rabbit genetics

Hematogenous reaction of rabbit bone marrow to endosteal penetrating titanium screws

[1]Bianca Fernea, [1]Aurel Damian, [1]Cristian Martonoș, [1]Adina F. Vidrean, [2]Aurelia Coroian, [3]Diana Mesaros, [1]Oana A. Pece

[1] Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; [2] Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; [3] Control Systems Engineering Department, University of Oradea, Oradea, Romania. Corresponding author: O. A. Pece, oanamastan@yahoo.com

Abstract. Bone repair after fractures or experimental defects is done with the active participation of osteoblasts that are formed from osteoprogenitor cells, originating from periosteum, endosteum and hematogenous marrow. The purpose of this study was to verify the relationships between the hematogenous marrow in the medullary canal and a titanium screw that penetrates the endosteum and comes into intimate contact with the marrow. Titanium screws 5 mm long and 2 mm in diameter were inserted into the femur of female rabbits. These screws penetrated the endosteum and penetrated about half their length into the medullary canal, coming into intimate contact with the hematogenous marrow. The intervention area was harvested one month post-implantation and processed for histological investigations. The results showed that the marrow presented a very good tolerance to the titanium implant, being arranged in direct contact with the surface of the screw. Moreover, the hematogenous marrow actively participates in bone proliferation, an aspect supported by the existence of bone formations in different stages of evolution in the marrow in the vicinity of the implant surface. In conclusion, hematogenous marrow exhibits very good tolerance to the titanium screw and stimulates osseointegration.
Key Words: bone marrow, cells, histology, implant, osseointegration, titanium screws.

Introduction. Bone repair processes after injuries or experimental defects require the mobilization of skeletal stem cells, which are also called osteoprogenitor cells. These cells differentiate into osteoblasts which are responsible for the synthesis of bone material, which they deposit at the site of the injury. Osteoprogenitor cells originate from several sources, such as periosteum, endosteum and hematogenous marrow (Colnot 2009).

The hematogenous marrow contains hematopoietic stem cells, stromal cells, endothelial cells, pericytes, fibroblasts, adipocytes, and numerous blood vessels (De Souza et al 2016; Chen et al 2018). Among the currently used implants, the screw-type ones are preferred because they ensure a very efficient anchorage in the bone and have the largest contact surface with the bone (Misch et al 2008; Colnot et al 2012). Regarding implant length, some researchers prefer long implants that provide a significantly larger contact surface at the bone-implant interface than short implants (Adell et al 1990; Lekholm et al 1994; Rao & Gill 2012).

If the implant is longer than the thickness of the bone cortex, then it penetrates the endosteum and penetrates into the cavities of the trabecular bone from the depth, coming into contact for a certain length with the bone marrow existing in these cavities. By inserting screw-type implants that exceeded the thickness of the cortical bone by approximately half their length, Pantor et al (2022) concluded that the bone marrow showed very good tolerance to the titanium implant during the first 7 days post-implantation. They did not identify immunological rejection reactions and no pathological processes or proliferative tendency of unwanted tissues such as fibrous connective tissue. The aim of this study was to verify the tolerance shown by the hematogenous marrow towards the titanium implant, one month after implantation in the femur of female rabbits.

Material and Method. The experiment was carried out between April and May 2021. Biological material was represented by 10 female domestic rabbits (*Oryctolagus cuniculus*), aged 12 months with an average weight of 4 kg. For testing we used titanium screws with a diameter of 2 mm and a length of 5 mm. The experiment had the approval of the Bioethics Commission, mentioned in document number 219 of 10.07.2020, within the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca.

Access to the femoral bone was created through the skin incision, followed by the muscle incision. With a 1.8 mm drill, a screw insertion hole was drilled in the middle area of the femur, then the screw was inserted by self-tapping. Suturing of the muscles and then the skin followed, and as a post-operative treatment, enrofloxacin 20 mg kg^{-1} and meloxicam 1 mg kg^{-1} were administered subcutaneously.

One month postoperatively, the animals were euthanized with pentobarbital at a dose of 60 mg kg^{-1} intravenously and then samples were collected from the intervention area for histological investigations. The harvested parts were fixed with 10% formalin for 7 days, then decalcified with trichloroacetic acid and embedded in paraffin. Sections with a thickness of 5 μm were stained with the Goldner trichrome method, and an Olympus BX41 microscope equipped with a digital camera was used for their examination.

Results and Discussion. One month after the insertion of the screws, at the interface of the marrow in the medullary canal with the surface of the screws, no immune rejection processes were detected, respectively it was not triggered foreign body reaction, and no local necrotic processes. On the contrary, the hematogenous marrow was in intimate relations with the surface of the screws, enveloping the coils and penetrating into the grooves between the coils, which they completely occupy (Figure 1).

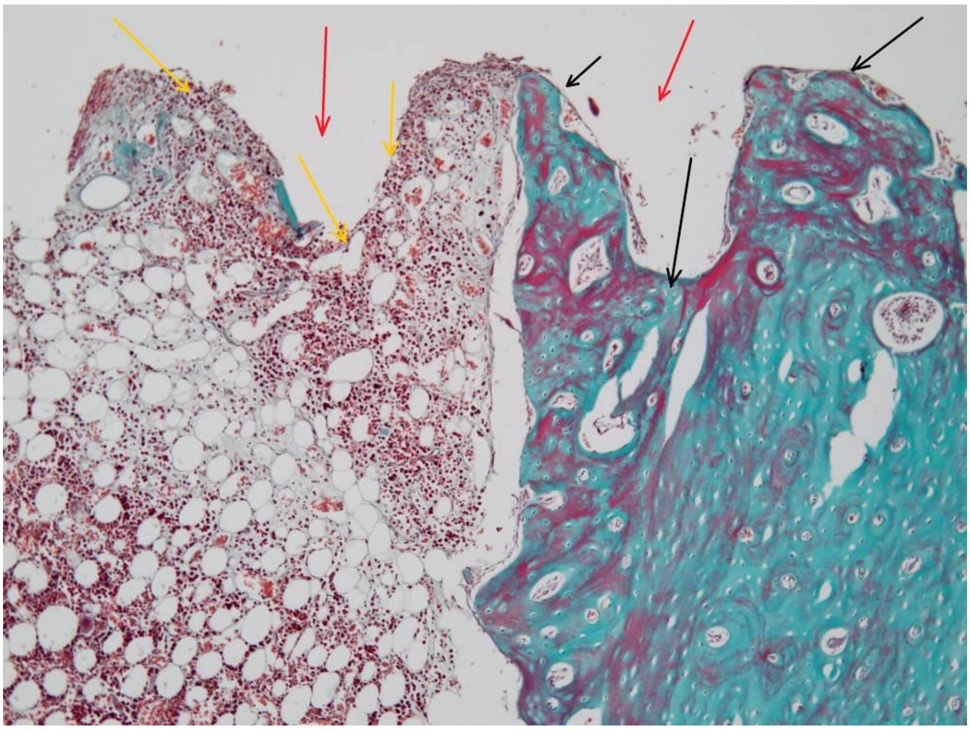

Figure 1. Screw interface with hard and soft tissues: red arrow - screw site; black arrow - screw/bone interface; yellow arrow - hematogenous screw/marrow interface (Trichrome Goldner, ob. 10x).

The marrow penetrated here consisted of a fine stroma in which were precursors of blood-figured elements in various stages of evolution. Moreover, the marrow in the immediate vicinity of the surface of the screws was very well vascularized, containing significantly more vessels and of a larger caliber than the marrow located at a distance from the intervention area (Figures 2, 3).

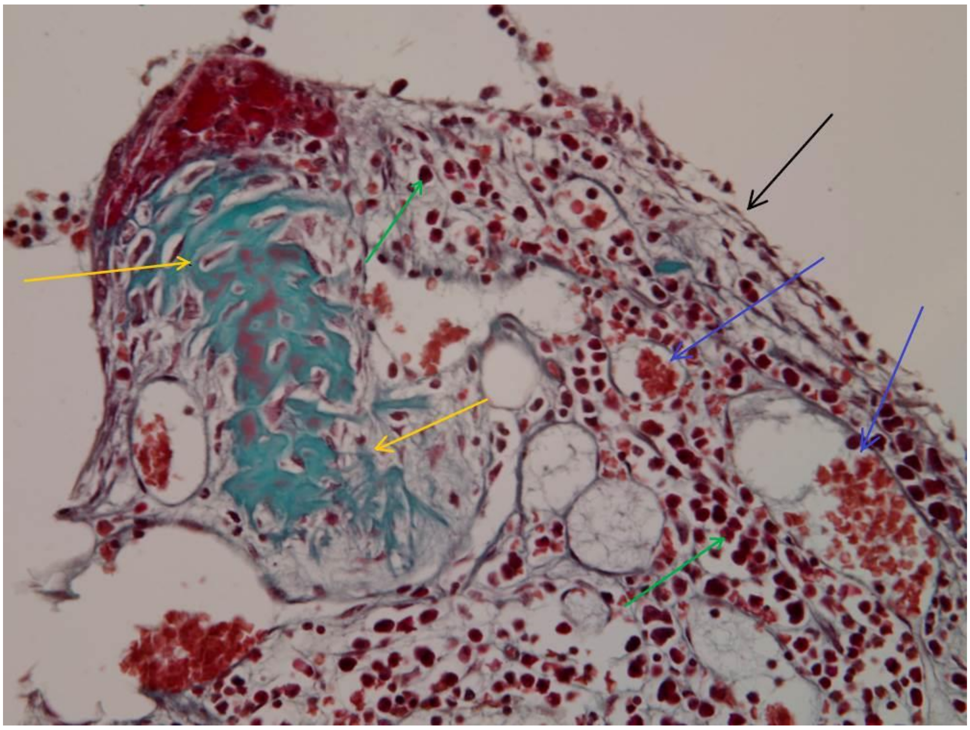

Figure 2. Hematogenous marrow on the interface: black arrow - contact with the screw surface; yellow arrow - newly proliferated bone formations; green arrow - precursors of blood figurative elements; blue arrow - blood vessels (Trichrome Goldner, ob. 40x).

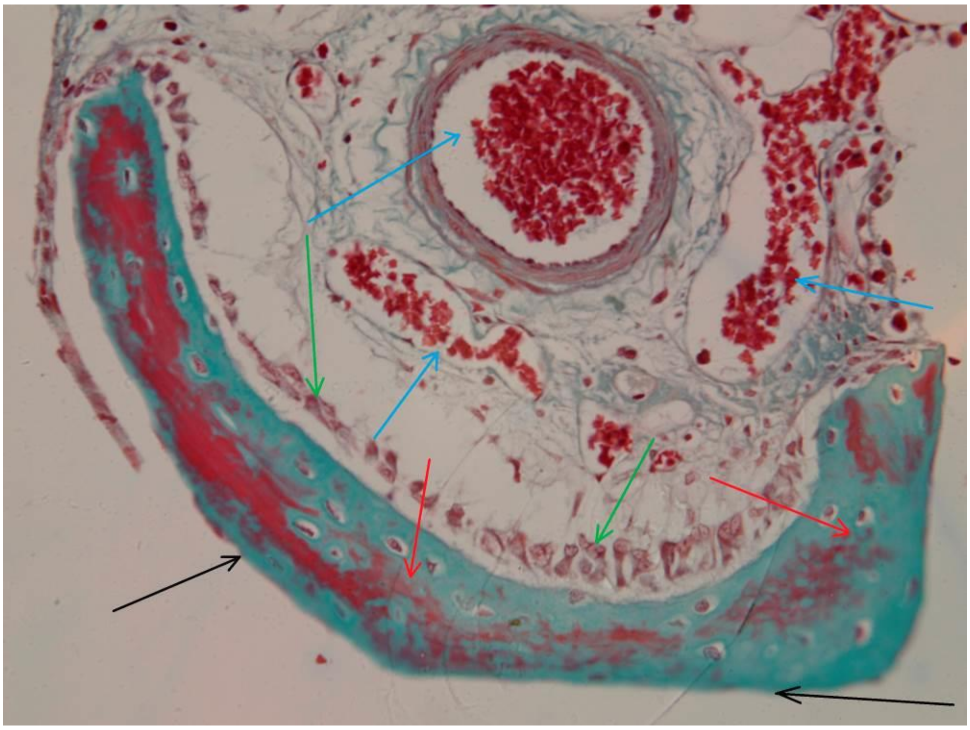

Figure 3. Hematogenous marrow on the interface: black arrow - contact with the screw surface; red arrow - newly proliferated bone; green arrow - osteoblasts; blue arrow - blood vessels (Trichrome Goldner, ob. 40x).

All these aspects suggest that the medulla in the vicinity of the surface of the screws was a very active one, although the number of figured elements here was not greater than in the medulla located at a distance (Figure 4), on the contrary, numerically they were somewhat less. This aspect suggests that the marrow also participates very actively in other events taking place in the area. One such event is that of bone proliferation to cover the screw interface as much as possible. This statement is supported by the presence in the stroma in the vicinity of the screws of some structures that suggest proliferation and organization of bone formations, most of them in a very early stage (Figure 2).

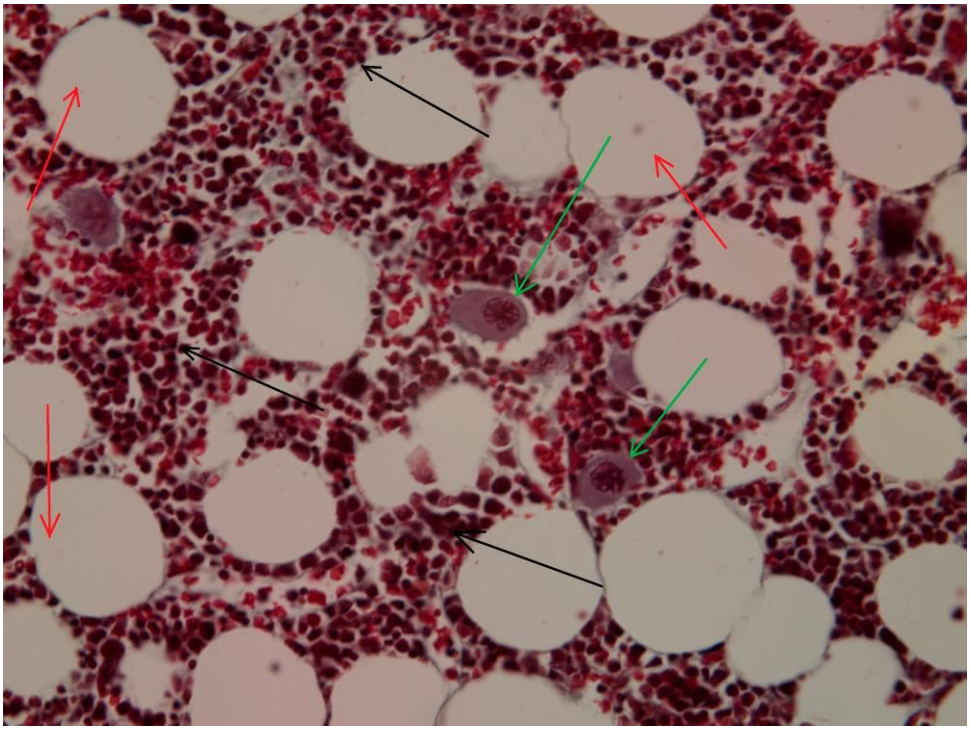

Figure 4. Hematogenous marrow in the vicinity of the screw surface: black arrow - precursors of blood figurative elements; red arrow - adipocytes; green arrow - megakaryocytes (Trichrome Goldner, ob. 40x).

In some situations there were also more organized and evolved bone formations, even with the appearance of trabeculae, especially in the grooves between the turns. In such situations, a particularly active osteoblastic mobilization was also observed, the origin of which could be from medullary precursors (Figure 3).

These aspects suggest a very active participation of the hematogenous marrow in the process of osseointegration of the screws, by providing the cells that participate in the processes of bone synthesis, namely osteoblasts and factors stimulating the formation of new bone on the surface of the screws. In this way, a complex of factors that support the osseointegration process are met, starting with the special tolerance that the hematogenous marrow exhibits when in contact with the titanium screw surface. The absence of any adverse reaction allows the nearby marrow to participate in the osseointegration process, which it supports and potentiates to an unrivaled level, knowing that the most effective stimulation factors are at the level of the marrow.

The animal body has remarkable potential to repair tissues damaged by accidents or surgery. The repair of damaged tissues is done by recruiting cells that directly participate in tissue regeneration, a very important source of such cells being the hematogenous marrow (Soltan et al 2007). Hematogenous marrow contains osteoblastic precursors that can differentiate into osteoblasts, cells directly involved in osteogenesis (Smiler & Soltan 2006). Studying the relationships between the hematogenous marrow and the surface of the titanium implant at 7 days post-implantation, Pantor et al (2022) found a very good tolerance to titanium. They found that the marrow in the immediate vicinity of the implant was perfectly functional and showed no reaction to the titanium implant. The aforementioned authors claim that the marrow not only tolerates the presence of the titanium screw, but provides optimal conditions for the initiation of reparative processes in a very short period of time and at a very high speed. The explanation would be that the hematogenous marrow in the medullary canal provided both osteoblasts and stimulatory factors. Similar results were obtained by other authors who studied the effect of hematogenous marrow on the osseointegration of titanium implants that penetrated the endosteum and entered the medullary canal. The period taken in the study was identical to that of the authors mentioned above and the results comparable (Ratiu et al 2022).

The results obtained by us show that even in the medium term (one month) the realities between the bone marrow and the titanium implant are of collaboration and not of rejection. This highlights the fact that the hematogenous marrow stimulates osteogenesis in the situation where the implant penetrates the endosteum. It is very important to state that this stimulation extends throughout the osseointegration process, which is a very big advantage. The stimulatory effects of hematogenous marrow on bone reparative processes have been known for a long time and have been extensively studied. Some authors stated that the marrow aspirate provides the growth factors necessary for both bone formation and angiogenesis (Barry & Murphy 2004).

Studies have used either fresh marrow aspirates or marrow cultures or components. In all variants, positive results were obtained, but it was found that the work required to obtain a certain marrow product can produce some changes in the more sensitive, more delicate components. These changes mean that marrow preparations, although very beneficial, do not quite have the potential of unexposed marrow for certain manipulations. Furthermore, the aspiration method is painful for donors, sometimes requiring general anesthesia and may be associated with adverse reactions (Bain 2005).

Comparing the two ways of stimulating osseointegration, namely the use of preparations from the marrow and the insertion of implants in direct contact with the marrow, we consider that the second way is more advantageous. The claim is based on the fact that in this context the marrow does not undergo changes because it is not subjected to manipulations nor is it moved from its natural environment.

Moreover, a very important aspect is given by the fact that the stimulation of osteogenesis extends throughout the whole period of this process, while marrow preparations provide stimulation for a limited period of time. The limitations of using this modality to stimulate osseointegration are due to the fact that it cannot be applied in all situations. It lends itself to application on healthy bones that have a deep cancellous component with areolae in which there is hematogenous marrow. In such bones, the use of long screws that penetrate the endosteum gives osseointegration advantages that are, to our knowledge, unmatched.

Conclusions. The insertion of titanium screws with a length that ensures penetration of the endosteum and direct contact with the hematogenous marrow, ensures special and very advantageous conditions for osteogenesis. Direct contact with the marrow makes the reparative processes benefit from very fast, direct and continuous stimulation. This stimulation extends throughout the period of osseointegration, which is an advantage that cannot be rivaled by any other method of stimulation.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Adell R., Eriksson B., Lekholm U., Brânemark P. I., Jemt T., 1990 Long-term follow-up study of osseointegrated implants in the treatment of the totally edentulous jaws. International Journal of Oral and Maxillofacial Implants 5(4):347-359.

Bain B. J., 2005 Bone marrow biopsy morbidity: review of 2003. Journal of Clinical Pathology 58(4):406-408.

Barry F. P., Murphy J. M., 2004 Mesenchymal stem cells: clinical applications and biological characterization. International Journal of Biochemistry and Cell Biology 36(4):568-584.

Chen K. G., Johnson K. R., Mckay R. D. G., Robey P. G., 2018 Concise review: conceptualizing paralogous stem-cell niches and unfolding bone marrow progenitor cell identities. Stem Cells 36(1):11-21.
Colnot C., 2009 Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. Journal of Bone and Mineral Research 24(2):274-282.
Colnot C., Zhang X., Knothe Tate M. L., 2012 Current insights on the regenerative potential of the periosteum: molecular, cellular, and endogenous engineering approaches. Journal of Orthopaedic Research 30(12):1869-1878.
De Souza L. E. B., Malta T. M., Haddad S. K., Covas D. T., 2016 Mesenchymal stem cells and pericytes: to what extent are they related? Stem Cells and Development 25(24):1843-1852.
Lekholm U., van Steenberghe D., Herrmann I., Bolender C., Folmer T., Gunne J., Henry P., Higuchi K., Laney W. R., 1994 Osseointegrated implants in the treatment of partially edentulous jaws. A prospective 5-year multicenter study. International Journal of Oral and Maxillofacial Implants 9(6):627-635.
Misch C. E., Strong J. T., Bidez M. W., 2008 Scientific rationale for dental implant design. In: Contemporary implant dentistry. 3rd edition. Misch C. E. (ed), Mosby Inc., Elsevier, pp. 200-232.
Pantor M., Rațiu C. A., Ciavoi G., Rațiu I. A., Maghiar A., Maghiar A. M., 2022 The hematogenous marrow tolerance when being in direct contact with the titanium implant. Acta Stomatologica Marisiensis 5(2):43-50.
Rao P. L., Gill A., 2012 Primary stability: the password of implant integration. Journal of Dental Implants 2(2):103-109.
Ratiu C. A., Ratiu I. A., Miclaus V., Pantor M., Rus V., Martonos C. O., Lacatus R., Purdoiu R. C., Gal A. F., 2022 The influence of haematogenous bone marrow on the early osseointegration of a titanium implant which penetrates the endosteum. International Journal of Morphology 40(1):188-193.
Smiler D., Soltan M., 2006 Bone marrow aspiration: technique, grafts, and reports. Implant Dentistry 15(3):229-235.
Soltan M., Smiler D., Prasad H. S., Rohrer M. D., 2007 Bone block allograft impregnated with bone marrow aspirate. Implant Dentistry 16(4):329-339.

Received: 19 November 2024. Accepted: 22 December 2024. Published online: 30 December 2024.
Authors:
Bianca Fernea, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: bianca-nicolasa.fernea@student.usamv-cluj.ro
Aurel Damian, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: aurel.damian@usamvcluj.ro
Cristian Martonos, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: CMartonos@Rossvet.edu.kn
Adina Florina Vidrean, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: adina.cioata@yahoo.com
Aurelia Coroian, Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: aurelia.coroian@usamvcluj.ro
Diana Mesaros, Control Systems Engineering Department, University of Oradea, Universităţii street, No. 1, Oradea, Romania, e-mail:dsas@uoradea.ro
Oana Andreea Pece, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania, e-mail: oanamastan@yahoo.com

How to cite this article:
Fernea B., Damian A., Martonoș C., Vidrean A. F., Coroian A., Mesaros D., Pece O. A., 2024 Hematogenous reaction of rabbit bone marrow to endosteal penetrating titanium screws. Rabbit Gen 14(1):41-46.