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Consolidation Structures Proliferated Around a Titanium Implant Implanted in the Female Rabbit Femur in an Orifice Smaller than the Screw Core

Las Estructuras de Consolidación Proliferaron Alrededor de un Implante de Titanio Colocado en el Fémur de un Conejo Hembra en un Orificio más Pequeño que el Núcleo del Tornillo

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SUMMARY: Orthopedic implants require effective osseointegration to function optimally and withstand weight-bearing and muscle forces. This study aimed to evaluate the process of osteogenesis induced by titanium screws implanted in the femur of female rabbits. Five domestic female rabbits were divided into two groups. Titanium screws were inserted into the femur diaphysis through drilled orifices smaller than the screw core. Specifically, a 1-mm hole was drilled, followed by the insertion of 2-mm self-tapping titanium screws using a screwdriver. After duration of six weeks, the animals were humanely euthanized, and histological and morphometric analyses were conducted. Histological examination revealed that the area adjacent to the bone wall was covered by a thin layer of newly formed bone tissue. In contrast, the periosteal and endosteal regions exhibited a thick layer of newly formed bone extending over the interface surface. This significant bone growth progressed outward in the periosteal area and inward in the endosteal area, leading to a remarkable expansion of the interface. Similar findings have been reported by other authors, who noted that the interface surface can even double in some instances, resulting in a fan-like appearance. The insertion of the titanium screw into a hole smaller than its core exerts excessive pressure on the bone, affecting the entire bone-implant interface. One consequence of this pressure is a decrease in the mechanical strength of the bone. In response, the body attempts to restore the preoperative strength by proliferating bone-strengthening formations. This newly formed bone extends laterally from the interface, significantly contributing to the engorgement of the bone wall in both the periosteal and endosteal regions.

KEY WORDS: Bone proliferation; Consolidation; Titanium implant.

INTRODUCTION

One of the factors in the success of osseointegrated implants is its stability (Albrektsson & Zarb, 1993). This stability consists of two main components: primary stability, which is essential upon implant insertion, and secondary stability, which gradually takes over from primary stability. Primary stability is achieved by anchoring the implant in the bone through direct contact between the implant surface and the bone walls of the insertion hole. It is important to note that this connection is mechanical rather than biological. Secondary stability starts with new bone making initial contact with the implant surface and is a biological process. In summary, overall stability involves a gradual decrease in primary stability and a gradual increase in secondary stability, with secondary stability replacing primary stability over time (Bosshardt *et al*., 2017).

The amount of new bone deposited around the implant and within the interface determines the increase in secondary stability. Sometimes, bone deposition and implant stability processes are not completely synchronized. For

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example, around 2-3 weeks after the initial procedure, there is a noticeable decrease in primary stability, and the rate of bone growth depends on various factors. Overall stability is at its weakest during this phase of the osseointegration process (Bosshard *et al*., 2017).

The process is slow because the bone remaining in the implant area needs to be resorbed before new bone can grow. In animal experiments, it's important to consider that in animals, the old bone near the implant starts to break down 1-2 weeks after the implantation. This information is relevant for accurately applying results from animal studies to humans (Berglundh *et al*., 2003). In humans, bone proliferation starts only after 2 weeks (Bosshardt *et al*., 2017). The bone proliferation processes are followed by remodeling, which gradually replaces the rapidly proliferated primary bone with the stronger secondary (lamellar) bone at the interface. The presence of remodeling processes is indicated by the appearance of primary and secondary osteons at about 6 weeks in animals (Bosshardt *et al*., 2017).

Bone remodeling is a process that occurs in all bones, including those surrounding implants (Puleo & Nanci, 1999). This process continues throughout life, with faster remodeling during intrauterine life and slower remodeling in adulthood. During intrauterine life, the goal of bone remodeling is to transform primary bone into secondary (haversian or trabecular) bone. In adulthood, bone remodeling aims to replace old bone components with new haversian and trabecular bone systems, adapting their architecture to the mechanical forces applied to the bone (Diculescu & Onicescu, 1987; Martin *et al*., 2008).

When an implant is inserted into the bone, the surgery can cause changes in the bone opening wall, which can be felt up to 1 mm deep under normal conditions (Liddell & Davies, 2018). It is important to calculate the diameter of the insertion hole so that the screw doesn't exert too much pressure on the bone wall. Additionally, leaving adequate space between the implant and the host bone may promote early peri-implant bone formation (Futami *et al*., 2000; Berglundh *et al*., 2003; Franchi *et al*., 2004).

If the orifice is too small, it creates additional pressure on the bone, which can amplify the lesions (Cha *et al*., 2015; Sasaki *et al*., 2015). Furthermore, close contact between the implant surface and the bone may result in poor bone proliferation (Futami *et al*., 2000) or even bone resorption (Zubery *et al*., 1999; Franchi *et al*., 2005).

This study aims to verify the bone's response around the intervention area through proliferation and remodeling processes, as a reaction to the weakening of bone strength caused by the insertion hole and the consequences resulting from the additional pressure due to insertion into a hole with a diameter smaller than the screw core.

MATERIAL AND METHOD

The test animals were five one-year-old female domestic rabbits weighing on average 4 kg. This experiment was performed with the consent of the institutional bioethics committee (Decision No. 289/390 of 03.06.2023). It was performed in accordance with the national legislation No. 215 of 2004. The rabbits had 2 mm diameter titanium screws inserted into a 1 mm hole. All screws inserted by self-tapping were titanium screws of 2 mm diameter and 5 mm length. Rabbits were anesthetized with a mixture of xylazine (Xylazin Bio 2%, 5 mg/kg, Czech Republic) and ketamine (Ketamidor, 50 mg/kg, Austria). For analgesia, buprenorphine (Buprecare, 0.05 mg/kg IM, United Kingdom) was used.

The surgical area was aseptically prepared and draped for surgery. A lateral femoral approach to the femur was performed and the femoral diaphysis was exposed. For the experimental model, a 1-mm hole was drilled in the femur diaphysis, and then 2-mm self-tapping titanium screws were inserted using a screwdriver into the drilled holes. The fascia lata was sutured with Monocryl 3-0 sutures in a continuous pattern, and the skin was sutured with Vicryl 3-0 sutures in an intradermal pattern. Meloxicam (1 mg/kg, subcutaneous) for 3 days and enrofloxacin (5-10 mg/kg, subcutaneous) for 5 days were administered postoperatively.

After 6 weeks postoperative, the animals were humanely euthanized and femoral diaphysis of each animal was prepared by cutting the femur at ~3 cm away from the implants. The samples were fixed in formalin 10% for 7 days, decalcified with trichloroacetic acid 7% and embedded in paraffin. Sections of 5 mm were obtained and stained using Goldner's trichrome method. Histological analysis was performed using an Olympus BX41 microscope and a digital camera Olympus E 330 (Fig. 1).

Morphometric assessments using ToupView software were performed to quantify the new proliferated bone at the interface level.

Statistical analysis. The results were expressed as means ± standard deviations (STD-P) and were analyzed by using Microsoft Office Professional Plus Excel 2016. Comparisons between groups were assessed by using Student's paired t-Test, with a two-tailed distribution. Significance was established at $p \leq 0.005$.

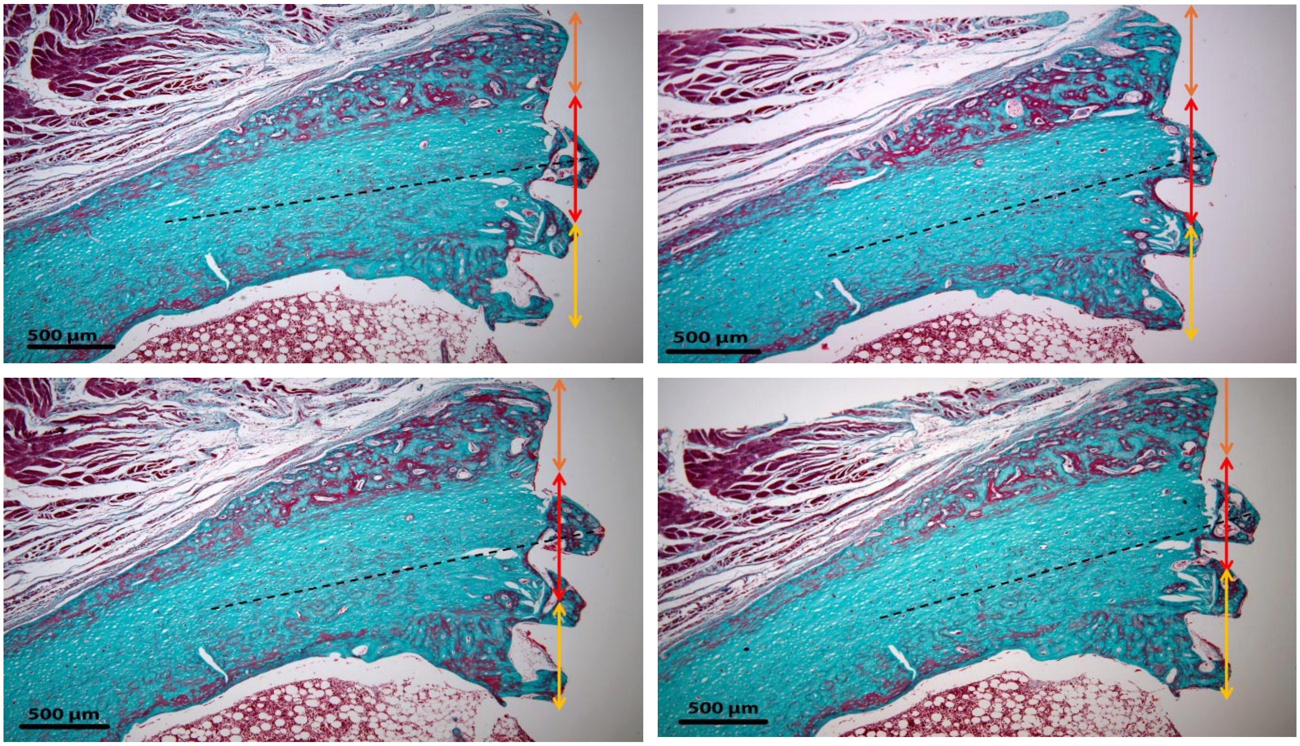

Fig. 1. The interface aspects in the female rabbit experimental models. Orange arrow - new proliferated bone in periosteal region; red arrow - compact bone; yellow arrow - new proliferated bone in endosteal region; blue intermittent line - total interface depth at 2000µm.

RESULTS

All the animals survived the surgery and recovered from anesthesia within 35–40 minutes. They returned to normal behavior and ate within 36 hours after the surgery. No complications were recorded during or after the surgery. The wound healed without issues, and the skin sutures were removed nine days after the surgery.

Six weeks after the insertion of the screw, researchers observed the presence of newly proliferated bone structures at the bone-implant interface. These structures were found at both short and long distances from the interface. The new bone was in direct contact with the implant surface, but there were variations in thickness and organization at different areas of the interface. The thickest layer of newly proliferated bone was found in the endosteal and periosteal regions of the interface. On the other hand, in the area to the right of the bone wall, the layers of newly proliferated bone were continuous but thin.

The bone around the implant has grown outward on the surface and extends a significant distance. Inside the bone, there are large structures that give the impression of further growth. The thickness of the bone varies around the implant. In some areas, the bone thickens due to growth from the outer and inner surfaces, while in other regions it thickens due to intense remodeling processes, forming dense structures that occupy a significant portion of the bone. The preponderance of these structures is situated on the opposing side of the surgical site. It is noteworthy that they can occupy a significant portion of the bone thickness, potentially extending to up to two-thirds of its total depth (Fig. 2).

In this study, we evaluated the bone proliferation in periosteal, osteal and endosteal regions relative to the total bone interface. Our analysis revealed statistically significant differences in both the osteal and endosteal bone proliferation metrics, indicating a noteworthy impact on the overall bone architecture (Table I). However, it is

Table I. Statistical relevance: Differences were considered statistically significant if $p \leq 0.005$.

	P values (1500 µm)	P values (1500 µm)	P values (2000 µm)
Periosteal	0.26	0.38	0.002
Osteal	0.001	0.0001	0.0004
Endosteal	0.0002	0.0001	0.001

important to note that no statistically significant differences were observed in the periosteal area. This suggests that while osteal and endosteal regions exhibit distinct proliferative responses, the periosteal area does not contribute to variations in bone proliferation in the same manner.

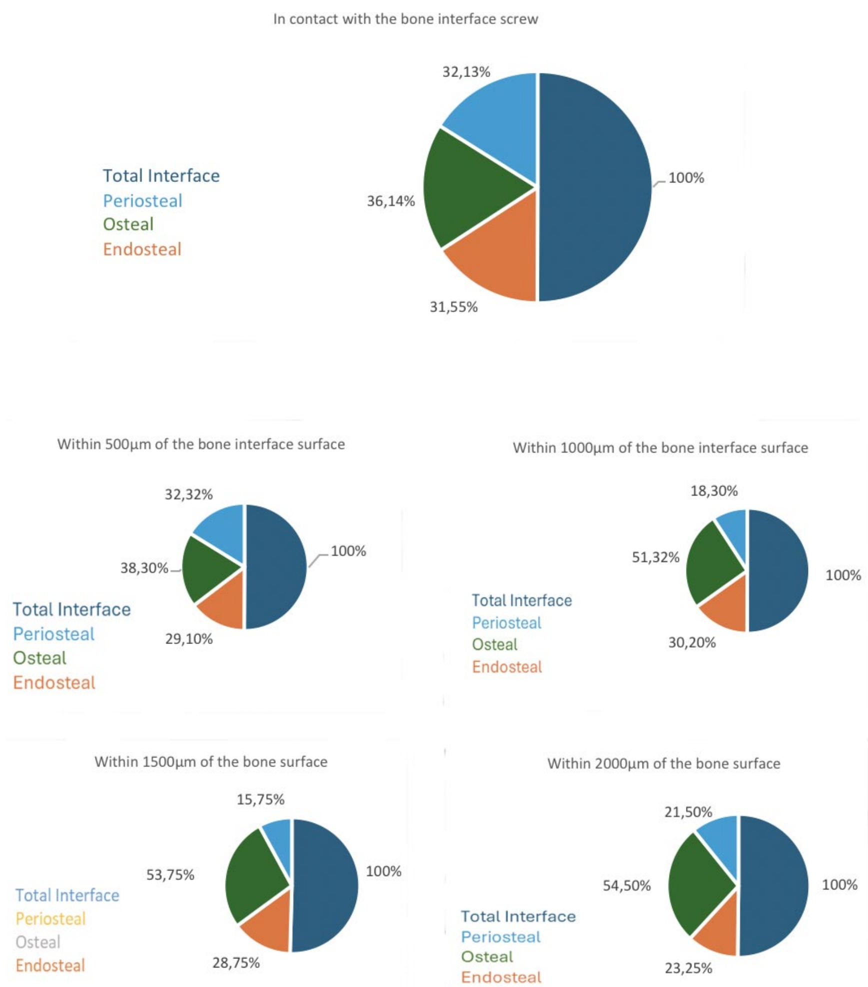

Fig. 2. Percentage expression of proliferated bone on the interface at 500 µm, 1000 µm, 1500 µm and 2000 µm, respectively.

DISCUSSION

Six weeks after the implant was placed, it has become mostly encased in newly formed bone tissue – directly in contact with the implant surface. The area in front of the bone wall is covered by a thin layer of new bone-grown tissue, while the periosteal and endosteal regions boast a thick layer of newly formed bone that extends over the interface surface. This significant growth extends outward in the periosteal area and inward in the endosteal area, resulting in a remarkable expansion of the interface. Interestingly, other authors have also observed similar phenomena, where the interface surface may even double in some cases, creating a fan-like appearance (Pantor *et al*., 2022a,b; Marcu *et al*., 2022).

The authors concluded that this is an adaptive consolidation reaction in response to a significant decrease in the mechanical strength of the bone wall at the insertion site. The thick layer of new bone proliferated in the periosteal and endosteal areas continues laterally from the interface to a great distance, with the caveat that its thickness decreases stepwise as it moves away from the interface. These thick layers of newly proliferated bone provide a significant thickening of the bone wall from the interface to the far distance from the interface. They contribute significantly to increasing the mechanical strength of the bone wall, which has been weakened by the maneuvers that preceded the implant insertion process.

Moreover, the newly proliferated bone with an endosteal starting point extends into the medullary cavity in the form of trabeculae polymorphic in size and degree of organization. There are also bony prominences of different shapes and sizes that project into the medullary cavity. From their appearance and structure, they give the impression that they have a clear tendency to further enlargement, so that new trabeculae can grow and, together with the existing ones, form a kind of trabecular scaffold anchored to the internal wall of the bone.

The structures around the surgical site increase the mechanical strength of the bone, even at a distance from the implant. There is a noticeable thickening in the bone wall opposite the implant insertion site, with structural changes affecting about two-thirds of the inner part of the bone wall. This area is characterized by the presence of many polymorphic osteons, indicating intense bone remodeling and thickening of the bone wall. In summary, the bone wall opposite the intervention area has experienced a decrease in overall strength, leading to thickening and remodeling to restore its mechanical strength, resulting in significantly stronger bone in that area.

According to some authors, there have been reports on the structures that form around titanium implants, close to the implant surface as well as extending some distance into the surrounding bone areas (Marcu *et al*., 2022; Ra?iu *et al*., 2022). However, we could not find any literature that mentions the presence of newly formed bone structures, such as trabeculae and protrusions, within the medullary cavity. We believe these structures formed due to the implant being inserted into a hole smaller than the screw core, which caused excess pressure and resulted in changes to the bone strength beyond the immediate implant site. The appearance of these new bone structures at a distance from the implant site can be seen as adaptive structures that help restore bone strength, both near the implant and further away.

CONCLUSIONS

Insertion of the titanium screw into a hole smaller than the core of the screw exerts excessive pressures on the bone, which are felt as far as the bone-implant interface. One of the consequences is a decrease in the mechanical strength of the bone so that the body strives to restore the strength it had before the operation by proliferating bone-strengthening formations. These formations are represented by newly proliferated bone in the periosteal and endosteal areas of the interface which greatly increase the surface area of the interface; newly proliferated bone extending laterally from the interface to a great distance in the periosteal and endosteal region causes the bone wall to become engorged; proliferation of branched bone trabeculae and bone protrusions in the medullary cavity; bone remodeling processes with the appearance of numerous osteomas, most of which are present in the wall opposite the insertion site.

SABOU, I.; GHERMAN (DRAGOMIR) M. F.; OBER, C.; MICLAUS, V.; RATIU, C.; OROS, N.; ALEXANDRU, B. & OANA, L. Las estructuras de consolidación proliferaron alrededor de un implante de titanio implantado en el fémur de una coneja hembra en un orificio más pequeño que el núcleo del tornillo. *Int. J. Morphol., 43(2)*:600-605, 2025.

RESUMEN: Los implantes ortopédicos requieren una osteointegración eficaz para funcionar de manera óptima y soportar el peso y las fuerzas musculares. Este estudio tuvo como objetivo evaluar el proceso de osteogénesis inducido por tornillos de titanio implantados en el fémur de conejas hembras. Se dividieron cinco conejas domésticas en dos grupos. Los tornillos de titanio se insertaron en la diáfisis del fémur a través de orificios perforados más pequeños que el núcleo del tornillo. En concreto, se realizó un orificio de 1 mm y, a continuación, se insertaron tornillos autorroscantes de titanio de 2 mm con un destornillador. Después de seis semanas, se sacrificó a los animales y se realizaron análisis histológicos y morfométricos. El examen histológico reveló que la zona adyacente a la pared ósea estaba cubierta por una fina capa de tejido óseo neoformado. Por el contrario, las regiones perióstica y endóstica presentaban una gruesa capa de hueso de nueva formación que se extendía sobre la superficie de la interfaz. Este importante crecimiento óseo progresó hacia fuera en la zona perióstica y hacia dentro en la zona endóstica, lo que dio lugar a una notable expansión de la interfaz. Otros autores han publicado hallazgos similares, que observaron que la superficie de la interfaz puede incluso duplicarse en algunos casos, lo que da lugar a una apariencia de abanico.La inserción del tornillo de titanio en un orificio más pequeño que su núcleo ejerce una presión excesiva sobre el hueso, lo que afecta a toda la interfaz hueso-implante. Una consecuencia de esta presión es una disminución de la resistencia mecánica del hueso. En respuesta, el cuerpo intenta restaurar la fuerza preoperatoria mediante la proliferación de formaciones que fortalezcan los huesos. Este hueso recién formado se extiende lateralmente desde la interfaz, lo que contribuye significativamente a la congestión de la pared ósea tanto en la región perióstica como en la endóstica.

PALABRAS CLAVE: Proliferación ósea; Consolidación; Implante de titanio.

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