



# EXPERIMENTAL SUBSTANTIATION OF OSTEOTRANSPLANT APPLICATION IN TRAUMATIC VERTEBRAL DEFECTS

V.V. Rerikh<sup>1,2</sup>, Yu.A. Predein<sup>1</sup>, A.M. Zaidman<sup>1</sup>, A.D. Lastevsky<sup>1</sup>, V.A. Bataev<sup>3</sup>, A.A. Nikulina<sup>3</sup>

<sup>1</sup>Novosibirsk Research Institute of Traumatology and Orthopaedics n.a. Ya.L. Tsvinyan, Novosibirsk, Russia

<sup>2</sup>Novosibirsk State Medical University, Novosibirsk, Russia

<sup>3</sup>Novosibirsk State Technical University, Novosibirsk, Russia

**Objective.** To analyze the features of bone tissue formation during plasty of vertebral body defect or fracture with an allogeneic bone graft in an experiment *in vitro*.

**Material and Methods.** Models of the vertebral body defect (fracture of the cranioventral part with penetration into the nucleus pulposus) were created in an experiment on 20 mini-pigs of the same age. Plasty of traumatic defects was performed with allogeneic bone graft or autologous bone. CT, histological, and spectrometric studies of microscopic specimens were carried out at 14, 30, 90, and 180 day. Reparative osteogenesis, X-ray density, Ca and P content, and microhardness were studied.

**Results.** After implantation of allogeneic bone graft, an organ-specific bone similar to the recipient's bone in morphological structure, X-ray density, mineral composition and microhardness, was formed on the 90<sup>th</sup> day ( $P = 0.01$ ). After transplantation of autobone, the regenerate formed by this day in the central part was in a phase of resorption and restructuring with lower indices of X-ray density, content of Ca and P, and microhardness ( $P = 0.01$ ).

**Conclusion.** After plasty of vertebral body traumatic defects with allogeneic bone graft, the organ-specific bone tissue is formed at an earlier time and reliably exhibits greater mineralization and strength.

**Key Words:** allogeneic bone graft, vertebral body plasty, vertebral fracture, autobone, microhardness, mineralization, spectrometry, osteogenesis.

Please cite this paper as: Rerikh VV, Predein YuA, Zaidman AM, Lastevsky AD, Bataev VA, Nikulina AA. Experimental substantiation of osteotransplant application in traumatic vertebral defects. *Hir. Pozvonoc.* 2018;15(4):41–51. In Russian.

DOI: <http://dx.doi.org/10.14531/ss2018.4.41-51>.

The compensation of bone defects resulting from mechanical injuries, congenital anomalies or surgical interventions continues to be an acute medical and social problem [1–15].

Bone tissue possesses a fairly high regenerative capacity, but in cases of severe traumatic injuries, especially in load-bearing areas, it is not sufficient for recovery [16–22].

Bone tissue defects caused by fractures of a vertebral body require reconstructive surgical interventions and implantation of various materials to restore the shape of the vertebral body and create favorable conditions for its consolidation [23–25].

The gold standard for plastic material is the recipient's own bone. The following hypothesis has been formulated: an allogeneic bone graft created *in vitro* can, after implantation into the bone bed, rearrange into the organ-specific bone in a shorter period of time with indices of

mineralization and strength not inferior to the autobone [18, 19].

The objective of the study was to analyze the features of bone tissue formation during plasty of vertebral body defect or fracture with an allogeneic bone graft in an *in vitro* experiment.

## Material and Methods

All experiments were carried out in accordance with the ethical standards governing animal experiments within the framework of international and Russian regulatory documents (European Convention on the Protection of Vertebrate Animals of 1986, the Order of the Ministry of Health of the Russian Federation No. 267 of 19.06.2003 «On approval of the regulations of Good Laboratory Practice»).

Allogeneic bone grafts were created on the basis of chondrografts made from cultured chondroblasts, which were

extracted from the vertebral body growth plate of a newborn minipig under sterile conditions [26]. Allogeneic bone graft is a conglomerate of osteogenic cells and matrix containing tissue-specific proteins of the pre-bone tissue, mineral components in the form of matrix vesicles and calcifications, alkaline phosphatase and vascular endothelial lining. The structural composition of the bone graft is similar to that of the embryonic bone tissue. Expression of type I collagen, fibronectin, osteonectin, CD 44 and isolectin B4, von Willebrand factor (markers of endothelial cells) have been detected in the bone graft using immunohistochemical methods. Type II collagen, aggrecan, was expressed in single cells in the central part of the graft, which is a sign of the ongoing process of transdifferentiation [27].

The formed grafts varied in size and ranged in volume from 6 to 8 mm<sup>3</sup>. In order to examine the osteogenesis on

the basis of allogeneic bone graft and to compare it with the osteogenesis on the basis of the autobone we conducted a study on twenty 6-month mini-pigs weighing 15–18 kg. The experiment was performed under general anesthesia. The anterior spine of the animal was mobilized after the approach to the lumbar vertebrae. The anterior longitudinal ligament was peeled off at three adjacent vertebral bodies. A cutter with a diameter of 5 mm was used to create a defect in the anteroposterior direction in two of them (L4, L5), closer to the cranial section, to the anterior wall of the spinal canal without opening the epidural space. The grafts were formed from the bone produced during the creation of traumatic defects and were used to completely fill the created defect in the distal vertebra (L5). The defect in the superior vertebra (L4) was loosely filled with allogeneic bone graft (Fig. 1).

The fracture was modeled on the superjacent vertebra (L3). The cranioventral angle was cut off using a chisel from bottom to top and from front to back along the front surface of the cranial part of the body, 7–8 mm off the limbus. The cut-off fragment was raised anteriorly, it became mobile, but remained connected to the fibrous ring. The pulpous nucleus penetrated into the defect. 2–3 allogeneic bone grafts were tightly placed between the fragment and the body. On the front, the defects were closed with a hemostatic sponge and the detached anterior longitudinal ligament. The vertebrae were marked with metal markers. The wound was closed by layered closure. A control X-ray of the lumbar spine was performed. Animals were withdrawn from the experiment according to the pre-approved schedule. The resulting macro specimens of the lumbar spine were subjected to macroscopic evaluation.

There were three homogeneous series of the experimental animals, which differed in the timing of the surgical interventions and the plastic material used.

The post-surgery observation was performed after 14, 30, 90, and 180 days. The results of the study were evaluated using X-ray radiography of the area

of intervention. CT scans were used to assess the bone block formation, radiographic density, and bone tissue quality.

The integration of plastic material in the recipient bed was evaluated according to the classification of Tan et al. [10] based on the analysis of CT scans:

- 1) complete fusion of the graft and the bed, with the continuity of both spongy and cortical bones;
- 2) partial fusion, the fusion of the graft and the bed along the periphery, however, a line of ongoing restructuring is still visible in the central part;
- 3) unipolar nonunion of the graft and bed in the cranial or caudal part, with the fusion of the graft and the bed in another part;
- 4) bipolar nonunion both on the caudal and on the cranial border of the graft and the bed, graft resorption.

The classification of Misch et al. [28] is the most suitable one for assessing the radiological density of the bone tissue since it reflects not only the descriptive characteristic of the bone tissue quality but also provides a quantitative estimate in Hounsfield units (HU):

D1 – >1250 HU (thick layer of compact bone, poor blood supply);

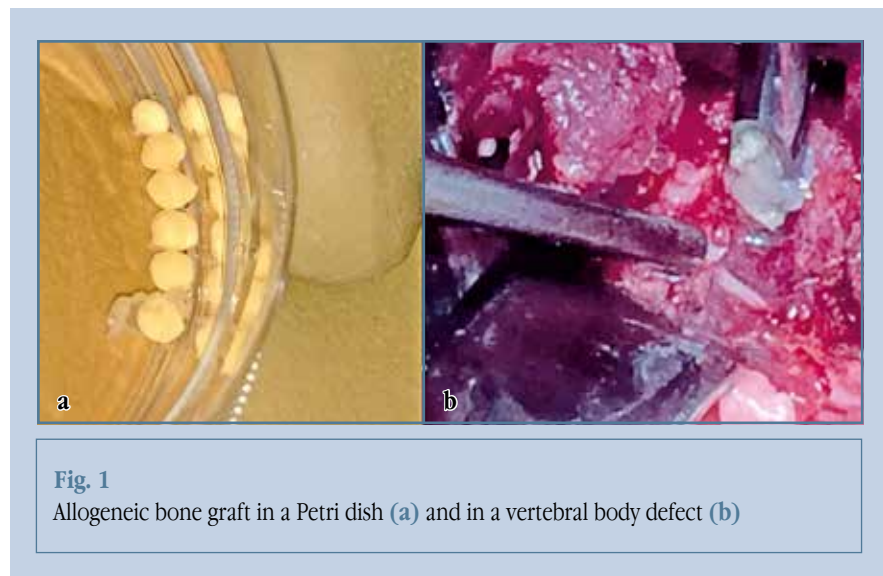
D2 – 850–1250 HU (thick bone, but with the porous compact layer and the pronounced spongy one, good blood supply);

D3 – 350–850 HU (thin bone with the porous compact layer, loose structure of the spongy layer, good blood supply);

D4 – 350 HU (loose spongy substance).

The morphological method was used to study the structure of the bone tissue of the regenerate and its cellular composition. For this purpose, the specimens for light microscopy were decalcified in trilon B and stained with hematoxylin-eosin according to van Gieson. The study of the microhardness of the formed bone and the recipient bone bed was carried out according to Vickers on a PMT-3 apparatus using the following formula:  $HV = 1.8544 \times p/d^2$ , where H is microhardness, p is the load on the indenter, d is the diagonal of the indenter footprint. The arithmetic average of the diagonal values of the indenter footprints on the trabeculae of the studied bone area was taken as a value of d in calculating the microhardness [29].

The analysis of bone mineral saturation is aimed at determining the content of Ca and P as the main components of the bone tissue, mainly represented by hydroxyapatite crystals [30], whose chemical composition corresponds to the formula  $Ca_{10}(PO_4)_6(OH)_2$ . The analysis of the mineral composition and the determination of Ca and P content was carried out on a Carl Zeiss EVO50 scanning electron microscope, since the spectroscopy provides greater accuracy.



**Fig. 1**  
Allogeneic bone graft in a Petri dish (a) and in a vertebral body defect (b)

Three areas were evaluated in all types of studies: the central (R1) and peripheral (R2) zones of the graft and the zone adjacent to the area of implantation (R3). To determine the study area, a circle was drawn around the area of implantation, whose radius was divided into two equal parts ( $R1, 2 = R_{total}/2$ ), where  $R1 = R2$ . Also,  $R1 = R2 = R3$ .

The studies were performed in the circles whose diameters were equal to values of R1, 2, 3 (Fig. 2–4).

The values recorded during the study were presented using descriptive statistics. Due to the small sample size, the interval variables are presented as non-parametric statistics, for which the medians and quartiles were calculated.

The comparison of groups based on quantitative indicators was performed using the unpaired Mann-Whitney rank nonparametric criterion. In the case of pairwise comparisons, the differences were considered statistically significant at a level of less than 0.05. In the case of multiple comparisons, the differences were considered significant when the Bonferroni adjustment was taken into account: for comparison of five pairs, the threshold level of alpha was taken to be  $0.05/5 = 0.01$ ; of six pairs -  $0.05/6 = 0.008333$ ; of seven pairs -  $0.05/7 = 0.00714$ .

Statistical analysis was performed using IBM SPSS Statistics software (version 21.0).

## Results

The CT examination of the macroscopic specimen demonstrated that on Day 90 the type 1 allogenic bone graft fusion with the bed occurred in all cases in series I and III of the experiment, while in series II, where the autobone was used for the bone block formation, the fusion could be characterized as types 3 and 4 by this time point. The complete formation of type 1 bone fusion had been achieved only on Day 180. Remarkably, in series I and II, changes in the X-ray density run in the opposite directions up to 30 days from the beginning of the experiment. The X-ray density of the newly formed bone tissue in series I and

III, where the allogenic bone grafts were used, gradually increased up to the final term of 180 days, while in series II this parameter constantly and significantly decreased during the first month, and only afterwards did it start to increase. After 180 days the X-ray density of the newly formed bone was the same in all series (Fig. 5).

When autobone was used (series II), the distribution of X-ray density with the increase at point 2 corresponded to the formation of bone tissue identical to that of the vertebral body in the zone adjacent to the recipient bed, and incomplete osteogenesis in the central part of the autobone and in the zone not in contact with the bed (Fig. 6). Therefore, osteogenesis using autobone proceeds from the periphery to the center. Statistical significance of the differences is confirmed by the ratio between the overall index of the X-ray density of the autografts and the values observed in the vertebral body, which are 1.28 times higher ( $P = 0.01$ ).

The indices shown in Fig. 5 are confirmed by morphological study, which revealed identical dynamics of the bone block formation.

After 90 days, the bone tissue of the trabecular structure was formed in the area of the cranioventral fracture of the vertebral body; the elements of the allogenic bone graft were not differentiated clearly. There was a continuous transition of both the bone trabeculae and the vessels following from the vertebral body through the area of plastic replacement to the cranioventral angle. The trabeculae formed a looped net with a tendency towards longitudinal direction. Notably, there was a thickening of the ventral compact plate. Osteoblasts were evenly spaced inside the bone trabeculae and there were no gluing lines. There were osteoblasts around the bone trabeculae, which indicates the formation of organ-specific bone tissue in the area of the former fracture. The space between the bone trabeculae was filled with myeloid bone marrow (Fig. 7a). Similar developments were observed at the same time in the area of filling the defect with the allogenic bone graft (Series I; Fig. 7b).

There were fragments of the autograft in the central zone of the graft; bone trabeculae were located in the connective tissue. There were also fragments of newly formed bone tissue, which was formed on the periphery, in the area of the recipient bed, and was closely adjoined to the area of the bone tissue defect. The space between the young bone trabeculae on the periphery is filled with bone marrow and vessels. Active osteogenesis continued at the junction of the recipient bed and the graft, while resorption and osteogenesis continued in the central part.

The morphological picture on Day 90 indicates the absence of complete bone tissue regeneration in the area of autobone grafting (Fig. 7c).

Microhardness values are the same at all points of the allogenic bone graft; the average difference between points 1, 2 and 3 was 0.53 HV ( $P = 0.392$ ;  $P = 0.695$ ;  $P = 0.569$  between points 1 and 2, 1 and 3, 2 and 3, respectively). The microhardness of the regenerate at this time point differs from the microhardness of the vertebral body on average 1.06 times (by 4.83 HV) in favor of point 4, which is statistically significant ( $P = 0.01$ ;  $P = 0.01$ ;  $P = 0.01$  for points 1, 2 and 3, respectively). Overall indices are 1.03 times (2.95 HV) lower than those for the vertebral body, which is statistically significant ( $P = 0.01$ ); however, it is a clinically insignificant difference which points to a completed process of bone

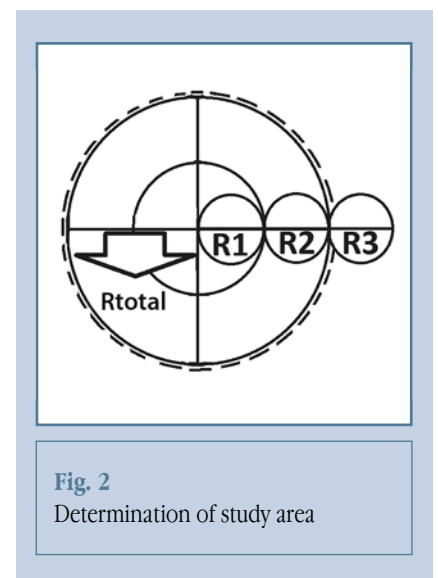


Fig. 2  
Determination of study area



mineralization in the area of allogeneic bone graft substitution (Table 1).

In series I, there were no significant differences in Ca/P values between points 1, 2 and 3 after 90 days ( $P = 0.857/0.959$ ;  $P = 0.474/0.755$ ;  $P = 0.348/0.783$  between points 1 and 2, 1 and 3, 2 and 3, respectively), which may indicate a uniform process of mineralization in all zones of allogeneic bone graft and the

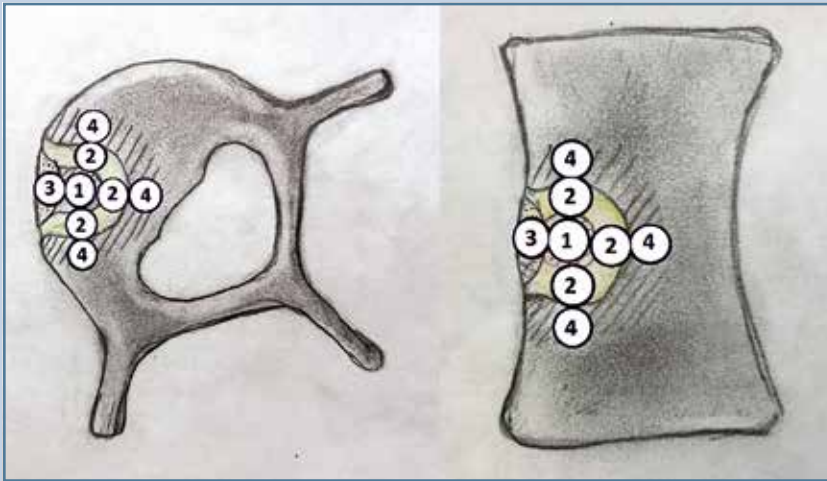
completion of the osteogenesis. After 90 days, there were no significant differences between the graft and vertebral body ( $P = 0.073/0.103$ ;  $P = 0.06/0.160$ ;  $P = 0.007/0.083$ ;  $P = 0.009/0.05$  for points 1, 2, 3 and overall indices, respectively). The only significant difference was observed for Ca spectrometry at point 3, which corresponds to 1.08%, however it was not

clinically significant in vivo conditions ( $P = 0.007$ ; Table 2).

On Day 90 after the surgery, the studies of Ca/P values in series II revealed their decreasing along with a distance of points from the recipient bed. The lowest amount of Ca/P was reported for points 1 (9.89 [9.11; 10.91]/6.89 [5.81; 8.03] %) and 3 (11.51 [10.84; 12.89]/7.36 [6.79; 8.25] %); the highest was reported for point 2 (14.79 [13.86; 15.21]/10.97 [10.42; 11.74] %), which is in the direct contact with the recipient's bed. In the vertebral body, these values are equal for point 4 (16.61 [15.19; 17.47]/13.87 [12.52; 14.9] %). The data obtained indicate a significant non-uniformity of the mineralization process during osteogenesis based on autograft ( $P < 0.008333$ ); in all cases, the differences obtained are statistically significant, except for point 1 vs. point 3 in P spectrometry, for which  $P = 0.119$ , which is a statistically insignificant; Table 3).

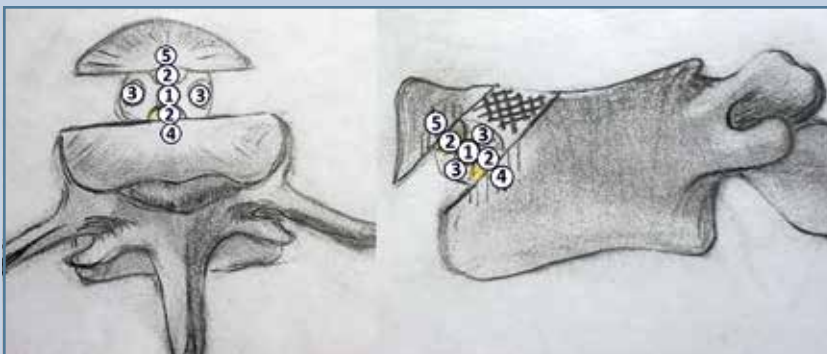
On Day 90 after the surgery, the highest values of microhardness in series II were observed for point 4 (81.8 [80; 83.4] HV) which denotes the vertebral body; point 2 was the second one in terms of its values, which differed only by 2.5 HV ( $P = 0.005$ ) from point 4 (statistically insignificant difference). The lowest values were observed for points 1 (64.7 [59.1; 67.4] HV) and 3 (69.9 [67.3; 74.9] HV) which are equally distant from the recipient bed; these values do not conform to the microhardness of the vertebral body, which indicates the ongoing process of bone tissue formation at the points located at a distance from the bone bed ( $P = 0.01$  and  $P = 0.01$ , respectively); the differences are statistically significant. In this case, the regeneration of a vertebral body defect proceeds from the feeding recipient bed to the central part of the autograft, and therefore the microhardness index at the central point is lower compared to points 2 and 3 ( $P = 0.01$  and  $P = 0.01$ , respectively); the differences in pairwise comparisons are statistically significant.

By Day 14 the values were distributed as following: The differences in the X-ray density of bone tissue at all points of the allogeneic bone graft were statis-



**Fig. 3**

Measurement areas for replacement of a vertebral body defect in series I and II: 1 – regenerate center; 2 – the edge of the regenerate adjacent to the bed; 3 – the edge of the regenerate, not adjacent to the bed; 4 – the edge of the recipient bed adjacent to the regenerate



**Fig. 4**

Measurement areas for fracture of the cranioventral vertebral angle in series III: 1 – regenerate center; 2 – the edge of the regenerate adjacent to the bed; 3 – the edge of the regenerate, not adjacent to the bed; 4 – the edge of the vertebral body adjacent to the regenerate; 5 – edge of the cranial ventral angle adjacent to the regenerate

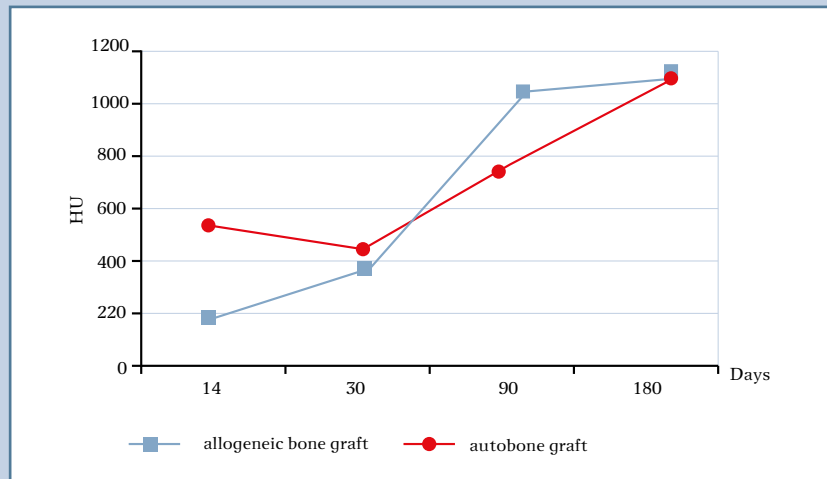


Fig. 5

Bone tissue density in series I and II

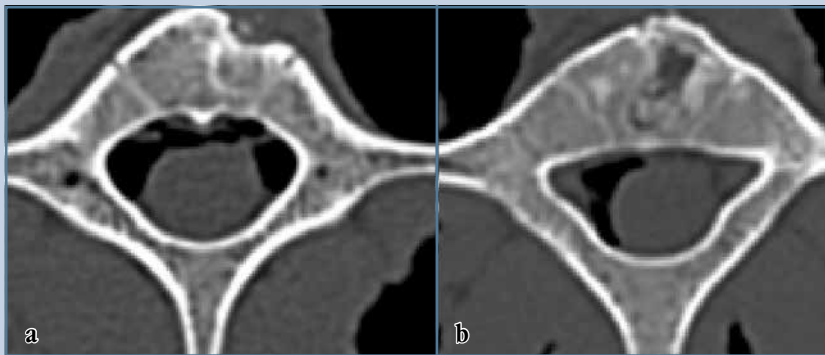


Fig. 6

Data of MSCT of vertebral body with bone tissue defect substituted with allogeneic bone graft (a) and with autobone (b), on Day 90

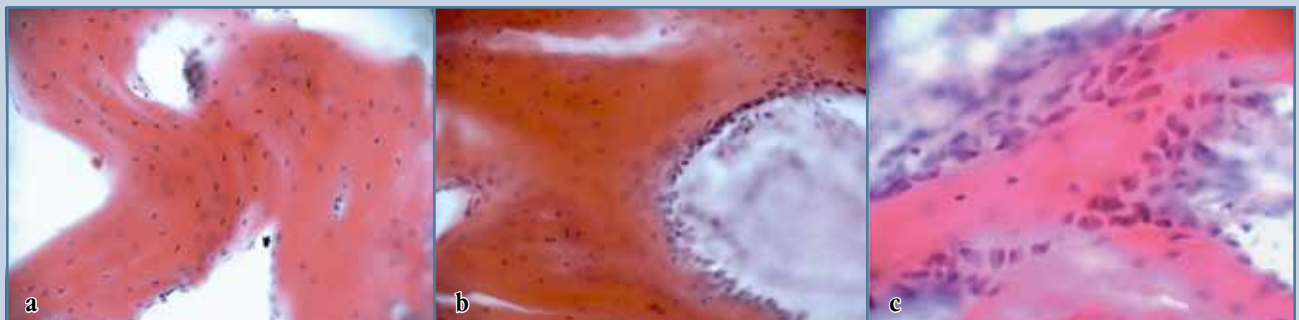


Fig. 7

Data of the light microscopy on Day 90 after the implantation, hematoxylin-eosin staining: a – in case of fracture of the cranioventral angle and plasty with allogeneic bone graft (series III); b – in case of regeneration of a vertebral body defect based on allogeneic bone graft (series I); c – in case of regeneration of a vertebral body defect on the basis of autobone (series II)

tically insignificant, not exceeding 8 HU ( $P = 0.637$ ;  $P = 0.842$ ;  $P = 0.892$  between points 1 and 2, 1 and 3, 2 and 3, respectively), which indicates relative homogeneity of the X-ray density inside the graft. Compared to the values for the vertebral body, the values for points 1, 2 and 3 were on average 6.04 times lower than the value at point 4 (1079 [1046; 1127] HU ( $P = 0.01$  for each point)); the differences obtained are statistically significant and indicate a non-conformity of the X-ray density of allogeneic bone graft. In confirmation, the regenerate density (180 [157; 200] HU at the time of the first control) which corresponded to the type 4 according to the Misch classification, had significantly lower values, with 5.99-fold difference relative to the vertebral body at point 4 ( $P = 0.01$ ); the differences are statistically significant.

The differences in X-ray density between points 4 and 5, corresponding to a fragment of the vertebral body, are considered separately. 14 days after the surgery, the differences were statistically insignificant and amounted only to 10 HU ( $P = 0.258$ ).

On Day 30, the X-ray density of the allogeneic bone graft increased in comparison with Day 14, and the localization of the area relative to the recipient bed does not affect the degree of osteogenesis process, which is confirmed by the absence of statistically significant differences between points 1, 2 and 3

( $P = 0.509$ ;  $P = 0.804$ ;  $P = 0.87$  between points 1 and 2, 1 and 3, 2 and 3, respectively). However, there is an insignificant tendency at point 2 to be ahead of other points of the allogeneic bone graft in terms of the X-ray density, if we evaluate the measurements in subsequent observations. The highest value of the X-ray density was observed at the point 4 (1030 [987; 1058] HU), which on average was 2.62 times higher than the values in the area of the allogeneic bone graft. The X-ray density of the vertebral body was

compared with the overall index for the regenerate (397 [349; 434] HU), which already corresponded to Misch type 3. The values of the overall density of the regenerate were 2.89 times lower than those at point 4 ( $P = 0.01$ ). It confirms the accuracy of values for points 1, 2 and 3 obtained in the above-mentioned separate analysis, in which the differences in pairwise comparison are statistically insignificant.

After Day 30, the difference in the values between points 4 and 5 was 28 HU,

which is higher than at the control after 14 days, however taking into account the subsequent results, we considered this to be a statistically insignificant difference ( $P = 0.141$ ).

After 90 days, the values of the X-ray density of the allogeneic bone graft continues to increase at all points with a slight predominance at point 2 (1076 [987; 1140] HU) over the points 1 (1009 [941; 1090] HU) and 3 (1020 [983; 1082] HU,  $P = 0.109$  and  $P = 0.166$ , respectively), which is a statistically insignificant difference. The differences between points 1 and 3 of the regenerate were only 11 HU ( $P = 0.741$ ), which is statistically insignificant. By Day 90, all values of the X-ray density of allogeneic bone graft at points 1, 2 and 3 conformed to those of the vertebral body bone tissue at point 4 (1075 [1004; 1124] HU,  $P = 0.042$ ;  $P = 0.867$  and  $P = 0.084$ , respectively), which is also a statistically insignificant result. The values of X-ray density show the leveling of values of the allogeneic bone graft and the vertebral body, indicating complete osteogenesis. Such conclusions are supported by comparing the overall density of the regenerate (1041 [972; 1098] HU) with the values for the vertebral body, where the 1.03-fold difference was seen as statistically insignificant ( $P = 0.107$ ) under the conditions of the present study.

On Day 90, the values of the X-ray density of the fragment leveled off compared with those of the vertebral body; the difference between them decreased 2.8 times and amounted to 10 HU. The differences are statistically insignificant ( $P = 0.731$ ).

After 180 days, the X-ray density of the allogeneic bone graft at points 1, 2 and 3 conformed to the density of the vertebral body (1056 [1031; 1130] HU) and on average even slightly exceeded the values at point 4 (by 26.33 HU) which were reduced by 19 HU, that is, they were 1.03 times higher ( $P = 0.545$ ;  $P = 0.423$  and  $P = 0.667$ , respectively), which is a statistically insignificant difference in all pairwise comparisons. There were no statistically significant differences between the values in the graft area ( $P = 0.627$ ;  $P = 0.958$ ;  $P = 0.735$  between

Table 1

Microhardness of the bone tissue in series I (90 days), HV

| Point 1               | Point 2               | Point 3               | Overall index          | Point 4              |
|-----------------------|-----------------------|-----------------------|------------------------|----------------------|
| 86.9<br>[83.1; 89.1]* | 87.5<br>[84.1; 89.8]* | 86.8<br>[84.4; 89.2]* | 88.95<br>[86.1; 91.2]* | 91.9<br>[89.1; 94.1] |

The values are presented as Me [Q25; Q75],  $n = 12$ ,  $P < 0.008333$ ;

\*the result is statistically significant relative to point 4.

Table 2

The data from spectrometric studies of the bone tissue in series I (90 days), %

| Study areas   | Examined component    |                      |
|---------------|-----------------------|----------------------|
|               | Ca                    | P                    |
| Point 1       | 17.72 [16.22; 18.72]  | 13.95 [13.16; 15.10] |
| Point 2       | 17.75 [16.59; 18.57]  | 14.08 [12.71; 15.84] |
| Point 3       | 17.09 [15.81; 18.20]* | 14.16 [12.71; 15.23] |
| Overall index | 17.44 [16.22; 18.57]  | 14.07 [12.94; 15.24] |
| Point 4       | 18.17 [17.32; 19.42]  | 14.54 [13.39; 16.71] |

The values are presented as Me [Q25; Q75],  $n = 12$ ,  $P < 0.008333$ ;

\*the result is statistically significant relative to point 4.

Table 3

Bone tissue spectrometry in series II (90 days), %

| Study areas   | Examined component        |                          |
|---------------|---------------------------|--------------------------|
|               | Ca                        | P                        |
| Point 1       | 9.89 [9.11; 10.91]*/**/** | 6.89 [5.81; 8.03]*/**    |
| Point 2       | 14.79 [13.86; 15.21]*/**  | 10.97 [10.42; 11.74]*/** |
| Point 3       | 11.51 [10.84; 12.89]*/**  | 7.36 [6.79; 8.25]*/**    |
| Overall index | 12.53 [10.02; 13.57]*     | 8.03 [6.89; 10.42]*      |
| Point 4       | 16.61 [15.19; 17.47]      | 13.87 [12.52; 14.90]     |

The values are presented as Me [Q25; Q75],  $n = 12$ ,  $P < 0.008333$ ;

\*the result is statistically significant relative to point 4.

\*\*the result is statistically significant inside the graft relative to point 3;

\*\*\*the result is statistically significant inside the graft relative to point 2.



points 1 and 2, 1 and 3, 2 and 3, respectively). The values for points in the regenerate area are on average 4.16 times closer to the fragment density than to the density of the vertebral body, which may indicate an acceleration of the repair processes in the indicated zones. The overall index of the X-ray density of the regenerate was 1083 [1035; 1128] HU, which corresponds to the Misch type 2; the 1.03-fold difference was found to be statistically insignificant relative to the values for the vertebral body ( $P = 0.01$ ).

The values in the fragment zone and in the region of the vertebral body had slightly higher differences than during the previous control (24 HU), but the difference remained statistically insignificant ( $P = 0.423$ ; Table 4).

90 days after the surgery, similar values were obtained from all points of the allogeneic bone graft and they reliably conformed to the bone tissue of the vertebral body at point 4 ( $P = 0.787$ ;  $P = 0.696$  and  $P = 0.432$  for points 1, 2 and 3, respectively). The microhardness of the former fragment also reliably conformed to the bone tissue of the vertebral body, which may indicate the consolidation of the fracture and restoration of an adequate blood supply to the cut-off fragment ( $P = 0.867$ ; Table 5).

The study of spectrometric data showed that Ca/P content of the vertebral body at point 4 was equal to that of the cranioventral angle at point 5 ( $P = 0.703 / 0.715$ ); no statistically significant differences were found in the pairwise comparison. Similarly, the amount of Ca/P in allogeneic bone graft at points 1, 2, 3 was not significantly different to that in the vertebral body, which may indicate the completed process of bone graft mineralization ( $P > 0.01$ ). The difference in Ca/P values within the region of plastic replacement by bone graft was estimated as insignificant ( $P = 0.342/0.774$ ;  $P = 0.677/0.223$ ;  $P = 0.195/0.115$  between points 1 and 2, 1 and 3, 2 and 3, respectively).

The data obtained by Ca spectrometry for the control regions of allogeneic bone graft, vertebral body and cranioventral angle did not reveal significant differences between them, which may indicate

uniform osteogenesis in all studied zones ( $P > 0.01$  for all points).

The data obtained by P spectrometry for the bone graft control regions have significant differences relative to the data for the vertebral body ( $P = 0.01$ ;  $P = 0.01$ ;  $P = 0.01$  for points 1, 2 and 3, respectively). The difference revealed by Ca spectrometry between overall values for the regenerate and those of the vertebral body turned out to be significant ( $P = 0.01$ ). However, they are not clinically significant in in vivo conditions (Table 6).

## Discussion

A comparative assessment of allogeneic bone graft with autograft has been conducted. It has been established that obvious significant results were achieved in all series in the study on Day 90 from the beginning of the experiment; the values of X-ray density and microhardness are directly proportional to each other.

Allogeneic bone graft was investigated in series I and III with different types of surgery. In the series with the formation of a bone defect in the vertebral body and its subsequent plasty by the bone graft (series I), the X-ray density and microhardness at the points inside the regenerate conformed to those in the vertebral body, which may indicate a completed process of osteogenesis ( $P > 0.01$ ). Such distribution of parameters in different surgery models indicates a complete formation of bone fusion and block, and indirectly about bone tissue mineralization, and uniform regeneration process in all parts of the bone graft, regardless of the proximity of the point to the recipient bed.

In series III, despite the penetration of the nucleus pulposus between the cranioventral fragment and the body, the plasty with allogeneic bone graft in the plane of the artifactual fracture ensured the fusion with the formation of the bone identical in qualitative and quantitative parameters to the vertebra.

In this case, the X-ray density continued to increase synchronously in direct proportion to the values of microhard-

ness, with a slight predominance towards the edge of the graft. Moreover, all values of these parameters at points 1, 2 and 3 on Day 90 were in line with the values of the bone tissue of the vertebral body ( $P > 0.01$ ), which indicates the consolidation of the fracture and the restoration of adequate blood supply in the cut-off fragment.

The rate of mineralization in the allogeneic bone graft was estimated spectrometrically. The Ca/P values inside the graft between the points in each series indicated that the process of osteorepair proceeds uniformly ( $P > 0.01$ ). The assessment of the values for a particular point under the conditions of different models of damage revealed the following:

- the directly proportional relationship between the content of Ca and P for X-ray density and microhardness;
- allogeneic bone graft retained the rate of mineralization, regardless of the modeled defect and the intervention performed.

According to Ca/P spectrometry, after 90 days the difference between values within the area of plastic replacement with the allogeneic bone graft and those of the vertebral body is estimated as insignificant ( $P > 0.01$ ), which indicates the completed process of mineralization.

Considering separately the data obtained for the use of the autograft in the model of vertebral body defect, we found that differences in the X-ray density between the graft and the vertebral body remained statistically significant ( $P < 0.01$ ) on Day 90. The distribution of X-ray density with its increase in the zone adjacent to the recipient bed, and the incomplete process of bone formation in the central part of the autograft and in the zone not in contact with the bed were observed. We can claim that the process of bone tissue formation proceeds from the periphery to the center. The change in microhardness was directly proportional to the X-ray density. The fact that we observed the same relationships when using a graft of a different nature testifies in favor of a link between these parameters. The microhardness values in this experimental model do not conform to that for the vertebral

Table 4

X-ray density of bone tissue in series III, HU

| Time after grafting, days | Point 1              | Point 2              | Point 3              | Overall index        | Point 4              | Point 5              | Type according to Misch et al. [27] classification |          |          |
|---------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|--|----------|----------|
|                           |                      |                      |                      |                      |                      |                      | regenerate   | vertebra | fragment |
| 14                        | 173<br>[157; 199]*   | 181<br>[158; 205]*   | 182<br>[157; 202]*   | 180<br>[157; 200]*   | 1079<br>[1046; 1127] | 1067<br>[982; 1092]  | 4  | 2        | 2        |
| 30                        | 387<br>[344; 423]*   | 410<br>[347; 434]*   | 384<br>[345; 444]*   | 397<br>[349; 434]*   | 1030<br>[987; 1058]  | 1002<br>[975; 1027]  | 3  | 2        | 2        |
| 90                        | 1009<br>[941; 1090]  | 1076<br>[987; 1140]  | 1020<br>[983; 1082]  | 1041<br>[972; 1098]  | 1075<br>[1004; 1124] | 1065<br>[1022; 1173] | 3  | 2        | 2        |
| 180                       | 1074<br>[1022; 1155] | 1090<br>[1030; 1130] | 1083<br>[1035; 1121] | 1083<br>[1035; 1128] | 1056<br>[1031; 1130] | 1080<br>[1048; 1110] | 2  | 2        | 2        |

The values are presented as Me [Q25; Q75], n = 22, P &lt; 0.00714;

\*the result is statistically significant relative to point 4.

body, which indicates the ongoing process of bone tissue formation ( $P < 0.01$ ). The distribution of values is clearly visible with an increase from the center to the periphery. The microhardness value in the central zone of the graft is less pronounced in relation to other areas. According to the data of Ca/P spectrometry, mineralization in the autograft is uneven. The data obtained after 90 days

show significant differences between the graft points and the vertebral body ( $P < 0.01$ ) and between the points inside the autograft ( $P < 0.01$ ). Remarkably, an experimental model using an autograft, rather than that using allogeneic bone graft, demonstrated a tendency for a directly proportional change in the indices of Ca and P spectrometry. The data suggests continued bone formation; min-

eralization is uneven, proceeding from the periphery to the center of the autograft by the type of creeping substitution, the absence of a complete bone block is established morphologically.

Osteogenesis in the autograft was significantly slower than in allogeneic bone graft ( $P < 0.01$ ). For example, for series I and II, it was possible to visualize the rate of formation of bone tissue using X-ray density, the distribution of which, as shown above, is identical to that for microhardness. In case of plasty of the defect (series I and II), after 30 days, the process of resorption in the peripheral part of the autograft begins accompanied by an increase in the bone graft density, which demonstrates the active process of bone block formation. Mathematically significant differences compared to the values of the vertebral body appeared with the higher density of the allogeneic bone graft ( $P < 0.01$ ). The described distinction is leveled out after 180 days.

## Conclusion

In the case of bone defect plasty with the allogeneic bone graft, the bone formation proceeds uniformly in all zones of the recipient bed, without statistically significant differences ( $P < 0.01$ ), according to the type of angiogenic osteogenesis, regardless of the damage model, and after 90 days it is completed ( $P < 0.01$ ). By this time the bone tissue

Table 5

Microhardness of the bone tissue in series III (90 days), HV

| Point 1              | Point 2              | Point 3              | Overall index        | Point 4              | Point 5              |
|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| 87.8<br>[85.2; 90.9] | 87.8<br>[85.2; 91.4] | 87.1<br>[85.1; 90.8] | 87.8<br>[85.2; 90.9] | 88.3<br>[84.6; 92.1] | 88.0<br>[86.0; 90.2] |

The values are presented as Me [Q25; Q75], n = 12, P &lt; 0,00714.

Table 6

Bone tissue spectrometry in series III (90 days), %

| Study areas   | Examined component   |                        |
|---------------|----------------------|------------------------|
|               | Ca                   | P                      |
| Point 1       | 16.66 [15.78; 17.27] | 12.97 [12.80; 13.35] * |
| Point 2       | 16.90 [16.48; 17.24] | 13.03 [12.38; 13.71] * |
| Point 3       | 16.63 [15.97; 17.14] | 12.70 [12.06; 13.18] * |
| Overall index | 16.67 [16.31; 17.20] | 12.90 [12.49; 13.48] * |
| Point 4       | 17.11 [16.52; 17.66] | 14.61 [13.04; 15.40]   |
| Point 5       | 17.04 [16.74; 17.56] | 14.63 [13.84; 15.66]   |

The values are presented as Me [Q25; Q75], n = 12, P &lt; 0,00714;

\*the result is statistically significant relative to point 4.



is formed and its quality, strength characteristics, microhardness index and mineral composition are identical to the bone tissue of the vertebral body ( $P < 0.01$  for each diagnostic criterion). A plasty of the defect with

the autograft revealed a statistically significant difference in bone tissue in the central zone in terms of quality, strength characteristics, microhardness and mineral composition, which were inferior to the bone tissue formed in the

peripheral zones of the recipient bed and the vertebral body.

*The study did not have sponsorship.*

*The authors declare no conflict of interest.*

## References

1. **Apicella A, Apicella D, Syed J, Aversa R.** Innovative biomaterials in bone tissue engineering and regenerative medicine. In: Tatullo M, ed., *MSCs and Innovative Biomaterials in Dentistry*. Springer International Publishing, 2017;63–84.
2. **Asghari F, Samiei M, Adibkia K, Akbarzadeh A, Davaran S.** Biodegradable and biocompatible polymers for tissue engineering application: a review. *Artif Cells Nanomed Biotechnol.* 2017;45:185–192. DOI: 10.3109/21691401.2016.1146731.
3. **Bastami F, Nazeman P, Moslemi H, Rezai Rad M, Sharifi K, Khojasteh A.** Induced pluripotent stem cells as a new getaway for bone tissue engineering: a systematic review. *Cell Prolif.* 2017;50(2). DOI: 10.1111/cpr.12321.
4. **Bose S, Tarafder S, Bandyopadhyay A.** Effect of chemistry on osteogenesis and angiogenesis towards bone tissue engineering using 3D printed scaffolds. *Ann Biomed Eng.* 2017;45:261–272. DOI: 10.1007/s10439-016-1646-y.
5. **Gordeladze JO, Haugen HJ, Lyngstadaas SP, Reseland JE.** Bone tissue engineering: state of the art, challenges, and prospects. In: Hasan A, ed., *Tissue Engineering for Artificial Organs: Regenerative Medicine, Smart Diagnostics and Personalized Medicine*. Wiley, 2017:525–551.
6. **Ng J, Spiller K, Bernhard J, Vunjak-Novakovic G.** Biomimetic approaches for bone tissue engineering. *Tissue Eng Part B Rev.* 2017;23:480–493. DOI: 10.1089/ten.TEB.2016.0289.
7. **Noori A, Ashrafi SJ, Vaez-Ghaemi R, Hatamian-Zaremi A, Webster TJ.** A review of fibrin and fibrin composites for bone tissue engineering. *Int J Nanomedicine.* 2017;12:4937–4961. DOI: 10.2147/IJN.S124671.
8. **Smith BT, Santoro M, Grosfeld EC, Shah SR, van den Beucken JJJP, Jansen JA, Mikos AG.** Incorporation of fast dissolving glucose porogens into an injectable calcium phosphate cement for bone tissue engineering. *Acta Biomater.* 2017;50:68–77. DOI: 10.1016/j.actbio.2016.12.024.
9. **Sethu SN, Namashivayam S, Devendran S, Nagarajan S, Tsai WB, Narashiman S, Ramachandran M, Ambigapathi M.** Nanoceramics on osteoblast proliferation and differentiation in bone tissue engineering. *Int J Biol Macromol.* 2017;98:67–74. DOI: 10.1016/j.jbiomac.2017.01.089.
10. **Tan GH, Goss BG, Thorpe PJ, Williams RP.** CT-based classification of long spinal allograft fusion. *Eur Spine J.* 2007;16:1875–1881. DOI: 10.1007/s00586-007-0376-0.
11. **Vasyliov R, Oksymets VM, Rodnichenko A, Zlatska AV, Gubar OS, Zubov DO.** Tissue engineering-based approach for restoration of combat related critical sized bone defects. *Cytotherapy.* 2017;19:S223–S224. DOI: 10.1016/j.jcyt.2017.02.311.
12. **Westphal I, Jedelhauser C, Liebsch G, Wilhelmi A, Aszodi A, Schieker M.** Oxygen mapping: Probing a novel seeding strategy for bone tissue engineering. *Biotechnol Bioeng.* 2017;114:894–902. DOI: 10.1002/bit.26202.
13. **Wu Q, Yang B, Hu K, Cao C, Man Y, Wang P.** Deriving osteogenic cells from induced pluripotent stem cells for bone tissue engineering. *Tissue Engineering Part B: Reviews.* 2017;23:1–8. DOI: 10.1089/ten.TEB.2015.0559.
14. **Yorukoglu AC, Kiter AE, Akkaya S, Satiroglu-Tufan NL, Tufan AC.** A concise review on the use of mesenchymal stem cells in cell sheet-based tissue engineering with special emphasis on bone tissue regeneration. *Stem Cells Int.* 2017;2017:2374161. DOI: 10.1155/2017/2374161.
15. **Zhang XY, Fang G, Zhou J.** Additively manufactured scaffolds for bone tissue engineering and the prediction of their mechanical behavior: a review. *Materials (Basel).* 2017;10:50. DOI: 10.3390/ma10010050.
16. **Brusko AT, Gaiko GV.** Contemporary ideas about the stages of reparative bone tissue regeneration in fractures. *Visnik ortopedii, travmatologii ta protezuvannya.* 2014;(2):5–8. In Russian.
17. **Dedukh NV, Sykal AA.** Bone repair under type 2 diabetes mellitus (experimental investigation). *Problemi osteologii.* 2015;18(4):12–18. In Russian.
18. **Zaidman AM, Kosareva OS, Shchelkunova EI, Korel AV, Sukhikh AV, Strokova EL, Ivanova NA, Rerikh VV, Predein YuA., Lastevsky DA, Ageeva TA, Gusev AF.** Experimental substantiation of application of three-dimensional bone graft for regeneration of bone tissue of various localization and histogenesis. *Modern Problems of Science and Education.* 2016;(6). URL: <https://www.science-education.ru/ru/article/view?id=25582>. In Russian.
19. **Rerikh VV, Lastevskiy AD, Sadovoy MA, Zaidman AM, Bataev AV, Predein YuA, Avetisyan AR, Romanenko VV, Mamonova EV, Nikulina AA, Semantsova ES, Smirnov AI.** Experimental verification of using nanostructured ceramic implants and osteograft. *AIP Conference Proceedings.* 2017;1882:020059. DOI: 10.1063/1.5001638.
20. **Drueke TB, Massy ZA.** Changing bone patterns with progression of chronic kidney disease. *Kidney Int.* 2016;89:289–302. DOI: 10.1016/j.kint.2015.12.004.
21. **Harris PE, Bouloux PM.** Metabolic bone disease. In: *Endocrinology in Clinical Practice*, ed. by P.E. Harris, P.M. Bouloux. CRC Press, 2014:243.
22. **Picke AK, Gordaliza Alaguero I, Campbell GM, Gl er CC, Salbach-Hirsch J, Rauner M, Hofbauer LC, Hofbauer C.** Bone defect regeneration and cortical bone parameters of type 2 diabetic rats are improved by insulin therapy. *Bone.* 2016;82:108–115. DOI: 10.1016/j.bone.2015.06.001.
23. **Chen C, Li D, Wang Z, Li T, Liu X, Zhong J.** Safety and efficacy studies of vertebroplasty, kyphoplasty, and mesh-container-plasty for the treatment of vertebral compression fractures: preliminary report. *PLoS One.* 2016;11:e0151492. DOI: 10.1371/journal.pone.0151492.
24. **Guo JB, Zhu Y, Chen BL, Xie B, Zhang WY, Yang YJ, Yue YS, Wang XQ.** Surgical versus non-surgical treatment for vertebral compression fracture with osteopenia: a systematic review and meta-analysis. *PLoS One.* 2015;10:e0127145. DOI: 10.1371/journal.pone.0127145.
25. **Stauff MP, Carragee EJ.** Vertebral compression fracture rules. *Spine J.* 2014;14:971–972. DOI: 10.1016/j.spinee.2014.03.005.
26. **Zaidman AM, Korel AV, Shchelkunova EG, Ivanova NA.** Method for producing three-dimensional bone graft. Patent RU 2574942. Appl. 11.11.2014; Publ. 10.02.2016. Bul. 4. In Russian.
27. **Zaidman AM.** New plastic material for the treatment of vertebral pathology and not only... *Hir. Pozvonoc.* 2018;15(2):91–97. In Russian. DOI: <http://dx.doi.org/10.14531/ss2018.2.91-97>.
28. **Misch CE, Meffert RM.** Implant quality of health scale: A clinical assessment of the health disease continuum. In: *Dental Implant Prosthetics*, ed. by C.E. Misch. St. Louis: Elsevier Mosby, 2005:596–603.
29. **Parshev SN, Polozenko NYu.** Microhardness of Materials. Volgograd, 2004. In Russian.
30. **Supova M.** Problem of hydroxyapatite dispersion in polymer matrices: a review. *J Mater Sci Mater Med.* 2009;20:1201–1213. DOI: 10.1007/s10856-009-3696-2.

## Address correspondence to:

Rerikh Viktorovich,  
Novosibirsk Research Institute of Traumatology  
and Orthopaedics n.a. Ya.L. Tsivyan,  
Frunze str., 17, Novosibirsk, 630091, Russia,  
VRerih@niito.ru

Received 09.07.2018

Review completed 16.07.2018

Passed for printing 01.08.2018

*Victor Viktorovich Rerikh, DMSc, Head of the Functional group, senior researcher, Novosibirsk Research Institute of Traumatology and Orthopaedics n.a. Ya.L. Tsivyan, Frunze str., 17, Novosibirsk, 630091, Russia; professor of traumatology and orthopedics in Novosibirsk State Medical University, Krasny pr., 52, Novosibirsk, 630091, Russia, VRerikh@niito.ru;*

*Yury Alekseyevich Predein, orthopedic traumatologist, Novosibirsk Research Institute of Traumatology and Orthopaedics n.a. Ya.L. Tsivyan, Frunze str., 17, Novosibirsk, 630091, Russia, predein.y.a@ya.ru;*

*Alla Mikhailovna Zaidman, DMSc, Prof., Head of the Functional group of Pathomorphology and Theoretical Research in Vertebrology in the Laboratory-Experimental Department, Novosibirsk Research Institute of Traumatology and Orthopaedics n.a. Ya.L. Tsivyan, Frunze str., 17, Novosibirsk, 630091, Russia, AZaydman@niito.ru;*

*Alexey Dmitrievich Lastevsky, orthopedic traumatologist, researcher, Novosibirsk Research Institute of Traumatology and Orthopaedics n.a. Ya.L. Tsivyan, Frunze str., 17, Novosibirsk, 630091, Russia, ALastevsky@niito.ru;*

*Vladimir Andreyevich Bataev, DSc in Technics, Prof, Head of the Department of Material Science in Mechanical Engineering, Novosibirsk State Technical University, K. Marksa pr., 20, Novosibirsk, 630073, Russia, rector@nstu.ru;*

*Aelita Aleksandrovna Nikulina, PhD in Technics, researcher of the Department of the Material Science in Mechanical Engineering, Novosibirsk State Technical University, K. Marksa pr., 20, Novosibirsk, 630073, Russia, aelita27@mail.ru.*