

9. Kurhaluk N, Tkachenko N. Melatonin and alcohol-related disorders. *Chronobiol Int.* 2020 Jun; №37(6):781–803. DOI: 10.1080/07420528.2020.1761372
10. Luo J. Effects of Ethanol on the Cerebellum: Advances and Prospects *Cerebellum.* 2015 Aug; 14(4):383–5. DOI: 10.1007/s12311-015-0674-8
11. Martínez-Salvador J, Ruiz-Torner A, Blasco-Serra A, Martínez-Soriano F, Valverde-Navarro AA. Morphologic variations in the pineal gland of the albino rat after a chronic alcoholisation process. *Tissue Cell.* 2018 Feb 02; 51: 24–1. DOI: 10.1016/j.tice.2018.01.004
12. Rocco A, Compare D, Angrisani D, Sanduzzi Zamparelli M, Nardone G. Alcoholic disease: liver and beyond. *World J Gastroenterol.* 2014 Oct 28; 20(40):14652–9. DOI: 10.3748/wjg.v20.i40.14652
13. Varadinova M, Lozanova Valcheva-Traykova M, Boyadjieva N. Effect of Circadian Rhythm Disruption and Alcohol on the Oxidative Stress Level in Rat Brain. *Am J Ther.* 2016 Nov/Dec; 23(6): 1801–5. DOI: 10.1097/MJT.0000000000000363
14. Yeroshenko GA, Shevchenko KV, Lisachenko OD, Vilhova OV, Yakushko OS, Skotarenko TA, Bilash VP. Ultrastructural remodeling of rat submandibular glands in chronic ethanol intoxication. *World of medicine and biology.* 2020; 3(73):175–8. DOI 10.26724/2079-8334-2020-3-73-175-178
15. Yeroshenko GA, Fedoniuk LYa, Shevchenko KV, Kramarenko DR, Yachmin AI, Vilkhova OV, Skotarenko TA. Structural reorganization of the rats' submandibular glands acini after the influence of 1% methacrylate. *Wiadomosci lekarskie.* 2020;73(7):1318–22. DOI: 10.36740/WLek202007102

Стаття надійшла 22.01.2021 р.

DOI 10.26724/2079-8334-2022-1-79-227-231

UDC 616.71-089.843 / .844: 615.461] 048.8

**V.A. Filipenko, K.S. Poplavska, O.D. Karpinskaya, M.Yu. Karpinsky**  
SI "Institute of Pathology of the Spine and Joints, prof. M.I. Sytenko National Academy  
of Medical Sciences of Ukraine", Kharkiv

## STUDY OF STRENGTH OF FUMERAL BONE OF RATS AFTER FILLING BONE TISSUE DEFECTS WITH BONE CEMENT BASED ON TRICALCIUM PHOSPHATE

e-mail: karolina.krivoru@gmail.com

There are bone defects that do not heal on their own, despite the ability of bone tissue to fully regenerate. These defects occur due to degenerative-dystrophic diseases, injuries, resections of tumors and tumor-like formations, metastatic lesions, as a result of the development of aseptic instability after primary arthroplasty. Autogenous material for transplantation is the most acceptable, but its use depends on the quality of the bone. Allografts have compensated for the shortcomings of autografts, but the risk of infection and ethical issues limit the use of this material. The group II cement that we offer retains the properties of tricalcium phosphate-based materials: high biocompatibility, osteoconductive and osteointegrative qualities, and, due to strengthening by HA whiskers, it has higher strength and slower biodegradation time.

**Key words:** bioceramics,  $\alpha$ -tricalcium phosphate, calcium phosphate cement, orthopedic surgery, bone regeneration.

## В.А. Філіпенко, К.С. Поплавська, О.Д. Карпінська, М.Ю. Карпінський ДОСЛІДЖЕННЯ МІЦНОСТІ СТЕГНОВИХ КІСТОК ЩУРІВ ПІСЛЯ ЗАПОВНЕННЯ ДЕФЕКТІВ КІСТКОВОЇ ТКАНИНИ КІСТКОВИМ ЦЕМЕНТОМ НА ОСНОВІ ТРИКАЛЬЦІЙФОСФАТУ

Існують дефекти кістки, які не загоюються самостійно, незважаючи на здатність кісткової тканини до повної регенерації. Ці дефекти виникли внаслідок дегенеративно-дистрофічних захворювань, травм, резекцій пухлин і пухлиноподібних утворень, метастатичних уражень, у результаті розвитку асептичної нестабільності після первинного ендопротезування. Аутогенний матеріал для трансплантації є найбільш прийнятним, але його використання залежить від якості кістки. Аллотрансплантати компенсували недоліки аутогенних трансплантатів, але ризик інфекційного ураження й етичні питання обмежують застосування цього матеріалу. Цемент II групи, який пропонуємо ми, лишив у собі характеристики, що притаманні матеріалам на основі трикальційфосфату: високі біосумісні, остеоіндуктивні й остеоінтегративні якості, та, за рахунок зміцнення гілками гідроксіапатиту отримав вищу міцність, і більш повільний час біодеградації. Ці характеристики як час перебування імплантованого матеріалу та його витривалість при навантаженні, напругу мають вплив на можливість здійснення навантаження в більш ранній післяопераційний період.

**Ключові слова:** біокераміки,  $\alpha$ -трикальційфосфат, кальцій-фосфатний цемент, ортопедичні операції, регенерація кістки.

*The study is an initiative.*

The relevance of the study of osteoplastic materials is due to the occurrence of such bone defects that do not heal on their own, despite the ability of bone tissue to fully regenerate. These include defects caused by degenerative-dystrophic diseases, injuries, resections of tumors and tumor-like formations, metastatic lesions, as well as in the case of aseptic instability after primary arthroplasty [1–3]. Autogenous material for transplantation is the most suitable, mainly because it meets certain requirements: it contains

osteogenic stem cells, has osteoinductive growth factors and plays the role of the osteoconductive matrix. However, its use is limited by the amount of material, reduced bone quality of some dystrophic diseases, additional injuries and the pain present in the donor site. [4, 5].

Allografts, having become available in the surgeon's arsenal, have partially compensated for the shortcomings of autografts. However, antigenic properties, the risk of infection (viral diseases), ethical issues limit the use of this material. Given that, allografts lose their osteoconductive and osteoinductive properties while being manufactured, sterilized and stored, they cannot be considered the "gold standard" [4]. Xenotransplants are characterized by a slowed-down reconstruction, compared to other bone materials as well as by the risk of infections of viral origin (Kreutzfeldt-Jakob, etc.) [6, 7].

Synthetic materials, such as calcium-phosphate bioceramics, have high biocompatible, osteoconductive and osteointegrative properties, and their composition is close to the bone matrix, which causes the gradual replacement of bone tissue [8, 9]. In addition, the variety of forms in which bioceramics (powder, granules, blocks of different porosity) are presented, expands the range of uses, even if defects of different configurations are present [8]. Due to the difficulties for the surgeon in filling defects of irregular shape, it has become important to create a material with osteoinductive and osteoconductive properties, which is introduced into the cavity in a liquid state and which hardens quickly while meeting the required strength values close to the bone. Unfortunately, the fragility and the low compressive strength of this material, as well as the rather rapid biodegradation, limit its use. It is expected that the addition of certain reinforcing impurities (in the form of HA whiskers) will significantly improve the mechanical properties of cement, slow down its degradation. It is desirable that such particles have bioactive properties similar to the quality of cement. A minor modification of the material can significantly change its properties, which makes it necessary to study the experimental biological properties of the studied material.

**The purpose** of the study was to determine the strength limits of the femurs of rats after filling their cavity defects with bone types of cement, based on  $\alpha$ -tricalcium phosphate at different times after surgery.

**Materials and methods.** In the laboratory of the biomechanics of the State Institution "Sytenko Institute of Spine and Joint Pathology at the National Academy of Medical Sciences of Ukraine" experimental studies of the strength of the femurs of rats after filling the cavity defects with bone cement were conducted at different times after surgery. The study was performed on 20 laboratory rats of the population of the Institute of Spine and Joint Pathology (ISJP). A cavity defect in the proximal part of the left femur was formed in all the rats with the dental boron. This defect was filled with bone cements based on tricalcium phosphate (TCP). Two types of bone cements were used:  $\alpha$ TCP was used for group I (comprising 10 rats), and  $\alpha$ TCP reinforced with hydroxylapatite (HA) whiskers were used for group II (comprising the other 10 rats). Animals were removed from the experiment after 1 month and 3 months after the surgery, 5 rats from each group.

The preparations of the operated and contralateral femurs of the laboratory animals were tested. The preparations of the femurs were tested for strength under the vertical compressive load. The loading scheme is shown in fig. 1.

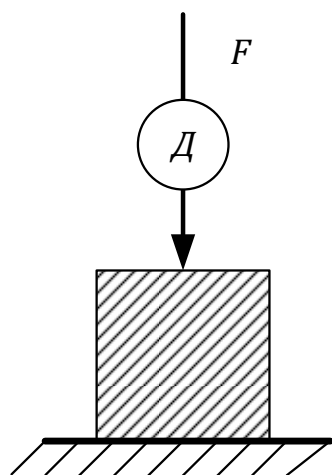


Fig. 1 Scheme of the experiment, F – compressive force; D – dynamometer

Also, the study of compressive strength was performed on samples of bone cement columns. In total, 5 samples of each type of cement were made. The samples were made cylindrical with the diameter of 5 mm and with the length of 10 mm. According to the results of tests of the cement samples, the strength limit was calculated according to the formula [9]: where  $F$  is the magnitude of the force at which the destruction of the sample we observed;  $S$  is the cross-sectional area of the sample.

During the tests, the values of the compressive force that provoked the destruction of the sample were recorded using the strain gauge SBA-100L and a CAS recording device type CI-2001A.

The test results were processed using statistical methods. The mean ( $M$ ), standard deviation ( $SD$ ) as well as the minimum and the maximum values of the experimental data samples for cement samples of different types and each of the groups of bone preparations of experimental animals were calculated. The comparison of the strength limits of the cement samples and the femoral preparations was performed using one-dimensional analysis of variance (ANOVA) and a posteriori Duncan test. The data processing was performed in the IBM SPSS Statistic 20.0 application package [11].

**Results of the study and their discussion.** The first stage of the study was the one of the compressive strength of dry samples of the bone cement. The cement samples were produced in the laboratory in the manner similar to other experiments: the dry sample was mixed with the solidifying liquid at the room temperature. To give the cement a shape suitable for the strength assessment experiment, it was placed in insulin syringes and then removed. The results of the tests showed that the cement samples with the addition of hydroxylapatite whiskers have statistically significantly higher strength ( $p=0.021$ ), as evidenced by the indicators of their strength limit –  $(15.41 \pm 1.93)$  MPa against  $(10.57 \pm 1.67)$  MPa of the cement samples not reinforced with hydroxylapatite whiskers.

The clear idea of the ratio of the values of the compressive strength of the bone cement samples can be obtained using the diagram shown in fig. 2.

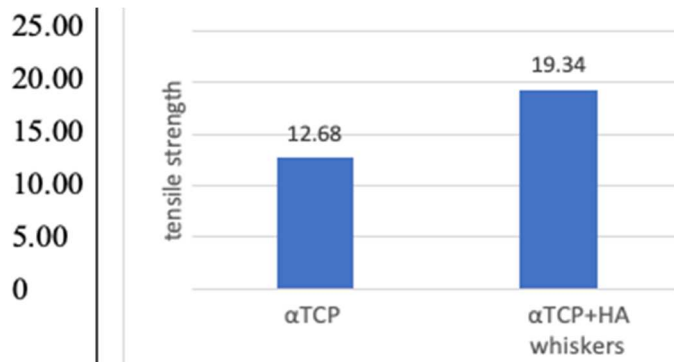


Fig. 2 The diagram of the strength limit of the bone cement samples.

The second stage of the work was performed to test the strength of the femoral preparations of the laboratory rats with bone defects, which is filled with the above-mentioned cements. The main parameters that characterize the suitability of the cement for practical use are the cement setting time and the compression strength. The CPC needs to set relatively slowly so that the surgeon has enough time to perform the implantation but at the same time it has to set quickly enough to prevent

the unjustified prolongation of the surgery. The setting time of TCP-based cement is usually several tens of minutes.

According to the studies, a month after filling the bone defect with cement, the strength of the operated bones is determined to be statistically significantly lower than intact bones in both groups. There was no statistically significant difference between the groups depending on the type of the cement ( $p=0.699$ ), but the average value of the destructive force for the second group of drugs with cement mixed with HA ( $204 \pm 40$ ) H is slightly lower than for the cements without it ( $214 \pm 40$ ) N.

In the diagram in fig.3 you can see the more detailed comparison of the magnitudes of the destructive force due to which the destruction of the femurs of the laboratory animals a month after filling bone defects with bone cements took place.

Three months after the surgery, there is a statistically significant difference ( $p=0.010$ ) in the strength of the intact and the operated bones in the first group of the cements. In the other group of animals, the difference in strength between the intact and the operated bones is determined at the significance limit ( $p=0.055$ ). Similar to the study a month after the surgery, in terms of the type of the cement used, the statistical significance of the strength was not detected in the groups of the preparations ( $p=0.932$ ), but the mean values of the destructive force are slightly higher in group I cements ( $230 \pm 70$ ) H against ( $226 \pm 70$ ) H in the second group.

To visually compare the magnitude of the destructive force at which the destruction of the femoral preparations of the laboratory animals three months after filling the bone defects with bone cements took place, a diagram was constructed, which is shown in fig. 4.

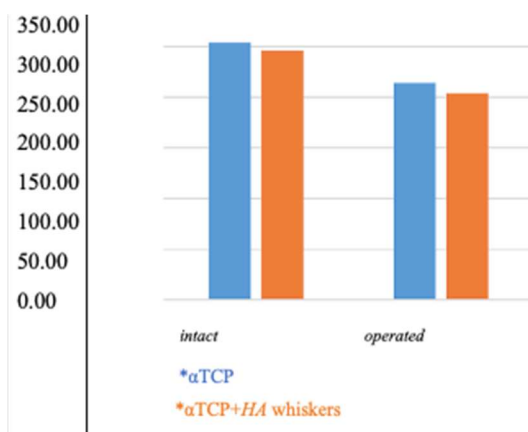


Fig. 3 Demonstrates the diagram of the values of the load at which the destruction of the femurs of the laboratory animals a month after filling bone defects with the bone cement took place.

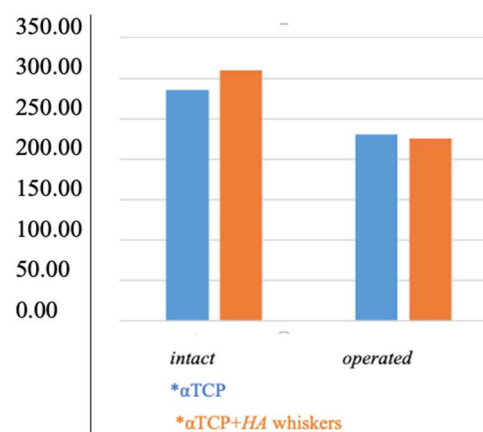


Fig. 4 Demonstrates the diagram of the values of the load at which the destruction of the femurs of laboratory animals three months after filling bone defects with TCP-based cements took place.

Table 1 shows the results of the comparative analysis of the changes in the strength of the preparations of the femurs of rats, depending on the time period after the surgery.

Table 1

**The evolution of changes in the strength of the preparations of the femurs of rats, depending on the time period after the surgery.**

| Group                                                                                          | duration | Destructive force, H |                     |                     |
|------------------------------------------------------------------------------------------------|----------|----------------------|---------------------|---------------------|
|                                                                                                |          |                      | intact              | operated            |
| $\alpha'$ -Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>                                     | 1 month  | (M±SE)               | 254±47              | 214±43              |
|                                                                                                | 3 month  |                      | 286±57              | 230±70              |
|                                                                                                | t, p     |                      | t=-0.968<br>p=0.361 | t=-0.436<br>p=0.674 |
| $\alpha'$ -Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub><br>reinforced by <i>HA whiskers</i> | 1 month  | (M±SE)               | 246±33              | 204±36              |
|                                                                                                | 3 month  |                      | 310±40              | 226±73              |
|                                                                                                | t, p     |                      | t=-2.764<br>p=0.025 | t=-0.603<br>p=0.563 |

The comparative analysis of the changes in the bone strength of the laboratory rats depending on the time period after the surgery showed that three months after the surgery, the average strength of both operated and intact bones increased, although the changes did not become statistically significant.

The studies have shown that the initial strength of the dry form of the bone cement columns is higher in the cement with the addition of HA whiskers, and is (15.41±1.93) MPa compared to (10.57±1.67) MPa of group I cement. This seems quite logical since the modulus of elasticity of HA (110 MPa) is by three times higher than the one of tricalcium phosphate (33 MPa) [10]. With regard to the strength of the operated bones, a statistically insignificant increase in the strength of intact bones can be attributed to the age-related changes in the laboratory animals. The preparations of the operated limbs at the first stage (1 month after surgery) are statistically significantly inferior to intact bones, but three months after the difference loses statistical significance. This proves the fact that at this stage, in the area where the defect of bone tissue was formed, a block appeared, whose properties are similar to bone tissue, ie there reparative processes took place. The fact that the average values of the destructive force for the femoral preparations of the defects filled with the cement from group I were slightly higher than those of the cement from group II with admixtures of HA whiskers, given the greater initial strength of the samples of cement II groups, although the difference was not statistically significant, it can be assumed that this is due to the different rate of the resorption of the cement, and their replacement by bone tissue. However, in order to confirm this assumption, we need to research the results of the morphological studies.

In the State Institution “Institute of Pathology of the Spine and Joints named after prof. M.I. Sytenko of the National Academy of Medical Sciences of Ukraine” we investigated the above-mentioned cement experimentally ( $\alpha$  TCP and  $\alpha$  TCP reinforced by HA whiskers) by testing the strength of the rat bones. We conducted the test on two terms: the term of one month and the term of three months. This time is sufficient to form an idea of the properties of the studied cement in the process of its degradation. From these indicators, it is established that due to the addition of HA whiskers, the cement changes its properties by increasing the load threshold, which is the expected result. While the materials with HA for bone replacement have high compressive strength and very slow biodegradation: the formed block is observed in radiography up to 10 years [1, 10], the lack of these important properties of the studied cement of group II is observed. While TCF ceramics have the fastest resorption, according to research in dynamics, this time is not sufficient to form a strong bone block [2, 9, 10]. The implantation of allografts in the dynamics shows good results, due to the fact that they can provide structural support. Although they do not have osteogenic properties, allografts eliminate several shortcomings of autogenous grafts, but they also have the potential to transmit viruses and other infectious agents [8, 9].

This permits to give certain recommendations for the protocol of the treatment of patients with the replacement of bone defects in the early post-operative period. The increase in strength in group II permits to recommend a dosed load on the operated limb without correcting its value. According to the above-mentioned results, it is established that for the period of one month after the surgery, the cement from the II group has the strength indicators higher than the cement of the I group. Three months after the surgery,

this difference is leveled between all the study groups. So we pointed to the reconstruction process and filling the defect with bone tissue.

### Conclusions

The studied cement from group II, with impurities of HA whiskers has the primary strength limit ( $15.41 \pm 1.93$ ) MPa, which is statistically significantly higher than the strength limit of the cement from group I ( $10.57 \pm 1.67$ ) MPa. One month after filling the defects of bone tissue with cements based on TCF, the strength of the operated bones is determined to be statistically significantly lower than the intact bones in both groups. There was no statistically significant difference between the groups in terms of the type of cement ( $p=0.699$ ). Three months after the surgery, the strength of both operated and intact bones increased, but the difference in strength of the intact and the operated bones is not statistically significant. Between the groups of the preparations of the operated bones, in terms of the type of the cement used, the statistical significance of strength was also not determined ( $p=0.932$ ). From the above-mentioned list, we can conclude that by reinforcing the cement by adding HA whiskers based on  $\alpha$ TKP, we increase its strength properties, and to some extent, we slow down the restructuring of the material implanted in the defect of the bone tissue. TCP-based materials are well restructured, but biodegradation is too rapid to form a strong block of bone-implanted material, and they have a fragile structure, which is extremely important for the patient in the postoperative period in terms of the load on the operated limb.

### References

1. Zyman ZZ, Kaltsiyfosfatni biomaterialy. Navchalnyj posibnyk. 2018:285. [in Ukrainian]
2. Kozlov, A.YU. Statisticheskiy analiz dannykh v MS Excel: Uchebnoe posobie. AYU Kozlov, VS Mhitaryan, VF Shishov. - Moskva: INFRA-M, 2021. - 320 c. [in Russian]
3. Pankratov AS, Lekyshvily MV, Kopetskyĭ YS, Kostnaya plastika v stomatologii i cheliustno-lytsevoĭ khirurgii. Osteoplastycheskiye materyaly: rukovodstvo dlia vrachey. 2011 : 272. [in Russian]
4. Tankut OV, Zamishchennia defektiv kistok pid chas totalnoho endoprotezuвання kolinnoho suhloba (ohliad literatury). Ortopedyia, travmatologiya i protezirovanie. 2019; 3: 97–103. doi: 10.15674/0030-59872019397-103. [in Ukrainian]
5. Filipenko VA, Vorontsov PM, Husak VS. Kistkova aloplastyka v razi reviziynoho endoprotezuвання kulshovoho suhloba za umov aseptychnoi nestabilnosti atsetabuliarnoho komponenta. Ortopedyia, travmatolohiya y protezyrovanye. 2020; 4: 5–11. doi: 10.15674/0030-5987202045-11. [in Ukrainian]
6. Bone grafts and substitutes market size, share and trends analysis report by material type (natural, synthetic), by application type (spinal fusion, craniomaxillofacial, long bone), by region, and segment forecasts, 2018–2025. [Online source]. 2018. Retrieved from <https://www.grandviewresearch.com/industry-analysis/bone-grafts-substitutes-market>
7. Hashiguchi K, Yamakawa Y. Introduction to finite strain theory for continuum elasto-plasticity. Wiley: 2012; 417 p.
8. Fillingham Y. Bone grafts and their substitutes. The Bone & Joint Journal. 2016; 98-B (1 Sup-pl A): 6–9. doi: 10.1302/0301-620X.98B.36350.
9. Laurencin C, Khan Y, El-Amin SF, Bone graft substitutes. Expert review of medical devices. 2006; 3 (1): 49–57. doi: 10.1586/17434440.3.1.49.
10. Sohn HS, Review of bone graft and bone substitutes with an emphasis on fracture surgeries. 2019; 23: Article ID : 9. doi: 10.1186/s40824-019-0157-y.
11. Tovar N, Gimbo R, Gangolli R, Witek I, Lorenzoni F, Marin C, et al. Modification of xenogeneic graft materials for improved release of p-15 peptides in a calvarium defect model. J Craniofac Surg. 2014; 25(1):70–76. doi: 10.1097/ScS.0b013e3182a2dfe7.

Стаття надійшла 15.01.2021 р.