საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 13, №2, 2019
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 13, no. 2, 2019

Medical Sciences

The Comparative Effect of Biomaterials on the X-Ray Picture of the Healing of the Defect of Compact Bone Tissue

Oleksii Korenkov

Department of Morphology, Sumy State University, Sumy, Ukraine

(Presented by Academy Member Nodar Mitagvaria)

ABSTRACT. **The aim of this work is to compare the influence of the main types of biomaterials on the x-ray picture of the healing of the defect of compact bone tissue. The experiment involved 96 white Wistar rats. In the middle third of the diaphysis of the femur there was reproduced perforated defect with a diameter of 2.5 mm, which in the animals of the 1st group was left to heal by a blood clot, in the animals of the 2^{nd} group was filled with β-tricalcium phosphate (β-TCP), in the animals of the 3^{rd} group – with natural hydroxyapatite (NHAp), and in the animals of the 4th group – with biocomposite material (synthetic hydroxyapatite/collagen SHAp/Col). The study of the healing of injured bones with the measurement of their optical density was carried out on the 15^{th}, 30^{th}, 60^{th} and 120^{th} day by computed tomography. It is established that during the whole period of the experiment the investigated biomaterials (except NHAp) are replaced by regenerate components. Rating from the lowest optical density to the largest on the $15\text{-}30^{th}$ day was represented by the site of the defect in animals of the 1st group (225±36 HU and 526±64 HU), sites of implantation of SHAp/Col (1165±31 HU, 1575±67 HU), β-TCP (1500±49 HU, 1544±56 HU), NHAp (2715±197 HU 2703±81 HU), and on the 60^{th} day by the area of the defect in animals of the 1^{st} group (1259±73 HU), the sites of implantation of β-TCP (1423±12 HU), SHAp/Col (1757±86 HU) and NHAp (2841±109 HU). On the 120^{th} day of the experiment, the optical density of the defect area of the animals of the 1^{st} group (1652±24 HU) has not reached the same indication as of the maternal bones (1762±33 HU), the site of implantation of NHAp (3000±81 HU) significantly exceeded it (1680±61 HU), and the sites of implantation of β-TCP (1762±60 HU) and SHAp/Col (1777±57 HU) equaled with it (1750±49 HU, 1714±44 HU). Thus, NHAp provides high and stable densitometrical properties of the defect area of compact bone tissue, and all other materials for the period of 120 days contribute to full restoration of the optical density of the injured bone.** *© 2019 Bull. Georg. Natl. Acad. Sci.*

Key words: computed tomography, optical density, reparative bone regeneration, β-tricalcium phosphate, hydroxylapatite, collagen

The search for effective means and methods of bone grafting to optimize reparative osteogenesis is one of the most urgent problems of modern traumatology. More and more new osteoplastic materials are actively introduced in clinical practice. They represent different forms of bone collagen, glycosaminoglycans, hydroxylapatite, tricalcium phosphate and their combinations. According to the literature, the latter demonstrate their efficiency, biocompatibility and safety [1, 2]. At the same time, information about other defining characteristics such as the comparative effect of biomaterials on the optical density of the regenerate components in the scientific literature for some commercial drugs is completely absent, and for others densitometrical indicators have a significant discrepancy (several times) [3-5]. The latter is probably due to the fact that the above characteristic is influenced by many factors, which include the size and shape of the implant, the size of the defect, the regenerative potential of bone tissue [6, 7]. Therefore, in our opinion, it is necessary to compare existing osteoplastic materials with each other under the same conditions in experimentally created bone defects. In addition, the available in scientific literature results of the study of the comparative effect of calcium-phosphate materials on the x-ray picture of the healing of bone defects are mainly obtained on the spongy bones, skull bones, and for some drugs there are no results for the compact bone tissue [8-10].

Taking this into account, the aim of our work is to compare the influence of the main varieties of biomaterials on the x-ray picture of the regenerative process in the experimental defect of compact bone tissue.

Material and Methods

The experiment involved 48 rats Wistar rats, which had the same age (8 months) and weight (about 250 g). The study was conducted in com-pliance with the rules of humane treatment of experi-mental animals and approved by the Commission on Bioethics of Sumy State University (Minutes №1/1 of 24.01.2018). With the help of dental boron, under general intramuscular anesthesia (acepromazine – 2.5 mg/kg, ketamine – 75 mg/kg) and under aseptic conditions, a defect was formed in the middle third of the femoral shaft with a diameter of 2.5 mm.

Further, depending on the biomaterials used, the animals were divided into 4 groups:

Group 1 (24 rats) – control, the bone defect was left to heal under blood clot;

Group 2 (24 rats) – the defect was filled with the β-tricalcium phosphate in the form of block (β-TCP, osteo-plastic material «chronOS™», Synthes, Switzerland);

Group 3 (24 rats) – the defect was filled with the natural hydroxyapatite of spongy substance of the tubular bones of cows (NHAp, osteoplastic material «cerabone®», Botiss, Germany) in the form of granules (2–2.5 mm);

Group 4 (24 rats) – the defect was filled with the biocomposite calcium-phosphate material based on the synthetic hydroxyapatite and collagen of the 1st type from the skin of cattle in the form of granules with a size of 3.5 mm (SHAp/Col, osteoplastic material «Collapan», Intermedapatyt, Russia).

The bone defect healing study with measurement of its optical density in Hausfield units (HU) was performed using Toshiba Activion (Japan) computed tomograph under General intramuscular anesthesia (ketamine – 50 mg/kg) on the 15th, 30th, 60th and 120th day after injury.

Statistical processing of the obtained digital values was performed using the Statistica 7.0 software package. The average values are presented as M±m, where M is the average value of the indicator, m is the standard error of the average value. The significance of differences between the indicators of the animals of the first, second, third and fourth groups, of the 15th, 30th, 60th and the 120th days was evaluated using Student t-test. Differences were regarded as statistically probable in the conditions of $p<0.05$.

Results and Discussion

The study found that one of the main radiological differences in the healing of bone defects in animals of all study groups was the difference in the dynamics of changes in optical density. Thus, taking into account the optical density index, it can be noted that on the 15th-30th day of the experiment in the rating from the lowest to the highest value, the fist place was occupied by the defect site, which was under the blood clot (225±36 HU, 526±64 HU), and then the SHAp/Col implantation site (1165±31 HU, 1575±67 HU), β-TCP (1500±49 HU, 1544±56 HU) and NHAp (2715±197 HU, 2703±81 HU) ($p<0.05$).

Table. Data of variation-statistical processing (M±m) of the optical density of the defect site and the maternal bone in Hounsfield units (n=12)

Group experiment	Metering area	Optical density in units of Hounsfield			
		Day after surgery			
		15	30	60	120
Group 1	The area of the defect	225±36^{2,3,4}	526±64^{2,3,4}	1259±73^{2,3,4}	1652±24^{3}
	Maternal bone	1729±109*	1746±45*	1738±51*	1762±33*
Group 2	The area of the defect	1500±49^{1,3,4}	1544±56^{1,3}	1423±12^{1,3,4}	1762±60^{3}
	Maternal bone	1619±31	1663±41	1597±55*	1750±49
Group 3	The area of the defect	2715±197^{1,2,4}	2703±81^{1,2,4}	2841±109^{1,2,4}	3000±81^{1,2,4}
	Maternal bone	1678±43*	1689±18*	1665±41*	1680±61*
Group 4	The area of the defect	1165±31^{1,2,3}	1575±67^{1,3}	1757±86^{1,2,3}	1777±57^{3}
	Maternal bone	1630±61*	1793±22*	1791±29	1714±44

Note. The sign ^{1} ($p<0.05$) shows significant difference in the optical density of animals of any group relative to the first, respectively the signs ^{2,3,4} show the significant difference of animals of any group relative to the second, third and fourth. The sign * ($p<0.05$) shows significant difference in optical density between the defect area and the maternal bone.

Thus, the sites of implantation of SHAp/Col, β-TCP and NHAp did not differ only in optical density, but on the 15th day of the experiment significantly exceeded the similar indicator of the regenerate of the animals whose defect was healed by a blood clot. The latter indicates that the optical density of the defect site was due to the density of the calcium-phosphate material implanted in its cavity. It should also be noted that on the 15th day of the experiment, the optical density of the defect site, which was exposed under the blood clot (225±36 HU), the implantation sites of SHAp /Col (1165±31 HU) and β-TCP (1500±49 HU) did not reach the same index of the maternal bone (1729±109 HU, 1630±61HU, 1619±31 HU), and the implantation sites of NHAp (2715±197 HU) significantly exceeded it (1678±43 HU) (Table).

Under this condition, on the 15th day of the experiment, the boundary between the defect site and the adjacent maternal bone was well traced in all experimental groups. However, this was particularly evident in animals whose defect healed under a blood clot and in animals with an implanted SHAp/Col. This is due to the fact that the regenerate of animals of the control group due to the very low optical density in the defect area was not visualized, and the density of the shadow of SHAp/Col was significantly lower than that of the maternal bone. In contrast to these experimental groups, the optical density of the implantation site of β-TCP and NHAp was visually closer to the same index of the maternal bone. However, due to the presence of small depressions on the outer surface of the cortical bone layer, clear contours of β-TCP and NHAp in the defect cavity and in the bone marrow canal, the site of their implantation was also well visualized (Fig. 1).

On the 30th day of the experiment, the optical density of the defect site of the control group animals increased by 2.3 ($p<0.05$) times compared to the 15th day of the experiment. Due to the increase in the optical density of the regenerate in the defect area, its contours began to be traced. However, due to the significant difference between the optical density of the regenerate (526±64 HU) and the maternal bone (1746±45 HU), the defect area, as before, was still well visualized and clearly delineated from the edges of the maternal bone. On the 60th day of the experiment there was an increase in the optical density of the injury site by 2.4 times ($p<0.05$) compared to the previous period. Despite this, the optical density of the regenerate (1259±73 HU) was significantly lower than in the maternal bone (1738±51 HU), due to which the place of the former injury on the computer tomogram could still be distinguished.

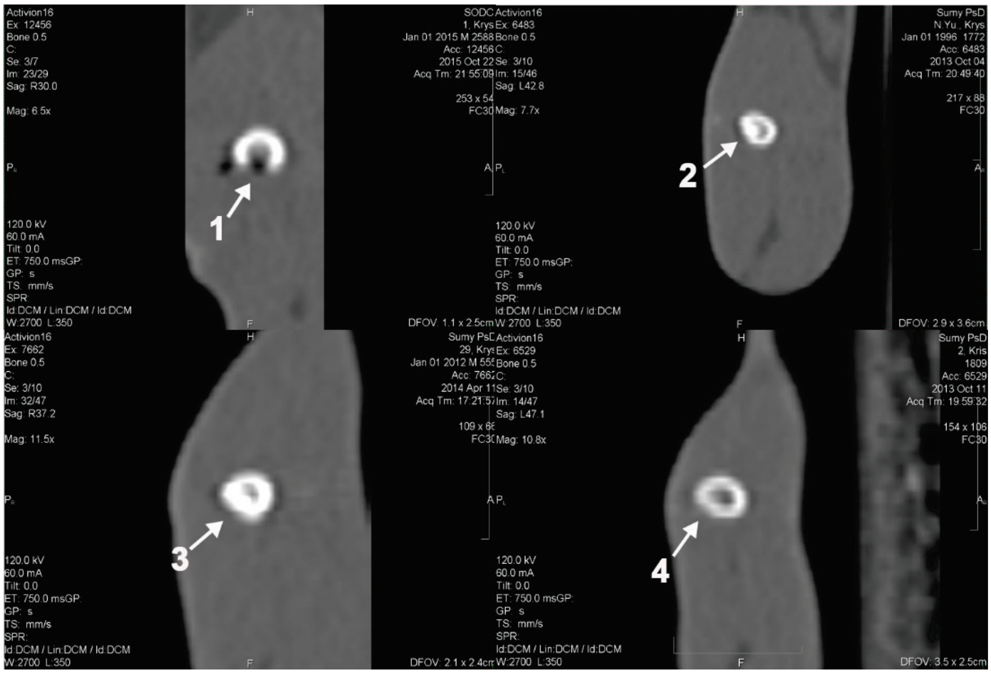

Fig. 1. Computed tomography of rat femurs on the 15th day after injury. The area of the defect in animals of the control group (1), and with the implanted β-TCP (2), NHAp (3), SHAp/Col (4).

The optical density of the SHAp/Col implantation site (1575±67) on the 30th day of the experiment was close to the same index of the maternal bone (1793±22), so that the visual boundary between them was almost not traced. The site of the previous injury could be distinguished only by the left part of the osteoplastic material in the medullary canal and a small indentation in the cortical bone plate. On the 60th day, the place of SHAp/Col implantation externally had a restored cortical layer, and its optical density (1757±86 HU) was equal to the maternal bone (1791±29 HU). However, due to the presence of a small thickening of the diaphysis from the medullary canal, the area of SHAp/Col implantation could still be detected.

Unlike the animals of the fourth group, the dynamics of changes in the optical density of the β-TCP implantation site had a wave-like dynamics. First, during the first month, the optical density remained practically unchanged (1500±49 HU and 1544±56 HU), and on the 60th day of the experiment, the densitometric index under study decreased (1423±12 HU), which, in our opinion, may indicate the predominance of implant resorption over bone tissue regenerate maturation. At the same time, these changes in the optical density of the β-TCP implantation site visually on a computer tomogram were accompanied by a gradual decrease in the implant size from the bone marrow canal and a decrease in the defect size. However, in the area of implantation of NHAp on the 30-60th day of the experiment, the optical density (2703±81 HU and 2841±109 HU) was unchanged and significantly surpassed the similar indicator of a maternal bone (1689±18 HU and 1665±41 HU). As a result, the contours of the implant were clearly traced both at the level of the defect and in the bone marrow canal (Fig. 2, 3).

Due to the presented dynamics of densitometric changes in the bone defect on the 60th day of the experiment, there were changes in the optical density rating. Thus, the β-TCP implantation sites moved from the third place on the 15th–30th day to the second, while the SHAp/Col implantation sites, on the contrary, from the second to the third place. Thus, on the 60th day of the experiment, the rating from the lowest to the highest optical density index was headed by the defect site, which was healed under the blood clot (1259±73 HU), the next was the β-TCP implantation site (1423±12 HU), SHAp/Col (1757±86 HU) and NHAp (2841±109 HU) ($p<0.05$).

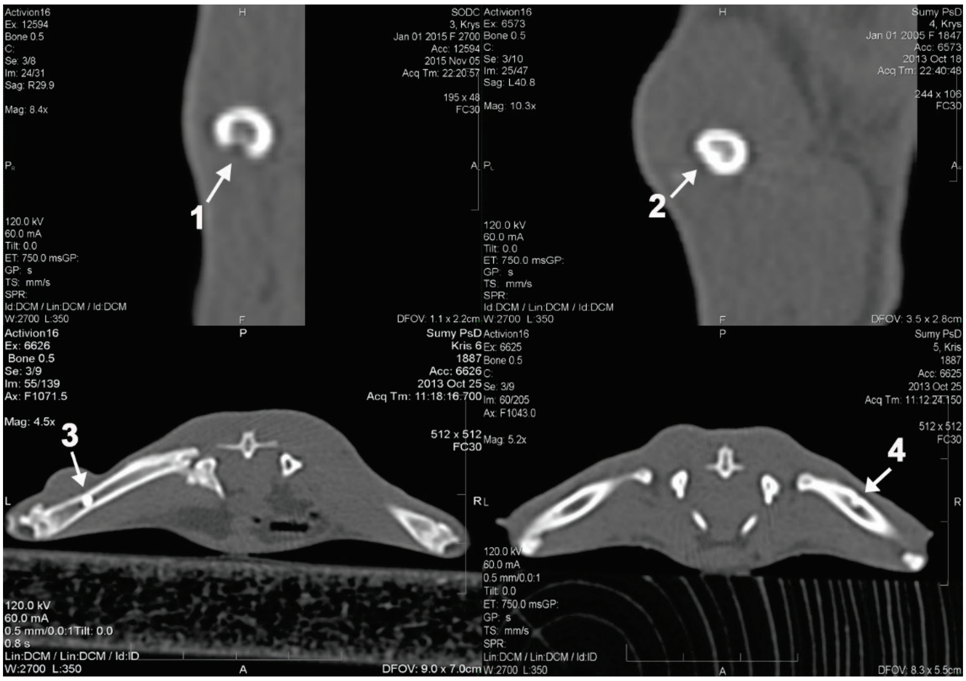

Fig. 2. Computed tomography of rat femurs on the 30th day after injury. The area of the defect in animals of the control group (1), and with the implanted β-TCP (2), NHAp (3), SHAp/Col (4).

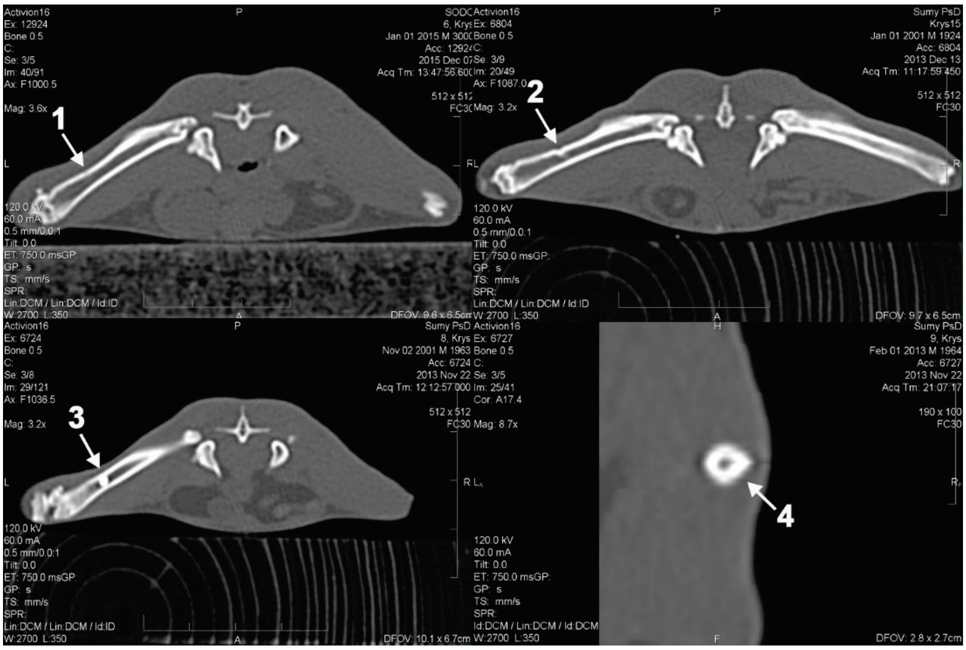

Fig. 3. Computed tomography of rat femurs on the 60th day after injury. The area of the defect in animals of the control group (1), and with the implanted β-TCP (2), NHAp (3), SHAp/Col (4).

On the 120th day of the experiment in the defect site, which was healed under the blood clot, and in the implantation site of the studied biomaterials (except HAp), there was a tendency to increase the optical density compared to the previous period and its approximation to the same indicator of the maternal bone or equaling with it. At the same time, the lowest optical density was observed in the regenerate of the control group animals (1652±24 HU), the highest – in the NHAp implantation site

(3000±81 HU), and in the implantation sites of β-TCP (1762±60 HU) and SHAp/Col (1777±57 HU) the densitometric index had no significant difference. At the same time, other researchers who carried out their measurements of the optical density of calcium phosphate implant sites mainly in experiments on the spongy bones and skull bones, demonstrate slightly different values of the densitometric index. So, Azevedo AS. et al. found out that the optical density of the proximal metaphysus of the tibia of New Zealand rabbits on the 90th day after implantation of β-tricalcium phosphate into the cavity of its defect did not have a significant difference with the same index of the defect site, which healed under the blood clot, and was 450±250 HU [3]. However Kamal M. et al. note that the optical density of the site of the defect of the alveolar process of the upper jaw of New Zealand rabbits in 8 weeks after implantation into its cavity of β-tricalcium phosphate is 3421±103.2 HU [5]. According to Tanaka T. et al., the optical density of the proximal epiphysis of the tibia in humans 2 weeks after implantation into the cavity of its defect of β-tricalcium phosphate (1010.9±81.1 HU) was much higher than in the adjacent to the area of implantation spongy bone (178.0±45.1 HU), and after 6 years (168.8±75.1 HU) –the same (174.9±69.3 HU) [4]. Thus, there is a significant difference in the optical density of the β-tricalcium phosphate implantation site between the results of the three research groups.

In our study on the 120th day of the experiment, the optical density of the defect site in the animals of the control group (1652±24 HU) approached, but did not reach the same index of the maternal bone (1762±33 HU) ($p<0.05$), which, according to the densitometric study, can be estimated as incomplete reparative osteogenesis. At the same time, in the animals whose defect healed under the blood clot, there was a complete restoration of the original shape of the bone, and visually on a computer tomogram it was difficult to detect the place of the former injury. However, in the area of β-TCP implantation in the last observation period the optical density (1762±60 HU) was equal to the same index of the maternal bone (1750±49 HU), which indicates the replacement of calcium phosphate material with well-mineralized bone tissue. At the same time, there was no complete restoration of the original form of the bone, because at the level of implantation of the biomaterial there was still a barely noticeable deepening or dense endosteal callus.

Data on the effect of biocomposite calcium phosphate materials on the density of the formed regenerate are also available in the scientific literature. Thus, the results of the study of Huang Z. et al. indicate that the optical density of the site of defect of the epiphysis of the femur of New Zealand white rabbits 12 weeks after implantation into its cavity of biomaterial (chitosan/mineralized collagenfibrils/ mesenchymal stem cells) amounted to 357±23 HU. At the same time, the above figure was 4.5 times greater than in the area of the defect, which healed under the blood clot (-100±10 HU) [8]. However, according to Danilkovich N. et al., the optical density of the defect site of the rabbit radius 3 months after implantation of the biomaterial (collagen-hydroxyapatite scaffold/ mesenchymal stem cells / platelet growth factors) was 2200±100 HU and exceeded the same index of the site of the defect, which healed under the blood clot (1800±100 HU) [12].

In our study, the optical density of the femoral shaft defect site on the 120th day after SHAp/Col implantation was 1777±57 HU. In addition, in the last period of the experiment, the densitometric index did not only approach, but also exceeded by 3.67% ($p>0.05$) the same index of the maternal bone (1714±44 HU), which together with the absence of deformation on the outer surface of the cortical bone layer indicates a complete restoration of the defect. At the same time, endosteal callus remained, which indicates the absence of a complete restoration of the initial form of the injured bone in the last period of observation (Fig. 4).

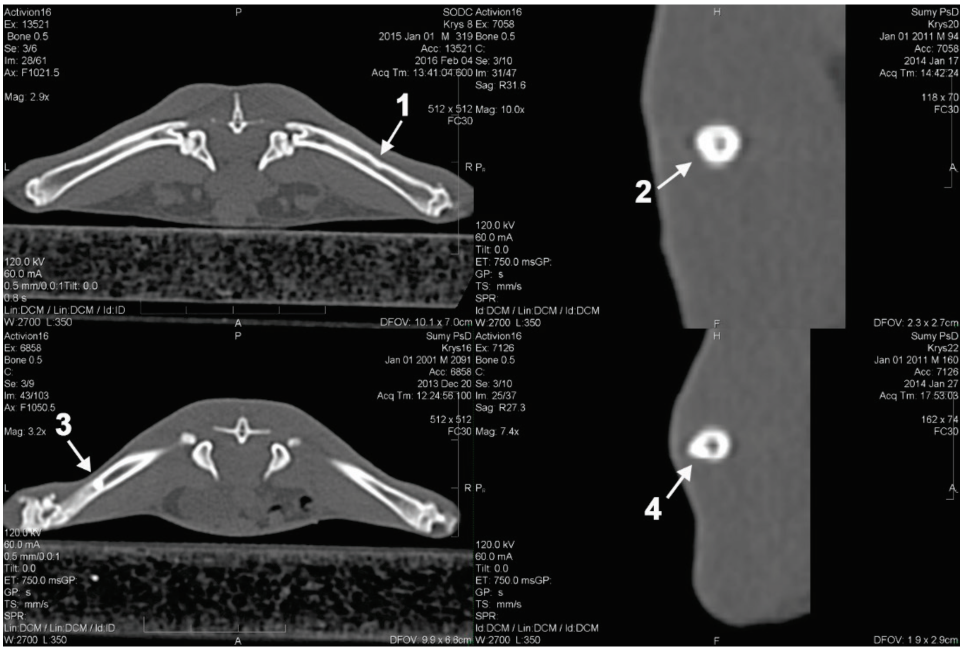

Fig. 4. Computed tomography of rat femurs on the 120th day after injury. The site of the restored defect in animals of the control group (1), and with the implanted β-TCP (2), NHAp (3), SHAp/Col (4).

The leader in optical density among the studied by us osteoplastic materials was the NHAp implantation site. The latter, according to the densitometric index under study, not only exceeded the implantation site of all other calcium phosphate materials studied by us, but also had an almost constant optical density throughout the entire duration of the experiment. For its part, the study of Zaccaria L. et al. [9] also indicate that the optical density of the area of the defect of human skull bones even 4 years after implantation of hydroxylapatite into its cavity has a high value (2800 HU). Son J. S. et al. [13] note that the optical density of the femoral shaft defect site of hounds 10 weeks after implantation into its cavity of hydroxylapatite has a much lower value (862±61 HU). In our study, during 120 days of the experiment, the NHAp implantation site maintained a high optical density (2703±81–3000±81 HU), which significantly exceeded the same index of the maternal bone (1665±41–1689±18). Due to this, there were no signs of NHAp replacement by regenerate components during the entire observation period.

Conclusion. Thus, NHAp provides high and stable densitometrical properties of the defect area of compact bone tissue, and all other materials for the period of 120 days contribute to the full restoration of the absolute optical density of the injured bone.

სამედიცინო მეცნიერებები

ბიომასალების შედარებითი გავლენა კომპაქტური ძვლოვანი ქსოვილის დეფექტების მკურნალობის რენტგენულ სურათზე

ო. კორენკოვი

სუმის სახელმწიფო უნივერსიტეტი, მორფოლოგიის დეპარტამენტი, სუმი, უკრაინა

(წარმოდგენილია აკადემიის წევრის ნ. მითაგვარიას მიერ)

წინამდებარე ნაშრომის მიზანია ბიომასალების მთავარი სახეობების გავლენის შედარება კომპაქტური ძვლოვანი ქსოვილის დეფექტების მკურნალობის რენტგენულ სურათზე. ექპერიმენტი ჩატარდა „ვისტარ“-ის ჯიშის ოთხმოცდათექვსმეტ ალბინოს ვირთაგვაზე. თეძოს დიაფიზის შუა მესამედში აღდგენილ იქნა 2,5 მმ დიამეტრის პერფორირებული დეფექტი, რომელიც პირველი ჯგუფის ცხოველებს შედედებული სისხლით სამკურნალოდ დუტოვეს, მეორე ჯგუფის ცხოველებს β-ტრიკალციუმფოსფატით შეუვსეს, მესამე ჯგუფის ცხოველებს – ბუნებრივი ჰიდროქსიაპატიტით (NHAp), ხოლო მეოთხე ჯგუფის ცხოველებს – ბიოკომპოზიტური მასალებით (სინთეტიკური ჰიდროქსიაპატიტი/კოლაგენი SHAp/Col). დაზიანებული ძვლების მკურნალობა და მათივე ოპტიკური სიმკვრივის დადგენა შესწავლილ იქნა მეთხუთმეტე, ოცდამეათე, მესამოცე და ასმეოცე დღეს კომპიუტერული ტომოგრაფიის გამოყენებით. დადგინდა, რომ მთელი ექსპერიმენტის განმავლობაში გამოკვლეული ბიომასალები (NHAp-ის გარდა) აღდგენილი კომპონენტებით ჩანაცვლდა. ცვლილება უდაბლესი ოპტიკური სიმკვრივიდან უმაღლესისკენ მეთხუთმეტე-ოცდამეათე დღეების შუალედში წარმოდგენილი იყო პირველი ჯგუფის ცხოველების დაზიანებული ადგილებით (225±36 HU და 526±64 HU), იმპლანტირებული უბნებით SHAp/Col (1165±31 HU, 1575±67 HU), β-TCP (1500±49 HU, 1544±56 HU), NHAp (2715±197 HU 2703±81 HU), ხოლო მესამოცე დღეს პირველი ჯგუფის ცხოველების დაზიანებული უბნით (1259±73 HU), β-TCP (1423±12 HU), SHAp/Col (1757±86 HU) და NHAp (2841±109 HU)-ის იმპლანტაციის უბნებით. ექსპერიმენტის ასმეოცე დღეს, პირველი ჯგუფის ცხოველების დაზიანებული ადგილების ოპტიკურმა სიმკვრივემ (1652±24 HU) ვერ მიაღწია იმავე მაჩვენებელს, რასაც დედის ძვლებმა (1762±33 HU), NHAp (3000±81 HU)-ის იმპლანტაციის უბანი მნიშვნელოვნად აჭარბებდა მას (1680±61 HU), ხოლო β-TCP (1762±60 HU) და SHAp/Col (1777±57 HU)-ის იმპლანტაციის უბნები მისი ტოლი იყო (1750±49 HU, 1714±44 HU). ამრიგად, NHAp უზრუნველყოფს კომპაქტური ძვლოვანი ქსოვილის დაზიანებული ადგილის მაღალ და სტაბილურ დენსიტომეტრიულ ხარისხს, და ასმეოცე დღეს ყველა მასალის ერთობლივი ზემოქმედება განაპირობებს დაზიანებული ძვლის ოპტიკური სიმკვრივის სრულ აღდგენას.

REFERENCES

1. Beniashvili R. M., Kulakov A. A., Gurin A. N., Grigor'yants L. A., Komlev V. S., Semkin V. A. (2017) Desnevaia i kostnaia plastika v dental’noi implantologii. 240 s. GEOTAR-Media (in Russian).
2. Kulakov A. A., Arzhantsev A. P., Abrahamyan S. V. (2018) Dental’naia implantatsiia: national’noe rukovodstvo. 400 s. GEOTAR-Media (in Russian).
3. Azevedo A. S., Sá M. J., Fook M. V., Neto P. I., Sousa O. B., Azevedo S. S., Teixeira M. W., Costa F. S., Araújo A. L. (2014) Use of chitosan and β-tricalcium phosphate, alone and in combination, for bone healing in rabbits. *Journal of Materials Science: Materials in Medicine*, **25**(2): 481–486.
4. Tanaka T., Kumagae Y., Chazono M., Kitasato S., Kakuta A., Marumo K. (2014) A novel evaluation system to monitor bone formation and β-tricalcium phosphate resorption in opening wedge high tibial osteotomy. *Knee Surgery, Sports Traumatology, Arthroscopy*, **23**(7): 2007–2011.
5. Kamal M., Andersson L., Tolba R., Al-Asfour A., Bartella A. K., Gremse F., Rosenhain S., Hölzle F., Kessler P., Lethaus P. (2017) Bone regeneration using composite non-demineralized xenogenic dentin with beta-tricalcium phosphate in experimental alveolar cleft repair in a rabbit model. *Journal of Translational Medicine*, **15**(1): 263–275.
6. Onoprienko G. A., Voloshin V. P. (2017) Mikrotsirkuliatsiia i regeneratsiia kostnoi tkani: teoreticheskie i klinicheskie aspekty. 184 s. BINOM (in Russian).
7. Pankratov A. S., Lekishvili M. V., Kopetsky I. S. (2011) Kostnaia plastika v stomatologii i cheliustno-litsevoi khirurgii. Osteoplastic materials: A Guide for Physicians. 272 s. BINOM (in Russian).
8. Huang Z., Chen Y., Feng Q. L., Zhao W., Yu B., Tian J., Li S. J., Lin B. M. (2011) In vivo bone regeneration with injectable chitosan/hydroxyapatite/collagen composites and mesenchymal stem cells. *Frontiers of Materials Science*, **5**(3): 301–310.
9. Zaccaria L., Tharakan S. J., Altermatt S. (2017) Hydroxyapatite ceramic implants for cranioplasty in children: a single-center experience. *Child's Nervous System*, **33**(2): 343–348.
10. Walsh W. R., Smitham P., Yu Y., Oliver R., Stewart D., Rawlinson J., Vizesi F., Irie H. (2007) Healing of a critical size defect in sheep using bone graft substitutes in block form. *Orthopaedic Research Society 53rd Annual Meeting Transactions,* San Diego, California, February 11–14, Poster No: 1433.
11. Osipenkova-Vechtomova T.K. (2017) Sudebno-meditsinskaia ekspertiza kostei. 272 s. BINOM (in Russian).
12. Danilkovich N., Derkachev V., Kosmacheva S., Potapnev M. (2016) Application of bone-marrow mesenchymal stem cells and platelet-derived growth factors for human osteogenic graft engineering. *5th International Conference on Tissue Engineering and Regenerative Medicine*, **7**(2): 155.
13. Son J. S., Appleford M., Ong J. L., Wenke J. C., Kim J. M., Choi S. H., Oh D. S. (2011) Porous hydroxyapatite scaffold with three-dimensional localized drug delivery system using biodegradable microspheres. *Journal of Controlled Release*, **153**(2): 133–40.

Received March, 2019