

**REGENERATION OF LONG SKELETAL BONE AFTER
IMPLANTATION OF OSTEOPLASTIC MATERIAL COLLAPAN
INTO ITS CAVITY DEFORMITY**

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ABSTRACT

The objective of the study was to investigate the quantitative and qualitative features of bone graft regeneration, the nature of its interaction with the osteoplastic material CollapAn and the rate of biodegradation of the implant in the deformity of the femoral shaft of rats. CollapAn was implanted into the deformity of the femoral shaft in 8 months old rats, followed by light microscopic morphometric and scanning electron microscopic analysis of its regeneration on the 60th and 120th day. Throughout the entire experiment, the study found no signs of necrosis and necrobiosis of osteocytes in the maternal bone adjacent to the site of implantation. On the surface and in the middle of the CollapAn particles, osteogenic cells and foci of osteogenesis were identified.

On the 60th day in the area of the deformity the experiment identified reticulo-fibrous and lamellar bone tissue, and on the 120th day only lamellar bone tissue with residue of osteoplastic material integrated in its structure and with a high content of primary and secondary osteoblasts and osteocytes. Gradual resorption of osteoplastic material and its replacement by bone tissue, whose ratio by 60th day of the experiment was 11.61±2.31% to 88.39±2.31%, and by 120th day 7.12±1.75% to 92.88±1.75%.

The microscopic dynamics of the bone deformity regeneration shows a high biocompatibility of the material CollapAn, its osteoconductive properties, good integration of osteoplastic material with bone tissue, and almost complete restoration from deformity on the 120th day.

KEYWORDS: CollapAn, hydroxyapatite, collagen, antibiotic, reparative osteogenesis.

INTRODUCTION

One of the important problems that podiatrists face in their practice is the regeneration of bone deformities. The high frequency of the reasons that lead to bone deformities, dictates the need to find the means that would ensure their full recovery [Zherdeev KV, 2007; Merkulov VN et al., 2009]. Transplantation of bone tissue, usually performed with this pathology, has a long history, which has made significant progress over the years. However, due to high incidence of complications that may occur during such operations, in recent years there has been increasing interest in the calcium phosphate osteoplastic materials and their combinations with collagen, which displace bone tissue from clinical practice [Zatsepin ST, 2001; Ger-

manov VG et al., 2006]. The similarity of the chemical structure of these drugs with bone tissue and inertia to biological tissues makes it possible to use them widely in lost bone tissue replacement [Barinov SM, Komnev VS, 2005; Pankratov AS et al., 2011; Korenkov AV, 2014].

Drug CollapAn began to be used in clinical practice since 1995 [Pankratov AS et al., 2011]. During this time, it demonstrated high biocompatibility and excellent performance in the treatment of bone deformities in orthopedics, traumatology, spinal surgery, dentistry and maxillofacial surgery [Barer GM et al., 2004; Snetkov AI et al., 2006; Zherdeev KV, 2007; Kavalersky GM et al., 2013]. However, despite sufficient evidence of the safety and biocompatibility of CollapAn, other definitive properties of the osteoplastic material, such as dynamics and the rate of biodegradation, the substitution of the newly formed bone at the implant site, have a significant discrepancy in the data, namely from 3 to 6 and

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more months [Bushuyev OM, 1999; Berchenko GN et al., 2006]. In addition, the majority of studies on CollapAn relate morphological studies of trabecular bone, which lacks the morphometric characteristics of histological preparations, microscopic and especially electron microscopic features of bone graft regeneration [Bushuyev OM, 1999; Iordanishvili AK et al., 2002; Berchenko GN et al., 2006; Snetkov AI et al., 2006; Zherdeev KV, 2007]. Therefore, the objective of our work is to use histological, morphometric and electron microscopic techniques to investigate the quality and quantity of bone graft regeneration, the nature of its interaction with CollapAn, the rate of biodegradation of the implant in the deformity of exactly the compact substance (diaphysis) of rat femurs.

MATERIALS AND METHODS

The experiment was performed on 12 white, 8 months old laboratory male rats weighing 250 ± 10 grams. It was approved by the Institutional Bioethics Committee in accordance with the principles of the Guide for the Care and Use of Laboratory Animals published by the US NIN (No. 85-25, revised in 1985) [Kozhemyakin YM et al., 2002]. Surgical intervention was performed under ketamine anesthesia (0.3-0.5 ml per 1 kg of rat weight) in aseptic conditions. Using 2.5 mm diameter dental drill at low rpm and with cooling, transcortical diaphyseal deformity was inflicted, which was filled with osteoplastic material CollapAn (Intermedapatit Company, Russia, registration number 2011/10304 SDF) without rigid fixation. The latter is an artificial granular biocomposite material, made from high-purity synthetic nanostructured hydroxyapatite, type 1 collagen from cattle split leather and antibiotics (lincomycin, gentamicin, etc.) (Fig. 1).

Further on the 60th and 120th days after surgery, animals were taken from the experiment by decapitation under deep ether anesthesia, followed by examination of the bones by light microscopy morphometrics and scanning electron microscopy. Scanning electron microscopy was performed using an electron microscope SEM 106-I (SEMI Company, Ukraine). Light microscopy of histological sections stained with hematoxylin and eosin was performed using OLYMPUS light microscope (USA) followed by photographing slides with digital cameras. Morphometric analysis was performed

using Video Test and Video Size image processing software [Sarkisov DS, Perov YL, 1996].

The latter was determining in the deformity the volume percent of bone tissue and residual osteoplastic material as the ratio of these constituents (%) to the total area of the deformity (100%) on the 60th and 120th day of the experiment.

In addition, the state of the structure adjacent to the site of implantation of maternal bone in order to establish or refute postoperative complications through the presence or absence of signs of necrobiosis and necrosis of osteocytes was investigated using these methods [Grigoryan AS, Toporkova AK, 2007]. The resulting digital values were processed statistically by calculating the arithmetic mean (M) and standard error (m). The significance of differences between the comparable values was evaluated using Student t-test using STATISTICA 5.1 for Windows computer program. Differences were considered significant at $p < 0.05$ [Lapach SN et al., 2000].

RESULTS

On the 60th day of the experiment the deformity area is filled with newly formed bone tissue with residue of osteoplastic material immured in its structure, covering an area of $88.39 \pm 2.31\%$ and $11.61 \pm 2.31\%$. The bone plate is preferably in a peripheral portion of the deformity, in its structure it contains a significant amount of secondary osteoblasts, osteocytes and is tightly attached to the parent bone edges. In the central parts of the deformity prevails reticulo-fibrous bone tissue whose

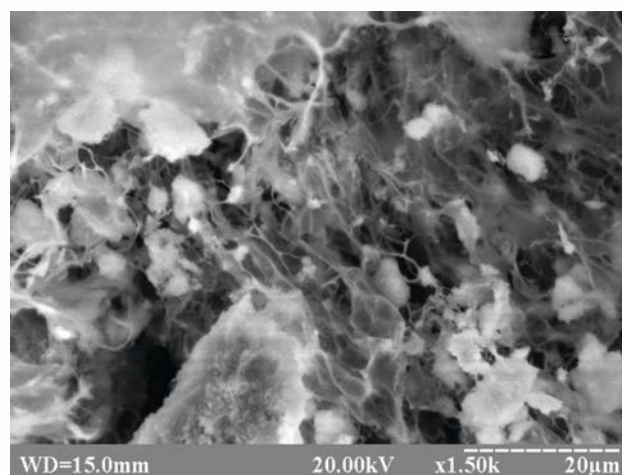


FIGURE 1. Microstructure of the inner surface of the granule of osteoplastic material CollapAn. Electronic scanning image. X 1500.

bone trabeculae surface and bone lacunae contain primary osteoblasts and osteocytes, while the intertrabecular spaces contain osteoplastic material residue undergoing resorption. Osteoplastic material residue in hematoxylin and eosin stain has the form of a structureless mass of sulfur, undergoes

gradual resorption, and contains osteogenic cells and small osteogenesis foci (Fig 2 a,b,c,d,e). In its turn, maternal bone shows the presence of bone lacunae with typical osteocytes.

On the 120th day reticulo-fibrous bone tissue is not detected in the bone deformity, and there is only

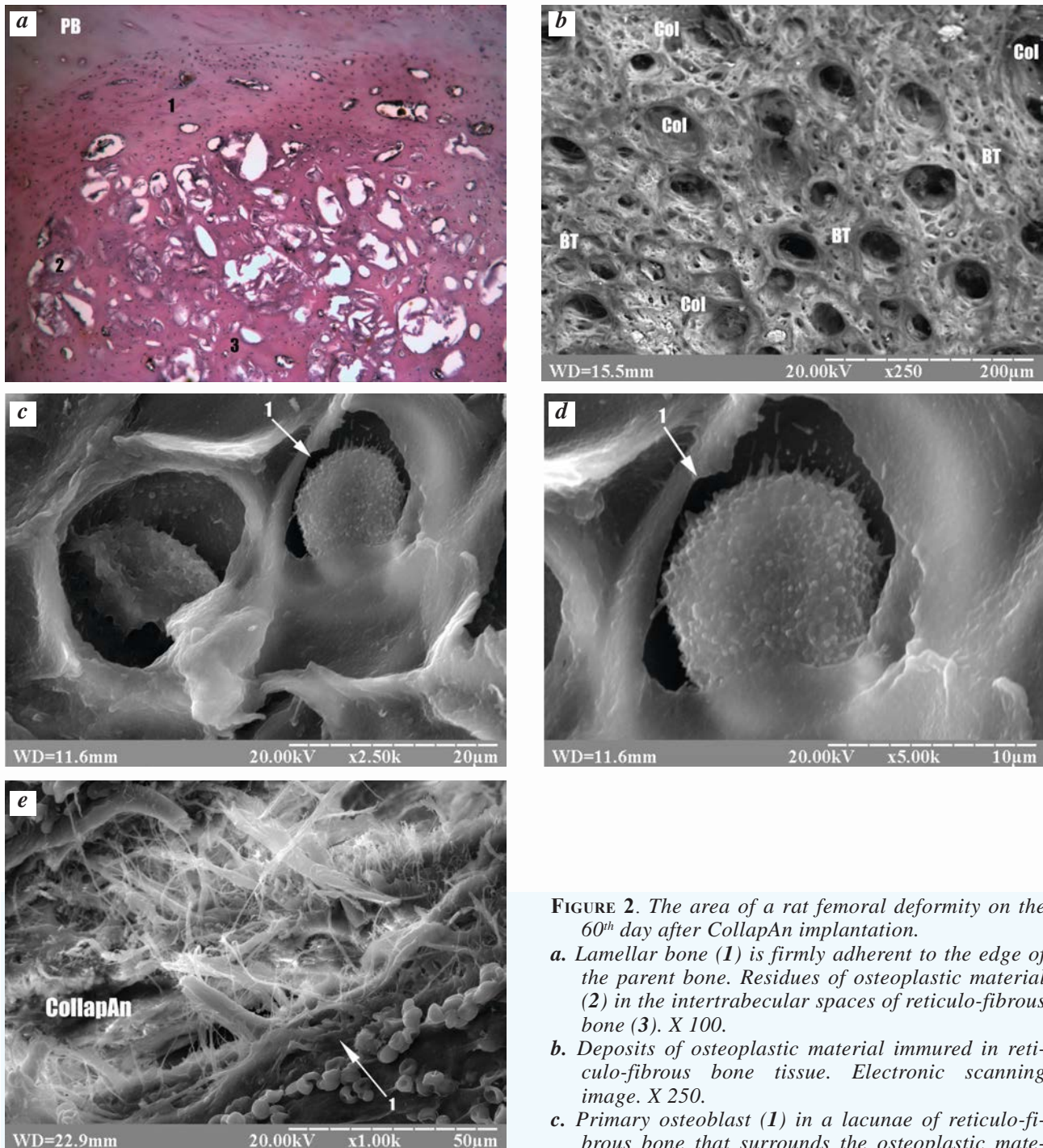


FIGURE 2. The area of a rat femoral deformity on the 60th day after CollapAn implantation.

a. Lamellar bone (1) is firmly adherent to the edge of the parent bone. Residues of osteoplastic material (2) in the intertrabecular spaces of reticulo-fibrous bone (3). X 100.

b. Deposits of osteoplastic material immured in reticulo-fibrous bone tissue. Electronic scanning image. X 250.

c. Primary osteoblast (1) in a lacunae of reticulo-fibrous bone that surrounds the osteoplastic material. Electronic scanning image. X 2500.

d. Previous photos only at higher magnification. Primary osteoblast (1) in a lacunae of reticulo-fibrous bone that surrounds the osteoplastic material. Electronic scanning image. X 5000.

e. Bone tissue of bone graft regeneration (1) surrounds osteoplastic material which is amenable to resorption. Electronic scanning image. X 1000.

secondary callus of lamellar (mature) nature. Evidence of mature nature of the newly formed bone graft regeneration is supported by its expressed lamellar structure, formed osteons, which are oriented along the axis of the bone, and by the bone located in the lacunae of the secondary osteocytes with long spikes. The arrays of bone tissue contain the residue of small CollapAn particles with osteogenic cellular elements, bone formation foci and occurrences of lysis phenomena (Fig. 3 a,b,c,d).

The area of newly formed bone tissue increased as compared with the previous follow-up period by 5.07% ($p>0.05$), and that of the osteoplastic material, on the contrary, decreased by 38.67% ($p>0.05$)

and was $92.88\pm 1.75\%$ in the first and $7.12\pm 1.75\%$ in the second case. A part of the parent bone adjacent to the edges of the deformity, as in the previous follow-up period, is characterized by the presence of lacunae with typical osteocytes.

Discussion

Microscopic examination of compact substance (diaphysis) rat femurs after osteoplastic material CollapAn implantation into their deformity found that it has a high biocompatibility, as demonstrated by the presence in the implantation site adjacent to the parent bone of lacunae with typical osteocytes, without signs of their necrobiosis and necrosis.

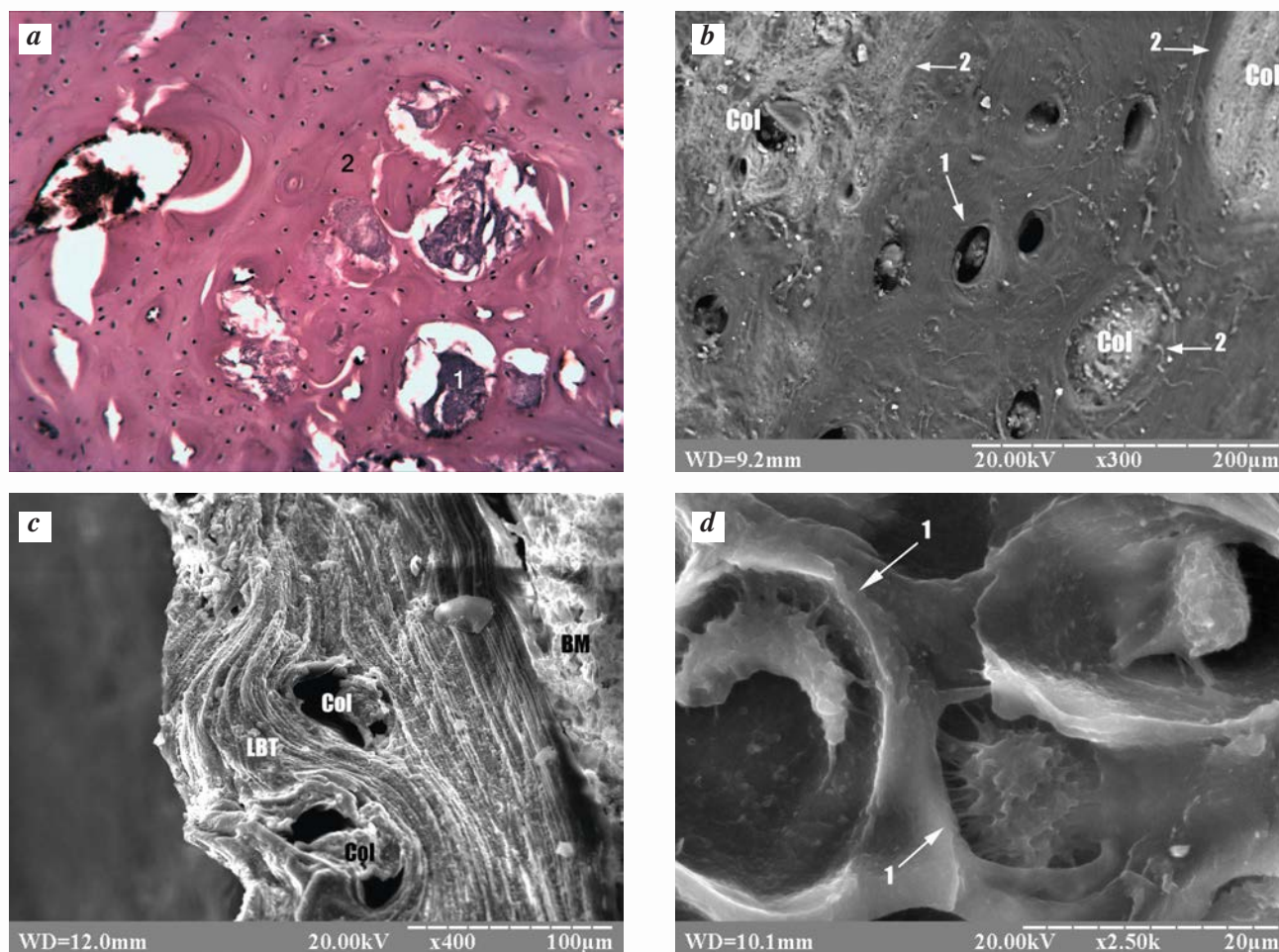


FIGURE 3. The area of a rat femoral deformity on the 120th day after CollapAn implantation.

- a. Deposits of osteoplastic material (1) are integrated in the bone plate of regenerated bone graft (2). X 200.
 b. Osteons (1) lamellar bone tissue with residue of immured osteoplastic material CollapAn in their arrays (2). Electronic scanning image. X 300.
 c. Residue of osteoplastic material CollapAn in the structure of lamellar bone tissue bone graft regeneration, next to which is the bone marrow. Electronic scanning image. X 400.
 d. Secondary osteocytes with spikes (1) comprising lamellar bone tissue that surrounds residues of osteoplastic material. Electronic scanning image. X 2500.

Most researchers also report high biocompatibility of CollapAn, not to mention that we did not find any work disproving it in literature data analysis [Bushuyev OM, 1999; Barer GM et al., 2004; Berchenko GN et al., 2006; Snetkov AI et al., 2006; Zherdeev KV et al., 2007; Pankratov AS et al., 2011; Kavalersky GM et al., 2013]. In addition, it was found that CollapAn shows osteoconductive properties, as indicated by the location of osteogenic cells on the surface and in the middle of a CollapAn particle and the formation of osteogenesis foci. It is known that the osteoplastic material that creates the necessary support for cells to attach, gives them the opportunity to share and maintain their differential phenotype, as osteogenic cells proliferate only when attached and spread on a solid substrate. Therefore, the use of osteogenic material by osteoplastic cells as a platform for attachment and generation of new bone tissue on the surface and in its cavities is the essence of osteoconduction [Jenkins MJ, 2011].

Microscopic examination also made it possible to establish the qualitative and quantitative parameters of bone tissue regeneration, and the amount of osteoplastic material. Thus, on the 60th day of the experiment the deformity area is filled with reticulo-fibrous and lamellar bone tissue, and on the 120th day only the lamellar one with a high content of primary, secondary osteocytes and osteo-

blasts and with residue of osteoplastic material integrated into their structure. Histological studies of other authors also show bone tissue neoplasm process in the deformity of femoral epiphysis with gradually lysed CollapAn particles. In this case, without indicating morphometric parameters Berchenko GN et al (2006) describe almost complete recovery of the deformity with small residue of implant particles on the 90th day of the experiment, while Bushuyev O.M. (1999) describes complete resorption of osteoplastic material and regression of the deformity in 6 months after the CollapAn implantation. In our study, we also observed a gradual resorption of osteoplastic material and its replacement by bone tissue regenerate. However, morphometric method made it possible even to set the amount of the implant and regenerated bone tissue, whose ratio on the 60th day of the experiment was $11.61 \pm 2.31\%$ to $88.39 \pm 2.31\%$, and on the 120th day – $7.12 \pm 1.75\%$ to $92.88 \pm 1.75\%$.

Conclusion

Thus, the microscopic dynamics of the bone deformity regeneration indicates high biocompatibility of the CollapAn material, its manifestation of osteoconductive properties, good integration of osteoplastic material into bone tissue and the resorption rate of the implant and bone tissue neof ormation, whose ratio on the 120th day was $7.12 \pm 1.75\%$ to $92.88 \pm 1.75\%$.

REFERENCES

1. Barer GM, Vavilov TP, Tumanova AC. [Use of biocomposite drug CollapAn containing various antibacterial inclusion on the surgical stage of complex treatment of periodontitis] [Published in Russian]. Dept. 2004; 10: 45-49.
2. Barinov SM, Komlev VS. [Bioceramics based on calcium phosphates] [Published in Russian]. Moscow. Publishing House Science. 2005. 308 p.
3. Berchenko GN, Kesyan GA, Urazgildeev RZ, Arsenyev IG, Mikelaishvili DS. [Comparative experimental morphological study of the effect of some calcium phosphate materials used in trauma and orthopedic practice to activate reparative osteogenesis] [Published in Russian]. Newsletter ESSC SB RAMS. 2006; 4(50): 327-333.
4. Bushuyev OM. [CollapAn use in treatment of chronic osteomyelitis] [Published in Russian]. Abstract. diss. cand. of medical sciences. Moscow, 1999. 20 p.
5. Germanov VG, Kovalersky GM, Cherkashov ZA, Semenov VA. [Osteoplastic surgery: from a bone transplant to modern biocomposite materials] [Published in Russian]. Medical aid. 2006; 4: 16-19.
6. Grigoryan AS, Toporkova AK. [Problems of integration of implants in bone tissue (theoretical aspects)] [Published in Russian]. Moscow. Publishing House Technosphere. 2007. 128 p.
7. Jenkins MJ. Polymers in biology and medicine. Moscow. The scientific world. 2011. 256 p.

8. *Zherdeev KV*. [Use of CollapAn implant gel in Pediatric Bone Pathology] [Russian]. Diss. cand. of medical sciences. Moscow. 2007. 139 p.
9. *Zatsepin ST*. [Bone pathology of adults. Guidelines for doctors] [Published in Russian]. Moscow. Medicine. 2001. 450 p.
10. *Iordanishvili AK, Gololobov VG, Baschenko YV, Sakharov NV*. [CollapAn – a modern optimizer of reparative osteogenesis. Ambulatory surgery] [Published in Russian]. Hospital-replacing technologies. 2002; 2: 6-8.
11. *Kavalersky GM, Protsenko AI, Nikuradze VK*. [CollapAn in surgery of injuries and diseases of the cervical spine] [Published in Russian]. Moscow. Typografiya HT-print. 2013. 107 p.
12. *Korenkov AV, 2014*. Evaluation of healing of the skeleton long bone experimental defect after implantation of osteoplastic material CALC-I-OSS® into its cavity by the method of computerized tomography. The New Armenian Medical Journal. 2014; 8, 4: 23-27.
13. *Kozhemyakin YM, Hromov OS, Philonenko MA, Sayfetdinova GA*. [Scientific and practical guidance on the content of laboratory animals and working with them] [Published in Ukrainian]. Kiev. Publishing House Avicenna. 2002. 319 p.
14. *Lapach SN, Chubenko AV, Babich PN*. Statistical methods in biomedical research using Excel. K. Morion. 2000. 320 p.
15. *Merkulov VN, Dorokhin AI, Omelyanenko NP*. [Impairment of consolidation in fractures of bones in children and adolescents. Methods of diagnosis and treatment] [Published in Russian] M. SCIENCE-PRESS. 2009. 264 p.
16. *Pankratov AS, Lekishvili MV, Kopecky IS*. [Bone grafting in dentistry and maxillofacial surgery. Osteoplastic materials: A Guide for Physicians] [Published in Russian]. M. BINOM. 2011. 272 p.
17. *Sarkisov DS, Perov YL*. [The microscopic technique. Moscow] [Published in Russian]. Publishing House Medicine. 1996. 542 p.
18. *Snetkov AI, Batrak SY, Frantov AR, Avakian AM*. [Applications of implant CollapAn in the clinic of pediatric bone pathology] [Published in Russian]. Biomaterials. 2006; 5: 2-4.