

The Influence of Haematogenous Bone Marrow on the Early Osseointegration of a Titanium Implant which Penetrates the Endosteum

La Influencia de la Médula Ósea Hematógena en la Osteointegración Temprana de un Implante de Titanio que Penetra en el Endostio

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SUMMARY: The utility of metallic bio-medical implants in osseous or dental affections is irrefutable. The paper aims to test the tolerance of the bone marrow to titanium implants. Titanium implants were inserted in the femur of 11-months old rabbits. The implants penetrated the endosteum, half of their length getting into the haematogenous bone marrow. Seven days after the insertion we collected bone fragments containing the implant. The CT exam revealed a significant decrease in the density of the bone at the interface with the implant and a more discrete one aloof from the insertion area. The histologic exam after 7 days revealed osseous reparatory processes only in the endosteal area from where it expanded on the surface of the implant which was inside the marrow. The presence and intensity of the osseous reparatory processes after only seven days post-implant demonstrates that the marrow actively participates in bone regeneration and implants osseointegration.

KEY WORDS: Osseointegration; Titanium implant; Endosteum, Haematogenous bone marrow.

INTRODUCTION

Ever since the notion of osseointegration was issued by Branemark, numerous researches were done which led to impressive progress in this field. In a relatively short period of time, a conclusion was drawn that titanium implants are a successful way to treat the patients partially or totally edentulous. This conclusion is based on numerous studies on experimental animals which investigated the phases of osseointegration in different types of titanium implants (Abrahamsson *et al.*, 2004). The proliferation of new bone around an implant depends on a great number of factors, some of which refer to the patient and others to the implant. The cells actively involved in the processes of osseous proliferation around the implants are the osteoblasts which are formed by the proliferation and differentiation of osteoprogenitor cells. The osteoprogenitor cells are cells of mesenchymal origin situated close to the osseous surfaces. Thus, they appear as disposed in the inner osteogenic layer of the periosteum and at the level of the endosteum (Gal & Miclaus, 2020).

At the bone level, there are growth factors that get involved in the synthesis of new bone tissue, acting upon the cells from the bone marrow and from the surface of the bone (Anitua, 2015). Increasingly, more researchers advocate the involvement of bone marrow in the healing of the tissues after injuries, bleeding or diseases. The *in vivo* transplant of adult stem cells in the bone marrow aspirate was successfully used to enhance osseous regeneration (Smiler & Soltan, 2007). The authors claim that the transplanted cells stimulate the proliferation of an initial unmineralized bone matrix (osteoid), and then initiate a mineralization process (Smiler, 1996). Some studies show that in order to reset the shape and the function of injured tissues, the autogenous bone grafts may be replaced with bone allografts combined with bone marrow aspirate (Smiler & Soltan, 2006; Soltan *et al.*, 2007). The bone marrow aspirate offers cells that can differentiate into bone cells and influence the tissues from the receiving and

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adjacent place, in the proliferation of new bone. Likewise, the bone marrow aspirate provides the necessary growth factors for both bone building and angiogenesis (Barry & Murphy, 2004; Zipori, 2004).

The aim of this study is to test the tolerability of bone marrow to a titanium implant that penetrates the periosteum and its degree of involvement in the process of early osseointegration of the implant.

MATERIAL AND METHOD

This experiment had the approval of the Ethics Committee of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania (approval no. 219/10.07.2020), in accordance with national (Law 43 of 2014) and European (EU Directive 63 of 2010) legislation. The experimental animals used in this study were 4 male rabbits 11 months old, weighing about 4.5 kg. The materials used were represented by titanium implants 5 mm long and 2 mm in diameter, which come under the recommendations of the literature (Wancket, 2015). We opted for this length for the implant to penetrate the periosteum of the femoral bone of the rabbit and to penetrate the medullar cavity with about half of its length. After the anaesthesia of the rabbits by intramuscular administration of xylazine 5 mg/kg + ketamine 40 mg/kg, we introduced a venous catheter in the external auricular vein for fluid therapy (5 ml/kg/h).

The preparation of the orifice for the insertion of the implants was made with a 1.8 mm drill, after which the implants were manually screwed. Postoperative, the animals were kept under anti-infectious protection with Enroxil 5 % (enrofloxacin) sc, 20 mg/kg, for 5 days, and Meloxicam, sc. 1 mg/Kg, for 3 days as an analgesic. After 7 days the animals were euthanized and the fragment containing the implant from the femur was gathered. The pieces were formalin-fixed 10 % for 7 days, then decalcified with trichloroacetic acid and included in paraffin. Sections 5 µm thick were made and colored with Goldner’s trichrome method. To examine the histological preparations we used an Olympus BX41 microscope fitted with an Olympus E-330 digital camera to catch the microscopic images.

For the computer tomography (CT) investigations, the bone segments were scanned with a CT scanner Siemens Somatom Scope 16 Slice. The scanning was done at an array window of 512X512, the thickness of the sections 1 mm, 130 kV, helical scanning, 0.45 spiral step with a 0.75 mm reconstruction of the sections.

RESULTS

CT Results. On the CT images the bone density was measured in different points of the bone relative to the implant at the interface, both right and left lateral from the implant and from distance (Fig. 1A). The bone density was also measured in the witness bone (Fig. 1B).

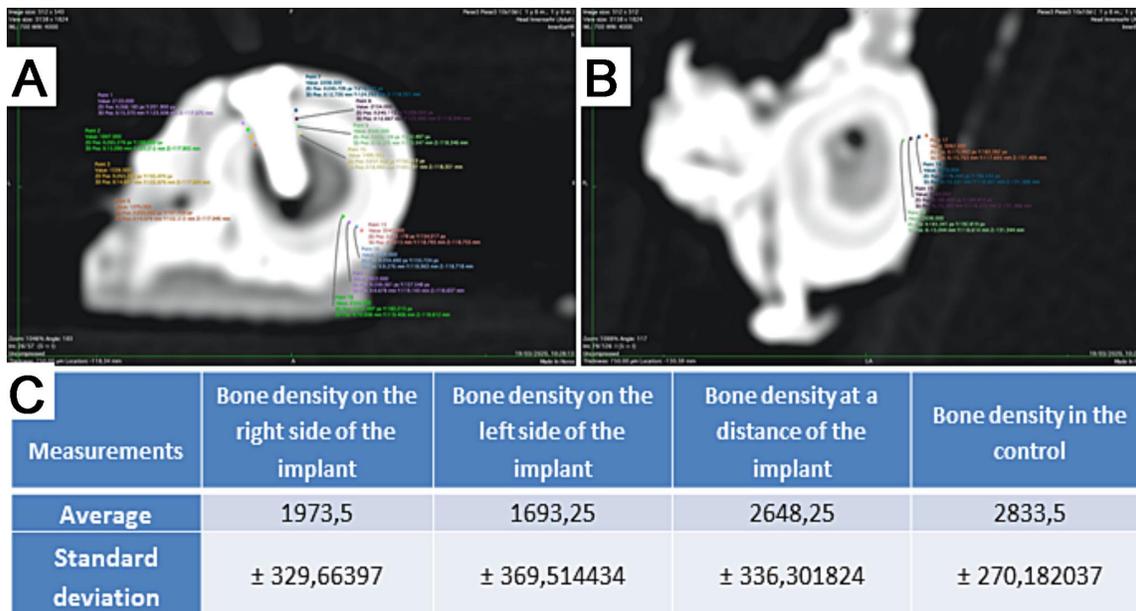


Fig. 1. A - CT image of the bone segment in which the titanium implant was inserted; B - CT image of the witness bone segment; C – Bone density in the intervention area as compared to the witness

The bone at the interface with the implant presented an average density of $1973,5 \pm 329,66$ HU (Hounsfield unit) on the right side of the implant and of $1693,25 \pm 369,51$ HU on the left side of the implant. The measurement of the density of the bone at distance from the interface revealed an average density of $2648,25 \pm 336,301$ HU. The average bone density of the witness bone was $2833,5 \pm 270,18$ HU (Fig. 1C). Note that the value of the bone density at the level of the measured areas decreases from the periosteum towards the endosteum.

Histological results. The histological sections include the whole intervention area so that they offer the possibility of assessing all the components that come in contact with the surface of the implant (Fig. 2A).

The suprapariosteal soft structures are in an active process of recovery following surgical trauma. Here and there, are relatively small areas occupied by fibrin networks that contain bone fragments left during the surgical intervention. The only reaction of the body to their presence is the osteoblastic mobilization needed for their gradual removal for the area to be invaded by newly formed structures.

In the periosteal area of the interface, we can notice only very discrete signs of bone proliferation can be highlighted, represented by the osteoid which can comprise here and there, some incipient sketches of trabeculae. The diaphyseal bone wall in direct contact with the implant presents a focal degeneration subsequent to the impingement on the zonal vascularisation at the moment of the surgical intervention. The spaces between the threads of the screw of the implant appear partially filled with fibrin networks which incorporate bone fragments from the milling and especially from the tapping (Fig. 2B).

On the central area of the bone-implant interface, seven days after the insertion of the implants, we cannot notice a certain beginning of bone reparatory processes. On the endosteal area of the interface, the situation is totally different as compared to the other areas. Here we can notice certain bone reparatory processes with a great tendency of expansion in three directions: towards the central area, lateral from the interface, and in the depth, covering a great part of the implant (Fig. 2C).

The fact that the bone reparatory processes started at the level of the endosteum is certified by the fact that the pitch of the screw in the endosteal area is filled with newly proliferated bone tissue (Fig. 2D). To support this information comes as well the presence of newly proliferated bone tissue in intimate contact with the endosteum and which expands laterally from the implant to a relatively great distance. From here, the reparatory processes gradually expand in-depth, so

that the next pitch contains both newly proliferated bone tissue and very young connective tissue. The next pitch contains some very young bone tissue with a discrete tendency of forming very thin osseous trabeculae together with the same connective tissue (Fig. 2E). The next pitch contains only that particular connective tissue formed by collagen fibres all of them oriented in the same direction through which the cellular component is well represented.

The bone tissue proliferated has the aspect of a very young trabecular bone formed by polymorph osseous trabeculae separated by wide areolar spaces. In these areolar spaces, there is a loose connective tissue that contains an impressive number of osteoblasts (Fig. 2F). Together with them, there is a small number of osteoclasts.

Through the young osseous trabeculae proliferated around the implant and the bone marrow there is a limited area occupied by very young loose connective tissue.

DISCUSSION

The CT investigations revealed certain aspects related to the correct positioning of the titanium implant into the osseous wall and the fact that it surpasses the thickness of the osseous wall by at least half of its length. In what concerns the density of the osseous tissue the measurement results showed the greatest decrease of the density in the bone at the interface of the implant and a certain decrease in density was noticed at a distance from the implant area.

The histological investigation confirmed that the body manifested a very good tolerance to the titanium implant this aspect being noticed at the level of both hard and soft tissues in the proximity of the implant. The osseous tissue on the bone-implant interface behaves differently in the first 7 days post-implant, depending on the area. At 7 days from the insertion of the implant, the involvement of the periosteum in the osseous reparatory processes is minimal, barely perceivable. Only incipient processes of osseous proliferation are noticed, manifested by the presence of osteoid and discrete trabecular structures which occupy very small areas in the close vicinity of the periosteum.

The central area of the bone-implant interface does not present, 7 days after the insertion of the implant, newly proliferated bone. On the endosteal part of the bone-implant interface, the osseous reparatory processes are very intense and already occupy an estimable area because the newly formed bone has already migrated in three directions, towards the surface of the implant (towards the central area of the bone

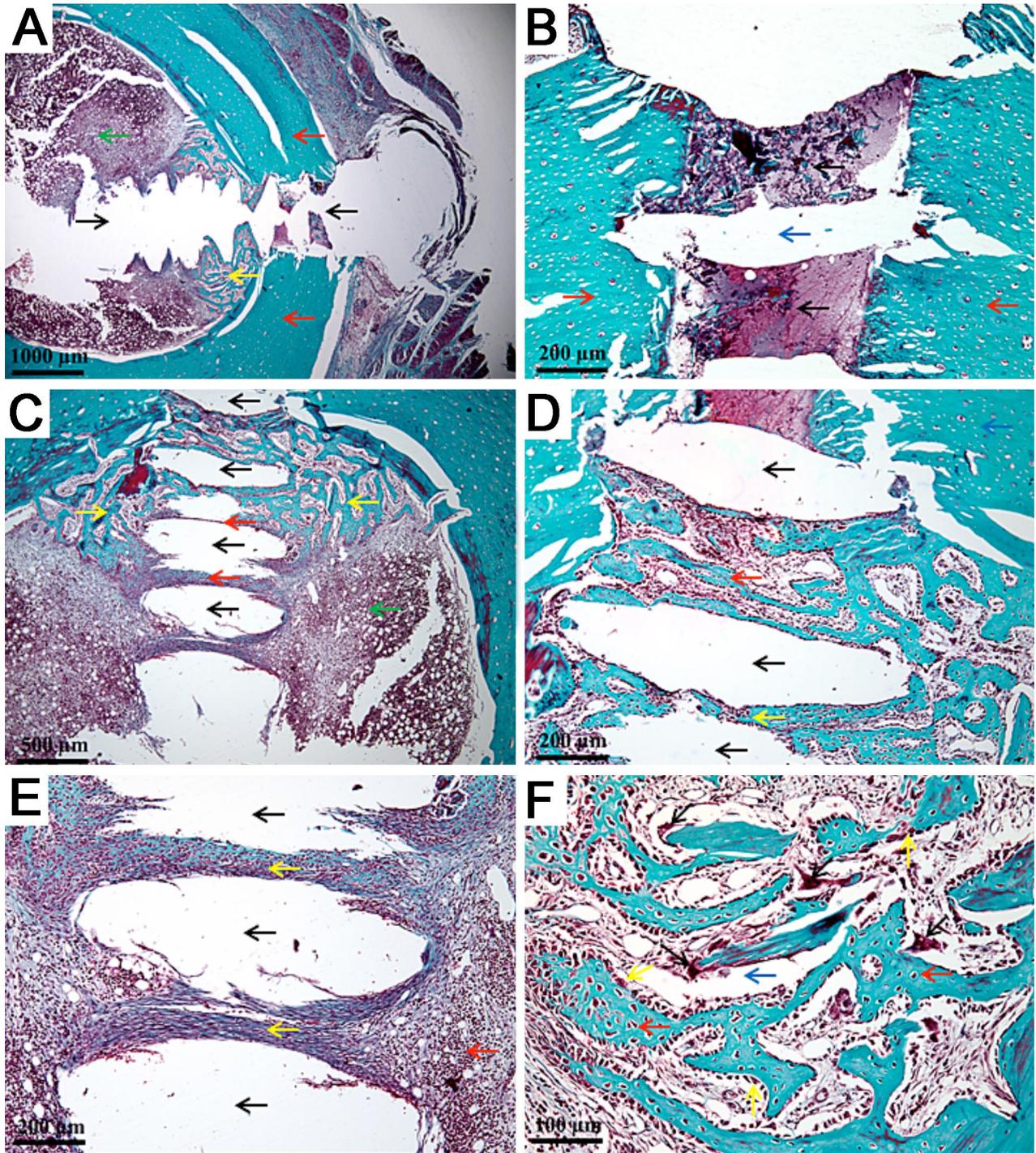


Fig. 2. Osseointegration stage after 7 days (Goldner's trichrome stain): A. Arrows: black – the place of the implant, red – osseous wall of the femur, yellow –spongious bone newly proliferated in the medullary cavity, green – bone marrow. B. Arrows: black – fibrin network containing bone fragments from the milling and tapping in the first two pitches of the screw; blue – the place of the screw; red – the osseous wall of the femur. C. Arrows: black – the place of the screw; red – materials proliferated in the pitches of the screw from the medullary cavity; yellow – newly proliferated spongious bone in the medullary cavity; green – bone marrow; D. Arrows: black – the place of the screw; red – areolar bone proliferated in the pitch of the screw next to the endosteum, yellow – areolar bone in the first pitch of the screw in the medullary cavity, blue – osseous wall of the femur; E. Arrows: black – the place of the screw, yellow – temporary connective tissue in pitches 2 and 3 of the screw in the medullary cavity, red – bone marrow; F. Arrows: red – osseous trabeculae with osteocytes, blue – areolae, yellow – osteoblasts, black - osteoclasts

defect), towards the interior of the medullary canal and on the medullary part of the endosteum on a quite large area around the bone defect. The presence of bone marrow in the proximity of the bone defect seems to have positive effects on osseous regeneration, most probably due to a rich vascularisation of the haematogenous bone marrow and the abundance of poorly differentiated cells (e.g. the perivascular pericytes, the mesenchymal stem cells). According to some studies, the osteogenic cell line can derive from perivascular pericytes (Schwarz *et al.*, 2007).

There are differences regarding the degree of maturation and consolidation of the proliferated materials along the part of the implant that penetrates the medullary cavity. The osseous material with the most advanced organisation is found in the pitch towards the endosteum which is totally occupied by immature cancellous bone. The next pitch is not more than 50 % filled with spongy bone the rest being connective tissue made of parallel arranged collagen fibres which seem to represent an early stage of bone-building. A similar structure was reported by other authors 4 days following implant insertion (Schwarz *et al.*). The next pitch is 30 % filled with very young bone (discrete trabecular structures), and the last pitch only contains the transitory connective tissue. Similar features were noticed by other authors as well, who concluded that bone remodelling started between the 4th and the 7th days (Schwarz *et al.*). Until the 4th day, they didn't notice direct contact between the screwed parts of the implant and the alveolar bone, the space left between the implant and the alveolar bone being filled with cellular debris and bone fragments resulted from the milling and tapping engulfed in a blood clot. After 4 days they noticed that the blood clot changes into a temporary connective tissue made up of regularly arranged collagen fibres (i.e., perpendicular to the surface of the implant). Other authors came to similar conclusions which also advocate that osteoblastic differentiation was initiated between the 1st and the 4th days in the newly formed connective tissue (Schwarz *et al.*). The fact that the process of bone-forming around the implant has already started during the first week after the insertion of the implant is advocated by other authors as well (Abrahamsson *et al.*). Some authors noticed minuscule areas of newly formed bone disposed between the alveolar bone and the surface of the implant one week after the insertion of the titanium implant in the mandible and maxillary on the dog. They found the newly formed bone has the tendency to organise itself in trabecular bone with wide areolae and lots of osteogenic cells (Schwarz *et al.*).

In our study, we found that by bone proliferation with the endosteal starting point, a new "osseous triangular structure" was formed. This "osseous triangular structure" offers secondary stability in the deep area of the implant. Some bioactive factors relieved by some cells in the haematogenous

marrow are likely to get involved. In an experiment on goats, 2 groups were used; in the first group individuals were inserted implants without growth factors while the second one got a thrombocyte concentrate (PRGF) in the created alveolus; at the same time before the insertion, the implant was introduced in PRGF. After 8 weeks, the implants were extracted together with the bone around them. With the implants inserted with PRGF, the bone remained attached to the whole length of the implant, while with the second group the bone got detached from the apical area. The conclusion was that the growth factors contained by PRGF stimulated the osseointegration of the titanium implants (Anitua, 2006).

For the reconstruction of some bone defects, the most proper method is the use of autogenous graft. Yet, the method has its limitations, such as the limited availability of tissues, the increased period of time and interventions especially in the case of reconstructions (Misch, 2011). Alternative methods were researched, from which the use of bone marrow aspirate was studied to fill the bone defects to stimulate the healing of fractures and to treat pseudoarthrosis (Lacerda *et al.*, 2009). Using a bone marrow aspirate for the treatment of bone defects on dogs, the improvement of bone formation was obtained (Niemeyer *et al.*, 2004). By using bone marrow aspirate inserted in collagen framing and tricalcium phosphate to reconstruct the bone defects, Niemeyer *et al.* noticed the differentiation of mesenchymal stem cells of the bone marrow in osteoprogenitor and osteoblast cells. In the intervention for the augmentation of the maxillary sinus, Sauerbier *et al.* (2010) used mesenchymal stem cells (MSC) collected from the bone marrow aspirate combined with particles of bovine osseous matrix and came to the conclusion that the method can offer a reliable foundation for dental implants.

In order to simulate the clinical situation of bone regeneration within the defects before the insertion of the implants, some authors inserted in the tibia of rabbit titanium implants 8.5 mm long and 3.75 mm in diameter. In the space created between the implant 3.75 mm in diameter and the decorticated bone 5 mm in diameter, they injected bone marrow centrifuge on the experimental group and blood clot on the witness group (Sauerbier *et al.*). Seven days after the surgery they could not notice clear regeneration processes, but after 21 days they found a significant bone formation and an interface contact with the surface of the implant. There was no difference in the bone repairing with or without bone marrow centrifuge. They concluded that for the correct regeneration of the bone in the defects around the implant the spontaneously formed blood clot is enough. They made out a case that the lack of statistical differences between the groups involved in the study may be related to the fact that the tibia does not have a medullary bone but a medullary canal (Sauerbier *et al.*). They didn't take into account the fact that inside the medullary ca-

nal of the rabbits' tibia there is haematogenous bone marrow. The implant they used was 8.5 mm long so that it came in direct contact with the marrow. The direct contact with the marrow decisively stimulated the osseointegration process on both groups aspect which led to the evenness of the results (Sauerbier *et al.*).

CONCLUSIONS

The titanium implant which penetrated the endosteum getting into the haematogenous bone marrow was well tolerated by both the tissues around the implant (periosteum, endosteum, bone tissue) and the haematogenous marrow. Seven days from the insertion of the implant we noticed osseous regeneration processes very discrete in the periosteal area, absent in the central part of the bone wall, and very active in the endosteal area. The newly proliferated bone at the level of the endosteum practically coats the surface of the implant which stands out in the medullary cavity-forming after 7 days a new osseous tissue with a triangular aspect which gives the implant a good stability. The bone proliferation reaction involves an impressive number of osteoblasts that originated from different sources like the osteoprogenitor cells of the endosteum, the perivascular pericytes in the marrow, the weakly differentiated cells in the marrow, proliferation which seems to be influenced by the growth factors produced in the haematogenous bone marrow as well. The involvement of the haematogenous marrow in the reparatory processes and in the process of early osseointegration of the titanium implants seems to be an important one, significantly speeding the osseointegration of the titanium implants in direct contact with it.

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RESUMEN: La utilidad de los implantes biomédicos metálicos en afecciones óseas o dentales es irrefutable. El documento tiene como objetivo probar la tolerancia de la médula ósea a los implantes de titanio. Se insertaron implantes de titanio en el fémur de conejos de 11 meses. Los implantes penetraron en el endostio y la mitad de su longitud penetró en la médula ósea hematogéna. Siete días después de la inserción, recolectamos fragmentos de hueso que contenían el implante. El examen de TC reveló una disminución significativa en la densidad del hueso en la interfaz con el implante y una más discreta alejada del área de inserción. El examen histológico a los 7 días reveló procesos de reparación ósea solo en el área endóstica desde donde se expandió en la superficie del implante que estaba dentro de la médula. La presencia e intensidad de los procesos de reparación ósea después de solo siete días del implante demuestra que la médula ósea participa activamente en la regeneración ósea y en la osteointegración de los implantes.

PALABRAS CLAVE: Osteointegración; Implante de titanio; Endostio; Médula ósea hematogéna.

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