās nelielā diapazonā. Eksperiments četru punktu liecē parādīja līdzīgu sakarību, bet vēl izteiktāk. Pamatojoties uz iegūtajiem datiem ir iespējams apgalvot ka materiāla stiprība tieši korelē ar tā blīvumu un porainību. Iegūto hidroksiapatītu turpmāk paredzēts izmantot mākslīgo biomateriālu radīšanai un kaulaudu defektu korekcijai.

Виктор Филипенков, Лаурис Рупекс, Иварс Кнетс, Наталия Бородаенко, Зилгма Ирбе, Лига Межмале, Инета Розенштрауха, Висвалдис Витиньш. Характеристики механических свойств костной ткани крупного рогатого скота и натурального гидроксиапатита.

Создание искусственных органов и заменителей биологических тканей относится к числу наиболее важных задач биомеханики. Различные биоматериалы, такие как оксид алюминия, гидроксиапатит, титан, сплавы Co-Cr-Mo и Ti-Al-V, метилметакрилат, полиэтилен, композиты на основе пористого наногидроксиапатита, коллагена и альгината и многие другие, играют важную роль в создании искусственных материалов для замещения костной ткани. В работе описана методика получения натурального гидроксиапатита из кости крупного рогатого скота при помощи термической обработки. Результаты экспериментов, проведенных методом инфракрасной спектроскопии показали, что белок из термически обработанных образцов костной ткани крупного рогатого скота удален практически полностью. Структура костной ткани изучена методом оптической микроскопии как до, так и после удаления из нее белка. Определены некоторые характеристики механических свойств костной ткани крупного рогатого скота как до, так и после депротеинизации. Определены плотность и пористость данных материалов. Таким образом можно утверждать, что новый метод удаления белка из костной ткани при помощи отжига при температуре ниже 415 ^{O}C позволяет сохранять полностью минеральную структуру кости с целью дальнейшего использования в качестве наполнителя для биокомпозитного материала. Из экспериментальных результатов так-же видно, что разрушающее напряжение костной ткани крупного рогатого скота значительно уменьшается после депротеинизации, а начальный модуль упругости меняется незначительно. Эксперименты на четырехточечный изгиб показали такую же зависимость, но в более выраженном виде. Таким образом можно утверждать, что прочность материала в значительной степени зависит и от его плотности и пористости. Предполагается полученный гидроксиапатит использовать при создании искусственных материалов для замещения костной ткани.