ORIGINAL ARTICLE

Vascularization of a bone stump

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ABSTRACT

Aim To study the character of blood circulation in the bone stump at tight and loose closure of the medullary cavity.

Methods Two series of experiments on 39 rabbits with mid-third femoral amputation and muscular plasty were carried out. In the 1st (experimental) series, the bone scapula was closed by thin cortical autograft taken from the epimetaphyseal area, and then the muscles were sutured, and in the 2nd – the scapula was closed by myoplasty only. Follow-up periods: 1st series – 7, 14, 21 days and 1, 3, 6 months, 2nd series – 1, 3, 6 months. Histological methods with infusion of vessels with ink-gelatin mixture and morphometry was used.

Results In the 1st series there was a rapid restoration of the disturbed macro- and microcirculation due to the reserve sources of blood circulation and the development of extravascular ways of microcirculation. In the 2nd series, blood circulation recovery was significantly slower and occurred mainly due to the development of extravascular microcirculatory pathways.

Conclusion The study established undeniable usefulness of tight closure of the bone marrow cavity during amputation.

Key words: amputation, bone graft, canal, microcirculation

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Original submission:
22 September 2023;
Revised submission:
24 October 2023;
Accepted:
06 December 2023
doi: 10.17392/1677-23

Med Glas (Zenica) 2024; 21(1):214-221

INTRODUCTION

Despite the large number of works devoted to amputations and post-amputation pain syndrome related to diseases and defects of stumps (1-7), there are only single reports (8,9) which cover the issues of reparative regeneration and blood circulation in the bone remnant of the future working organ.

To date, there have been no in-depth studies of blood circulation during amputation. Mechanisms of compensation of circulatory disorders in bone tissue have not been studied, the role of the intersected nutrient artery system in stump formation has not been elucidated, the influence of the density of the medullary cavity closure on the nature of blood circulation in the bone stump has not been studied. To nourish the thick cortical diaphyseal plate of tubular bone, sufficient blood flow through narrow intraosseous and medullary vessels is required, which requires increased intraosseous pressure in the medullary canal; its value should exceed the normal level of intratissue pressure (4,10,12,13). Opening of the medullary cavity leads to a drop in intraosseous pressure (12,13), and hence, to impaired blood circulation of the bone residual limb. Data on the vascularisation of the bone stump after amputation are mainly presented in the literature (14,15). To date, however, there are no studies covering the effect of the closure of the medullary canal on the blood supply to the bone stump.

The aim of this study was to investigate the character of blood circulation of a bone stump at tight and loose closure of the medullary canal at amputation.

MATERIAL AND METHODS

Material and study design

Two series of experiments on 39 rabbits with amputation of the middle of the third femur and muscle grafting were conducted in the vivarium of the Vinnytsia National Medical University named after M.I. Pirogov (Vinnytsia, Ukraine) throughout the year of 2022. In the 1st (experimental) series, a thin cortical autograft taken from the epimetaphyseal area and the muscles were stitched over it, and in the 2nd (control) series, the bone sawbone was closed with stitched muscles. Follow-up periods included: in the 1st series – 7, 14, 21 days, as well as 1, 3, 6 months, in the 2nd series – 1, 3, 6 months. Histological method with infusion of vessels with ink-gelatin mixture was used.

Experiments were performed in accordance with the principles of humane treatment of animals set forth in the European Community directives (86(609) EEC) and the Helsinki Declaration on Humane Treatment of Animals.

This research has been approved by the Regional Ethics Committee of the Scientific and Research Institute of Rehabilitation of National Pirogov Memorial Medical University, Vinnytsia, Ukraine (approval No 3/2023).

Methods

In all experiments ink pouring of vessels after administration of lethal dose of hexenal was used. After appropriate wiring, 15-30 mkm thick sections were made and stained with hematoxylin and eosin and van Gieson picrofuchsin. Enlightened sections of 100-150 mkm thickness were also prepared.

The morphometry was performed using digitized images of the preparations on a Pannoramic scanner (3DHISTECH, Hungary) using the image analysis programs Orbit Image Analysis and Pannoramic Viewer. The relative volume of vessels and tissue structures in the bone regenerate was determined. The number of cells, intercellular substance, and the percentage of ossified and non-ossified structures were counted.

Statistical analysis

The statistical method based on analysis of variance (ANOVA) was used to analyse biomedical information by determining the differences between the mean (±SD) samples for the two observation groups. The $p<0.05$ was used as significant difference.

RESULTS

1st (experimental) series – 24 observations

Term 7 days, 3 observations. The vascular network of the cortical diaphyseal layer was sharply full of blood. Individual vessels were significantly dilated. There was insignificant avascularity of small areas. In some places the vascular network was irregular. There were multiple cysts

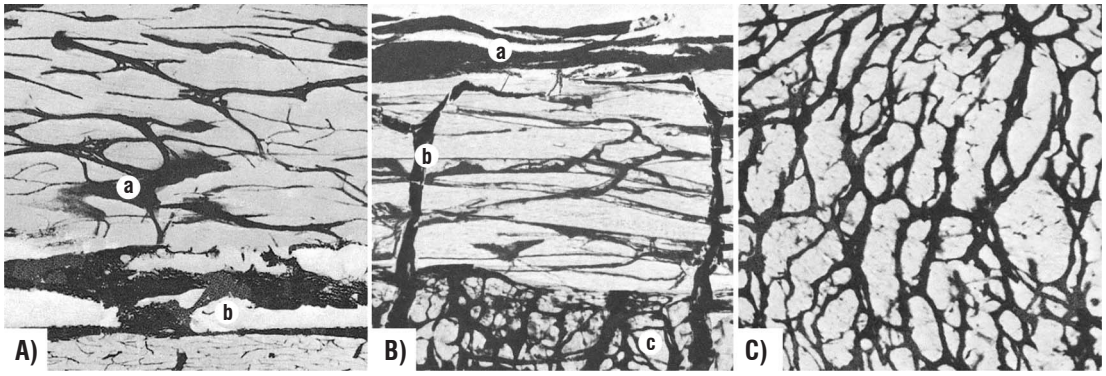

Figure 1. A) Sharp dilation of the microvessels of the cortical diaphyseal layer: a) extensive cysts of the medullary cavity; b) term 14 days; B) Significant dilation of the periosteal vascular network: a) cortical diaphyseal layer; b) medullary cavity; c) term 1 month; C) Full-thickness of the vascular network of the medullary cavity; term 1 month (Microphotographs, enlightened slices ×50)

and sinusoids in medullary cavity. Large vessels were sharply dilated. Vessels of soft tissues and periosteum were full of blood. Periosteum was thickened, containing multiple microcysts. Vascular canals were enlarged at the place of contact of the autograft with the diaphyseal cortical layer. The gap between the fragments was filled with fibroreticular tissue.

Term 14 days, 3 observations. The enlightened sections showed revascularization of the cortical diaphyseal layer. Avascular areas were absent. The vascular canals of the cortical diaphyseal layer were sharply dilated with the phenomena of pronounced vascular permeability (Figure 1A). They contained enlarged diameter proliferating microvessels or finely petrified newly formed capillaries. The vessels in the bone canals were located wall-to-wall. The large vessels of the medullary cavity were significantly dilated. In the distal and proximal parts of the medullary canal oedematous fatty bone marrow and oedematous loose fibrous tissue with cystic cavities and sinusoids were presented. Due to oedema, the medullary contents were pushed into the middle part of the canal. A large number of anastomoses revealed between the vessels of the soft tissue, periosteum, medullary cavity and haversus system. In the contact zone of the graft with the cortical diaphyseal layer, areas of fibroreticular tissue filling the gap, intermedial callus beams and osteoclasts were detected.

Term 21 days, 3 observations. Regional hypervascularization, increased vascular permeability of the microcirculatory bed persisted. In the endosteal bone formation zone a large number of sinusoidal capillaries, tissue cysts, the walls of which contain a network of dilated microvessels were found. The vascular branches of the feeding artery in the proximal part of the medullary canal were dilated. Individual large tissue cysts were found here. Phenomena of oedema of the medullary contents were less presented. The interfragmentary space was filled with a dense network of vessels associated with the vascular network of the medullary cavity, cortical diaphyseal layer and tissues surrounding the end. The tissue of the forming regenerate at the level of the end surface vascularized by a fine- and medium-cell vascular network of interbody spaces. In the centre of the endosteal, regenerate vessels were located longitudinally. The bone graft was fused to the diaphyseal layer by the periosteal, endosteal and intermedial calluses. Osteoblasts and osteoclasts were detected in osteoblastic tissue of interosseous spaces. There was an intensive resorption of autograft bone tissue and its replacement by rather mature bone tissue.

Term 1 month, 5 observations. The vascular canals of the cortical diaphyseal layer were significantly enlarged. The abundant medullary and periosteal vascular network was preserved (Figure 1B). Dilation of the venous vessels was noted. The space between the resorbable graft and the file was filled with cellular-fibrous tissue rich in osteoblasts and sinusoidal capillaries, which ingrow from the periosteal, intramedullary networks and haversus vessels. A large number of anastomosing vessels connecting vessels of the periosteum and surrounding soft tissues with the microcirculatory network of the medullary cavity was found. In its proximal part, a fatty bone marrow with dilated main vessels and a

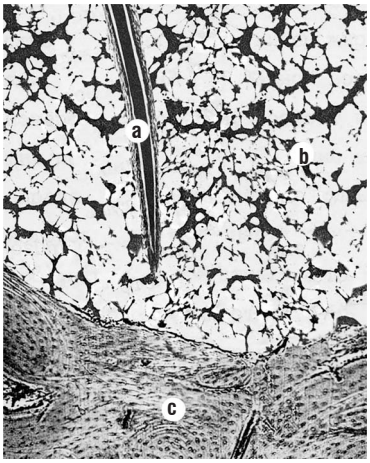

Figure 2. Reduced feeding artery (a) among adipose bone marrow (b), bone lamina (c) (Microphotographs; hematoxylin and eosin staining ×90)

full-blooded vascular network was found (Figure 1C). In the distal part of the medullary canal, the adipose bone marrow with small interlayers of fibrous tissue was presented. The bone lamina consisted mainly of mature bone tissue. The vessels of the periosteum and surrounding soft tissues were dilated.

Term 3 months, 5 observations. The vascular canals of the cortical diaphyseal layer remained enlarged. In the superficial layers of the cortical diaphyseal layer a longitudinal network of wide vessels with a clear connection to the vascular network of the medullary cavity was formed. They contained an abundant number of capillaries connected with the periosteal and intramedullary network. The periosteal vessels did not differ from the norm. The vascular network of the medullary cavity was close to normal. In its distal part single sinusoidal vessels were noticed. Microcirculatory changes of compensatory character were completed. The feeding artery in the distal part of the medullary canal was narrowed and reduced (Figure 2).

Term 6 months, 5 observations. The phenomena of microcirculatory channel rearrangement subside. The microcirculatory network of the cortical diaphyseal layer and the medullary canal acquire angioarchitectonics close to normal.

2nd (control) series – 15 observations

Term 1 month, 5 observations. In all observations, avascularity of the end of the cortical diaphyseal layer over 1-2 cm was detected. Above this zone there was a dilation of a part of Haversian and Folkman channels. Many of them lacked cellular elements (Figure 3A). The vascular network was impoverished and irregular. Part of the vessels was sinusoidal with the phenomena of carcass diffusion into the perivascular space (Figure 3B). There was a parietal arrangement of capillaries due to perivascular oedema. In the distal part of the medullary canal, focal avascularity of bone marrow was found, its replacement by loose fibrous and fibroreticular tissue impregnated with carcass. The lumen of large medullary vessels exiting into the fibrous-tissue fringe was visible. The contours of their walls were indistinct. Also, a large number of cysts associated with irregular microvascular network was found. Irregular filling of vessels with its diffusion into surrounding tissues was revealed. In the proximal part of the medullary canal the bone marrow was partially replaced by oedematous loose fibrous tissue with a large number of sinusoidal vessels, multiple cysts and feeding artery lumen. On the side of the medullary canal and periosteum, sinusoidal capillaries had grown into the fibrous tissue.

Term 3 months, 5 observations. In two observations, a cone-shaped, two cylindrical, and one club-shaped bone stump was formed. The medullary canal in cone-shaped stumps was closed by immature osteon-bulbar tissue. Avascularity of the end of the bone stump was preserved. Vessels

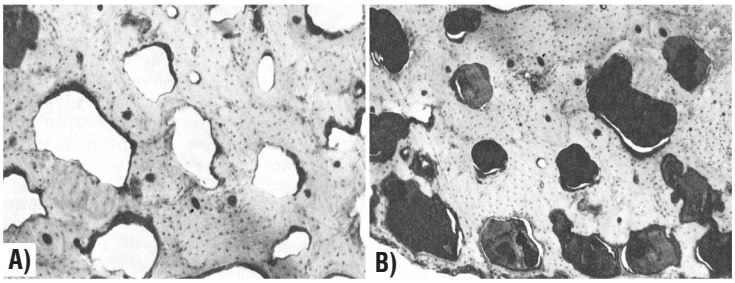

Figure 3. A) Absence of vessels and tissue elements in a part of vascular canals of bone; B) Perivascular accumulation of carcass in dilated vascular canals (Histotopograms of transverse sections; hematoxylin and eosin staining ×50)

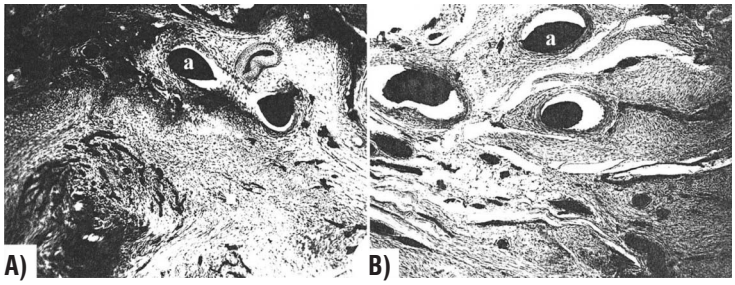

Figure 4. A) Immature bone structures, closing the medullary canal, formed around the large branches of the feeding artery, passing into the fibrous tissue fringe (a); B) The fibrous tissue fringe containing large branches of the feeding artery filled with ink (a) (Microphotographs; hematoxylin and eosin staining ×90)

of periosteum and surrounding soft tissues were dilated. The beginning of ingrowth of sinusoidal capillaries from the vascular network of the medullary canal and cortical diaphyseal layer into this zone were noted. Proximally, the vascular canals of the cortical diaphyseal layer were enlarged for a considerable extent. Paucity of the vascular bed and absence of cellular composition in the haversal canals were revealed; they contained altered microvessels of sinusoid type with the phenomenon of carcass diffusion into the perivascular space. In the centre of the medullary canal oedematous loose fibrous tissue, large tissue cysts with enlarged microvessels in the walls were found. Peripheral oedema of medullary contents was observed. Oedema was in interstitial spaces, microvessels in the form of sinuses. Between the beams of endosteal bone formation lumens of carcass-filled dilated branches of the feeding artery, passing from the medullary canal to the fibrous tissue fringe were found (Figure 4A, B). It revealed foci of loose fibrous tissue among the dense fibrous tissue around the feeding artery and its branches. In the proximal part of the medullary canal, oedematous adipose bone marrow with foci of avascularity and wide areas of carcass-soaked loose fibrous tissue, multiple cysts with enlarged microvessels in the walls and sinusoidal vessels were determined for a considerable extent. There were also sharply dilated nutrient artery and its branches.

Term 6 months, 5 observations. Moderate dilation of part of the vascular canals of the cortical diaphyseal layer was preserved; sinusoidal capillaries were detected in them. Some canals and interbody spaces of the endosteal bone formation contained a finely looped network of narrow capillaries. Most osteocytes were with well stained nuclei. The phenomena of perivascular oedema was preserved. Large tissue cysts, sinusoidal vessels, dilated branches of the feeding artery were found in the distal and proximal parts of the medullary canal, mostly filled with fatty bone marrow. The diameter of the feeding artery was smaller than in the previous observations. A perforation of the endosteal regenerate by branches of the feeding artery with their emergence into the surrounding soft tissues was noted.

According to morphometry (Table 1), in the experimental group, compared with the control group, maximum vascularisation ($p<0.05$) was observed at one month, which contributed to the

Table 1. Quantitative characteristics of the structural elements of the bone regenerate of the amputation stump

Groups	Terms of observation (month)	Mean±SD of the number samples					
		Vessels	Fabric structures	Fabric structures		Intercellular substance (structures)	
				Cells	Intercellular substance	Ossified	Non-ossified
I group (bone-plastic amputations) (n=15)	1	21.2±2.78	78.4±1.82	19.8±2.39	76.6±4.04	45.8±1.64	53.8±1.98
	3	14.8±1.92	83.2±1.79	22.4±2.7	76.8±2.39	68.2±2.17*	31.8±3.7*
	6	9.8±1.89†‡	89.4±2.51†‡	11.9±2.28†‡	80.4±7.02	88.4±3.97†‡	10.8±3.89†‡
II group (myo-plastic amputations) (n=15)	1	12.4±1.14	86.2±0.84	13.2±2.05	83.6±2.88	31.2±2.17	67.6±1.82
	3	21.4±2.30*	77.6±2.61*	20.4±1.82	79.4±1.52	48.3±3.71*	51.2±3.63*
	6	13.2±1.79†	85.6±1.52†	14.4±2.71	85.2±2.77	57.8±2.59†‡	40.6±2.51†‡
PI, II		<0.05	<0.05	>0.05	>0.05	<0.01	<0.01
Intact bone (n=10)		7.18±0.51	92.8±2.71	10.3±1.69	89.7±2.67	~99.9	~0.1

*p<0.05 significant between 1 and 3 months of follow-up; †p<0.05 significant between 3 and 6 months of follow-up; ‡p<0.05 significant between 1 and 6 months of follow-up

rapid formation of the bone closure plate. In the group with myoplastic amputation, the maximum vascularisation process was observed with a long delay only at three months. It is quite logical that the formation of bone structures in the experimental group significantly exceeded ($p<0.01$) the corresponding indicators of the control group.

DISCUSSION

The circulatory network of long tubular bones is a single dynamic system with great compensatory capabilities (14). The intersection of muscles, periosteum, microvasculature of the gavernous and volcanic systems of the cortical diaphyseal layer and bone marrow vessels causes significant changes characterised by temporary avascularisation of bone and marrow tissue and long-term heterogeneity of the microvasculature (14).

Compensation of circulatory disorders is partially carried out by including reserve sources of blood circulation - the vascular bed, vessels of the periosteum and surrounding soft tissues, the development of anastomoses, the existing bone microcirculatory bed, collateral circulation (15-18). We have found that the restoration of impaired microcirculation occurs through the same type of changes: regional hypervascularization, increased capacity of the terminal vascular bed with sinusoidal change of capillaries and increased vascular permeability, formation of multiple tissue microcysts associated with the microvascular network. These changes are aimed at eliminating circulatory disorders and providing metabolic needs of the regenerating tissues by maximizing the use of extravascular microcirculatory pathways.

According to our study, the remodelling of the vascular network of the bone stump of the 1st and 2nd (experimental and control) series differed significantly. At tight closure of the medullary cavity there was a significant, sharply pronounced expansion of the vascular network of the cortical diaphyseal layer, medullary cavity, large branches of the feeding artery, soft tissue vessels and periosteum, rapid development of anastomoses. Such simultaneous and powerful inclusion of all possible compensation pathways in the process of revascularization prevents prolonged and significant avascularisation of the cortical diaphyseal layer and medullary contents. Small microplots of cortical layer avascularity disappeared already after 14 days. Avascularity of the adipose bone marrow was not observed in this series.

The study found that by almost one month, the phenomena of cortical and bone marrow revascularisation had returned to normal. In place of the resorbable graft a bone lamina was formed, which acquired a compact structure by 3 months of age. Such rapid formation of the plate and restoration of closure of the medullary cavity is possible only at a certain level of intraosseous circulation (19). We found large vessels at the end of the stump were absent; the reparative processes were completed. The state of the cortical diaphyseal layer changed insignificantly. There were moderately pronounced reparative processes with small bone resorption, mainly in the area of the file and along the endosteal and periosteal surfaces. However, the main contours of the tubular bone and the characteristic structure of the compact tissue remained unchanged. In the distal and proximal parts of the medullary canal, the fatty bone marrow with a normal microcirculatory network was mainly preserved.

Our results showed progressive depletion of the bone microvascular network in the control group, impaired vascular permeability, development of perivascular oedema, absence of proliferative processes in the bone and bone marrow tissues, progressive bone tissue resorption along the vascular channels, endosteal and periosteal surface with spongification, rarefaction and atrophy of the cortical diaphyseal layer, the formation of an inferior bone closure plate and degenerative-dystrophic changes in the cortical diaphyseal layer. Fatty bone marrow was replaced by loose fibrous tissue.

The findings differ slightly from those of Hansen-Leth C. study (11). The author used a thick full-layer cortical bone as a graft, which impaired blood circulation at the edge of the blade, could not provide tight closure of the canal and took a long time to reconstruct, which, of course, affected the results of the experiments. According to our study, the blood supply of the contents of the medullary canal and the cortical diaphyseal plate was carried out predominantly by the medullary network. The periosteal vessels, anastomosing to the intramedullary network, participate in the blood supply of only the most superficial layers of the compact

bone. It should be noted that the Hansen-Leth C. study (11) suggested that closure of the medullary cavity during amputation in clinical conditions is obviously useful; the graft used in our experiments was thin, it was located along the edge of the fillet, did not disturb blood circulation, provided tightness and fast rearrangement.

In other studies (14, 20) relating to blood circulation in the bone stump, the authors did not pay attention to the necessity of closing the opened medullary cavity during amputation; as a result, a wide range of various disorders of microcirculation and regeneration were observed in the postamputation period, similar to the data of the control group obtained in our experiments.

In contrast to the known (14,20), the present work is the first to focus on the creation of the medullary cavity hermeticism and elimination of gaping of the crossed powerful intraosseous vessels. Closure of the medullary canal with a dense bone cortical plate promoted rapid restoration of homeostasis and rational osteogenesis.

In conclusion, the study established the undoubted usefulness of tight closure of the bone marrow cavity during amputation.

FUNDING

The study was conducted within the framework of the research work “Stress bone injuries due to limb combat injuries” (No: 0123U100170), funded by the Ministry of Health of Ukraine with the state budget.

TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

REFERENCES

1. Ahmed A, Bhatnagar S, Mishra S, Khurana D, Joshi S, Ahmad SM. Prevalence of phantom limb pain, stump pain, and phantom limb sensation among the amputated cancer patients in India: a prospective, observational study. Indian J Palliat Care 2017; 23:24-35.
2. Ahuja V, Thapa D, Ghai B. Strategies for prevention of lower limb post-amputation pain: A clinical narrative review. J Anaesthesiol Clin Pharmacol 2018; 34:439-49.
3. Allami M, Faraji E, Mohammadzadeh F, Soroush MR. Chronic musculoskeletal pain, phantom sensation, phantom and stump pain in veterans with unilateral below-knee amputation. Scand J Pain 2019; 19:779-87.
4. Beverly M, Murray D. Factors affecting intraosseous pressure measurement. J Orthop Surg Res 2018; 13:187.
5. Bosanquet DC, Glasbey JC, Stimpson A, Williams IM, Twina CP. Systematic review and meta-analysis of the efficacy of perineural local anaesthetic catheters after major lower limb amputation. Eur J Vasc Endovasc Surg 2015; 50:241-9.
6. Buch NS, Qerama E, Brix Finnerup N, Nikolajsen L. Neuromas and postamputation pain. Pain 2020; 161:147-55.
7. Buchheit T, Hsia HJ, Cooter M, Shortell C, Kent M, McDuffie M, Shaw A, Buckenmaier CT, Van de Ven T. The impact of surgical amputation and valproic acid on pain and functional trajectory: results from the veterans integrated pain evaluation research (VIPER) randomized, double-blinded placebo-controlled trial. Pain Med 2019; 20:2004-17.
8. Buntragulpoontawee M, Pattamapaspong N, Tongprasert S. Multiple neuromas cause painful "jumping stump" in a transfemoral amputee: a case report. Int J Low Extrem Wounds 2016; 15:271-3.
9. Dumanian GA, Potter BK, Mioton LM, Ko JH, Cheesborough JE, Souza JM, Ertl WJ, Tintle SM, Nanos GP, Valerio IL, Kuiken TA, Apkarian AV, Porter K, Jordan SW. Targeted muscle reinnervation treats neuroma and phantom pain in major limb amputees: a randomized clinical trial. Ann Surg 2019; 70:238-46.
10. Filipowska J, Tomaszewski KA, Niedźwiedzki Ł, Walocha JA, Niedźwiedzki T. The role of vasculature in bone development, regeneration and proper systemic functioning. Angiogenesis 2017; 20:291-302.
11. Hansen-Leth C. Investigations of the vascular changes following fmputation on rabbits. Acta Orthop Scand Suppl 1982; 197:1-89.
12. Shevchuk VI, Bezsmertnyi YO, Bezsmertna HV, Dovgalyuk TV, Jiang Y. Reparative regeneration at the end of bone filing after ostoplastic amputation. Wiad Lek 2021; 74:413-7.
13. Shevchuk VI, Bezsmertnyi YO, Bezsmertna GV, Dovgalyuk TV, Jiang Y. Peculiar features of regeneration at the end of bone filing after amputation of a limb. World of Medicine and Biology 2021; 1:229-34.
14. Lavrishcheva GI, Onoprienko GA. Morphological and clinical aspects of reparative regeneration of supporting organs and fabrics. Medicine 1996; 206.
15. Bezsmertnyi Y, Shevchuk V, Jiang Y. The influence of post-amputation pain syndrome and intraosseous main vessels on the formation of limb bone stump. OTP 2023; 12:20-5.
16. Rollo G, Bonura EM, Falzarano G, Bisaccia M, Ribes Iborra J, Grubor P, Filipponi M, Pichierri P, Hitov P, Leonetti D, Russi V, Daghino W, Meccariello L. platelet rich plasma or hyperbaric oxygen therapy as callus accellerator in aseptic tibial non union. Evaluate of outcomes. Acta Biomed 2020; 10:91-4.

17. Rollo G, Luceri F, Falzarano G, Salomone C, Bonura EM, Popkov D, Ronga M, Pica G, Bisaccia M, Russi V, Grubor P, Franzese R, Peretti GM, Meccariello L. Effectiveness of teriparatide combined with the Ilizarov technique in septic tibial non-union. Med Glas (Zenica) 2021; 18:287-92.
18. Rollo G, Luceri F, Pichierri P, Giaracuni M, Bisaccia M, De Gabriele S, Puce A, Bonura EM, Popkov D, Peretti GM, Meccariello L. Reliability of S.A.R.A. (sterilization and reimplantation autograft) technique in long bone open fractures. J Biol Regul Homeost Agents 2020; 34:223-30.
19. Onoprienko GA. Vascularization of bones in fractures and defects. Medicine 1995; 224.
20. Bezsmertnyi YO, Shevchuk VI, Jiang Y, Bezsmertna HV, Bezsmertnyi OY. Bone stump formation against the background of post-amputation pain syndrome. Polish Annals of Medicine 2022; 29:148-54.