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Reinforcing structures proliferated around a titanium implant inserted into the femur of female rabbits, in a hole smaller than the screw core

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SHORT COMMUNICATION

Abstract
One of the basic conditions for the clinical success of osseo-integrated implants is implant stability (Albrektsson and Zarb, 1993). It comprises two components, the primary stability that should be ensured at the time of insertion and the secondary stability, which gradually replaces the primary stability. The primary stability is ensured by the direct contact between the implant surface and that of the bone walls of the insertion hole. Secondary stability begins with the first position of new bone on the implant surface. The aim of the present study was to verify the reinforcing structures proliferated around the titanium implant inserted into the femur of 5 adult female rabbits, in a groove smaller than the screw core diameter. The results suggested that inserting the titanium implant into a hole with a smaller diameter than the screw core produces excessive pressure on the bone, which is felt up to a distance from the bone-implant interface. Consequently, mechanical strength of the bone is decreasing and proliferating bone consolidation formations appears on both periosteum and endosteum areas. Additionally, it was observed that newly proliferated bone extends laterally from the interface to a great distance, causing thickening of the bone wall. The observed structures were represented by branched bone trabeculae and bony protrusions into the medullary cavity, together with bone reshuffling processes with the appearance of numerous osteons, most of which are present at the level of the wall opposite the insertion area.
Keywords: titanium implant, bone proliferation, strengthening structures

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INTRODUCTION
One of the basic conditions for the clinical success of osseo-integrated implants is implant stability (Albrektsson and Zarb, 1993). It comprises two components, the primary stability that should be ensured at the time of insertion and the secondary stability which gradually replaces the primary stability. The primary stability is ensured by anchoring the implant to the bone, respectively the direct contact between the implant surface and that of the bone walls of the insertion hole. It should be noted that this bond is mechanical and not biological. Secondary stability begins with the first position of new bone on the implant surface, and its nature is biological. In other words, total stability is represented by primary stability that gradually decreases and secondary stability that gradually increases, to replace primary stability (Bosshardt et al. 2017). Secondary stability increases in proportion to the amount of new bone deposited both in contact with the implant surface and in the depth of the interface. There are also times when the

two processes do not synchronize completely, such as at 2-3 weeks when the loss of primary stability is evident and that of bone proliferation depends on several factors, so total stability is weakest at this point in the osseointegration process (Bosshardt et al. 2017). This is because the old (remaining) bone in the depth of the interface must first be reabsorbed to make room for the newly proliferated one, and this process is relatively slow. In order for the results obtained in animal experiments to be extrapolated as accurately as possible to humans, it should be taken into account that resorption of old bone in the vicinity of the implant begins at 1-2 weeks in animals (Berglundh et al., 2003), while in humans it begins only after 2 weeks (Bosshardt et al., 2011). The processes of bone proliferation continue with those of remodeling, which aim to gradually replace the rapidly proliferated primary bone on the interface with secondary bone (lamellar) with clearly superior resistance. The presence of remodeling processes is confirmed by the appearance of primary and secondary osteons, approximately 6 weeks in animals (Bosshardt et al. 2017). Bone remodeling is not only present in the bone proliferated around implants but is a process that takes place in all bones (Puleo and Nanci, 1999). Remodeling is present throughout life and proceeds faster in intrauterine life and slower after adult bone constitution. The goal of bone remodeling in intrauterine life is the transformation of primary bone into secondary bone (haversian or trabecular). The remodeling of adult bones aims to replace worn bone components with new haversian systems and bone trabeculae, whose architecture is adapted to the mechanical forces exerted on the bone (Diculescu and Onicescu, 1987; Martin, 2008).

The surgery required to insert an implant into the bone is traumatic and is followed by certain changes in the bone hole wall, which can be perceived under normal conditions up to 1 mm depth (Liddell and Davies, 2018). The diameter of the insertion hole must be calculated in such a way that the screw does not exert too much pressure on the bone wall. Furthermore, having adequate space between the implant and the host bone may be useful for early peri-implant bone formation (Futami et al., 2000; Berglundh et al., 2003; Franchi et al., 2004).

If the hole is too small, additional pressure is created on the bone that can amplify bone damage (Cha et al., 2015; Sasaki et al., 2015). Close contact between the implant surface and bone also occurs, which can cause poor bone proliferation (Futami et al., 2000) or even bone resorption (Zubery et al., 1999, Franchi et al., 2005).

The aim of this study was to verify the response of the bone around the intervention area through proliferation and remodeling processes, in reaction to the weakening of the bone strength following the insertion hole and the consequences resulting from the additional pressure resulting from its insertion into the hole with a diameter smaller than the screw core.

MATERIALS AND METHODS

The biological material used in this experiment was represented by 5 females of rabbits of common breed, aged one year and average weight of 4 kg. Orthopedic screw implants with a diameter of 2 mm and a core of 1.5 mm were inserted into the femur following the following protocol: anesthesia with xylazine 5 mg/kg + ketamine 40 mg/kg, grooming the intervention area, skin incision, muscle incision, highlighting the femoral bone, drilling a hole with a drill bit with a diameter of 1mm, inserting screws by self-tapping, suturing tissues in the intervention area.

Postoperative treatment consisted of an analgesic (Meloxicam, sc. 1 mg/kg, for 3 days) and preventive antibiotic therapy (Enroxil 5% sc, 20 mg/kg, for 5 days). At the end of the experimental period (6 weeks), the animals were euthanized and the intervention area was collected for histopathological examinations. The collected samples were introduced for fixation in 10% formalin for 7 days, after which they were decalcified with trichloracetic acid for 4 weeks and finally included in paraffin. Sections with a thickness of 5 micrometers were performed and after stained with the Goldner's trichrome method. For the examination of histopathological preparations, we used an Olympus BX41 microscope equipped with Olympus E-330 digital camera.

RESULTS AND DISCUSSIONS

After 6 weeks from the insertion of the screws was observed the presence of newly proliferated structures both at the level of the bone-implant interface and at a short and large distance from the interface. On the interface is present new proliferated bone in direct contact with the implant surface, but with differences in thickness and stage of organization, from one area to another. The thickest layer of newly proliferated bone is in the endosteum and periosteum areas of the interface, while on the area near the bone wall, the layer of newly proliferated bone is continuous but thin. The proliferated bone in the periosteum area extends outwards on the implant surface but also laterally from the interface in the form of a well-represented layer, up to a great distance. The one in the endosteum area also extends laterally from the interface but also inside the medullary cavity, where it forms either large trabeculae or polymorphic protrusions that leave the impression that they tend to expand further. Moreover, on the circumference of the bone there are differences in thickness from one area to another. In some areas the bone wall is thickened by periosteal and endosteal bone proliferation, but in other areas it is due to intense bone remodeling processes with the appearance of polymorphic and dense osteons, which sometimes occupy more than half the thickness of the bone wall. The greatest number of such osteons are present on the opposite side of the intervention area, where they can occupy up to 2/3 of the thickness of the bone wall.

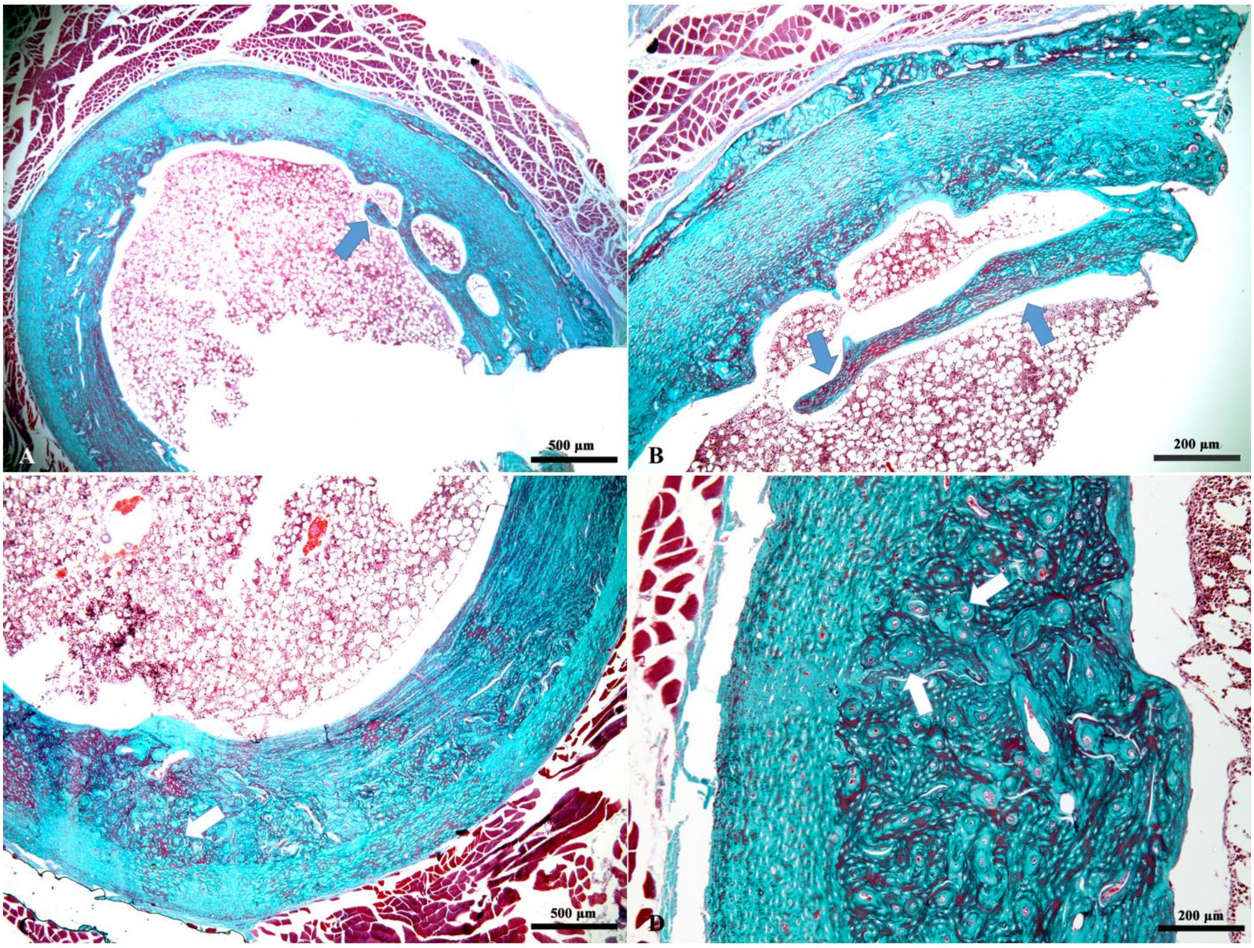

Figure 1. Newly proliferated bone structures (Goldner's trichrome staining); **A -** proliferation in the endosteum area that extends inside the medullary cavity, forming polymorphic protrusions that leave the impression that they tend to expand further (blue arrow) (4X magnification); **B** – large trabeculae in the medullary cavity (blue arrows) (10X magnification); **C -** intense bone remodeling processes with the appearance of polymorphic and dense osteons (white arrow) (4X magnification); **D -** polymorphic osteons that occupy more than half the thickness of the bone wall – detail (white arrows) (10X magnification).

6 weeks after implant insertion, it is largely clothed with newly proliferated bone tissue, arranged in direct contact with the implant surface. If the interface area next to the bone wall is covered only with a thin layer of newly proliferated tissues, the periosteal and endosteal areas are covered with a thick layer of newly formed bone extending across the interface surface. Its significant expansion on the interface, outwards in the periosteal area and inwards in the endosteal area, leads to a significant increase in the interface. This aspect has also been pointed out by other authors who have found that in some situations the interface surface area can even double, taking on the typical fan aspect (Pantor et al. 2022; Mark et al., 2022). The authors concluded that it was an adaptive reinforcement reaction in response to a significant decrease in the mechanical strength of the bone wall in the insertion area. The thick layer of newly proliferated bone in the periosteal and endosteal areas is continued laterally from the interface to a long distance, with the specification that its thickness decreases gradually as it moves away from the interface. These thick layers of newly proliferated bone ensure significant thickening of the bone wall, starting at the interface and up to a long distance from it. They contribute significantly to increasing the mechanical strength of the bone wall, weakened by the workmanship that accompanied the implant insertion process. Moreover, newly proliferated bone with endosteal starting point extends into the medullary cavity in the form of polymorphic trabeculae both in size and degree of organization. There are also bone protrusions of various shapes and sizes, projecting into the medullary cavity. After their appearance and structure, they give the impression that they have an obvious tendency to expand further, so that by growing them, new trabeculae can be created which, together with the existing ones, form a kind of trabecular scaffold anchored to the internal wall of the bone. These structures also contribute to increasing the mechanical strength of the bone around the intervention area, up to a great distance from the implant bone interface. An obvious thickening is present at the level of the bone wall opposite the implant insertion area, with obvious structural change affecting about 2/3 of the internal part of the

bone wall. The characteristic structural appearance for this area is dominated by the presence of a very large number of polymorphic osteomas, which proves that the thickening of the bone wall occurred as a result of intense bone remodeling processes. In other words, the bone wall opposite the intervention area fully felt the decrease in total bone strength and reacted to restore it, by thickening the bone wall but also by zonally reshuffling the bone to one with significantly higher mechanical resistance than that initially had by the bone in this area.

Structures for mechanical reinforcement of the area around titanium implants have been reported by other authors, with reference to those in the immediate vicinity of the interface, but also to the lateral extension to a certain distance of the proliferated bone in the periosteal and endosteal areas (Marcu et al., 2022; Raţiu et al., 2022). What we did not find reported in the consulted literature is the presence of newly proliferated bone formations with the appearance of trabeculae and protrusions in the medullary cavity. We consider that their appearance is given by the fact that the insertion of the implant into the hole smaller than the screw core was accompanied by pressures above a certain limit, which caused changes in the bone and weakening the bone strength up to a great distance from the intervention area. The appearance of these mechanical consolidation structures up to a distance from the implant insertion area represent adaptive structures capable of restoring the mechanical strength of the bone, both at the interface level and at a distance from it.

CONCLUSIONS

Inserting the titanium screw into the hole smaller than the screw core exerts excessive pressure on the bone, which is felt up to a distance from the bone-implant interface. One of the consequences is the decrease in the mechanical strength of the bone, so the body makes efforts to restore the resistance it had before the intervention, by proliferating bone consolidation formations. These formations are represented by newly proliferated bone on the periosteal and endosteal areas of the interface that greatly increase the area of the interface; newly proliferated bone extending laterally from the interface to a great distance in the periosteal and endosteal areas causing thickening of the bone wall. The proliferation of branched bone trabeculae and bony protrusions into the medullary cavity was observed, together with bone reshuffling processes with the appearance of numerous osteons, most of which are present at the level of the wall opposite the insertion area.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest

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