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Morphometric analysis of bone vascular channels during the biointegration of autologous bone grafts

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Abstract

Vascularization of the transplanted bone tissue is a key factor for success and precedes the formation of bone tissue. Vascularized bone grafts have been widely used in bone transplantation for their efficiency. Maturation of the bone tissue at the place of the transplant involves the change in the vascular patterning, from plexiform irregular vascular networks, to regular, polygonal networks following the structure of osteons. Seven dogs were performed bone transplantation and intravenous injection with China ink in order to highlight the network of blood vessels during bone formation at the place of the transplant. The area occupied by blood vessels increased during osteogenesis, and their length decreased in evolution, suggesting a maturation of the vascular pattern.

Keywords: bone transplantation, blood vessels, morphometric analysis, maturation of vessels, osteogenesis.

Introduction

Vascularization of the bone transplant has been regarded long-time as a key factor for the success of the bone transplantation; its importance has been highlighted in 1960 by Trueta J and Amato VP [1], which had studied the endochondral ossification during experimentally induced ischemia. Moreover, it is already known that blood vessels form first in the new bone, followed by the development of the bone matrix and the Haversian systems.

Angiogenesis is necessary in bone development in almost all of the normal and pathological conditions, from normal bone development and growth in length [2], to bone repair [3, 4] or mechanically stimulated osteogenesis [5].

During bone transplantation, vascularized bone implants tend to be used widely in clinical practice, with excellent results especially in pathologic conditions that involve local ischemia and necrosis [6–9]. The molecules that are involved in angiogenesis, namely vascular endothelial growth factor and fibroblast growth factor have non-angiogenic functions as well, being known to stimulate the proliferation of cells of mesenchymal origin. Thus, they not only vascularize the implant but also contribute to the growth and development of bone tissue and stimulate the implant cells [10].

The growth of the vascular network of the bone implant and of the newly formed bone can be studied by the identification of endothelial cells, active and proliferating, by immunohistochemistry, or by intravital injection of different types of macromolecules into the blood system, followed by the visualization of vascular lumens filled with ink.

Materials and Methods

We conducted research on a group of seven dogs, which, after the approval of the Ethics Commission of the Faculty of Veterinary Medicine, Timisoara, Romania, were performed transplantation of bone tissue from the iliac crest to previously created defects in radius or in the femoral shaft. The microscopic examinations were performed at one week, 14 days, 21 days, four weeks, six weeks and until 6–8 months of their histological evolution. Prior to sacrifice the animals, they were performed an intravenous injection of China ink. After prelevation of the specimens, they were decalcified with hydrochloric acid, followed by washing, fixation in 10% buffered formalin and sectioning in different incidences.

Examination of the specimens was performed with a Nikon Eclipse i80 microscope, followed by acquiring of microscopic images using a Nikon DS-U2 camera. The images were analyzed with ImageJ software. After obtaining a binary image, the area, the number and the length of the blood vessels were counted and analyzed.

Results

Microscopic sections

The samples were from the limit between the transplant and recipient bed, through the entire complex structure. The first aspect that stands out is a massive vascular invasion, which covers the entire structural complex, but with distinct features for the receptor and the graft (Figure 1). The receptor bone tissue has larger spaces, which usually contain larger diameter vessels, and independently of them, there is a true vascular invasion made up of glomeruli, with very fine vascular elements. Vessels are usually arranged on the walls of channels in the bone. Transplanted bone graft presents as a highly vascularized structure, observing irregular bone trabeculae with variable thickness. The trabeculae have a microporous structure, and contain very small diameter vessels. In the big trabeculae, their structure has a spongy appearance, with alternating spaces of all sizes. The large generally have irregular shapes, but there are many smaller cavities, with well-defined edges, bone deposition sites, but without osteons.

In the transition zone, several bridges between the transplant and the receptor bone can be observed, denoting a process of incorporation.

The junction between the receptor and the graft is crossed by a vascular network, well represented by thick trunks, accompanied by numerous fine filaments. This “vascular strip” is fragmented by bone trabeculae that link the graft to the receptor.

The vascular border is intimately linked to the receptor and the graft network. The vessels from the graft have a radial growth pattern, suggesting massive penetration from the receptor marrow cavity. The vessels may open on the surface but links with periosteal network are few in number and weak, which suggests an invasion and centrifuged revascularization. There is a hierarchy configuration: large vessels have dominant radial directions, closing regular polygonal spaces (Figure 2). These polygonal areas are crossed by finer vessels. At the confluence of major vessels, true vascular crossroads form, usually located in large intra-osseous spaces (Figure 3).

The receptor has the appearance of a normal vascular arrangement, with channels located predominantly in the long axis of the bone with oblique anastomotic systems forming elongated polygons. In areas close to the graft, enlarged channels may be seen. Graft vascular invasion is also noted, with vessels without well-defined orientation, often with plexiform appearance on cross sections, indicating that at this level the graft and the receptor lack the vascular integration at this stage (Figure 4). However, there are areas, which suggest some trends longitudinal orientation amid a fine network, completely disordered (Figures 5 and 6).

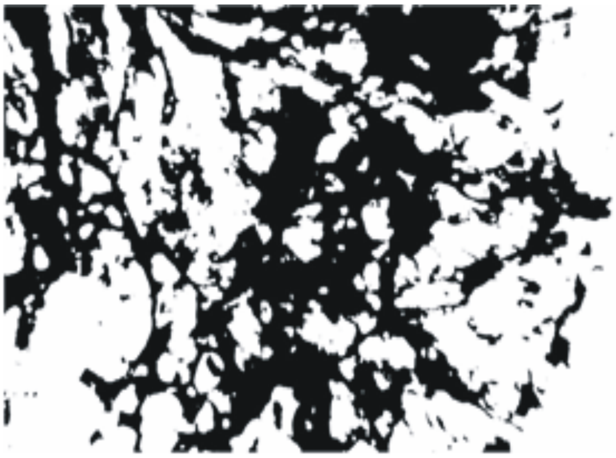

Figure 1 – ***Cross section at the limit between the bed and transplant, binary edited for morphometric analysis (×200).***

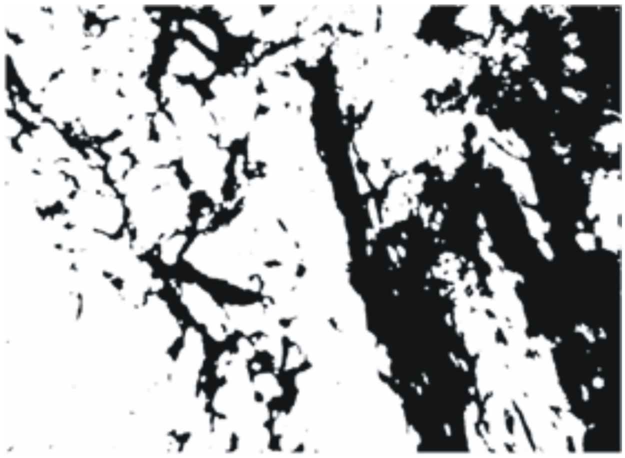

Figure 2 – ***Cross section of both transplant and receptor, binary edited for morphometric analysis (×200).***

Figure 3 – ***Cross section of the transplant recipient bed, binary edited for morphometric analysis (×40).***

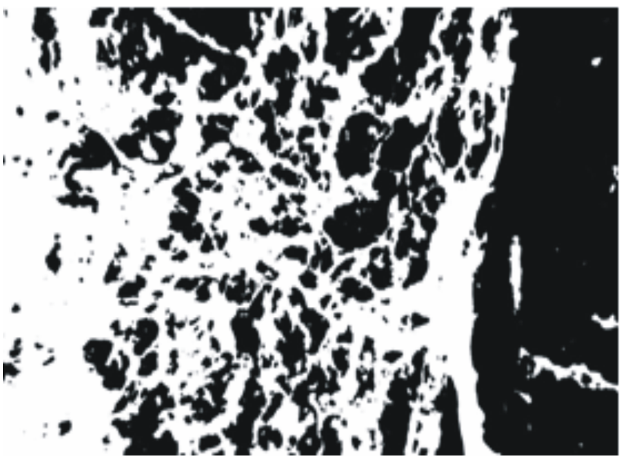

Figure 4 – ***Longitudinal section at the limit between the transplant and the recipient, binary edited for morphometric analysis (×200).***

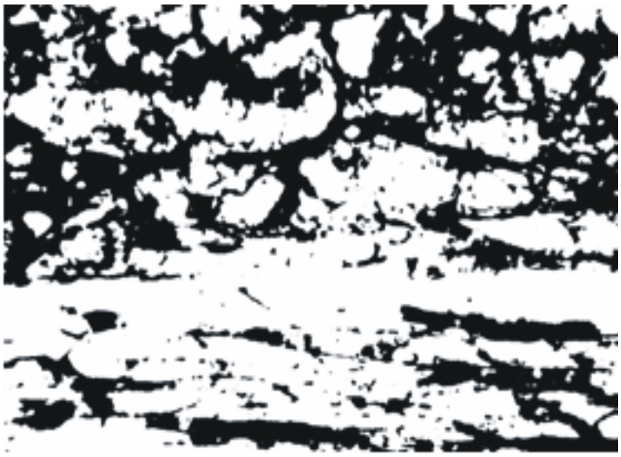

Figure 5 – ***Longitudinal section at the junction between recipient bed and transplant, binary edited for morphometric analysis (×200).***

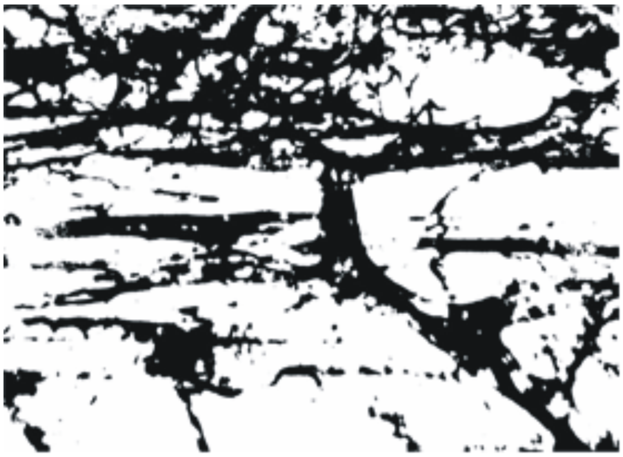

Figure 6 – ***Detail of previous figure (×400).***

Morphometric analysis

The average number of objects counted was 1472.5±769.9378763. The total area occupied by blood vessels increased in evolution but without statistical significance, from 371.7161093±129.6263727 μm^2 to 470.0880049±104.8651361 μm^2.

The average length of the blood vessels decreased in evolution, from 117.5065609±24.01554547 µm to 62.8227574±18.22018101 µm, suggestive for a more organized vascular pattern with a fine network of vessels with longitudinal and oblique arrangement, same as in the normal Haversian bone.

Discussion

Our research aimed to highlight the vascular bed evolution in autogenous bone transplants, on a longer interval, up to six months.

The vascular development in the newly formed bone is exuberant and starts from the recipient, as expected. The vascular pattern is initially chaotic, with plexiform arrangement of blood vessels in the graft, as well as in the newly formed bone. The recipient serves as a source for blood vessels, fact that is consistent with others results [11–13].

The vascular development was certainly improved in the situations where we were able to maintain the integrity of the periosteum of the graft, in which case the blood vessels developed more quickly than in the grafts without periosteum.

The vascular development precedes the bone formation; we have noticed a significant vascular network in the connective tissue between the graft and the recipient (data not shown).

To the best of our knowledge, this is the first computer-aided morphometric analysis of the blood vessels network development in bone grafts and implants. The injection method has the advantage that can be applied even on small experience animals and resists decalcification, unlike immunohistochemistry, which is sensitive to aggressive decalcifying agents.

Decalcification with Ethylene Diamino Tetra Acetate (EDTA) been extensively used with immunohistochemistry on small animals [14–17] with satisfactory results for different vascular markers and angiogenesis stimulators. However, for our purpose, this method has the advantage of being simple and reliable in experimented hands, also labeling all types of blood vessels and allowing thick sections to be used.

Conclusions

There is a massive vascular invasion of the graft–receptor complex formed mostly by newly formed vessels of small type and glomeruli. The large vessels have predominantly radial direction. The area occupied by the blood vessels increased in evolution. The length of the newly formed blood vessels decreased in time, consistent with the network maturation.

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