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Experimental morphologic and radiologic study of the integration of bone grafts into the host tissue and of the dynamics of the graft–receptor interface

HORAŢIU POMPILIU PETRESCU[1)], GABRIEL DINU[2)], GHEORGHE NODIŢI[3)], MARCEL BERCEANU-VĂDUVA[1)], DANA CRISTINA BRATU[4)], DINU VERMEŞAN[1)]

[1)]*Department of Orthopedics and Trauma, Imaging and Urology, "Victor Babeş" University of Medicine and Pharmacy, Timisoara, Romania*

[2)]*Department of Orthopedics and Anesthetics, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

[3)]*Department of Surgery II, "Victor Babeş" University of Medicine and Pharmacy, Timisoara, Romania*

[4)]*Department of Pedodontics–Orthodontics, "Victor Babeş" University of Medicine and Pharmacy, Timisoara, Romania*

Abstract

Bone is the second tissue in terms of number of transplants after blood. There is an increased trend of incidence of severe bone lesions with comminuted fractures, with significant lack of substance, as well as an increased incidence of cancer types combined with therapeutic advances in recent decades, allowing for large surgical interventions that affect the bones and create significant defects in bone and contribute to the overall increase in the number and complexity of bone transplants. Autografts may be used singly or in various combinations, with significantly better effects than other implant materials. Use of autografts is limited by complications from the receptor site, mainly related to infections and undetectable necrotic areas on initial microscopic examination, which prevent proper incorporation of autografts, but also those of the donor situs. The aim of the study was to assess the integration of tibial bone grafts into the femur of Wistar rats by radiologic exam and histological evaluation. We concluded that the fixing of the graft to the host tissue may be subject to some microenvironment influences. The presence of the periosteum on the grafts is certainly an asset during transplantation. We confirm once again that the ability of transplanted periosteum of osteoformation and reactivation. Our observations regarding the contribution of bone marrow endorse the view of its active role in bone formation.

Keywords: bone grafts, integration, osteogenesis, periosteum, bone transplant.

Introduction

Bone is the second tissue in terms of number of transplants after blood [1]. The increasing dynamism of life in the Western world and lifestyle that involves sports and increasingly large-scale use of motorized vehicles increase the incidence of severe bone lesions with comminuted fractures with significant lack of substance. Also, the increasing incidence of cancer types combined with therapeutic advances in recent decades, allowing for large surgical interventions that affect the bones and create significant defects in bone contribute to the overall increase in the number and complexity of bone transplants. Although there is an abundance of biomaterials widely used as bone implants, especially in dentistry, bone grafts retain their usefulness, either as autografts or as xenografts. Although widely used in orthopedic practice, bone grafts, especially the massive ones, are charged with failure rate of 25–35% in short-term and up to 60% in long-term (10 years) [2]. Currently there are different types of bone grafts useful, from biodegradable materials impregnated with different molecules with chemotactic effect for different selected cell types, three-dimensional printing produced cellularized xenografts immersed in culture medium containing mesenchymal stem cells from models obtained by computer tomography and autografts grown *in vitro* until they reach the required shape and size, with subsequent implantation [3]. Viable bone grafts can be created from cylindrical decellularized cancellous bone xenografts of the desired shape obtained by computer tomography and three-dimensional reconstruction and then populating the xenografts with mesenchymal stem cells that differentiate into osteoprogenitor cells capable of synthesis of mineralized bone [4]. Autograft may be used singly or in various combinations, with significantly better effects than other implant materials. Thus, in a retrospective study of acetabular reconstruction in 165 patients, the increased usefulness and efficiency of bone grafts to those of polyethylene was demonstrated [5]. The combination of autograft bone and chondrocytes harvested during the initial assessment arthroscopy and cultured for six weeks, suspended in fibrin gel, was useful in a study of nine patients with severe traumatic injuries of the knee joint. Method resulted in significantly improved therapeutic outcomes without adverse effects [6]. Better incorporation of autograft can be stimulated by various cytokines that stimulate osteogenesis or by magnetic field, as demonstrated by an experimental study that shows the stimulation of bone formation by applying biomagnetic materials in bone cavities surgically created in the femoral diaphysis of Wistar rats [7].

Use of autograft is limited by complications from the receptor site, mainly related to infections and undetectable

necrotic areas on initial microscopic examination, which prevent proper incorporation of autograft, but also those of the donor situs, particularly if it is the iliac crest [8]. But it is preferable to use bone grafts, including allografts, which, even if they are inconsistent in terms of histocompatibility, can be frozen and used successfully without complications, as evidenced by recent experimental study, in which model after tibial allografts freezing they were randomly transplanted between the experimental group rats without side effects [1]. Especially in bone fractures with less significant lack of bone substance, as the radial head articular comminuted fracture, the treatment with β-calcium triphosphate granules produced no statistically significant differences compared to patients treated with internal fixation, in a recent prospective study [9].

Clarification of histological and radiological evolution and their correlation for lack of bone fractures treated with autograft simply could lead to significant improvement in the rate of incorporation thereof, both on short and long term and to shorten treatment time with significant socio-economic effects.

Materials and Methods

We operated on a batch of 60 Wistar rats, animals from the Clinics of the Faculty of Veterinary Medicine, Timişoara, Romania.

General anesthesia in rats was obtained it with Inactin for 2–3 mg/100 g body-weight. Given the fragility of bones in rats, we adopted an appropriate technique to obtain grafts. We used two types of transplants, autografts and homografts, with the source (pelvic bone). We chose instead to transplant animal batches proximal third of the tibia, and the other third to the distal femur. Septic complications appeared in four rats, all of whom succumbed along the way. Deaths during surgery in rats occurred in eight individuals. We performed the collection of necessary parts for histological analysis at regular intervals of 7, 14, 21, 45, 60 days to 6–8 months. They were preserved in 10% formalin, followed by preparation for histological processing.

For histology and histochemistry, we used the method of decalcification with 5% and 10% Trichloracetic Acid. The method is longer but allows fine microscopic examination. The specimens were embedded in paraffin and sections of 7 μm in size were made by classical procedures. Preparations thus treated were subjected to staining. Hematoxylin–Eosin staining does not allow satisfactory results after the decalcification procedures we have used.

We tried to execute a series of radiographs from time to time following and radiological aspects in the evolution of the fresh homogeneous transplantation material, making some correlation between the histological and radiological aspects (Figures 1 and 2).

Results

Regarding the experimental results on rats, we analyzed the histological characteristic relations on one hand in graft-recipient bed, which with homogeneous integration of the transplant to the host, and, on the other hand, the aspects that characterize lack of bed homografts integration.

After implantation of a spongy graft, the homograft-proximal third of the tibia complex radiographic appearance (Figure 3) shows the deformation of the left tibia in the upper third, to form an old fracture consolidated with angled position of the fragments. The callus there is a strong compact bone structure, achieving a true osteoma aspect. The medullar canal is obstructed maintained at this level through a process of endostosis both proximal and distal to the implant. The existence of the transplanted graft in the condensed structure of newly formed bone can be appreciated in terms of radiographic examination.

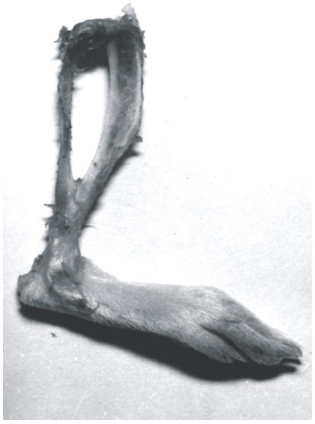

Figure 1 – ***Macroscopic aspect of the bone homograft–receptor structural complex of the tibial proximal third at 45 days.***

Figure 2 – ***Macroscopic aspect of the bone homograft–receptor structural complex of the tibial proximal third at 75 days.***

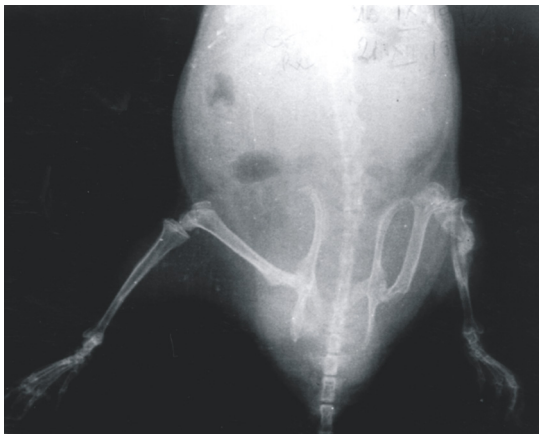

Figure 3 – ***Radiographic aspect at 75 days from homograft transplantation.***

The microscopic study (Figure 4) shows that the grafted fragment is formed at the periphery of the periosteum and muscle elements. The periosteum is continuous on one side of the graft, with a newly formed covering, probably made up predominantly of young cells. Internal to the periosteum, the cartilage is an area that extends laterally beneath the covering. This band of cartilage thickness is reduced gradually, while reducing the number of cells in the cell covering. Its structure becomes less clear, cartilage cells with vesicular aspect becoming less in number. The central mass graft consists of bone trabeculae in various degrees of building and resorption and vascular-connective tissue or remaining myeloid located in inter-trabecular areas.

Although all structures above also shows graft by their morphological and tinctorial characters a degree of viability, however, seems that the graft is fixed by the surrounding tissue only in the deep areas (Figure 4, arrow). In this area, between trabeculae of bone tissue of the graft and the recipient bed there are elements of young connective tissue. New structures that appear seem to be generated in the presence of bone debris from the periosteum elements as well as elements belonging to multipotent bone marrow cell population.

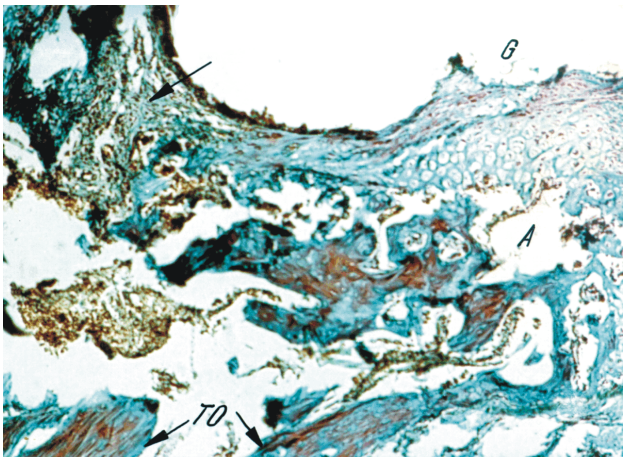

Figure 4 – ***The graft (G) is formed from periphery to the centre, from the connective covering, cartilage and spongy bone. In some of the bone cavities (A) the disappearance of the connective-vascular elements and bone marrow can be noticed, consecutive to a necrosis process. The graft is fixed to the receptor in a limited area (arrow) where the appearance of a predominantly cellular connective tissue can be noticed. This newly formed tissue seems to organize by proliferation of the cells from the graft periosteum and with the participation of the multipotent elements from bone marrow (MO) that is present in the area. TO: Bone trabeculae of the host bone. Trichrome staining, 40×.***

As evidenced by radiographic image of the case (Figure 3), the graft implanted developed an adjacent lump whose size exceeds the normal side. The study shows that lateral growths (Figures 5–8) on the periphery a covering conjunctiva, which goes deep from place to place, a zone of cell proliferation or continue with a chondroid tissue aspect. Based on this growths consists of compact bone, whose lamellae are disposed in eddies around the small irregular spaces without precise guidance, filled with vascular tissue and sometimes myeloid tissue.

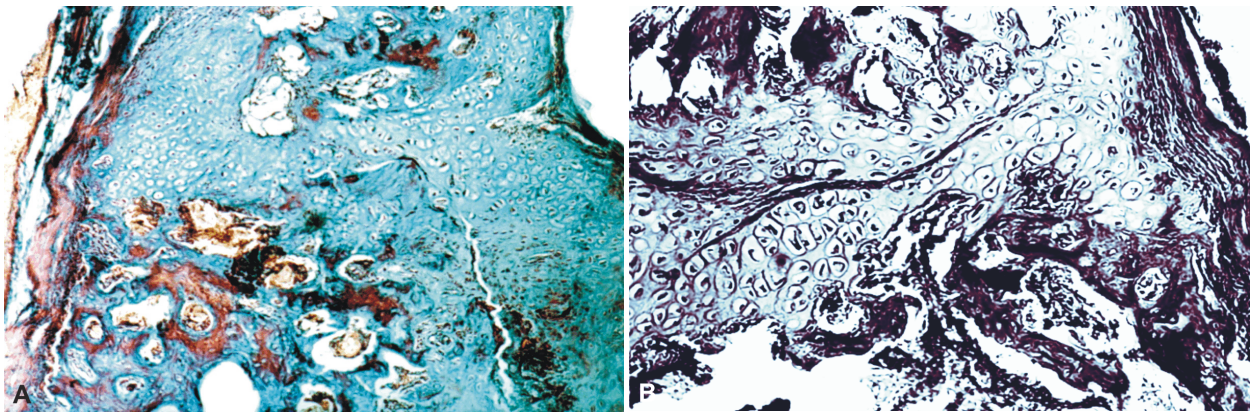

Figure 5 – ***(A and B) Various aspects from the structure of the tibial lump that is located near the transplant. Interpenetration of spongy tissue is observed, with the cartilage perichondrium and periosteum intense histogenetic activity. The images illustrate the covering connective that possibly in some areas forms bone, and cartilage in others. Trichrome staining: (A) ×40; (B) ×200.***

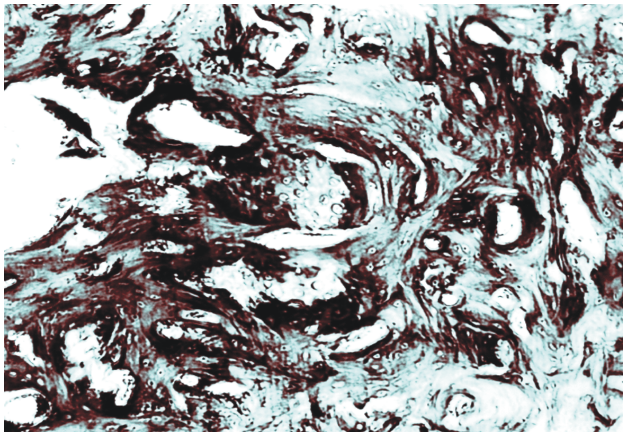

Figure 6 – ***Aspect of cancellous bone from the inner part of compact bone adjacent growth. Trichrome staining, ×40.***

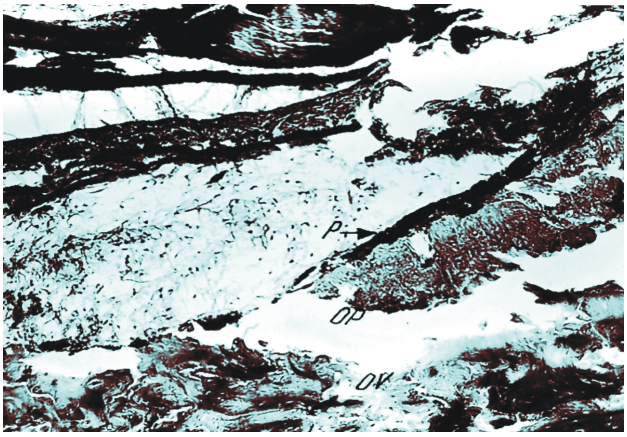

Figure 7 – ***Periosteum with low potential of bone formation, at fracture site of the tibia of rats. Between newly formed bone tissue the periosteum (PO) and old bone (OV) have not made any junction. Trichrome staining, ×40.***

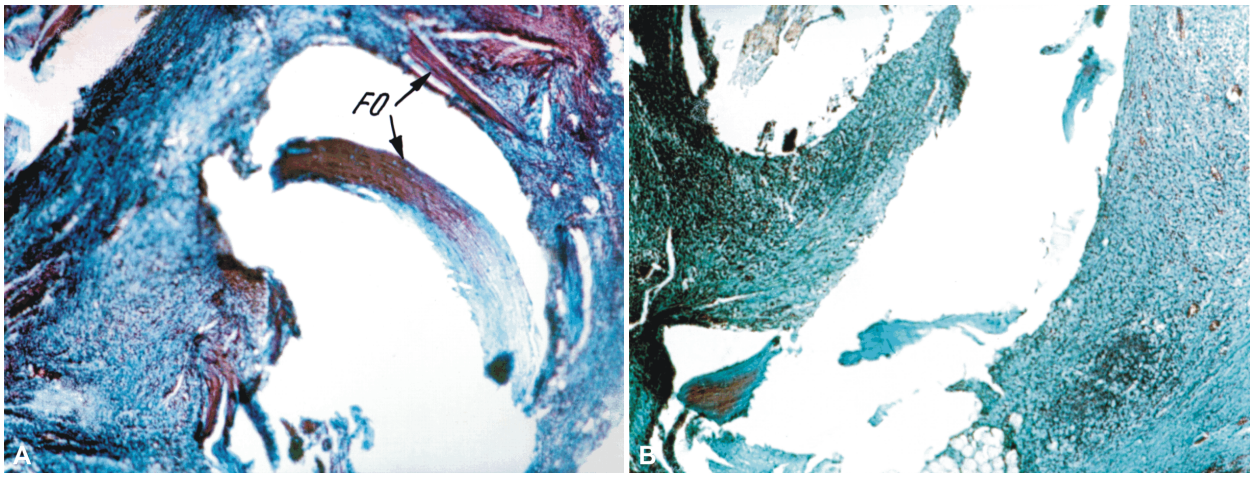

Figure 8 – ***(A and B) Various aspects of bed reaction receptor (rat tibia) to transplant bone (iliac bone homografts), the remaining bone fragments off (FO) or included in the tissue response of the bed. Appearance at 30 days. Trichrome staining, ×40.***

Between the compact bone of the tibia and the lump there is a solid weld, cut in some sections by a large blood vessel, oriented parallel with the tibia compact bone growths.

Interposed between the casing and compact shin, this trabecular mass consists of bony growths in varying degrees of resorption and vascular and myeloid tissue, more or less well preserved and chondroid looking tissue. It is constructed from a homogeneous hyaline substance, with weak basophilia and cartilage cells of various sizes, which give the appearance of vesicular cartilage. Cartilage cells have different morphological aspects, reflecting variations in their functional state. All these morphological outgrowth of chondrocytes found suggest that the grafted fragment is similar to those of the cartilage of the tibia conjugation, where there is a well-defined orientation and coordination.

Based on the issues found new and literature data, the graft was an irritating element and of stimulation of host tissue elements.

Discussion

Experimental results reveal microscopic aspects, some more, some less known about bone-cartilage transplants integration into the receptor.

The variety of aspects reported reflects structural modalities of tissue transplantation integration to host with the two contrary sides of a unique process of ossification.

The classical theory assumes that bone grafts are temporary implants. They undergo necrosis and early resorption in cancellous bone graft compared to the compact type.

In adult rats, homografts, we see the possibility of fixing the graft to the host tissue possibly subject to some microenvironment influences. That the parts grafted, given our experiences were fresh and with preserved periosteum, is certainly an asset in addition to their setting.

It is known, however, that homografts of fresh bone are not always able to contribute to osteogenesis due to reaction of rejection of the host tissue, as shown in our experiments.

We want to insist, however, on two ways resorption: that involving osteoclasts found in the area where intertrabecular spaces containing viable or myeloid conjunctive-vascular tissue and resorption with autolysis with osteolysis of bone trabeculae. We noticed this option in some areas where the connective tissues, myeloid as well as young connective tissues are missing or necrotic in the spongy or surrounding areas.

Lytic degradation of the bone is of course a consequence of depolymerization of trabecular bone matrix, with some probable trabecular osteocytes contribution by their enzymatic activity.

We found little information in the literature regarding the possibility of reactivation of osteocytes from the bone trabeculae during bone resorption. If released from bone cavities the osteocytes can start secretor activity. Other authors insist that osteocytes as well as chondrocytes are irreversible differentiated cells. Also, osteocyte survival is possible in all series where we used fresh bone, whether it was self-or homogeneous, but no information on their morphology nor the possibilities of metaplasia has been found.

Regarding the role of periosteum, sufficient data in the literature insists on fixing possibilities for transplants who maintain periosteum, and one recent experimental study has shown that the preservation of the periosteum produces significantly greater bone neoformation in the graft-recipient interface in rats as compares to those who underwent decortication (removal of periosteum), regardless of the type of graft used (compact or spongy) [10].

Our observations confirm once again that the ability of transplantated periosteum of osteoformation and reactivation. It worth note that these potentials are not as significant as that of host periosteum or the same intensity in all areas of grafted periosteum. Thus, in the bed adjacent to receptor site the transplanted periosteum has a higher bone formation capacity compared to more remote areas of the vitalized receiving tissue site. This gradient can be explained by the increased metabolic interrelations and exchanges that are created in the border area between transplant and host to more remote areas.

Intense proliferation of periosteum and penetration

into the adjacent cancellous bone and marrow cavity are more difficult to assess, unlike proliferation of bone graft cells and integration of the bone vascular channels [11, 12]. The most probable explanation is that their penetration site may be the least resistance site of the graft area, whose disintegrating elements could be easily invaded. We base this assertion on the observation of muscle fiber debris and blood and bone elements in the invaded bone structure. Regardless of the method of achieving, maintaining or creating vascularity, it significantly improves survival and graft incorporation situ the receiver. Thus, in an experimental study in animals it has been used saphenous vascular pedicle receiver femur graft from donor rats with or without vascular pedicle, the most effective were the autotransplants with preserved vascular pedicle, which produced and bone significantly higher, and presented and pronounced angiogenesis with vascular pedicle compared with heterotransplants who had similar characteristics in this regard [13]. In addition, even experimental study that was created by immersion of autograft carpal avascular necrosis in cyanoacrylate and freezing them, autoplastic radius with vascular pedicle was able to form bone and a revascularized graft [14].

Our observations regarding the contribution of bone marrow endorses the view of its active role not only in bone formation but also the formation of periosteum bone forming elements and fibrous tissue that fosters links with other surrounding tissues. In addition, there is a documented ability of endosteal bone marrow to produce both osteoblasts and chondrocytes from periosteum, which produces only osteoblasts [15]. Our research concerns the role of bone marrow from the receptor, both in autografts and homografts, highlighting the role of periosteum and marrow to activate local fixation of the graft.

Incorporation of the grafted bone into the receptor is accelerated by treatment with osteogenetic cytokines, leading to the formation of bone tissue neoformation that help strengthen the fracture [2, 16–18].

The use of allografts from implantable biomaterials is also controversial, with inferior or similar bone grafts, dependent on the heterograft architecture [19].

The presence of cartilage in the fracture was and is still discussed. Some authors argued that during the processes taking place in the healing of the fracture, the periosteum has osteogenetic abilities and forms a cartilaginous precursor of bone callus.

We signaled the occurrence of cartilage in lumps or compact masses, in various places, but closely related to the presence in these areas of hemorrhagic content during its organization or resorption. In our opinion, cartilage cells were differentiated from young connective cells present in these areas, the cellular elements of bone tissue adjacent areolas and from the reactivation of trabecular osteocytes during bone resorption.

Conclusions

The fixing of the graft to the host tissue may be subject to some microenvironment influences. The presence of the periosteum on the grafts is certainly an asset during transplantation. We confirm once again that the ability of transplantated periosteum of osteoformation and reactivation. Our observations regarding the contribution of bone marrow endorse the view of its active role in bone formation.

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Corresponding author
Horaţiu Pompiliu Petrescu, Assistant Professor, MD, PhD, Department of Orthopedics and Trauma, "Victor Babeş" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timişoara, Romania; Phone +40724–372 754, e-mail: lalusha87@yahoo.com

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