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The hematogenous marrow tolerance when being in direct contact with the titanium implant.

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Abstract

Introduction: The titanium implant represents a foreign body for the organism in which it is inserted, and it will never be accepted as an intrinsic structure. However, research has shown that it is very well supported both by soft and hard tissues, including here the limitrophe implant tissues. Aim of the study: The aim of this study was to test the tolerability towards the titanium implant inserted in direct contact with the hematogenous marrow. Material and methods: Titanium implants with the length of 10 mm and the diameter of 2 mm were inserted in the diaphysis of the femora bone of 10 male rabbits with the age of 10 months. At these particular dimensions, the implant exceeded the depth of the bone wall with more than half its length, taking direct contact with the hematogenous marrow from the marrow channel. Results: Seven days after the implants insertion, the histologic examination revealed the fact that the hematogenous marrow had a very good lenience towards the titanium implant, meaning that it did not lead to any immunological rejection reactions, nor to pathological processes or the tendency of proliferation of unwanted tissues like conjunctive fibrous tissue. In these conditions, the pre-implantation reparatory processes fully benefited of the hematogenous marrow support, which, not being altered in any way, offered cells and stimulant factors, of the same intensity, throughout the entire experimental period. Conclusions: The high tolerance of the hematogenous marrow to the titanium implant assures special conditions for the peri-implantation reparatory processes which take place at a speed that cannot be competed against by other stimulant modalities. This stimulant modality of reparatory processes cannot be applied in all situations, but only when working on healthy bones that contain hematogenous marrow, therefore the practical applicability relates especially to the dental interventions of implant prosthesis.

Keywords: hematogenous marrow, titanium implant, bone proliferation.

Introduction

Throughout our lives, we suffer injuries resulted from accidents or surgical interventions, which are remediated through the lesion healing process, due to the remarkable potential of the human body to repair itself. The healing and lesion repair processes imply the recruitment and proliferation of cells capable of re-establishing the original structure and function of the tissues. An important source of such cells is the bone hematogenous marrow, and studies show its implication in the healing process after injuries, bleeding or diseases [1].

The hematogenous marrow can be found in the cavities of the long and flat bones, having the capacity of being cut and transplanted. The utility of the marrow transplant is conferred by the fact that it contains numerous adult stem cells. Even though it is very unlikely, some authors claim that the adult stem cells might contain the same clinical potential as the embryonal stem cells, which would represent a huge advantage because it would eliminate the ethical and practical problems related to the preparation and use of the embryonal stem cells [2].

The marrow stem cells include hematopoietic stem cells, stromal marrow cells (mesenchymal stem cells) and multipotent adult cells. The hematopoietic stem cells differentiate towards different types of blood cells, whereas the stromal cells can differentiate towards adipocytes, chondrocytes, osteoblasts and other conjunctive tissue cells. Therefore, the transplant of marrow cells has the potential to contribute to the development of hematopoietic and osteogenesis cells [3]. In other words, the living cells of the bone marrow contribute to the bone development through osteogenesis.

The bone marrow contains osteoblast progenitors that can differentiate in mature

osteoblasts, that can directly contribute to the process of osteogenesis. Bone marrow suction was successfully used to improve bone regeneration; the transplanted cells initiate the development of an unmineralised bone matrix (osteoid) and they start the process of bone matrix mineralisation through accumulation of hydroxyapatite [4].

The bone marrow can be cut through suction from the stern, anterior iliac bone or posterior iliac bone. The technique of autogenous bone marrow suction and implantation does not imply major complications. The complementary procedures can be conducted as ambulatory ones with the patient under oral sedation and local anaesthesia, intra-venous sedation or general anaesthesia. Using the implantation of the suctioned marrow in the bony defects, a significant bone regeneration was obtained [3]. It must be stated that marrow suction provides the growth factors necessary for bone development, as well as angiogenesis [5].

There are a lot of advantages in using the autologous bone marrow suction for the treatment of bony defects, the mesenchymal stem cells being able to spontaneously differentiate into in vitro osteoblasts [6,7]. In experimental studies, it was demonstrated that the bone marrow stromal cells can form in vivo authentic bone, as well as the fact that they can form in vivo adipocytes [8]. It seems that the plasticity is more extended, meaning that some authors claim that the bone marrow contains myogenous, neurogenic and hepatogenic progenitors [9,10]. What needs to be considered is the fact that the suction method is painful for the donors, and it sometimes needs to be done under general anaesthesia and may be associated with side effects [11].

The marrow suction can be combined with different framing (collagen I, tricalcium phosphate, hydroxyapatite) in order to contribute to the acceleration of the bone healing process. From a surgeon's point of view, there are many advantages associated with the clinical application in one stage of the bone marrow concentrate. The immediate transplantation of bone marrow concentrate can prevent complications that might appear due to the low quality of the transplanted cells, like pre-aging (telomer contraction), reduced viability or the tendency to differentiate towards other types of cells, processes that might occur in case of propagation. Moreover, for this procedure, the infection risk is relatively reduced through the deduction of the ex vivo period of time [12].

Following a series of cases, some authors concluded that the bone marrow suction was able to facilitate the healing of massive bone loss. The same authors point to the fact that a cost-benefit analysis should be conducted in order to see whether the application in one stage of the bone marrow suction reduces the hospitalisation period and the additional costs involving stationary or necessary personnel for the ex-vivo transplant [6].

Some authors claim that the administration modality influences the results, claiming that the use of a recently suctioned bone marrow is more indicated than the preparations that have gone through different procedures, which can affect parts of the transplanted cells [12].

The use of the hematogenous marrow through the bone reparatory process stimulation has been long studied, either as a recently suctioned bone, or under the form of medullary components or cultures. All the studied variants revealed the beneficial effect of the hematogenous marrow, but there is one thing which needs to be highlighted, the fact that the procedures necessary for obtaining a certain marrow product may lead to certain alterations of the more sensitive, delicate components. In other words, the marrow concentrates are very useful, but they do not have all the qualities of the marrow which was not previously exposed to certain procedures.

Some authors have even gone further and tested the osteo-inductive potential of the hematogenous marrow over a titanium implant which penetrates the marrow cavity, having direct contact with the marrow. The results obtained by them were very encouraging [13,14].

Given the fact that it was proven the important effect of the titanium implants inserted in direct contact with the hematogenous marrow over the osteointegration, we aimed at investigating the possible side effects that might occur when the titanium implant is in direct contact with the marrow channel.

Material and methods

The materials used were titanium implants, self-drilling screw type, 5 mm in length and 2 mm in diameter (Bio Micron®-Cluj, Romania).

The biologic material was represented by 10 male rabbits, common race, aged 10 months. The study was approved by The Banat Bioethics Commission of Agricole Sciences and Veterinary Medicine "Regele Mihai I al României", no. 124/02.06.2022. The rabbits' accommodation throughout the whole experiment was made at the temperature of 20-240C and natural light with a light-darkness cycle of approximately 12/12 hours. The food used was standardised grained fodder, and the water was fresh and unlimited.

The process of narcosis was undertaken through intramuscular administration of xylazine 5 mg/kg + ketamine 40 mg/kg (Bioveta®-Czechia), followed by the placing of a venous catheter on the external auricular venae and the animal was connected to a fluid-therapy mechanism. After 7 days, the animals were sacrificed and the area that contained the implant was cut and immediately introduced in formalin 10 % for histologic fixation (Roth®-Germany). At the end of the stabilisation period, the pieces were decalcified with trichloroacetic acid (Roth®-Germany), dehydrated with ethyl alcohol (Chemical Company®-Iasi, Romania) in progressive concentration, clarified in 1-Butanol (Roth®-Germany), included in paraffin (Roth®-Germany), sectioned at 5 micrometres and coloured using the Tricrom Goldner method (Hematoxilin, Fuchsin acid [Rubin S], Orande G, Tungstophosphoric acid hydrate, Light green yelowish, Merk®-Germany and Xylidin Ponceau 2R - Roth®-Germany). The examining of the histologic concentrates was made using an Olimpus BX41 microscope containing an image digital camera type E-330.

Results

The histological exam revealed that the implant exceeded the endosteum, penetrating the femoral marrow cavity with three and a half spires, therefore it had initially been in direct contact with the hematogenous marrow, over more than a half its length. After 7 days, approximately half of the intra-marrow implant portion is already covered in new bone, looking like a young bone in full proliferation process and visible tendency of extending towards depth on the implant surface (figure 1).

In the progression area, what can be noticed is young conjunctive tissue with a very specific aspect which contains numerous cells, especially osteoblasts and even some very discreet lines of bone trabecula (figure 2). The aspect suggests that its evolution is directed towards a bone tissue and not towards a fibrous conjunctive tissue. Towards its final area, the implant is covered with a thin layer of young conjunctive tissue which continues without demarcation towards the hematogenous marrow of the marrow channel (figure 3).

The meadow situated next to the titanium implant contains progenitors of the sanguine figurate elements, in different stages of evolution, progenitors on all lines being highlighted (granulocyte, lymphocyte, monocyte, thrombocyte) (figure 4). These aspects suggest that the marrow situated next to the titanium implant is perfectly functional and does not react in any way to the presence of the titanium implant. Moreover, the large implant surface covered after only 7 days from the implant insertion of newly proliferated tissue, present only in the implant portion inserted in the marrow cavity, demonstrates the fact that the reparatory processes began in the endosteum area and evolved rapidly.

The high speed at which the new bone was proliferated can only partially be attributed to the endosteum, the fact that this proliferation was significantly stimulated being obvious.

This proliferation was initiated by the endosteal cells and benefitted from significant help from the hematogenous marrow, through the osteoblast progenitors and the stimulating substances present there. The stage to which the peri-implantation proliferation process evolved during a period of only 7 days from the titanium implant insertion, suggests the fact that the marrow did not react to the contact with the titanium implant as if it were a foreign body, through immunologic rejection mechanisms. If such mechanisms had been initiated, a foreign body reaction would have taken place, with the appearance of multinuclear giant cells that would try isolating and eliminating the titanium implant. Such multinucleate cells were not identified, grouped or isolated. Additionally, what needs to be mentioned is the fact that all the tissues proliferated at the interface between the implant and the area initially occupied by the hematogenous marrow, represent stages of new bone formation. The proliferation of conjunctive tissue with fibrosis tendency cannot be noticed. These aspects highlight the fact that the hematogenous marrow develops a high tolerance towards the material out of which the implant is made (titanium), meaning that it did not activate hostile reactions to its presence, which is nevertheless a foreign body for the organism.

The acceptance of the implant by the hematogenous marrow assured the optimal conditions for the activation of the reparatory processes within a very short period of time and at very high speed.

This rapid bone proliferation could not have taken place without the direct contact of the marrow with the marrow channel, which provided both osteoblasts and stimulating factors. If the marrow did not suffer any alterations due to the direct contact with the implant, the process of osteointegration benefited from the best possible conditions and that was the standpoint for the speed at which the reparatory processes took place. From this point of view, the results are so good, that they exceed those obtained from the use of suctioned marrow or hematogenous marrow concentrates, even though the specialised literature bristle of positive results obtained after their use, in many circumstances.

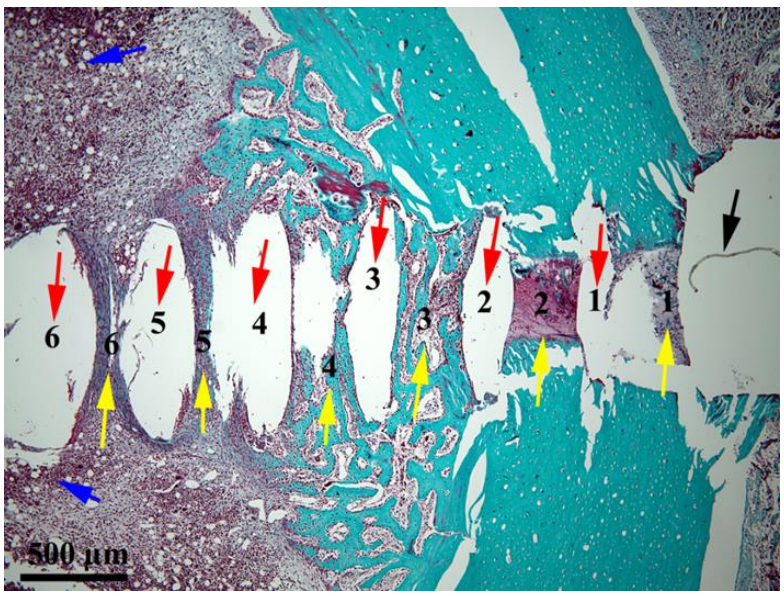

Figure 1. The aspect of the implantation area after 7 days from the implant insertion. Black arrow – the implant unthreaded screw collar; red arrow – implant spires (1-6); yellow arrow – the proliferated material in the implant socket (1-6); blue arrow – hematogenous marrow (Tricrom Goldner) 205

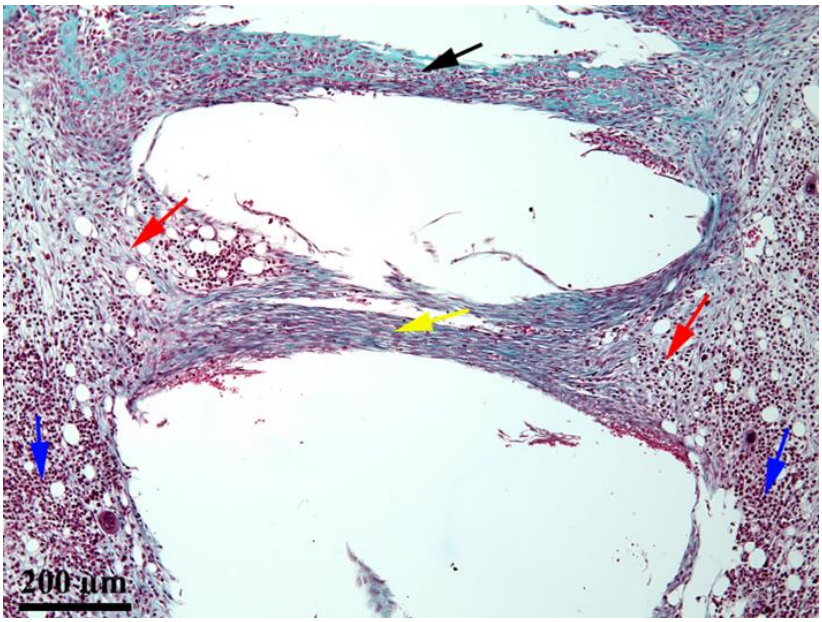

Figure 2. The interface between the 5-6 implant spires and the hematogenous marrow: black arrow – proliferated material in socket 5; yellow arrow - proliferated material in socket 6; red arrow – new conjunctive tissue proliferated on the interface; blue arrow – hematogenous marrow (Tricrom Goldner) 208

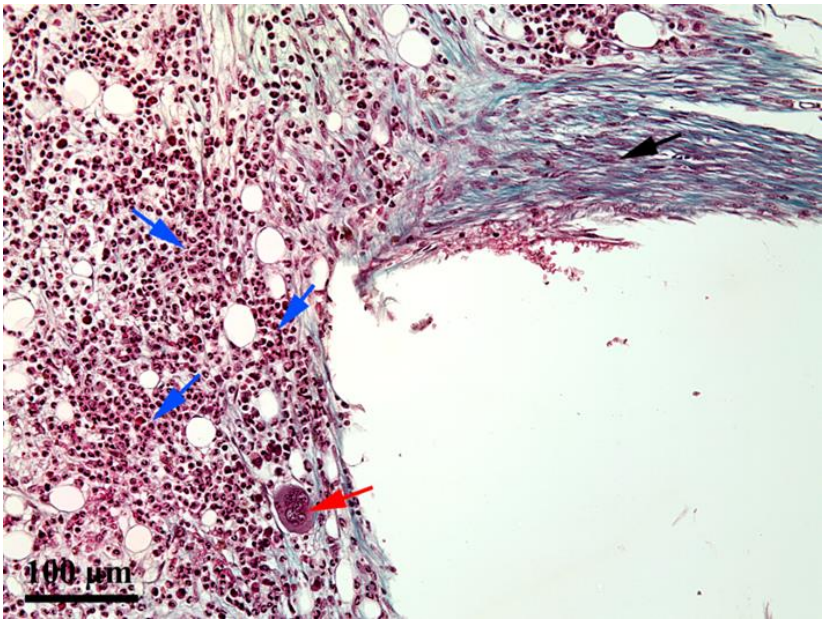

Figure 3. The interface between implant spire 6 and the hematogenous marrow – detail; black arrow – the proliferated material in socket 6; red arrow – megakaryocyte; blue arrow – progenitors of sanguine figurate elements in different evolution stages (Tricrom Goldner) 210

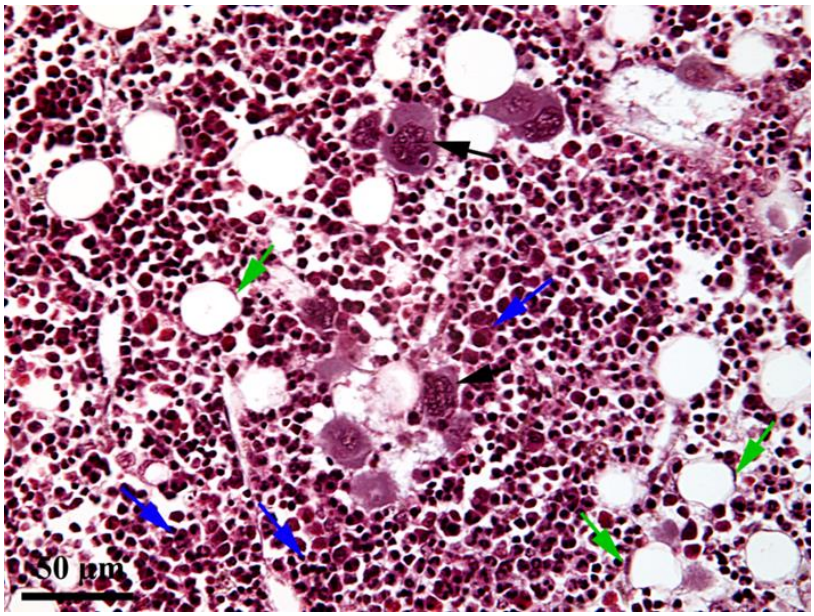

Figure 4. The hematogenous marrow; black arrow – megakaryocyte; blue arrow- progenitors of sanguine figurate elements in different evolution stages, green arrow – adipocyte (Tricrom Goldner) 217

Discussions

Considering a number of 18 patients, some authors reached the conclusion that the local injection of the autogenous bone marrow is a safe and efficient treatment method for the simple bone cyst, but sometimes repeated injections are necessary [15].

The growth and transplant of undifferentiated bone marrow cells are efficient procedures, but they are also complex and expensive. The bone marrow suction is much facile, fact which lead to its study, both clinically and in the laboratory, for the purposes of filling the bony defects, of stimulating the fracture healing and of pseudarthrosis treatment [16]. In order to reconstruct the bony defects, some authors combined the marrow suction with collagen and tricalcium phosphate fields, and they noticed different degrees of differentiating and maturing of mesenchymal bone marrow stem cells into osteoprogenitor and osteoblast cells osteoblast [17].

The percutaneous injection of the bone marrow suctioned represented the study object on experience animals. Some authors injected the suctioned marrow in the fifth day after osteotomies and induced bony defects, on 41 adult rabbits. The marrow was injected immediately after suction, due to the fact that the number of viable cells decreases as time passes. After 2, respectively 3 weeks, the callus volume was significantly greater on the animals that received suctioned marrow compared to those that have not. After having run the histological and radiologic examination, the conclusion was that the percutaneous inoculation of bone marrow improved the healing process of ostectomies and defects within 4 weeks [18]. Other researchers investigated if the bone marrow administered percutaneous determines the growth of bone production or if it has any effect over the early fracture healing. The tested parameters were represented by the callus transversal sectioned area, the braking resistance, the tension resistance and the callus volume at the fracture place. Two weeks after the administration, the four parameters, especially the callus volume, were significantly bigger ($0{,}001 < P < 0{,}005$) in case of marrow injected bones compared to those injected with physiological serum. After four weeks, all four parameters were significantly higher in the bones injected with suctioned marrow compared to those which were not [19].

The bone marrow was used both clinically and experimentally, combined with bone, in order to increase the graft osteogenesis capacity [19]. A big advantage associated with the use of suctioned marrow is the fact that it is available in relatively large quantities. Another advantage is the fact that the administration of the suctioned marrow can be done at a high degree of precision (exactly in the fracture area) based on imagistic information. This fact is of outmost importance especially in certain clinical situations, like infections, for example [19].

The autologous bone marrow concentrate was also tested on human patients with fractured inferior limbs. It is a well-known fact that in the case of inferior limb fractures several risks might arise, such as delays in the bone union or even the non-union of the fractured epiphyses, situation in which the autologous graft is indicated. Some authors claim that the most promising treatment would be the percutaneous injection of a suctioned or even concentrate of autologous bone marrow. They verified this method on 43 cases of open tibia fractures, with initial surgical treatment. In 23 cases (53,5%) when the autologous bone marrow concentrate was used, positive results were obtained [20]. Sugaya and colab. [21] used this concentrate in 17 cases of pseudo-arthrosis (ten femoral, five tibial, one humeral and one ulnar) and they obtained a rate of success of 76% %. Other authors reported even higher success rates (88%) for cases of tibial pseudo-arthrosis [22], or even higher than 94% [23].

In most cases, the bones that suffer an intervention present bigger or smaller modification, and in these cases, the use of suctioned marrow or some marrow concentrates represents the best reparatory process stimulation method. The modality we presented here offers results that are superior to those obtained when using suctioned marrow or marrow concentrates, but it must be mentioned that it is a bit more specific and can be applied only in some situations. It can be applied only on healthy bones that have cavities with hematogenous marrow, suitable especially in the cases of dental interventions referring to implant prothesis.

Conclusions

The insertion of titanium implants in direct contact with the hematogenous marrow was proven as an extremely efficient method of stimulation of the peri-implant reparatory processes, due to the fact that the marrow had a high tolerance towards the material out of which the implant was made. In this context, the reparatory processes fully benefited from rapid, direct and continuous marrow support, as osteoblast and stimulant factor supplier.

Conflict of interest: None to declare.

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