JOURNAL OF OSSEOINTEGRATION
periodontics and prosthodontics

Adaptive structures proliferated in the rabbit shoulder after 8 weeks from the insertion of a titanium implant

T. MARCU[1], A. F. GAL[2], C. A. RAȚIU[3], A. DAMIAN[2], I. A. RATIU[3]

[1]University of Medicine and Pharmacy "Carol Davila" Bucharest, Faculty of Dental Medicine, Bucharest, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
[3]University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania

TO CITE THIS ARTICLE
Marcu T, Gal AF, Rațiu CA, Damian A, Ratiu IA. Adaptive structures proliferated in the rabbit shoulder after 8 weeks from the insertion of a titanium implant. J Osseointegr 2022;14(3):180-184.

DOI 10.23805/JO.2022.14.24

KEYWORDS Implant, Bone-implant interface, Adaptive structures.

ABSTRACT

Aim A special place in the research related to implantology is held by those who study the process of osseointegration of implants. The proper development of the osseointegration process is crucial for the survival rate of the implant. The chances of long-term survival are related to the structure of the bone in which the implant is inserted.

Materials and methods In three 10-month-old male rabbits, self-tapping titanium implants with a diameter of 2 mm were inserted in the femoral shaft. After 8 weeks, the rabbits were stuk and the fragment of the femur containing the implant was collected. The collected pieces were fixed in Stieve mixture, embedded in paraffin, and the 5 µm sections obtained were stained with the Goldner trichrome method.

Results The microscopic examination revealed that the bone proliferated around the implant had two starting points, the periosteum and the endosteum. The newly proliferated bone from the periosteal level gradually extends to the middle area of the interface, outward over a certain distance and subperiosteal over another distance. The proliferated bone at the endosteal level extends to the middle area of the interface, towards the medullary canal to a certain depth, but also subendosteal to a certain distance. Moreover, the newly proliferated bone shows a clear tendency to reshape into Haversian bone, at a clearly higher level above the existing structure in the bone distant from the implantation area. This aspect shows the tendency of a bone with greater strength than the rest of the bone to form around the implant.

Conclusions The increase of the contact surface at the bone-implant interface as well as the proliferation of a bone with high resistance are the result of an adaptive reaction to restore the resistance of the area, weakened following the trauma caused by the insertion of the implant.

INTRODUCTION

Although modern dental implantology is less than 50 years old, there are permanent and persistent concerns for obtaining implants with the best possible qualities to improve the practical activity in this new yet important field for humanity (1). Researches in this field have brought a lot of information about the design of implants, the materials they are made of and their insertion technique. Special concerns in this regard still exist today and will exist for many more years (2). A special place in the research related to implantology is held by those who study the process of osseointegration of implants. The proper development of the osseointegration process is crucial for the survival rate of the implant. The chances of long-term survival are related to the structure of the bone in which the implant is inserted.

In a dense bone, the development of the osseointegration process can be estimated, but in low-quality bone, it is difficult to estimate because primary stability is debatable (3). Comparing the primary stability of implants in different bone qualities (4), statistically higher ISQ values were found for implants inserted into type II bone with 1 mm cortical bone. Supporting the same idea, some authors claim that from their point of view bone quality is more important for primary stability than the implant design (5). In a study on 85 patients, Turkyilmaz and McGlumphy (6) found a direct and significant correlation between bone density and dental implant stability. Miyamoto et al. (7), who argue that the stability of the dental implant is positively associated with cortical thickness, reported similar results. Moreover, some authors claim that the proliferated bone around the implant is largely related to the bone in which the implant is inserted. They claim that the bone proliferated around the implant has superior quality in the mandible compared to the maxilla (8, 9). There are differences between the maxilla and mandible regarding bone supply, which makes the survival rate higher in the case of implants inserted in the mandible as compared to those in the maxilla (10, 11). The appearance is more pronounced if we refer to the posterior maxilla where the cortical bone is thinner than in the mandible, and the trabecular bone thicker (12, 13).

Some researchers claim that during the osseointegration process, the more resistant the bone, the better the ability to prevent the proliferation of fibrous tissues on the bone-implant interface (14, 15). Many researchers evaluate the bone's quality and strength mainly by mineral density (16, 17), but others argue that other factors such as bone metabolism, cell activity, intercellular matrix, vascularization a.s.o. are to be taken into consideration (18). Given that the testing of implant osseointegration is mostly done on experimental animals, we decided to check the bone structures proliferated around a titanium implant inserted in the rabbit femur which surpasses its length by half, the thickness of the osseous wall. We also decided to check the surface occupied by the newly proliferated bone in direct contact with the surface of the implant ant the type of the herein proliferated bone.

MATERIAL AND METHODS

The study complied with the Declaration of Helsinki and was approved by the Bioethical Committee of USAMV (University of Agricultural Sciences and Veterinary Medicine, Bucharest, Romania), nr. 219/10.07.2020 according to national (Law nr. 43/2014) and European (E.U. Directive nr.63/2010) regulations.

The biological material used in this study was three 10-month-old male rabbits with an average weight of approximately 4 kg. Titanium implants with a diameter of 2 mm and a length of 5 mm were inserted in the rabbit femur by self-tapping. We opted for these dimensions to fit into the existing recommendations in the literature regarding the adaptation of the implant dimensions to the size of the experimental animal used. Data from the literature show that in rabbits the size of the implants used is limited to a maximum of 2 mm in diameter and 6 mm in length (19, 20). We chose this thickness in order not to surpass the maximum admitted thickness, and this length for the implant to penetrate the marrow duct by about half of the length in order for it to come in direct contact with the bone marrow.

The animals were sacrificed after 8 weeks and the area with the implant was harvested for histological investigations. After having been fixed for three days in a Stieve mixture, the pieces were dehydrated with ethyl alcohol, clarified with butyl alcohol (1-Butanol), and embedded in paraffin. Sections with a thickness of 5 µm were stained with the Goldner trichrome method and examined on microscope (Olympus BX41), equipped with a digital camera for capturing histological images. The processing of histological images was done with Adobe Photoshop 2020 software.

RESULTS AND DISCUSSION

The histological processing allowed intact preservation of the structures of the host, both those on the bone-implant interface and those on the implant-supraperiosteal tissue and implant-bone marrow interface. The lack of any rejection reaction certified the fact that the implant was well tolerated by the body, ensuring very good conditions for its osseointegration (Fig. 1). The aspect is illustrated first of all by the fact that on the bone-implant interface, bone tissue has proliferated directly, which, although not yet completely remodelled to the secondary bone, is in an advanced phase of remodelling and consolidation. The completion of the osseointegration process requires a certain amount of time, bone proliferation, and reshaping being processes that evolve slowly over several months. At this point in the experiment, the implant is intimately covered with bone tissue on the entire bone-implant interface, without the interposition of other tissues (fibrous, cartilaginous) even on limited areas. The type of bone at the bone-implant interface is different depending on the area, being in a more advanced stage of proliferation and reshaping in the periosteal and endosteal areas as compared to the central area.

In case of accidental or experimental bone defects, the repair processes have two starting points, namely the periosteum and the endosteum, because we can find here cells with the potential for proliferation and differentiation to osteoblasts, which synthesize bone matrix. In the case of osseointegration of implants, from these two starting points, the repair processes gradually expand to occupy step by step the entire interface. The investigation carried out by us shows that the speed at which the repair processes take place in the two starting points is not identical, there being relatively large differences 8 weeks after the insertion of the implants. More precisely, the repair processes with endosteal starting point have clearly outperformed those with periosteal starting point, although they are also very active. The proliferated bone at the two points extends practically in three directions each. The periosteal level extends on the surface of the implant both to depth and to the surface, but also subperiosteal over a relatively long distance (Fig. 2). The extension to the surface and the subperiosteal one determines the significant thickening of the upper half of the bone-implant interface. The proliferated bone at the endosteal level extends to the inside of the interface, to the medullary and subendosteal canal, causing the obvious thickening of the deep half of the interface (Fig. 3). This bone proliferation causes the implant to be coated with

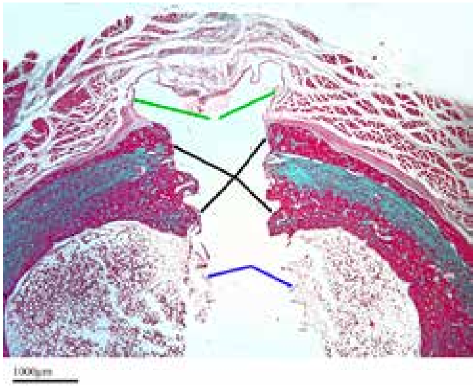

FIG. 1 Implant insertion area: black arrow, bone-implant interface; green arrow, implant interface - supraperiosteal soft tissues; blue arrow, implant interface - bone marrow.

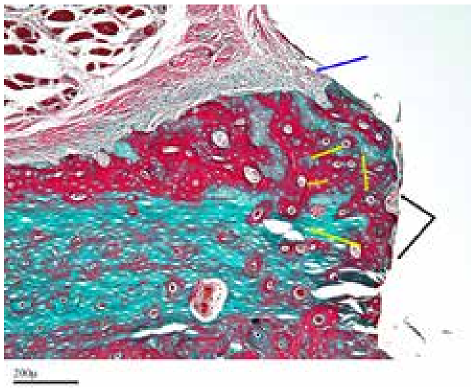

FIG. 2 Bone-implant interface, periosteal half: black arrow - bone-implant interface; yellow arrow - polymorphic Haversian systems; blue arrow – periosteum.

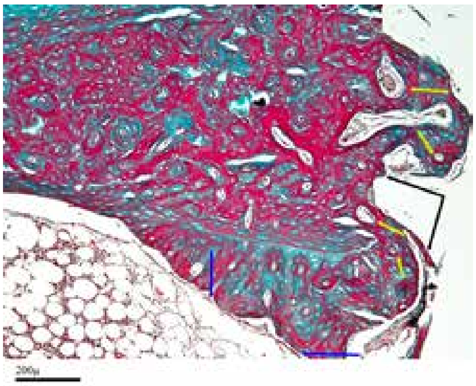

FIG. 3 Bone-implant interface, half endosteal: black arrow - bone-implant interface; yellow arrow - polymorphic Haversian systems; blue arrow - the endosteum.

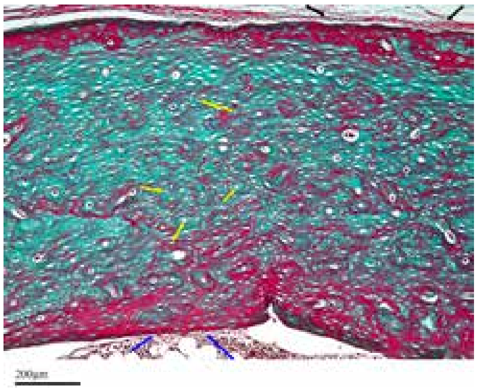

FIG. 4 Diaphyseal bone distant from the implantation area: black arrow - periosteum; yellow arrow - small and rare Haversian systems; blue arrow - the endosteum.

a newly formed bone on a surface much larger than the initial thickness of the bone in which the implant was inserted. The extension on the surface of the implant both to the surface and in depth makes the interface gradually take on the appearance of a fan, which ensures a very efficient coating of the implant with newly formed bone, on a surface significantly larger than the thickness of the osseous wall.

The extension of the proliferated bone to the large surface bone-implant interface shows the body's special tolerance to the titanium implant, but at the same time suggests that titanium seems to have a stimulating effect on bone proliferation during osseointegration. The aspect is maintained by the fact that the newly proliferated bone migrated on the surface of the implant covering a large part of the area which surpasses the thickness of the diaphysis wall and there is a tendency towards further expanding. There is also a possibility for the implant area which surpasses the thickness of the diaphysis bone to be gradually coated on the whole surface of the newly formed bone, but further investigations are required in order to clarify this aspect.

The proliferated bone around the implant gradually goes into reshaping processes towards the secondary, lamellar bone, which ensures a superior resistance to the area. The process is demonstrated by the emergence of Haversian systems in increasing numbers, most in the endosteal area of the interface, but also in the periosteal. It should be noted that both the density of the Haversian systems and their size are significantly higher than in the bone at a certain distance from the place of intervention. The processes of reshaping towards the secondary bone are still far from being completed, but there is a clear tendency to form around the implant a bone with a much higher resistance than that of the bone in which the implant was inserted (Fig. 4).

More than 40 years ago, some researchers asserted that a direct contact between the implant and the bone is only possible if the implant is made of ceramics not of metal (21). The successful use of metallic implants was introduced by the School of Goteborg led by Brånemark, who experimented a titanium implant and defined the term osseointegration (22).

The direct proliferation of osseous tissue in direct contact with the titanium implant was deeply researched. By experimenting a titanium implant inserted in the proximity of the knee joint on dog and humans, some authors observed a direct bone- implant contact along the entire periphery of the implant (23). Furthermore, some researchers studied the bone-implant interface on the electronic microscope and also found that the newly formed bone coats the entire surface of the implant. They suggested that it might exist certain direct chemical bonds between the bone and the titanium (24). The same coating of the entire surface of the titanium implant by the newly formed bone was noted in studies on rats, with the remark that between 1.5-2.5 months it has the characteristics of a cancellous bone, and after three months it has the morphological characteristic of a compact bone (25). After studies involving titanium implants inserted in the femoral and tibial diaphysis on sheep, Franchi and colleagues (26) noted that after three months from the implantation they were almost completely covered in compact bone. The same proliferation of compact bone formed up of concentric plates laid around the Havers canels were reported in studies on dogs (27). In case of implants inserted in humans, the researchers also noted the formation of dense lamellar bone formed of well organised concentric plates (24). While studying the osseointegration on healthy osseous tissue, some authors came to the conclusion that it is less important whether the implant was inserted in a cortical bed or in a primary cancellous bed, as there is a strong tendency of "corticalization" of the cancellous bone around the metallic implants (28).

If we compare the results that we obtained with those existing in the literature, we observe that they greatly overlap. Thus, the obvious tendency of the evolution of proliferated bone around the titanium implants towards a compact bone with increased resistance that we observed is also advocated by a large number of authors. The proliferation of osseous tissue in direct contact with the surface of the titanium implant is also advocated by all the authors we conferred with. Everybody advocate the same thing that is the fact that step by step, the implant is coated on the whole surface with newly proliferated bone. We have to point out that they came to these conclusions in the case of implants totally inserted into the bone. None of them checked if the bone proliferates on the surface of the bone which highly surpasses the thickness of the bone in which it was inserted, to get into some cavities, as in our case, the marrow canal. We observed that although the area of the implant which gets into the marrow canal comes in direct contact with the bone marrow and not with the bone, it nonetheless tends to be gradually coated with osseous tissue; an aspect which we think is due to the special qualities of the titanium which is very well tolerated by both the soft and hard tissues. More precisely, the implant assures a base for osseous proliferation in direct contact with its surface, and the progressive migration (sliding) of the bone on the area of the implant which surpasses the thickness of the osseous wall. This aspect seems to have a special practical character and we believe that it can be used in the medical practice to obtain a bone-implant interface significantly larger than the initial thickness in which the implant is inserted, especially when it comes to bones with minute thickness or with decreased resistance.

The observed aspects highlight the fact that the proliferation and bone remodelling around the titanium implant ensured its gradual coating, on a significantly larger surface than the thickness of the diaphyseal wall, with bone whose resistance clearly exceeded that of the rabbit femoral bone. The large surface of the bone-implant interface and the strong bone that was formed ensured a very good anchoring of the implant. The structures that achieved this objective appeared as a result of the effort to adapt the intervention area to the situation created by the insertion of the implant.

CONCLUSIONS

The newly proliferated bone migrates to the implant surface, but also extends to its extraosseous portions so that the interface gradually takes on the appearance of a fan, very effectively covering the implant on the surface significantly larger than the initial bone thickness.

The new bone that proliferated around the implant shows a clear tendency to reshape to the secondary bone with more and larger Haversian systems than in normal femoral bone, so that in the end it will have greater resistance than the bone had at the time of implant insertion.

The coating with newly proliferated bone with an increased strength, on the surface significantly larger than the wall thickness of the femoral bone, make up adaptive structures that ensure the restoration of the mechanical strength of the area and the very good stability of the implant.

Conflict of interest
None

Competing interests
The authors declare that they have no competing interests.

Funding
No funding was received.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors contribution
All authors read and approved the final manuscript.

Teodora Marcu, Cristian Adrian Ratiu, Ioana Adela Ratiu: Conception and design, drafting the article and final approval of the version to be published.
Florin Adrian Gal, Aurel Damian: Analysis and interpretation of data, revising the article and final approval of the version to be published.

REFERENCES

1. Guda T. Current trends in dental implants. J Korean Assoc Oral Maxillofac Surg 2014;40(2): 50–60.
2. Seth S, Kalra P. Effect of dental implant parameters on stress distribution at bone-implant interface. Inter J Sci Res 2013;2:121–124.
3. Yoon HG, Heo SJ, Koak JY, Kim SK, Lee SY. Effect of bone quality and implant surgical technique on implant stability quotient (ISQ) value. J Adv Prosthodont 2011;3:10–15.
4. Anil S, Aldosari AA. Impact of bone quality and implant type on the primary stability: an experimental study using bovine bone. J Oral Implantol 2015;41(2):144-8.
5. Akca K, Akkocaoglu M, Comert A, Tekdemir I, Cehreli MC. Bone strains around immediately loaded implants supporting mandibular overdentures in human cadavers. Int J Oral Maxillofac Implants 2007;22:101–109.
6. Turkyilmaz I, McGlumphy EA. Influence of bone density on implant stability parameters and implant success: A retrospective clinical study. BMC Oral Health 2008;8:32 .
7. Miyamoto I, Tsuboi Y, Wada E, Suwa H, Iizuka T. Infl uence of cortical bone thickness and implant length on implant stability at the time of surgery – Clinical, prospective, biomechanical, and imaging study. Bone 2005;37:776–780 .
8. Turkyilmaz I, Tozum TF, Tumer C. Bone density assessments of oral implant sites using computerized tomography. J Oral Rehabil 2007;34:267–272.
9. Turkyilmaz I, Soganci G. Rationale for Dental Implants. Current Concepts in Dental Implantology. IntechOpen Limited: London; 2015. p 1-23.
10. Jemt T, Stenport V. Implant treatment with fixed prostheses in the edentulous maxilla. Part 2: Prosthetic technique and clinical maintenance in two patient cohorts restored between 1986 and 1987 and 15 years later. Int J Prosthodont 2011;24:356–362.
11. Jemt T, Stenport V, Friberg B. Implant treatment with fi xed prostheses in the edentulous maxilla. Part 1: Implants and biologic response in two patient cohorts restored between 1986 and 1987 and 15 years later. Int J Prosthodont 2011;24:345–355.
12. Jacobs R. Preoperative radiologic planning of implant surgery in compromised patients, Periodontol 2000 2003;33:12–25.
13. Shibli JA, Mangano C, Mangano F, Rodrigues JA, Cassoni A, Bechara K, Ferreia JD, Dottore AM, Iezzi G, Piattelli A. Boneto-implant contact around immediately loaded direct laser metalforming transitional implants in human posterior maxilla. J Periodontol 2013;84:732–737.
14. Sennerby L, Meredith N. Implant stability measurements using resonance frequency analysis: biological and biomechanical aspects and clinical implications. Periodontol 2000 2008;47:51–66.
15. Elias CN, Rocha FA, Nascimento AL, Coelho PG. Influence of implant shape, surface morphology, surgical technique, and bone quality on the primary stability of dental implants. J Mech Behav Biomed Mater 2012;2(16):169–180.
16. Paschalis EP, Tatakis DN, Robins S. Lathyrism-induced alterations in collagen cross-links influence the mechanical properties of bone material without affecting the mineral. Bone 2011;49:1232–1241.
17. Fratzl P, Gupta H, Paschalis E, Roschger P. Structure and mechanical quality of the collagen-mineral nano-composite in bone. J Mater Chem 2004;14:2115–2123.
18. Molly L. Bone density and primary stability in implant therapy. Clin Oral Implants Res 2006;17(suppl 2):124–135.
19. Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG. Animal models for implant biomaterial research in bone: a review. Eur Cells Mater 2007;13:1–10.
20. Wancket LM. Animal models for evaluation of bone implants and devices: comparative bone structure and common model use. Veterinary Pathology 2015;52(5): 842-850.
21. Muster D, Champy M. Le Probleme d'interface os-biomattriaux. Actualités Odonto-Stomatologiques 1978 ;32(121):109-24.
22. Branemark PI. Introduction to Osseointegration. In: Branemark PI, Zarb G, Albrektsson B. Tissue integrated Prostheses, Osseointegration in Clinical Dentistry. Quintessenz: Berlin; 1985. p. 11-76.
23. Carlsson L, Röstlund T, Albrektsson B, Albrektsson T, Brånemark PI. Osseointegration of titanium implants. Acta Orthopaedica Scandinavica 1986;57:4, 285-289.
24. Albrektsson T, Branemark PI, Hansson HA, Lindstrom J. Osseointegrated implants, requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand 1981;52:155-170.
25. da Silva Melloa AS, dos Santos PL, Marquesi A, Queiroz TP, Margonar R, de Souza Faloni AP. Some aspects of bone remodeling around dental implants. Rev Clin Periodoncia Implantol Rehabil Oral 2016. http://dx.doi.org/10.1016/j.piro.2015.12.001.
26. Franchi M, Orsini E, Trirè A, Quaranta M, Martini D, Piccari G, Ruggeri A, Ottani V. Osteogenesis and morphology of the peri-implant bone facing dental implants. Scientific World Journal 2004;4:1083-1095.
27. Koch FP, Weng D, Kramer S, Biesterfeld S, Jahn-Eimermacher A, Wagner W. Osseointegration of one-piece zirconia implants compared with a titanium implant of identical design: a histomorphometric study in the dog. Clin Oral Impl Res 201 ;350–356. doi: 10.1111 / j.1600-0501.2009.01832.x.
28. Breine U, Branemark PI. Reconstructruction of alveolar jaw bone. Scand J Plast Reconstr Surg 1980;14(1):23-48.

