ORIGINAL ARTICLE

REPARATIVE REGENERATION AT THE END OF BONE FILING AFTER OSTOPLASTIC AMPUTATION

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ABSTRACT

The aim: To study the role and place of bone grafting in the formation of bone stump after amputation.

Materials and methods: 3 series of experiments were carried out on 44 rabbits with amputation of the thigh in the middle third and stump grafting using osteoplastic hermetic closure of the canal with a thin cortical plate (series I), closure of the canal with a spongy bone (series II), and loose closure of the canal with a cortical graft located at the entrance to the canal at an angle of 30° (III series). Observation period: 1, 3, 6 months. Histological examination method with vascular filling with 10% mascara-gelatin mixture.

Results: In series I, in the majority of observations, a stump of a cylindrical shape with a bone locking plate of an osteon-beam structure and normalization of intraosseous microcirculation was formed. A slight displacement of the graft caused a violation of microcirculation. In series II, organotypic stumps were formed in all observations. In series III, incomplete closure of the bone marrow cavity led to sharp microcirculatory disorders and the course of the reparative process with pathological bone remodeling.

Conclusions: The parameters of the favorable course of the reparative process and the formation of the organotypic bone stump are the safety of its cylindrical shape, the presence of a compact bone structure, normalization of intraosseous microcirculation.

KEY WORDS: amputation, bone grafting, reparative regeneration, intraosseous microcirculation

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INTRODUCTION

Amputation of a limb with the intersection of soft tissues, blood vessels, nerves, skin 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 [1], treatment of pain [2-8], and various modifications of known amputation methods. Unfortunately, such fundamental questions as stump healing, features of reparative processes, and factors influencing their course were not reflected in these works. According to the data of [9], unsatisfactory outcomes of bone stump healing were observed 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 [10]. 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 periosteal vessels is damaged, which penetrate from the periosteum into the compact substance of the bone through the Folkman and Haversian canals 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 the bone and is divided into proximal and distal branches, which branch into many small vessels, in the medullary canal. 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 are distributed along the bone and feed the inner two-thirds of the thickness of the cortical layer of the diaphysis.

In addition, amputation due to a breach of hermeticity in the medullary canal, it 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, this pressure 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, when the stump is formed after amputation, a closure bone plate must be formed, which ensures hermetic bone marrow cavity. Initially, the filing of the bones were covered with a fascia flap – the fascioplastic method. Subsequently, and until now, in amputation surgery, the myoplastic method is used – stapling of antagonist muscles under the filing of the bone. It should be noted that neither the first nor the second methods involve closing the bone marrow cavity. Their use is achieved only shelter of the truncated bone and the subsequent expectation of wound healing. The influence of these methods of plastics on reparative processes in the bone stump is very insignificant. Considering this circumstance, osteoplasty of the end of bone filing in various versions was applied. We expected that this technique should help improve blood circulation and improve reparative regeneration at the end of filing.

THE AIM

The aim was to to study the role and place of bone grafting in the formation of bone stump after amputation.

MATERIALS AND METHODS

3 series of experiments on 44 rabbits were conducted. 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 highly with an acute razor. The vessels were ligated with catgut. The bone was sawn aperiostally with a hand saw. In series I, during stump amputation, stump plastic surgery was performed using a bone-plastic hermetic closure of the canal with a thin cortical plate. In the second series, the canal was closed with a spongy bone; in the third series, the canal was closed loosely with a cortical graft located at the channel entrance at an angle of 30°.

Observation period was 1, 3, 6 months. Histological examination method with vascular filling with 10% mascara-gelatin mixture. Before removing from the experiment, the animal was intra-arterially injected with 5 thousand units of 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 experiments were carried out in accordance with the principles of humane treatment of animals, set out in European Community directives (86 (609) EEC) and the Helsinki Declaration on the Humane Treatment of Animals.

RESULTS

First episode. The experimental group made a tight closure of the filing with a thin cortical plate.

The 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 revealed sparse network of endosteal bone beams, along the lower edge of which is a locking plate. It consisted of mature and not quite 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 normally.

In two observations, a slight resorption of the cortical diaphyseal plate in the filing area 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.

The duration of 3 months, 6 observations. The stump shape retained the shape of the diaphysis in all observations. The thickness of the cortical diaphyseal layer saved all over. Bone cortical locking plate consisted of mature bone tissue. The mascara-filled vessels of the microcirculatory network corresponded to the norm. The reparative process is complete.

The 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. It has the characteristic structure of a compact bone with a longitudinal arrangement of vascular channels. 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 not quite mature. In the proximal bone marrow cavity, adipose bone marrow. The microcirculatory network here corresponds to normal bone microcirculation. In the distal vessels of the sinusoidal type, sinusoids. The reparative process in this observation is not completed.

Series II – closure of the medullary canal with a trabecular bone, 15 observations.

The duration of 1 month, 5 observations. The bone stump shape in all preparations is cylindrical (Fig. 1, 2, 3) with a locking bone plate of the osteon-beam structure (Fig. 4). Cortical diaphyseal plates are well contoured all over. In the inter-beam spaces, the adipose bone marrow with mascara-filled microvessels close to the vessels of normal bone. In the proximal part of the medullary canal, adipose bone marrow with a characteristic microvascular network.

The duration of 3 months, 5 observations. The shape of the bone stump is cylindrical. The base of the stump is flat. In all cases, the cortical diaphyseal plate maintains a normal structure. The bone locking plate of the osteon-beam structure is represented by mature bone tissue. The condition of

Fig. 1. Histotopogram of a cylindrical stump. Hematoxylin and eosin staining. X6.

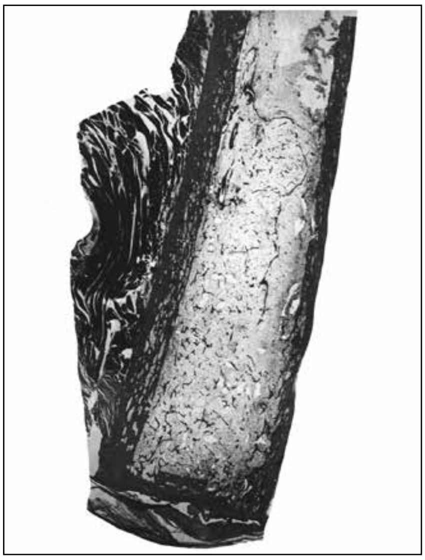

Fig. 2. Histotopogram of a cylindrical stump with sponged cortical diaphyseal plate. Hematoxylin and eosin staining. X6.

Fig. 3. Histotopogram of a cylindrical stump. Hematoxylin and eosin staining. X6.

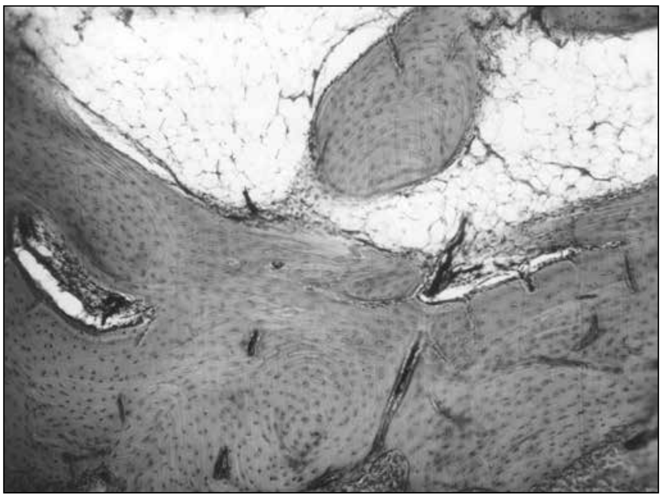

Fig. 4. Microphotograph. Bone locking plate of osteon-beam structure. Hematoxylin and eosin staining. X90.

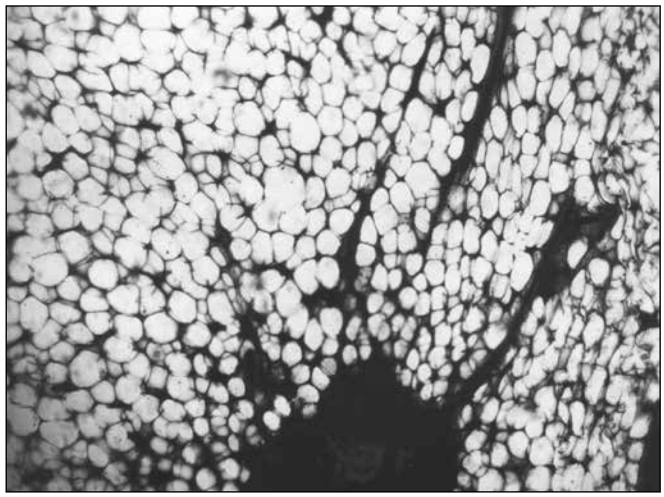

Fig. 5. Microphotograph. Bone marrow with mascara-filled microvessels in the proximal bone marrow canal. A large branch with mascara-filled venous sinus. Hematoxylin and eosin staining. X90.

the bone marrow in the terminal and proximal regions is normalized (Fig. 5, 6). The reparative process is complete.

The duration of 6 months, 5 observations. In all observations, the morphological picture was identical with the previous period.

Series III – bone grafting with a thin graft implanted in the medullary canal to a depth of 0.3 cm, 10 observations.

The duration of 3 months, 5 observations. The stump shape is sharply deformed. In four preparations, the stump form is conical. The cortical diaphyseal plate in these observations is unevenly thinned, there are its breaks and focal rarefication. In the conical part, the elongated bone beams forming it are revealed. There are no bone beams near the apex of the cone. There is dense and loose fibrous tissue with the inclusion of primitive rare bone beams and a diffuse arrangement of lymphoid-plasma cells. In one observation, a narrowing of the diameter of the stump in the lower part and closure of the medullary canal with a violation of the structure of the diaphysis are determined. In the preserved part of the medullary canal, the bone marrow is replaced by a swollen, loose fibrous tissue with a large number of sinusoidal vessels filled with mascara and tissue cysts. The gaps of the branches of the feeding artery filled with mascara are also revealed. Among the structures of endosteal bone formation, foci of immature bone tissue are revealed.

The duration of 6 months, 5 observations. In all observations, the stump was characterized as sharply deformed, conical. Three of them showed fractures of the cortical diaphyseal plate (Fig. 7, 8). At the end of the stump, the end sections of the cortical diaphyseal plate below and slightly above the level of the breaks underwent partial resorption

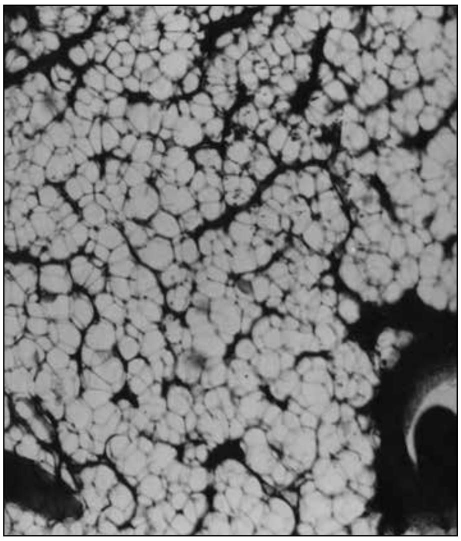

Fig. 6. Microphotograph. Adipose bone marrow of the end of the stump with mascara-filled vessels of the microcirculatory channel. The state of intraosseous microcirculation is close to normal. Hematoxylin and eosin staining. X90.

Fig. 7. Histotopogram of a cylindrical stump with a cone-shape end and a break in the cortical diaphysial plate. Hematoxylin and eosin staining. X6.

Fig. 8. Histotopogram of cone-shape stump. Hematoxylin and eosin staining. X6.

starting from the periosteal surface and replaced by newly formed immature bone tissue along the endosteal surface. Large contours were determined by its contours. In the medullary canal from its end and proximal over a large extent, loose edematous fibrous tissue with many large tissue cysts and thin-walled vessels of large diameter filled with mascara, as well as branches of the feeding artery, is revealed. In the medullary canal in the area of resorption of the diaphyseal plate in the edematous loose fibrous tissue, diffusely located macrophages, lymphoid and plasma cells are visible. A large number of ink-filled sinusoids is detected. The connective bone plate consists of immature bone tissue, not always expressed. Thus, even in the long term, in the reparative process at the end of the stump of the bone against the background of disturbed intraosseous microcirculation, pathological rearrangement of bone tissue took place.

The completeness of the reparative process was not noted in any observation of this series.

DISCUSSION

The formation of a bone stump after amputation involves the creation of an organotypic organ, which in its physical and physiological parameters should approach the bone normally. It is permissible to assume that in this organ the form that is provided by the cortical diaphyseal layer, its normal structure and adequate intraosseous circulation must be preserved. The latter largely depends on the level of intraosseous pressure, which should be sufficient to push blood through the vascular tubules of the cortical diaphyseal plate. Since depressurization of the bone marrow cavity occurs during amputation, the need to close it becomes obvious. Until recently, this was done by blocking the canal with fascia or muscle. However, in fact, it was the overlap of the bone filing, and not the gaping bone marrow cavity, in which it was necessary to restore the disturbed hermeticism. Studies have confirmed this assumption. In experiments on the use of various options for osteoplastic closure of the medullary canal with additional fixation of muscles by the end of the filing, various healing results were revealed. At the same time, we were convinced that the main constants of the created organ were: the preservation of the normal form and structure of the stump of the bone, the presence at the end of the bone cortical closure plate, the normalization of intraosseous microcirculation, and the completeness of the reparative process. In the first series of experiments, for a month and beyond, while maintaining the cylindrical shape of the stump at its end, in most cases, a bone locking plate was formed. Within a month, it was not quite mature, and subsequently – mostly mature, osteon-beam structure. The cortical diaphyseal plate changed slightly. Intraosseous microcirculation returned to normal. The slight displacement of the grafts that took place at the stage of development of the technique in several cases violated the nature of the reparative process. Immediately there was a slight resorption of the edge of the cortical diaphyseal plate, and not quite mature bone beams were revealed in the bone marrow canal along the endostal surface. In its distal section, single tissue cysts were detected. The base of the stump became beveled. The bone locking plate consisted of not quite mature bone tissue. It can be concluded that any, even a minor violation of the technique of performing amputation leads to significant changes in the reparative process.

The good stump formation results obtained in the second series of experiments are explained by the tight closure of the canal with a spongy graft, which is deprived of the possibility of displacement. In all the observations of this series, cylindrical stumps were obtained with a bone locking plate made of compact bone tissue at the end of the filing, normalization of intraosseous microcirculation and complete completion of the reparative process. Bone grafts in tissue regenerates were not detected in any case.

Lack of density of closure of the canal and even slight destruction of the bone marrow by a cortical graft placed in the plane of the filing at an angle 30°, it does not ensure the integrity of the bone marrow cavity. In the formation of the stump revealed large deviations from the above morphological standards of previous experiments. In all the observations of this series, the studied stump was characterized as sharply deformed. Serious violations of intraosseous microcirculation were revealed. Significant resorption of the cortical diaphyseal plate with its fractures led to changes in the shape of the stump. Bone closure plate is not formed in any case. The completion of the reparative process did not occur. This condition was regarded as a pathological rearrangement of bone tissue.

CONCLUSIONS

1. The parameters of the favorable course of the reparative process and the formation of the organotypic bone stump are the safety of its cylindrical shape, the presence of a compact bone structure, normalization of intraosseous microcirculation, and the completeness of the reparative process already by 1 month.
2. The most important factor in the optimal course of the reparative process at the end of the stump of the bone after amputation at the level of the diaphysis is the tight closure of the opened bone marrow cavity with a thin cortical or spongy graft.
3. Bone grafting with a cortical and spongy graft, provided that the bone marrow canal is tightly closed, contributes to the rapid restoration of intraosseous circulation and the formation of an organotypic stump.
4. The lack of hermetic closure of the bone marrow cavity leads to significant violations of the intraosseous circulation and reparative process.

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Conflict of interest:
The Authors declare no conflict of interest.

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