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Peculiarities of Bone Regeneration in Cases of Bio-Oss® and Autological Bone Graft Use – Experimental Study

Specyfika regeneracji kostnej w przypadkach zastosowania Bio-Oss® i wszczepu kości autologicznej – badanie doświadczalne

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Bone tissue regeneration remains one of the most important issues of maxillofacial surgery. Restoration of the native structure of bone after osteoplasty is directly related to the process of revascularization, because the growing of blood vessels into the graft is a precondition for transport of osteogenic cells, growth factors necessary for further osteogenesis.
Objectives. To determine the peculiarities of bone tissue regeneration after osteoplasty with BioOss® and compare it with autological bone graft.
Material and Methods. Experimental study was conducted on 60 white rats (males, aged 6–8 month, weight 270––380 g). The area of dorsal surface of the shinbone was used for implantation. After opening access to the bone surface, one 2 mm-diameter defect was formed on each shinbone. One of those bone defects was filled with osteoplastic material and the opposite was healing under a blood clot. After that the wound in soft tissues was closed in layer by layer. The osteocalcin level in blood serum was determined by ELISA employing N-MID Osteocalcin® ELISA (IDS) test system(enzyme immunological test for the quantitative measurement of osteocalcin). The peculiarities of revascularization were studied by histologic method. Histologic specimen were stained using Schmorl technique (thionine with phenol trinitrate) and natural hematoxylin-eosin.
Results. Mean values of osteocalcin concentration after BioOss implantation ranged from 2 ± 0.06 ng/mL to 3.65 ± 0.09 ng/mL; after autological bone graft transplant – from 1.88 ± 0.09 ng/mL to 2.2 ± 0.09 ng/mL. In the case of Bio-Oss use, the most active revascularization processes and the highest level of osteocalcin were registered on day 60. In the case of autological bone graft use, the quantity of blood vessels increased steadily and equally during the whole period of the experiment.
Conclusions. According to the obtained results, the use of autological bone graft and Bio-Oss osteoplastic material increases bone formation and revascularization activity and improves the bone structure unlike when healing took place under the blood clot (**Dent. Med. Probl. 2014, 51, 4, 458–467**).

Key words: bone regeneration, bone substitute materials, revascularization, osteocalcin.

Streszczenie

Wprowadzenie. Regeneracja tkanki kostnej pozostaje jednym z ważnych zagadnień w chirurgii stomatologicznej oraz szczękowo-twarzowej. Odbudowa naturalnej struktury kości po osteoplastyce bezpośrednio zależy od ponownego unaczynienia, ponieważ naczynia wrastające w wszczep zapewniają transport komórek osteogennych i czynników wzrostu niezbędnych do następowej osteogenezy.
Cel pracy. Ocena specyfiki regeneracji tkanki kostnej po osteoplastyce z użyciem materiału Bio-Oss® i porównanie jej z regeneracją po przeszczepie kości autologicznej w modelu doświadczalnym.
Materiał i metody. Badanie doświadczalne przeprowadzono u 60 białych szczurów (samców, w wieku 6–8 miesięcy, o wadze 270–380 g). Wszczepu dokonywano na powierzchni grzbietowej kości goleniowej. Po uzyskaniu dostępu do powierzchni kości preparowano 2 mm ubytki na obu kościach goleniowych. Jeden z nich był wypełniany

materiałem autogennym lub ksenogennym, a ubytek po przeciwnej stronie pozostawiano do wygojenia naturalnego poprzez skrzep krwi. Następnie rany były zamykane warstwa po warstwie. Stężenie osteokalcyny w surowicy krwi oznaczano badaniem immunoenzymatycznym (ELISA) z użyciem testu N-MID Osteocalcin®. Przebieg rewaskularyzacji oceniano histopatologicznie. Wycinki były barwione metodą Schmorla (tionina z trójazotanem fenolu) oraz naturalną hematoksyliną i eozyną.

Wyniki. Średnie stężenie osteokalcyny po użyciu Bio-Ossu wahało się od 2 ± 0,06 ng/ml do 3,65 ± 0,09 ng/ml, a po zastosowaniu kości autologicznej od 1,88 ± 0,09 do 2,2 ± 0,09 ng/ml. Po zastosowaniu Bio-Ossu w okresie 60-dniowej obserwacji stwierdzano bardzo aktywny proces neoangiogenezy z większymi stężeniami osteokalcyny w surowicy krwi. Po implementacji kości autologicznej rewaskularyzacja następowała równomiernie przez cały okres trwania eksperymentu.

Wniosek. Wypełnienie ubytku kostnego przeszczepem autologicznym lub materiałem ksenogennym Bio-Oss powodowało nasilenie osteogenezy i rewaskularyzacji oraz dawało poprawę regeneracji kostnej w odniesieniu do gojenia samoistnego poprzez skrzep krwi (**Dent. Med. Probl. 2014, 51, 4, 458–467**).

Słowa kluczowe: regeneracja kości, substytuty tkanki kostnej, rewaskularyzacja, osteokalcyna.

Restoration of bone tissue is one of the important issues of reconstructive surgery and dentistry. A large number of reconstructive surgical operations which involve bone grafting or the use of bone osteoplastic materials are conducted annually [1–11]. An important aspect for the understanding bone regeneration in different cases is the study of the formation of bone extracellular matrix [4–7].

A large variety of bone osteoplastic materials of different kind and with different properties and composition are widely used as opposed to the autological bone graft which involves additional trauma in the place of bone transplant withdrawal [9, 10].

Present-day biomaterials are intended for filling bone defects and should be osteoinductive, as well as provide for blood vessel budding [2, 5]. The blood vessels budding into the bone transplant or bone osteoplastic material is one of the indicators of bone formation process.

One of the sensitive markers of bone tissue metabolism is osteocalcin (vitamin K-dependent non-collagen protein), which is usually localized in the extracellular matrix and constitutes 25% of non-collagen matrix. Changes of its concentration in blood reflect metabolitic activity of bone tissue osteoblasts. Over 90% of the synthesized osteocalcin in young people and 70% in mature people is included into bone matrix, the rest goes to the blood flow. Both intact osteocalcin and its big N-MID-fragment circulate in blood [6, 12–14].

The aim of this experimental study was to determine the peculiarities of bone tissue regeneration after osteoplasty with BioOss® and compare it with autological bone graft.

Material and Methods

In the course of research the following materials were used: 1) Bio-Oss® (Geistlich): a bone mineral of natural origin received from animal bones; 2) autological bone graft (withdrawal of autological bone graft was made from dorsal surface area of the shinbone of the experimental animal, opposite to the transplantation area).

Experimental study was conducted on 60 white rats (males, aged 6–8 month, weight 270––380 g), which were divided into 2 groups (30 rats in each group). The surgery was performed under ether narcosis in aseptic conditions. Having depilated the surgical area, we made a cut in the area of dorsal surface of the shinbone, opened access to the bone surface and formed two defects using spheric bur, one 2 mm-diameter defect on each shinbone, following the anatomic features of shinbone structure of the experimental animals. One of those bone defects was filled with osteoplastic material and the opposite was used for control (healing took place under a blood clot). The wound in the soft tissues was taken in layer by layer. The Vicryl 4-0 (Ethicon) sutures were used for fasciae and muscles and Prolene 5-0 (Ethicon) sutures was used for skin. In the course of the experiment, we have implanted Bio-Oss®, and autological bone graft into the dorsal surface area of the rat shinbone in order to further research the dynamics of osteocalcin concentration change in blood of experimental animals and revascularization of the new bone tissue. The peculiarities of revascularization were studied by the histologic method. Histologic specimens were stained using the Schmorl technique (thionine with phenol trinitrate) and natural hematoxylin-eosin. The Schmorl technique involves the following stages: 1) histological samples are being placed into the thionine solution (one part of thionine solution saturated in 50% alcohol solution and 10 parts of distilled water) for 10 min; 2) rinsing in distilled water; 3) placing the samples in concentrated aqueous solution of phenol trinitrate for 0.5–1 min; 4) rinsing in distilled water; 5) differentiating in 70% ethanol until the stain excretion is terminated (during 5–10 min); 6) dehydration in 96% ethanol, illumination using carbol-xylene (solution of phenol and xylene or toluene in proportion

1 : 4 or 1 : 5), xylene. The natural hematoxylin-eosin staining technique involves: 1) removal of paraffin using toluene (3–5 min); 2) the samples are put into the ethanol of different concentrations (96% ethanol – 3 min; 80% ethanol – 3 min; 70% ethanol – 3 min; distilled water – 5 min); 3) staining with hematoxylin – 7–10 min; 4) rinsing in distilled water – 5 min; 5) differentiating in 1% hydrochloric acid solved in 70% ethanol; 6) rinsing in distilled water; 7) rinsing in 0.5% solution of ammonia; 8) staining with aqueous solution of eosin (0.5 – 1 min); 9) rinsing in distilled water (3 times); 10) rinsing in one portion of 70% ethanol (2 min) and two portions of 96% ethanol (2 min) to remove excessive eosin; 11) illumination in solution of phenol and xylene or toluene in proportion 1 : 4 or 1 : 5) – 1 min; 12) final dehydratation in 2 portions of xylene or toluene – 2 min.

To measure osteocalcin concentration, the blood serum of experimental animals was used. Blood sampling was carried out on the 14th, 30th, 60th, and 90th day. All the blood sampling was made at the same time of the day, due to the natural daily fluctuations of osteocalcin level. Further measurement was performed using the method of enzyme-linked immunoassay employing N-MID Osteocalcin® ELISA (IDS) test system (is an enzyme immunological test for the quantitative measurement of osteocalcin, an indicator of osteoblastic activity in human serum and plasma).

Light microscopy and microphotographing of hystological samples were performed using an OLYMPUS CX 41 microscope and an OLYMPUS C-5050 camera. Morphometry at the tissue level was performed using morphometric program for DP-SOFT microscope OLYMPUS CX 41.

For the statistical estimation of the results the Student's t-test, Mann-Whitney U-test (M-W) and Kolmogorov–Smirnov two-sample test (K-S) was used. Statistical significance was set a $p < 0.05$.

The scientific study was approved by the Ethical Board of animal experiment (Ethical Board of Danylo Halytskyi Lviv National Medical University, Ukraine, protocol №1, 23.01.2012)

Results

In cases of Bio-Oss use mean values of osteocalcin level in blood serum ranged from 2 (± 0.06) ng/mL to 3.65 (± 0.09) ng/mL (on the 14th day of the experiment the value was – 2 ± 0.08 ng/mL, on 30th – 3.05 ± 0.14 ng/mL ($p < 0.001$), on 60th – 3.65 ± 0.09 ng/mL ($p < 0.01$) and on 90th – 2.3 ± 0.07 ng/mL ($p < 0.01$) (M ± m) (Fig. 1, Table 1).

Peak values were observed on the 60th day of the experiment – 3.65 ± 0.09 ng/mL. Increased concentration of osteocalcin in blood serum of experimental animals was present until day 60 and decreased gradually in the period from 60th to 90th day.

In samples where autological bone graft was transplanted the rate of osteocalcin levels remained at a constant level (on the 14th day of the experiment the value was – 2.17 ± 0.12 ng/mL, on 30th – 1.88 ± 0.09 ng/mL

Table 1. Osteocalcin level fluctuation after osteoplasty with BioOss® and autological bone graft transplantation (M – mean; m – mean error; K–S – Kolmogorov-Smirnov test; M–W – Mann-Whitney test)

Tabela 1. Wahanie stężenia osteokalcyny po osteoplastyce z zastosowaniem Bio-Oss® oraz autogennych przeszczepów

Period (days) Okres (dni)	Bio-Oss®(Group 1)	Autological bone graft (autogenny kostny materiał) (Group 2)	Group 1 vs. Group 2
	Osteocalcin level(ng/mL)(M ± m)	Osteocalcin level(ng/mL) (M ± m)	
14	2 ± 0.08	2.17 ± 0.12	p > 0.05; K–S: p > 0.05; M–W: p > 0.05
30	3.05 ± 0.14; p < 0.001; K–S: p < 0.01; M–W: p < 0.01	1.88 ± 0.09; p < 0.05; K–S: p < 0.05; M–W: p < 0.05	p < 0.001; K–S: p < 0.01; M–W: p < 0.01
60	3.65 ± 0.09; p < 0.01; K–S: p < 0.01; M–W: p < 0.01	2.2 ± 0.09; p > 0.05; K–S: p > 0.05; M–W: p > 0.05	p < 0.001; K–S: p < 0.01; M–W: p < 0.01
90	2.3 ± 0.07; p < 0.01; K–S: p < 0.05; M–W: p < 0.05	2.2 ± 0.11; p > 0.05; K–S: p > 0.05; M–W: p > 0.05	p > 0.05; K–S: p > 0.05; M–W: p > 0.05

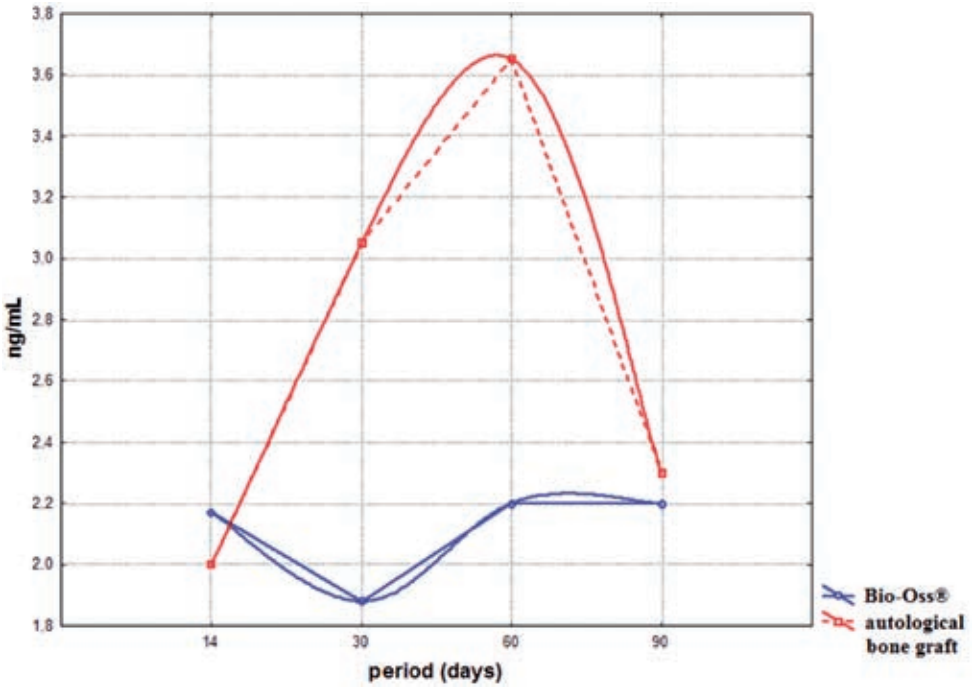

Fig. 1. Osteocalcin level fluctuation after osteoplasty with BioOss® and autological bone graft transplantation

Ryc. 1. Wahanie stężenia osteokalcyny po osteoplastyce z zastosowaniem Bio-Oss® oraz kości autogennej

($p < 0.05$), on 60th – 2.2 ± 0.09 ng/mL ($p > 0.05$) and on 90th – 2.2 ± 0.11 ng/mL ($p > 0.05$) (M ± m) (Fig. 1, Table 1). As no significant fluctuations of the osteocalcin level were noticed, the bone formation dynamic was physiological and took place without any increases.

25–30 μm² (25.88%) vessels prevailed on the histological bone tissue samples in the cases of Bio-Oss® use on the day 14, the number of 20–25 μm² vessels was 17.06% and the number of 15–20 μm² vessels was 10.0%; on the 30th day the 15–20 μm² (23.48%), 20–25 μm² (27.27%) and 25–30 μm² (31.06%) vessels prevailed; on the 60th day the 15––20 μm² (52.73%) and 10–15 μm² (24.55%) vessels prevailed, the number of 20–25 μm² vessels was 13.64%; on the 90th day the 15–20 μm² (41.11%) and 10–15 μm² (33.33%) vessels prevailed, the number of 20–25 μm² vessels was 12.22% (Fig. 2) (Table 2)

15–20 μm² (43.09%) and 10–15 μm² (29.26%) vessels prevailed on the histological bone tissue in the cases of autological bone graft transplantation on the 14th day, the number of 20–25 μm² vessels was 16.36%; on the 30th day the 15–20 μm² (30.53%) and 10–15 μm² (36.26%) vessels prevailed, the number of 5–10 μm² vessels was 17.18%; on the 60th day the 15–20 μm² (45.54%) and 10–15 μm² (29.54%) vessels prevailed; on th 90th day the 15––20 μm²(37.9%) and 10–15 μm² (34.68%) vessels prevailed (Fig. 3) (Table 2).

15–20 μm² (40.81%), 10–15 μm² (35.06%) vessels prevailed on the histological bone tissue in samples where healing took place under the blood clot on the 14th day, the number of 20–25 μm² vessels was 10.66%; on the 30th day – 15–20 μm²(43.75%) and 20–25 μm²(26.82%) vessels prevailed, the number of 10–15 μm² was 13.54% and 25–30 μm² vessels – 12.76%; on the day 60 – 15–20 μm²(34.45%) and 10–15 μm² (30.08%) vessels prevailed, the number of 5–10 μm² vessels was 17.48% and 20–25 μm² – 12.08%; on the 90th day – 15–20 μm² (36.1%) and 20–25 μm² (27.01%) vessels prevailed, the number of 10–15 μm² vessels was 16.1% (Fig. 4) (Table 2).

Prevalence of connective tissue and cartilage after osteoplasty and after healing under the blood clot was observed on the histological samples of the bone tissue on the 14th day. On the 30th day in the cases of Bio-Oss and autological bone graft use, the areas of immature bone tissue and centers of active bone formation were observed.

In cases where healing took place under the blood clot much more areas of immature bone were observed, the number of osteoblasts and activity of bone formation was significantly lower. On the 60th and 90th day in the cases of Bio-Oss and autological bone graft use, more active bone formation and larger number of osteocytes was noted; however, the remains of immature bone also were observed.

In samples where healing took place under the blood clot, the bone formation was less intensive.

In the case of Bio-Oss use revascularization was more intensive, new blood vessels formed faster, becoming centers of bone formation. The most active revascularization processes took place on the 60th day after the osteoplasty, when the highest number of newly formed small vessels (10–15 μm², 15–20 μm², 20–25 μm²) was registered. The highest level of osteocalcin also was registered on the day 60 (Fig. 1, 2). In the case of autological bone graft transplantation, the quantity of blood vessels

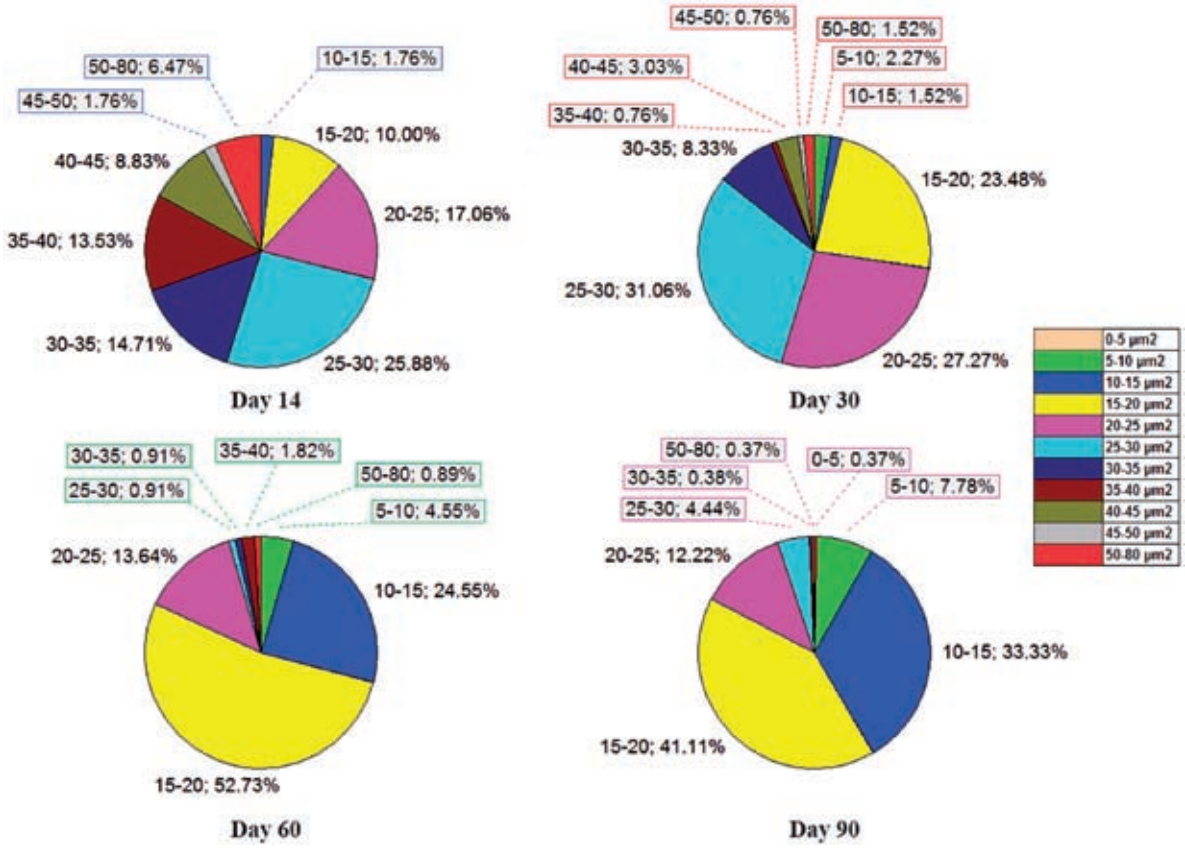

Fig. 2. Percentage change in value of vessels of different caliber in case of Bio-Oss® use

Ryc. 2. Procentowe zmiany liczby naczyń krwionośnych o różnej średnicy w przypadkach zastosowania Bio-Oss®

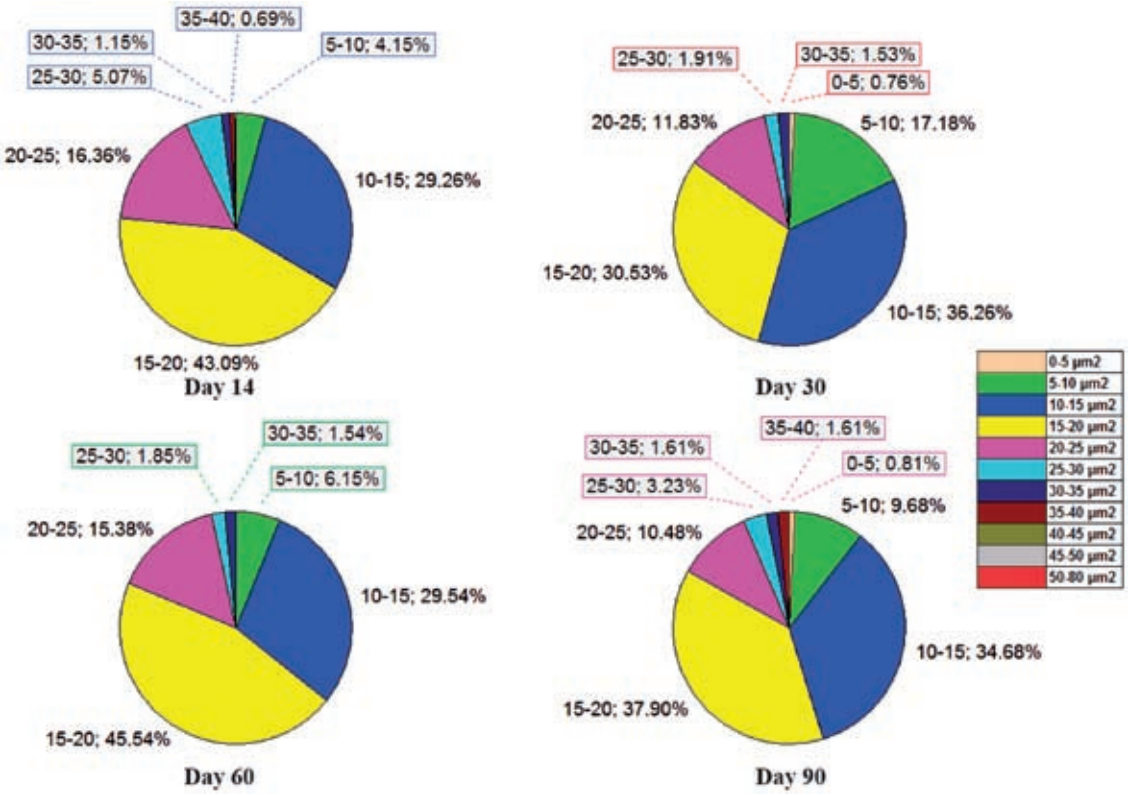

Fig. 3. Percentage change in value of vessels of different caliber in case of autological bone graft use

Ryc. 3. Procentowe zmiany liczby naczyń krwionośnych o różnej średnicy w przypadkach użycia przeszczepu autogennego

Table 2. Percentage of vessels of different caliber after osteoplasty using Bio-Oss®, autological bone graft and after heeling beneath the blood clot (p – relative value; m_p – mean error of relative value, K–S – Kolmogorov-Smirnov test; M–W – Mann-Whitney test)

Tabela 2. Odsetek naczyń krwionośnych różnego kalibru po osteoplastyce z zastosowaniem Bio-Oss®, autogennych przeszczepów oraz gojenia pod zakrzepem

	Bio-Oss® (Group 1)				Autological bone graft (Autogenny kostny materiał) (Group 2)				Under the blood clot (Gojenia pod zakrzepem) (Group 3)			
Period – days (Okres – dni)	14	30 $p < 0.001$; K–S: $p < 0.001$; M–W: $p < 0.001$	60 $p < 0.001$; K–S: $p < 0.001$; M–W: $p < 0.001$	90 $p < 0.001$; K–S: $p < 0.001$; M–W: $p < 0.001$	14	30 $p < 0.001$; K–S: $p < 0.001$; M–W: $p < 0.001$	60 $p < 0.01$; K–S: $p < 0.001$; M–W: $p < 0.05$	90 $p < 0.01$; K–S: $p < 0.001$; M–W: $p < 0.001$	14	30 $p < 0.001$; K–S: $p < 0.001$; M–W: $p < 0.001$	60 $p < 0.05$; K–S: $p < 0.001$; M–W: $p < 0.05$	90 $p < 0.001$; K–S: $p < 0.001$; M–W: $p < 0.001$
Blood vessel area (x), μm^2 (Powierzchnia naczyń krwionośnych)	% (percent of the total number of vessels) (odsetek ogólnej liczby naczyń krwionośnych)				% (percent of the total number of vessels) (odsetek ogólnej liczby naczyń krwionośnych)				% (percent of the total number of vessels) (odsetek ogólnej liczby naczyń krwionośnych)			
	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$	$P \pm m_p$
$0 < x \leq 5$	0	0	0	0.37 ± 0.37	0	0.76 ± 0.54	0	0.81 ± 0.80	0.28 ± 0.20	0.26 ± 0.26	0	0.26 ± 0.26
$5 < x \leq 10$	0	2.27 ± 1.30	4.55 ± 1.99	7.78 ± 1.63	4.15 ± 0.96	17.18 ± 2.33	6.15 ± 1.33	9.68 ± 2.66	8.13 ± 1.02	0.26 ± 0.26	17.48 ± 1.93	3.9 ± 0.99
$10 < x \leq 15$	1.76 ± 1.01	1.52 ± 1.06	24.55 ± 4.10	33.33 ± 2.87	29.26 ± 2.18	36.26 ± 2.97	29.54 ± 2.53	34.68 ± 4.27	35.06 ± 1.79	13.54 ± 1.75	30.08 ± 2.33	16.1 ± 1.87
$15 < x \leq 20$	10.0 ± 2.30	23.48 ± 3.69	52.73 ± 4.76	41.11 ± 2.99	43.09 ± 2.38	30.53 ± 2.85	45.54 ± 2.76	37.9 ± 4.36	40.81 ± 1.84	43.75 ± 2.53	34.45 ± 2.41	36.1 ± 2.45
$20 < x \leq 25$	17.06 ± 2.89	27.27 ± 3.88	13.64 ± 3.27	12.22 ± 1.99	16.36 ± 1.78	11.83 ± 2.00	15.38 ± 2.00	10.48 ± 2.75	10.66 ± 1.16	26.82 ± 2.26	12.08 ± 1.65	27.01 ± 2.26
$25 < x \leq 30$	25.88 3.36	31.06 ± 4.03	0.91 ± 0.91	4.44 ± 1.25	5.07 ± 1.05	1.91 ± 0.85	1.85 ± 0.75	3.23 ± 1.59	4.07 ± 0.74	12.76 ± 1.70	4.37 ± 1.04	9.09 ± 1.47
$30 < x \leq 35$	14.71 ± 2.72	8.33 ± 2.41	0.91 ± 0.91	0.38 ± 0.37	1.15 ± 0.51	1.53 ± 0.76	1.54 ± 0.68	1.61 ± 1.13	0	1.04 ± 0.52	1.03 ± 0.51	2.86 ± 0.85
$35 < x \leq 40$	13.53 ± 2.62	0.76 ± 0.76	1.82 ± 1.27	0	0.69 ± 0.40	0	0	1.61 ± 1.13	0.42 ± 0.24	1.04 ± 0.52	0.26 ± 0.26	2.34 ± 0.77
$40 < x \leq 45$	8.83 ± 2.18	3.03 ± 1.49	0	0	0	0	0	0	0.42 ± 0.24	0.26 ± 0.26	0.25 ± 0.25	0.78 ± 0.45
$45 < x \leq 50$	1.76 ± 1.01	0.76 ± 0.76	0	0	0.23 ± 0.23	0	0	0	0.05 ± 0.08	0	0	0.26 ± 0.26
$50 < x \leq 80$	6.47 ± 1.89	1.52 ± 1.06	0.89 ± 0.90	0.37 ± 0.37	0	0	0	0	0.1 ± 0.12	0.27 ± 0.26	0	1.3 ± 0.58

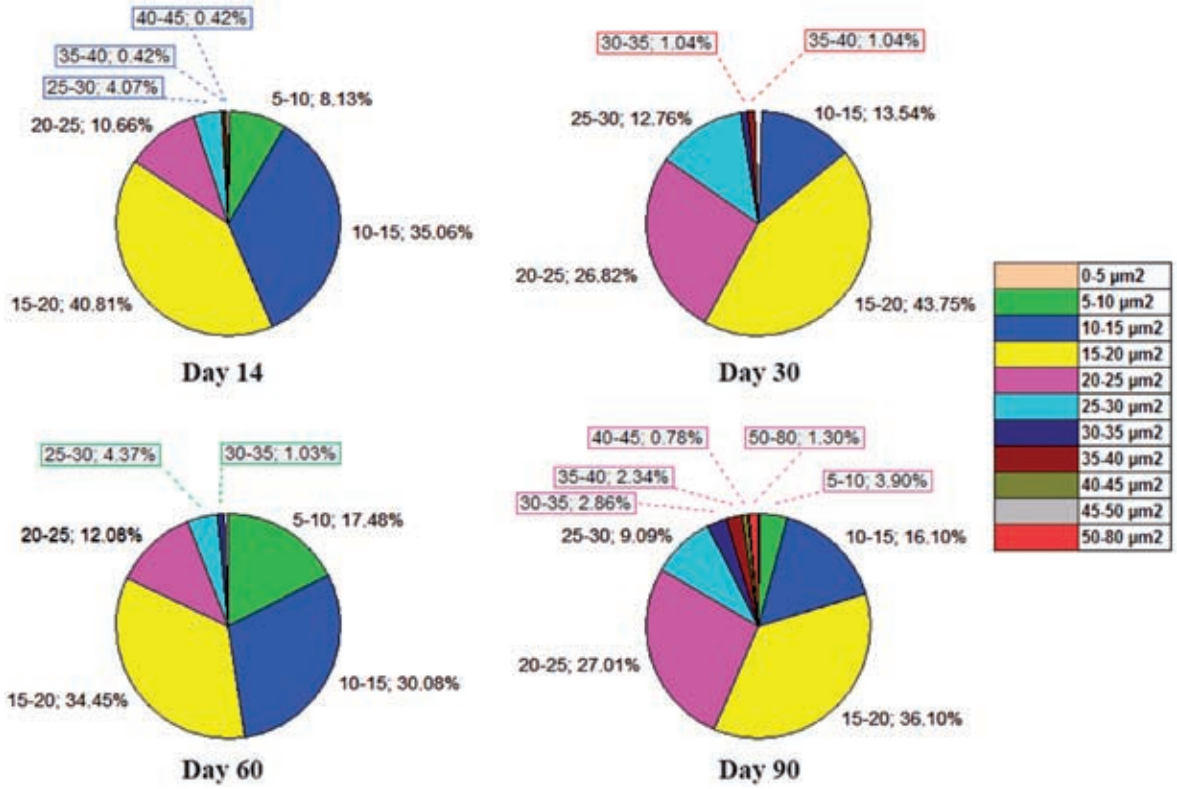

Fig. 4. Percentage change in value of vessels of different caliber in case when healing took place under the blood clot

Ryc. 4. Procentowe zmiany liczby naczyń krwionośnych o różnej średnicy w przypadkach gojenia samoistnego poprzez skrzep krwi

increased steadily and equally during the whole period of experiment (90 days), which was also indicated by the osteocalcin levels (Fig. 1, Fig. 3). In the samples where healing took place under the blood clot, the highest quantity small vessels was registered on the 60th and 90th day (Fig. 4).

Discussion

Osteocalcin is an indicator of the calcification stage of bone formation process. As it has got a very short time of decay in blood serum (about 5 min) it may be considered as an actual osteoblast activity index [6]. Some studies demonstrate that osteocalcin is a marker of late osteoblast differentiation [6, 15].

During the bone extracellular matrix mineralization, synthesis of osteocalcin and other calcium-binding proteins is initiated. In cultures in which mineralization is delayed, expression of osteocalcin is also delayed [12, 16, 17].

In the cases of Bio-Oss use, the highest level of osteoblast activity was on day 30 and 60, with a decrease on day 90. The 30th and 60th day rise may be a consequence of Bio-Oss bone matrix resorption with further formation of a new bone.

In the cases of autological bone graft use, no significant fluctuation of osteocalcin level was noticed, which may be connected with the peculiarities of autological bone graft integration process.

The quantity of small vessels and the dynamic of growth of different caliber vessels is one of the indicators of the revascularization process. The increase of small vessel quantity in bone regeneration area may be the criterion for the assessment of the revascularization process at a certain point of time and gives the opportunity to compare the rate of germination of blood capillaries into the implantation area. The osteocalcin level in blood serum measured at a certain point of time shows the osteoblasts activity, which is also strictly connected with the revascularization process.

As the blood vessels measurement and the osteocalcin levels in the blood serum show, the highest level of bone formation dynamic after osteoplasty with Bio-Oss was in the period from day 14 to 60.

During that period, the quantity of 0–25 µm^2 vessels changed significantly from 28.82% (day 14) to 95.47% (day 60).

In the cases of autological bone graft use, the bone formation took place steadily, without sharp rises of osteocalcin levels and increases of revascularization dynamic speed. The quantity of 0–25 µm^2 vessels changed slightly from 92.86%

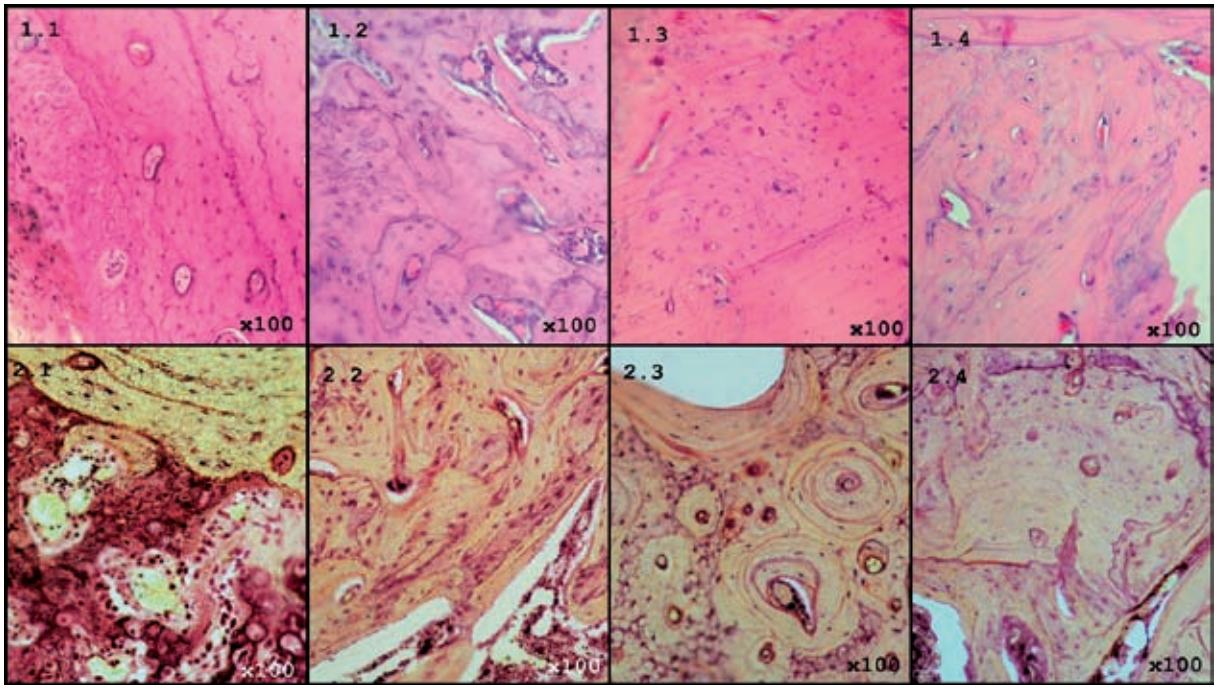

Fig. 5. The dynamics of revascularization after the osteoplasty with Bio-Oss® (stained by natural hematoxylin-eosin: 1.1 – 14 days; 1.2 – 30 days; 1.3 – 60 days; 1.4 – 90 days; stained by Schmorl technique: 2.1 – 14 days; 2.2 – 30 days; 2.3 – 60 days; 2.4 – 90 days)

Ryc. 5. Dynamika rewaskularyzacji po przeszczepie Bio-Oss® (barwienie naturalną hematoksyliną i eozyną: 1,1 – 14 dni, 1,2 – 30 dni, 1,3 – 60 dni, 1,4 – 90 dni; barwienie metodą Schmorla : 2,1 – 14 dni, 2,2 – 30 dni, 2,3 – 60 dni, 2,4 – 90 dni)

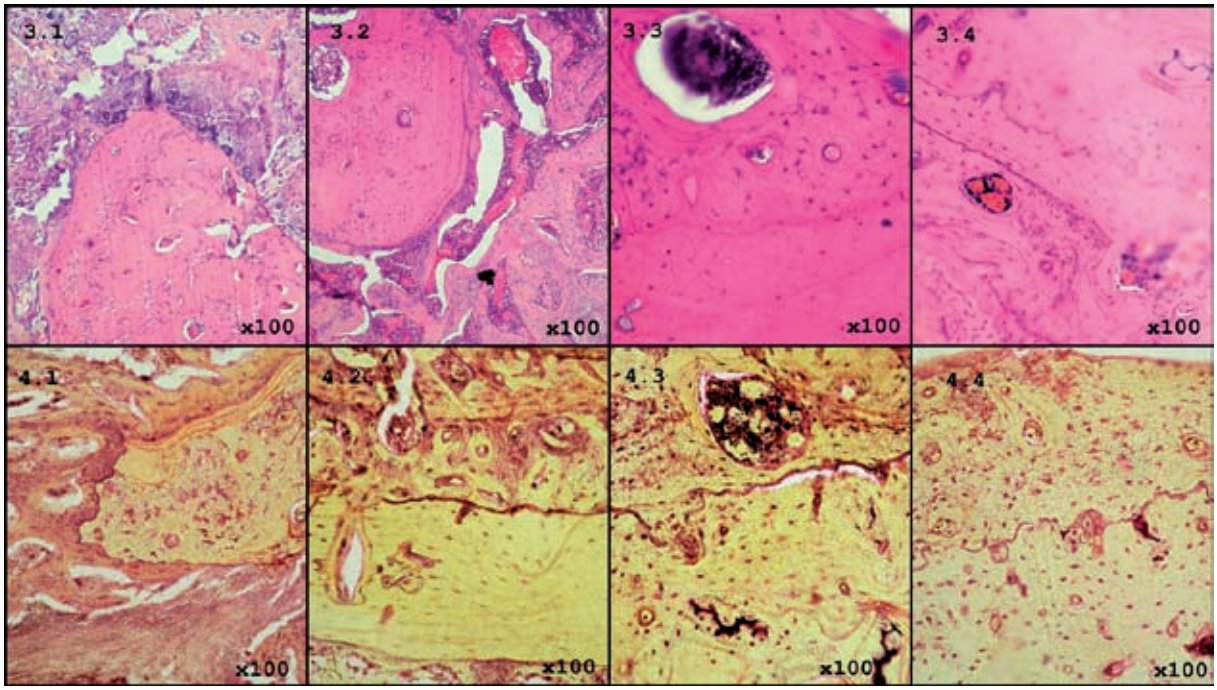

Fig. 6. The dynamics of revascularization after the autological bone graft transplant (stained by natural hematoxylin-eosin: 3.1 – 14 days; 3.2 – 30 days;3.3 – 60 days; 3.4 – 90 days; stained by Schmorl technique: 4.1 – 14 days; 4.2 – 30 days; 4.3 – 60 days; 4.4 – 90 days)

Ryc. 6. Dynamika rewaskularyzacji po przeszczepie kości autogennej (barwienie hematoksyliną i eozyną: 3,1 – 14 dni, 3,2 – 30 dni, 3,3 – 60 dni, 4 – 90 dni; barwienie metodą Schmorla : 4,1 – 14 dni, 4,2 – 30 dni, 4,3 – 60 dni, 4,4 – 90 dni)

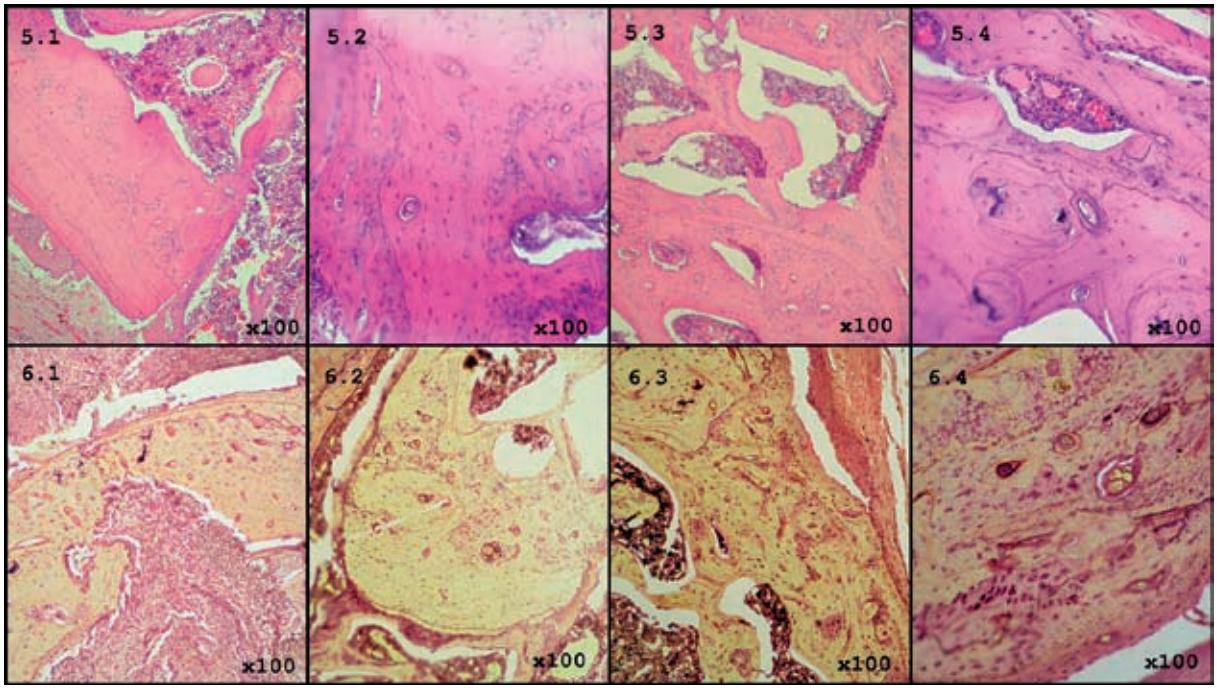

Fig. 7. The dynamics of revascularization after healing under the blood clot (stained by natural hematoxylin-eosin: 5.1 – 14 days; 5.2 – 30 days; 5.3 – 60 days; 5.4 – 90 days; stained by Schmorl technique: 6.1 – 14 days; 6.2 – 30 days; 6.3 – 60 days; 6.4 – 90 days)

Ryc. 7. Dynamika rewaskularyzacji po gojeniu wyłącznie poprzez skrzep krwi (barwienie hematoksyliną i eozyną: 5,1 – 14 dni, 5,2 – 30 dni, 5,3 – 60 dni, 5,4 – 90 dni; barwienie techniką Schmorla : 6,1 – 14 dni, 6,2 – 30 dni, 6,3 – 60 dni, 6,4 – 90 dni)

(day 14) to 93.55% (day 90), although the highest quantity of 0–25 μm^2 vessels was noticed on day 60 (96.61%).

A much lower growth dynamic was observed and the percentage of vessels was changed slightly during the experiment in samples where healing took place under the blood clot. During the whole period, the quantity of small vessels (0–25 μm^2) decreased from 94.94% (day 14) to 83.37% (day 90).

Statistical estimation of the inside-group and between-group differences show: 1) in cases of Bio-Oss use there was a significant rise of osteocalcin level by day 30 and day 60 and a significant decrease by day 90 (Table 1); 2) in the cases of autological bone graft use, there was a significant decrease of osteocalcin level by day 30 and an insignificant increase by day 60 and 90 (Table 1); 3) a significant difference of osteocalcin level was noticed compared to Bio-Oss and autological bone graft use on day 30 and 60 (Table 1); 4) in the cases of Bio-Oss use, there was a significant rise of small vessels quantity by day 30 and 60 (Table 2); 5) in the cases of autological bone graft use, there was a slight change in the quantity of small vessels by day 30 (Table 2); 6) in the cases when healing took place under the blood clot, there was a slight fluctuation of small vessels quantity with a minor rise on day 60 (Table 2).

According to the obtained results, the use of autological bone graft and Bio-Oss osteoplastic material increases bone formation and revascularization activity and improves the bone structure unlike when healing took place under the blood clot.

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