

5. Sobol LV, Popadynets' OH Morfofunktsionalni osoblyvosti endokarda shlunochkiv, mahistralnykh arteriy u normi ta pry diyi riznomanitnykh faktoriv. Ohlyad literatury. Halyskyi likarskyi visnyk. 2019; 2(26):48–55. [in Ukrainian]
6. Tataruchuk LV, Hnatyuk MS, Konovalenko SO, Yasinovskyy OB. Kilkisna morfolohichna otsinka vikovykh osoblyvostey remodelyuvannya arteriy yayechna u doslidnykh tvaryn. Visnyk naukovykh doslidzhen. 2017; 1:134–7. [in Ukrainian]
7. Chekalina NI, Kazakov YuM, Petrov YeYe. Mekhanizmy ateroskleroza: osnovy patohenetichnoyi terapiyi. Visnyk problem biolohiyi i medytsyny. 2014; 4:27–32. [in Ukrainian]
8. Shevchuk TI, Piskun RP, Vasenko TB. Zmina morfometrychnykh kharakterystyk sudyn sertsya pry eksperymentalnyi dyslipoproteyidemiyi. Svit medytsyny ta biolohiyi. 2017; 3(61):154–157. [in Ukrainian]
9. Demirci T, Sahin E. Heart and stress a morphometric and light microscopic study in a rat model. Averroes Europ Medic J. 2017 Aug;2(4):1–9.
10. Lekhan VN, Rudiy VM, Shevchenko MV, Nitzan Kaluski D, Richardson E. Ukraine: Health system review. Health Systems in Transition. 2015;17(2):1–154.
11. Silva JML, Nagato AG, Reis RB, Nardeli CR, Abreu FP. Morphometric analysis of the coronary arteries: a study of the external diameters. J Morphol Sci. 2016;3(33):138–41.
12. Tsitovskiy MN, Mateshuk-Vatseba LR. Ultrastructural organization of the aortic wall in the white rat. International Scientific and Practical Conference «World Science». 2018;3.2(9):32-37.

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### PECULIAR FEATURES OF REGENERATION AT THE END OF BONE FILING AFTER AMPUTATION OF A LIMB

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In order to evaluate the reparative processes at the end of the bone after amputation, 4 series of experiments were carried out on 64 rabbits with filing plastic cortical plate, its combination with miodesis, fascia cover and stitching of antagonist muscles. Timing of observation: 1, 3, 6 months. Examination method: histological with vessel filling in ink-gelatin mixture. It was established that in the first two series the formation of the bone closing plate and intraosseous circulation were restored by 1 month. In the series with myoplasty and fascioplasty, the shape of the residual limb was changed, the bone closing plate was absent, and the normalisation of intraosseous circulation did not occur even in remote lines. The preservation of the cylindrical shape of the residual limb, the normalisation of intraosseous circulation and the completion of the reparative process are indicators of favourable residual limb formation.

**Key words:** amputation, fascioplasty, muscle plastic, bone grafting, reparative regeneration.

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### ОСОБЛИВОСТІ РЕГЕНЕРАЦІЇ НА КІНЦІ ОПИЛУ КІСТКИ ПІСЛЯ АМПУТАЦІЇ КІНЦІВКИ

З метою оцінки репаративних процесів на кінці кістки після її ампутації проведено 4 серії дослідів на 64 кроликах з пластикою опилу кортикальною пластинкою, поєднання її з міодезом, укріплення фасцією і зшивання м'язів-антагоністів. Терміни спостереження: 1, 3, 6 місяців. Метод дослідження: гістологічний з наливкою судин туш-желатиновою сумішшю. Встановлено, що в перших двох серіях формування кісткової замикаючої пластинки і внутрішньокісткова циркуляція відновлювалися до 1 місяця. У серіях з міопластиком і фасціопластиком форма кукси змінювалася, кісткова замикаюча пластинка була відсутня, нормалізація внутрішньокісткової циркуляції не наступала навіть у віддалені строки. Збереження циліндричної форми кукси кістки, нормалізація внутрішньокісткової циркуляції і завершеність репаративного процесу є показниками сприятливого формування кукси.

**Ключові слова:** ампутація, фасціопластика, м'язова пластика, кісткова пластика, репаративна регенерація.

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Amputation of a limb with the intersection of soft tissues, blood vessels, nerves, bones significantly violates the static-dynamic stereotype, which contains a potential threat of various complications. In addition, such an intersection substantially complicates the formation of a rational relationship between these anatomical formations in the future organ of support and movement. In recent years, researchers have focused on such particular issues of amputations as predicting wound healing [6], treatment of pain [3], various modifications of known methods of amputation [4, 5, 7], description of malformations and diseases of the stump [1, 2, 8, 11]. Unfortunately, such fundamental questions were not reflected in these works, as the healing of the stump of the bone, the features of reparative processes and factors that influence their course. According to the data of, unsatisfactory outcomes of bone stump healing were noted in 97.1 % of the examined, and the formation of a functional bone stump within 1–1.5 months after amputation was

noted in only 10 % of cases. Such disappointing results of operations prompted us to experimentally study the features of reparative regeneration at the end of bone filing.

Amputation leads to a sharp change in the blood supply to the bone. The system of periosteum vessels is damaged, which penetrate from the periosteum to the compact substance of the bone through the Folkman and Haversian channels and feed the outer third of the cortical layer. The intersection of the periosteum and bone is accompanied by damage to the feeding artery. In the middle of the diaphysis, it, being one of the muscle branches, penetrates into the bone and is divided into proximal and distal branches in the bone marrow canal, which branch into many smaller vessels. The latter, in the form of precapillaries and postcapillaries, penetrate into the inner layers of the cortical plate through the system of Folkman channels, and along the bones of the Haversian channels, they are distributed along the bone and feed the inner two-thirds of the thickness of the cortical layer of the diaphysis.

Due to a breach of hermetic disorders in the medullary canal, amputation causes a drop in pressure necessary for pushing blood through the vessels of the narrow vascular channels of the cortical plate. In the formation of the future stump, it should be higher than the level of interstitial pressure, which will ensure the pushing of blood through narrow intraosseous vessels. Considering that the tubular bone is normally closed, during the formation of the stump after amputation, a closure bone plate should be formed, which ensures hermetic bone marrow cavity. In practice, initially and until now, filing bones are covered with a fascia flap – the fascioplasic method. Later, the clinic began to use the myoplastic method – stitching under the filing of bone antagonist muscles. It should be noted that neither the first nor the second methods involve closing the bone marrow cavity. Their use is achieved only by coverage of the truncated bone and the subsequent expectation of wound healing. The influence of these methods of plastics on reparative processes in a bone cult is very doubtful. Given this circumstance, a myodesis was applied, which provided for fixation to the filing of the bone through the drilled holes of the truncated muscles, followed by tight stitching of their ends over the filing. We hope that this technique should help improve blood circulation and reparative regeneration at the end of filing.

**The purpose** of the study was to study the features of reparative processes at the end of the bone after its amputation using various methods of plastic stump.

**Materials and methods.** 4 series of experiments were carried out on 64 adult rabbits. Amputation of the right hind limb in the middle third was performed under intravenous thiopental anesthesia. A 1 % solution of novocaine was perineurally injected into the nerve trunks and crossed high with an acute razor. The vessels were ligated with catgut. The bone was sawn aperiostally with a hand saw. In the first (main) series, the method of myodesis, which we tested in the clinic, was applied – attaching the crossed muscles to the end of the stump through the drilled holes and tightly stitching their edges under the end surface. In the second (experimental) series, the end was closed with a thin cortical plate. In III (control), a fascioplasic coverage of bone filing was performed. In IV (control), the antagonist muscles were stitched together under filing.

In all experiments, ink filling of vessels was used. Before removing from the experiment, the animal was intraarterially injected with 5 thousand units heparin in saline, after 15 minutes a lethal dose of hexenal was rapidly administered intravenously and the abdominal aorta was ligated. Below the ligature, a cannula was introduced from the system for intra-arterial injection, fixing it in the lumen of the vessels, and a 10% mascara-gelatin mixture was filled. After a day, the femur in the hip joint was isolated, a visual assessment of the relationship of the soft tissues with the bone was given, after which the thigh stump was freed from the soft tissues, leaving them only along the end surface. The drug was fixed in a 12 % formalin solution and decalcified with a 5 % nitric acid solution. From the obtained preparation of the whole bone stump of the femur, the articular end was cut off. The remaining plot was taken for research. A sagittal section was made through the middle of the bone, which was poured into a block of integoidin. Sections 15–30 mkm thick were stained with hematoxylin and eosin according to Van Gieson. The obtained histotopograms were studied using light microscopy. The state of the microcirculatory network of the bone and muscles filled with the mascara-gelatin mixture was evaluated, and the density of the location of the vessels and their diameter were taken into account “without special measurements”.

While studying a bone stump, we drew attention to changes in the cortical diaphyseal plate, the shape of the end section and the nature of its constituent structures, the presence of a connective bone plate, and the condition of the bone marrow canal tissue in its proximal and distal sections. We studied the nature of the soft tissue coating of the end surface of the stump.

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

**Results of the study and their discussion.** Experimental group – myodesis, 15 observations. Within 1 month (5 observations), the stump end shape in 4 observations is cylindrical, in one – round. In all observations, an endosteal bone regenerate was formed at the end of the sawn bone, connecting the inner edges of the cortical diaphyseal plate. On the lower edge of the regenerate, a bone locking plate of the osteon-beam structure was determined, consisting in most cases of mature, and in one of not quite mature bone tissue. The inter-beam spaces in the medullary canal are filled with adipose marrow. The vessels in it are close to normal. The thickness of the cortical diaphyseal plate in 4 cases remained almost unchanged, and in 1 case its partial resorption at the end was noted.

Term of 3 months, 5 observations. In 4 observations, the stump end shape is cylindrical. The bone locking plate is located horizontally, consists of mature bone tissue. The thickness of the cortical diaphyseal plate is uniform in the distal and proximal parts. At the end of the stump in the inter-beam spaces and in the proximal section, the adipose bone marrow with the mascara-filled vessels close to normal. In one observation, the end of the stump is rounded due to resorption of the cortical diaphyseal plate. The endosteal regenerate in this observation consisted of not quite mature bone tissue. Rare sinusoidal vessels and single tissue cysts in the medullary canal.

Term of 6 months, 5 observations. In 4 observations, the stump end shape is cylindrical. The bone locking plate consists of mature bone tissue. Cortical diaphyseal plate of uniform thickness. In all departments of the medullary canal, adipose bone marrow with a normal microcirculatory network. In one observation, the shape of the end of the stump has changed due to the expansion of the diameter. The formation of a periosteal regenerate was noted, which merged with the beams of the endosteal regenerate. The bone locking plate consisted of mature bone tissue of an osteon-beam structure. The vessels of the microcirculatory network in the distal are close to normal.

In conclusion of the section, it should be noted that in the majority of observations of this series, as a result of the reparative process, stumps were formed that retained the shape of the diaphysis with a locking bone plate, which already a month later consisted of mature bone tissue of an osteon-beam structure. Microcirculation in such a cult was approaching normal. In some observations, apparently due to uneven muscle tension, resorption of the cortical diaphyseal plate was noted, and the shape of the end of the stump changed from oblique to roundness and expansion. The bone locking plate in these observations consisted of not quite mature bone tissue. Intraosseous circulation to a certain extent differed from that normal.

The experimental group – bone grafting with hermetic closure of the canal with a thin cortical graft – 19 observations.

Duration of 1 month, 7 observations. The shape of the end of the stump in five observations remained cylindrical. The thickness of the cortical diaphyseal layer in the distal and proximal parts is the same. At the end of the filing, a bone cortical locking plate of an osteon-beam structure is formed from rather mature bone tissue. In the inter-beam spaces of the end of the stump and in the proximal sections of the stump, the microcirculatory network corresponded to that of the diaphysis in the norm.

In two observations, a slight resorption of the cortical diaphyseal plate in the filing region was noted, and its thickness even far from the end was uneven. The end of the stump is beveled. The bone locking plate consisted of not quite mature bone tissue. In the medullary canal near the filing, single sinusoids were detected. In the proximal section, adipose bone marrow with a characteristic microcirculatory network for the diaphysis. It should be noted that in these two observations in the postoperative period there was a displacement of the bone graft.

Duration of 3 months, 6 observations. The stump shape retained the shape of the diaphysis in all observations (fig. 1).

The thickness of the cortical diaphyseal layer is preserved throughout. Bone cortical locking plate consisted of mature bone tissue. The mascara-filled vessels of the microcirculatory network corresponded to the norm (fig. 2). The reparative process is complete.

Duration of 6 months, 6 observations. In 5 observations, the stump end shape is cylindrical. The bone pinch plate consisted of mature bone tissue. The cortical diaphyseal plate is basically uniform in thickness. In the proximal and distal sections, the adipose bone marrow with a microcirculatory network corresponding to the bone is normal. In one observation, the shape of the end of the stump has slight sloping due to the resorption of the cortical diaphyseal plate at the end. The bone tissue of the bone pinch plate is mature. In the proximal bone marrow cavity, adipose bone marrow. The microcirculatory network here corresponds to normal bone microcirculation. In the distal section, single vessels of the sinusoid type. The reparative process is complete.

The control group was fascioplasty, 15 observations.



Fig. 1. Histotopogram of a cylindrical stump with some roughness of the contours of cortical diaphyseal plate. Hematoxylin and eosin staining.  $\times 6.0$ .



Fig. 2. Microphotograph. Vessels of the microcirculatory network of the fatty bone marrow at the end of bone filing are masticatory-filled. Hematoxylin and eosin staining.  $\times 90$ .

Duration of 1 month, 5 observations. In two observations, a cylindrical stump with focal resorption of bone tissue of the cortical diaphyseal plate was noted. The ends of the filing have notches. There is no bone locking plate. Beams of endosteal regenerate of various maturity. In the distal section, the microcirculatory network is represented by a large number of sinusoidal vessels and tissue cysts. A large number of carcass-filled vessels pass from the bone marrow canal through the regenerate to the fibrous-tissue fringing. In three observations, the end of the stump is club-shaped thickened due to large periosteal regenerates located much higher. Complete resorption of the cortical diaphyseal plate, which is lost in the tissue of the periosteal regenerate, is noted. Endosteal regenerate consists of a network of immature bone beams. There is no bone locking plate. In the inter-beam spaces, wide vessels of the sinusoid type, cysts. Masticatory-filled vessels exit from the medullary canal into the fibrous tissue border.

Duration of 3 months – 5 observations. As a result of the reparative process, irregularly formed stumps were formed. In two cases they were conical, in three – club-shaped. Extensive resorption of the cortical diaphyseal plate over a significant extent was noted. The closure of the bone marrow canal with an endosteal regenerate from immature bone tissue. Bone locking plate is not formed. In the bone marrow contents, there are sinusoidal vessels and tissue cysts. In the soft tissues surrounding the bone stump, there are many vessels penetrating from the bone marrow canal into the soft tissues surrounding the stump.

Duration of 6 months – 5 observations. In all observations, a stump of cylindrical shape was formed, with extensive resorption of the cortical diaphyseal plate. In 4 of them, breaks are noted in the zone of greatest resorption. The endosteal regenerate at the end of the stump is represented by bone beams of various maturity. The completeness of reparative regeneration was not observed in any case. Large branches of the feeding artery pass between the beams of the endosteal regenerate from the bone marrow canal into the soft tissue bordering (fig. 3).

Myoplasty, 15 observations. Duration of 1 month – 5 observations. In all observations, the diameter of the distal stump is widened due to the formation of periosteal regenerates. The cortical diaphyseal plate in the distal section underwent significant resorption. Three observations have its breakdown. In the proximal spongy. The end of the stump is filled with regenerate from beams of various degrees of maturity. Sinusoidal vessels are determined. The branches of the feeding artery are found, passing into the fibrous tissue bordering the stump.

Duration of 3 months – 5 observations. In all observations, club-shaped stumps were obtained due to the formation of extensive periosteal regenerates, resorption of the cortical diaphyseal plate, and their fusion with endosteal bone formation (fig. 4). The latter is represented by bone beams of varying degrees of maturity. In two preparations above the regenerates – resorption of the cortical plate and its fractures. In the proximal and distal parts of the medullary canal, a significant number of sinusoidal vessels are present. Between the beams of endosteal bone formation, gaps of the branches of the feeding artery are revealed. Bone locking plate is not formed. The completion of the reparative process is not observed.



Fig. 3. Microphotograph. Endosteal bone regeneration near the end of the stump with the presence in the bone marrow spaces of multiple gaps of the feeding artery filled with marrow and branches of the venous sinus. Hematoxylin and eosin staining.  $\times 78$ .



Fig. 4. Histotopogram of the residual limb with enlarged periosteal regenerate end. Hematoxylin and eosin staining.  $\times 6$ .

Duration of 6 months – 5 observations. In all experiments, club-shaped stumps were formed with extensive periosteal regenerates along the lateral surfaces. Extensive resorption of the cortical diaphyseal plate with its rearrangement, in two cases with breaks above the end of the stump. The bone locking plate is formed in only one observation. In the remaining four, it is represented by a regenerate of immature bone tissue.

Generalisation of the data obtained made it possible to determine the optimal morphological parameters of the bone residual limb. These parameters are: retention of the cylindrical shape of the residual limb throughout the entire period of observation, rapid formation of the bone endplate already by 1 month, normalisation of intraosseous circulation, completion of the reparative process.

To this date, the vast majority of studies have assessed the results of the reparative process at the end of the residual limb by soft tissue healing [3, 4] and the presence of the bone closure plate [6, 7, 8]. Due to the narrow focus of these studies, consisting in the radiological description of the results, the problem of reparative regeneration at the end of the filing and the factors affecting it could not be covered in its full scope. The downside of these works is the lack of scientific analysis of the factors influencing the processes of osteogenesis and evaluation of residual limb grafting techniques in terms of reparative regeneration. The studies we have carried out make it possible to fill this gap. We must agree with the opinion [1, 4, 9] on the importance of bone grafting for residual limb functioning and prevention of pain syndrome. However, the authors rely on empirical judgement without looking deeper into microcirculatory issues, which are paramount.

The ability to quickly restore impaired intraosseous microcirculation during amputation appears only with plastic tight closure of the channel and the restoration of impaired intraosseous pressure. Such recovery occurs with a favorable course of the reparative process by the month, when the process of formation of the bone pinch plate is completed and the reparative processes die out. The microcirculatory network in this case corresponds to the bone normal. At the end of the stump in the adipose bone marrow, the vessels are defined as small branches, larger vessels were not detected. The main morphological constants of the stump persist throughout the observation period. Violation of the hermetic closure of the bone marrow cavity does not contribute to the rapid restoration of intraosseous pressure and microcirculation. The reparative process is disturbed, a large number of tissue cysts and sinusoids that are not characteristic of the diaphysis appear in the intraosseous vasculature. Uneven muscle tension during plastic surgery has a serious effect on the processes of reparative regeneration, which is clearly seen in some experiments of the first series and especially with fascioplasty and myoplasty. Due to the violation of microcirculation, the course of the reparative process was distorted, which did not end even by the six-month period. Due to insufficient overlapping of the medullary canal, the main vessels were not obliterated, expanded, occupied a significant part of the lumen. The end of the stump, even remotely, was filled with regenerate from immature bone tissue with the inclusion of lumens of the nourishing artery that exits into the surrounding soft tissue.

The experience of the surgeon and the rightness of the plastic shaping procedure on its formation play an extremely important role in obtaining a functional residual limb [9].

## Conclusions

1. Miodesis and osteoplastic closure of the bone marrow cavity opened during amputation with a thin cortical graft, ensuring its tightness, create conditions for the normal course of reparative processes and their completion already in the early stages.

2. The indicators of the favorable formation of the bone stump are the preservation of the cylindrical shape, normalization of the intraosseous circulation, the presence of a bone locking plate, and the completeness of reparative processes.

3. Myoplasty and fascioplasty do not ensure the integrity of the bone marrow cavity, as a result of which there is a violation of microcirculation and the course of the reparative process.

4. The consequence of imperfect plastic surgery is a change in the shape of the bone stump, the absence or incomplete formation of a bone locking plate from immature bone tissue, microcirculation disturbance, and the branches of the supply artery entering the soft tissues surrounding the stump.

*Prospects for further research are to study of the effect of various muscle tension on the formation of bone stump.*

## References

1. Vasilyev A Ju, Yegorova EA, Smyslenova MV. Luchevaya diagnostika izmeneniy kul'tey nizhnikh konechnostey pri protezirovaniy [Radiation diagnosis of changes in the stump of the lower extremities during prosthetics]. *Klinicheskaya meditsina*. 2013; 5(1):51–7. [in Russian]
2. Bezsmertnyi YO, Shevchuk VI, Grushko OV, Tymchyk SV, Bezsmertna HV, Dzierzak R, et al. Information model for the evaluation of the efficiency of osteoplasty performing in case of amputations on below knee. *Proc. SPIE 10808, Photonics Applications in Astronomy, Communications, Industry, and High-Energy Physics Experiments 2018*, 108083H (1 October 2018); doi: 10.1117/12.2501558.
3. Kahle JT, Highsmith MJ, Kenney J, Ruth T, Lunseth PA, Ertl J. The effectiveness of the bone bridge transtibial amputation technique: A systematic review of high-quality evidence. *Prosthet Orthot Int*. 2017 Jun; 41(3):219–226; doi: 10.1177/0309364616679318.
4. Lewandowski LR, Tintle SM, D'Alleyrand JC, Potter BK. The utilization of a suture bridge construct for tibiofibular instability during transtibial amputation without distal bridge synostosis creation. *J Orthop Trauma*. 2013 Oct; 27(10):e239–42; doi: 10.1097/BOT.0b013e31828d2c67.
5. Malloy JP, Dalling JG, El Dafrawy MH, Bustillo JS, Reid JS. Tibiofibular bone-bridging osteoplasty in transtibial amputation: case report and description of technique. *J Surg Orthop Adv*. 2012 Winter; 21(4):270–4.
6. Plucknette BF, Krueger CA, Rivera JC, Wenke JC. Combat-related bridge synostosis versus traditional transtibial amputation: comparison of military-specific outcomes. *Strategies Trauma Limb Reconstr*. 2016 Apr; 11(1) –:5 11; doi: 10.1007/s11751-015-0240-4.
7. Ranz EC, Wilken JM, Gajewski DA, Neptune RR. The influence of limb alignment and transfemoral amputation technique on muscle capacity during gait. *Comput Methods Biomech Biomed Engin*. 2017 Aug; 20(11):1167–1174; doi: 10.1080/10255842.2017.1340461.
8. Taylor BC, Poka A. Osteomyoplastic Transtibial Amputation: The Ertl Technique. *J Am Acad Orthop Surg*. 2016 Apr; 24(4):259–65; doi: 10.5435/JAAOS-D-15-00026.
9. Tintle SM, Keeling JJ, Forsberg JA, Shawen SB, Andersen RC, Potter BK. Operative complications of combat-related transtibial amputations: a comparison of the modified burgess and modified Ertl tibiofibular synostosis techniques. *J Bone Joint Surg Am*. 2011 Jun 1; 93(11):1016–21; doi: 10.2106/JBJS.J.01038.
10. Tosun B, Selek O, Gok U, Tosun O. Medial gastrocnemius muscle flap for the reconstruction of unhealed amputation stumps. *J Wound Care*. 2017 Aug 2; 26(8):504–507; doi: 10.12968/jowc.217.26.8.504.
11. Vaso A, Adahan HM, Gjika A, Zahaj S, Zhurda T, Vyshka G, et al. Peripheral nervous system origin of phantom limb pain. *Pain*. 2014 Jul; 155(7): 1384–91; doi: 10.1016/j.

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