

Osseointegration of zirconium dental implants three months after insertion in rabbit femur. Histopathological study

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Abstract

Implantology is a highly researched field with a constant concern in finding and studying new implant materials. Lately, zirconium has become a very attractive alternative to the detriment of titanium, but the research results were sometimes contradictory. Thus, we considered as opportune to study the osseointegration of zirconium dental implants in rabbit femur, three months after insertion. The biological material was represented by five rabbits and the experimental protocol was approved by the Ethics Committee of the University of Oradea, Romania. The implants (zirconium ceramic dental implants: 5 mm length, 2.6 mm diameter) were inserted in the femur under controlled conditions, after creating a bone defect. The animals received the appropriate postoperative care. Three months later, the implantation area was harvested and processed for histological examination. The assessment of the osseointegration process of the zirconium implants showed that they were very well tolerated by the host organism that did not trigger any rejection processes. Approximately 80% of the compromised bone was replaced with newly formed bone in advanced stages of remodeling and consolidation. The proliferated bone near the implants acquired a structure similar to the rabbit diaphyseal bone, but with higher density and size of the osteons. The stage reached by the osseointegration process three months after the insertion of the implants, ensures a good consolidation of the implants that supports the prosthetic structures, which are to be built on them.

Keywords: implant, osseointegration, rabbit, zirconium.

Introduction

The idea of replacing compromised teeth with implants is not of recent date, old information about such concerns already exists. Among the known dental implants that have been fully integrated into the bone is the one made from seashell by ancient Mayans, over 2000 years ago [1]. Interest in the use of biomaterials for implantation in hard tissues got higher and increased with time. Dental implants have many advantages over transition crowns, bridges or prostheses. They allow the creation of support structures for temporary or permanent prostheses, with the preservation of adjacent dental structures. Implants can provide much more stability to prostheses, especially to the fixed and large-scale ones, such as total dental prostheses [2], thus increasing the life quality of patients [3]. Furthermore, in the case of dental implants, it is not necessary to consider the risk of recurrent caries as in other procedures, such as onlay, crowns, and bridges procedures [4]. In order to be well tolerated by the body and incorporated in the hard tissue, it is mandatory for the implants to have an adequate primary stability [5, 6] and also possess a series

of specific properties mainly related to biocompatibility, implant construction and biomechanics. In the 1960s, Brånemark *et al.* used titanium implants in animal models, without suspecting the importance of discovering them at that time. For five years long, the clinical results of titanium implants utilization were quite poor, with a 50% failure rate [7], and only later did they realize it was a prosthetic material with special qualities.

In recent years, there is an increasing concern in finding new prosthetic materials with a high degree of biocompatibility [8, 9]. One of the materials considered as promising in this regard is zirconium, the use of which has been a high concern since 1990. The first zirconium abutment was manufactured in 1997. Due to its low thermal conductivity and elasticity, low affinity for the bacterial plate, a great biocompatibility and last but not least due to the white color, zirconium has become a very attractive alternative to the detriment of titanium [10]. Titanium, due to its dark color (gray), has aesthetic disadvantages especially in patients with thin gingival mucosa [11].

In the dental field, only a few clinical trials have been published regarding the use of zirconium as a dental abutment material. Thus, Glauser *et al.* reported 54 implants placed on 27 patients with zirconium contraforts and ceramic crowns, and followed them up for four years [12]. Some authors consider it inferior to the titanium from certain points of view, claiming that for similar roughness, zirconium does not allow a dental plaque construction as easy as titanium [13]. Others report that during the process of osseointegration, titanium abutments induced a more severe inflammatory reaction than zirconium [14]. Animal studies have shown that zirconium implants are comparable to titanium in terms of biocompatibility and osseointegration [10]. A highly important aspect is that zirconium does not have carcinogenic or mutagenic effects [15].

Because there are no long-term studies, some authors believe that zirconium implants may be an alternative to titanium only after checking the resistance in time and the survival rate of these ceramic implants [10, 16].

Aim

This study aims to investigate the osseointegration of zirconium implants in the femur of the rabbit, under light microscopy, and to identify the type of bone proliferated at the implant–bone interface at three months after insertion.

Materials and Methods

To assess the osseointegration process of zirconium implants, a histopathological study was performed on five common breed rabbits. The experimental study was conducted at the University of Medicine and Pharmacy of Oradea, Romania. It was approved by the Ethics Committee of the same University. Throughout the experiment, the rabbits have benefited from appropriate and constantly controlled conditions: 20–24°C and natural light, with a dark light cycle of about 12/12 hours. The animals were fed throughout the experimental period with standardized granulated food and benefited of fresh water *ad libitum*. The postoperative protocol included antibiotic treatment and strict monitoring of the intervention site and the general health of rabbits.

Threaded zirconium ceramic dental implants were used, with the following dimensions: 5 mm in length and 2.6 mm in thickness (Figure 1). Their construction was simple because we did not intend a later loading.

Prior to surgery, the rabbits were given Ketamine (0.5 mL/kg body weight) and Xylocaine (0.4 mL/kg body weight), both administered intramuscularly. Then, an experimental bone defect was performed in the middle of the femoral diaphysis with the dental drill, under continuous irrigation with sterile physiological saline solution to avoid overheating of the bone tissue. The implants were then inserted by screwing with a special key. The intervention area was sutured and the animals received the appropriate postoperative care. Postoperatively, Enrofloxacin was administered (1.8 mL/rabbit/day), three days subcutaneously.

Three months later, a new surgical intervention was performed to harvest fragments of the femur in the implantation area. The pieces were fixed in 10% buffered formalin for 10 days, then decalcified with trichloroacetic

acid and embedded in paraffin. Sections of 5-μm thickness were cut and stained with the Goldner's Masson trichrome method. This staining procedure clearly differentiates the mature osseous matrix, which stains green, from woven bone, which appears red [17, 18].



Figure 1 – Zirconium dental implants: 5 mm length, 2.6 mm diameter.

Results

Three months after inserting the zirconium implant, the entire the bone–implant interface is occupied by woven bone with different degrees of consolidation and remodeling (Figure 2). The proliferated bone is in close contact with the implant surface, covering a surface larger than the normal thickness of the diaphyseal bone (Figure 3) (fan-like aspect). In the opposite area to the implant, the diaphyseal wall is formed of non-Haversian lamellar bone tissue in the periosteal and endosteal areas, while in the area between the two (periosteal and endosteal zones), there are Haversian systems (osteons) with plexiform lamellar bone tissue in between. Most of the Haversian systems are small (2–3 lamellae), but there are medium sized ones (4–6 lamellae) and rarely large Haversian systems (more than seven lamellae) (Figure 4).

The whole interface is occupied by woven bone, also containing residual bone areas, filling the spaces between the bone structures undergoing processes of proliferation and differentiation to mature bone. The ratio between the two types of bone is in favor of the newly proliferated bone, with smaller or larger differences from one area to another. Proliferation and reshaping processes are very active at the bone–implant interface, being at a somewhat more advanced stage in the subperiosteal and subendosteal areas (Figures 5–7).

The state of repair and bone remodeling processes up to this point of the experiment are different from one area to another, which clearly shows that they are not yet completed. There are areas where the woven bone appears relatively dense, but the non-Haversian bone still predominates, although there are some osteons present (Figure 8). In other areas, the osteons are numerous, but in an early stage of organization, and there are some areas occupied by osteoid (Figure 9). Reshaping processes towards a Haversian bone are very active in most areas, with the presence of small (2–3 lamellae), medium (4–6 lamellae) and large (more than seven lamellae) osteons (Figures 10 and 11).

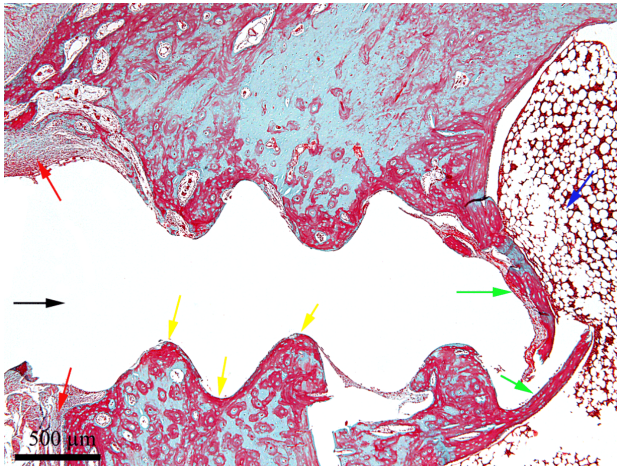


Figure 2 – Implant area (Goldner's Masson trichrome staining, $\times 40$): black arrow – implant area; blue arrow – medullary canal; yellow arrow – bone–implant interface; red arrow – periosteum; green arrow – proliferated bone, extending over the tip of the implant.

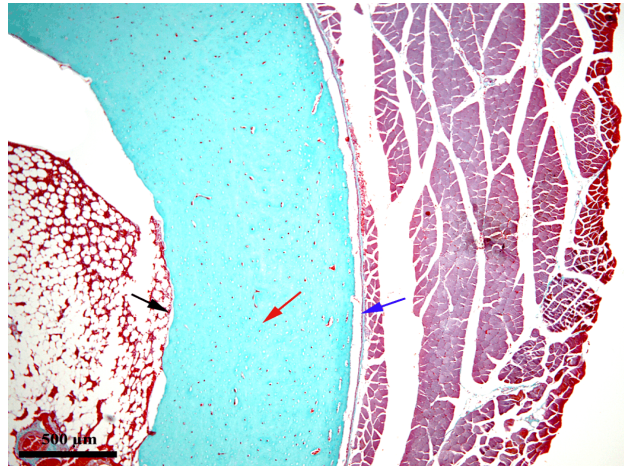


Figure 3 – Opposite area to the implant (Goldner's Masson trichrome staining, $\times 40$): black arrow – endosteum; red arrow – diaphyseal bone; blue arrow – periosteum.

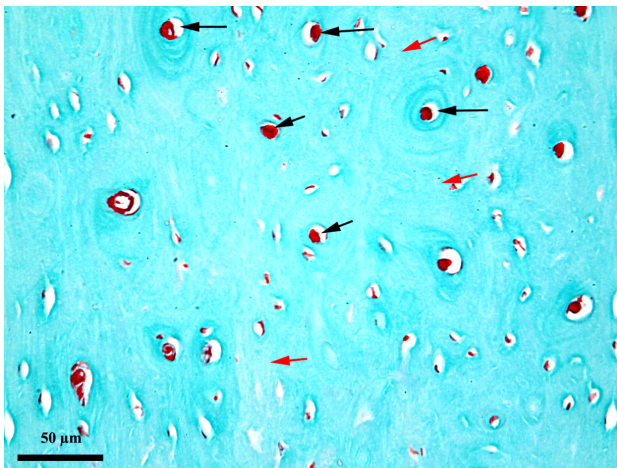


Figure 4 – Opposite area to the implant – detail (Goldner's Masson trichrome staining, $\times 400$): black arrow – Haversian systems; red arrow – non-Haversian lamellar bone.

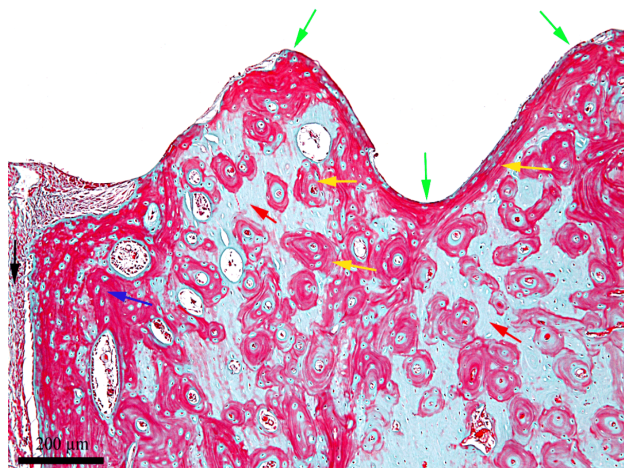


Figure 5 – Subperiosteal area (Goldner's Masson trichrome staining, $\times 100$): black arrow – periosteum; blue arrow – subperiosteal woven bone; yellow arrow – woven bone; red arrow – residual bone; green arrow – bone–implant interface.



Figure 6 – Central area of the diaphysis (Goldner's Masson trichrome staining, $\times 100$): black arrow – woven bone between the screw threads of the implant; blue arrow – woven bone on a screw thread of the implant; yellow arrow – blood vessels; red arrow – residual bone.

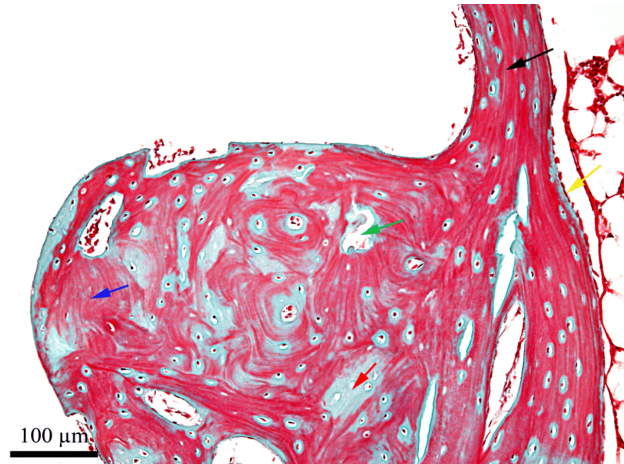


Figure 7 – Subendosteal area (Goldner's Masson trichrome staining, $\times 200$): black arrow – woven bone extended over the tip of the implant; blue arrow – woven bone in the endosteal region; yellow arrow – endosteum; red arrow – residual bone; green arrow – resorption lacuna in the residual bone.

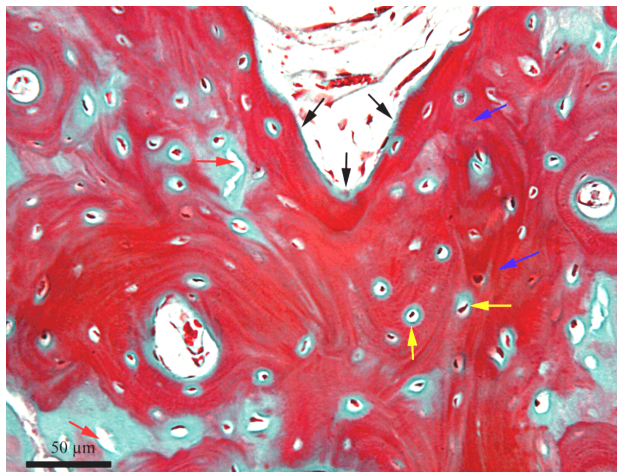


Figure 8 – Subperiosteal area – detail (Goldner's Masson trichrome staining, ×400): black arrow – bone–implant thread interface; blue arrow – woven bone; yellow arrow – osteocytes in osteoplasts; red arrow – small lacunae in the residual bone.

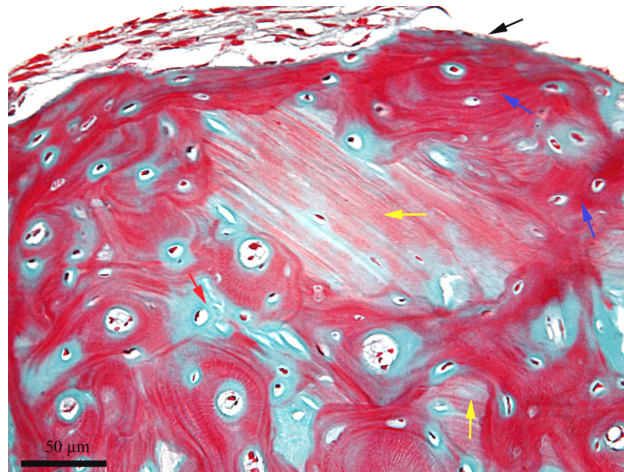


Figure 9 – Central area of the diaphysis – detail (Goldner's Masson trichrome staining, ×400): black arrow – implant interface; blue arrow – woven bone; yellow arrow – osteoid; red arrow – residual bone.

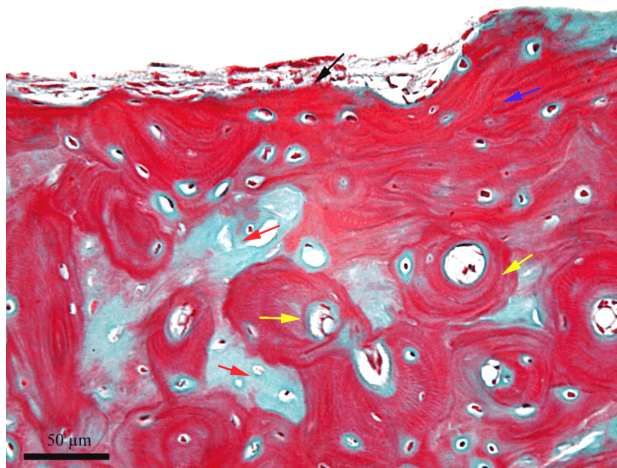


Figure 10 – Subendosteal area – detail (Goldner's Masson trichrome staining, ×400): black arrow – bone–implant interface; blue arrow – woven bone; yellow arrow – osteons; red arrow – residual bone.

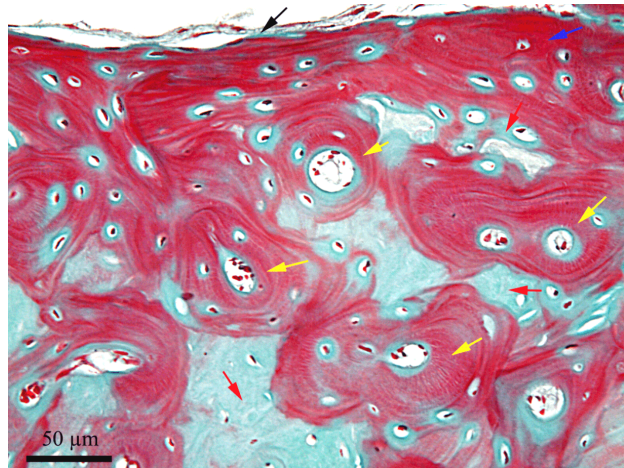


Figure 11 – Central area of the diaphysis – detail (Goldner's Masson trichrome staining, ×400): black arrow – bone–implant interface; blue arrow – non-Haversian bone; yellow arrow – polymorphic osteons; red arrow – residual bone.

From the periosteum to the medullary canal, the bone–implant interface is occupied to a very large extent (80%) by the newly proliferated bone (with differences from one area to the other), and the rest is represented by residual bone, which is to be replaced in a relatively short time. The thickness of the area occupied by woven bone at the bone–implant interface is not the same across the entire surface, being significantly larger in the periosteal and endosteal areas than in the medial area. The aspect is absolutely normal, given the fact that the repair processes had the periosteum and endosteum as their starting point, from where they gradually expanded to the central part. The fact that the newly formed bone is thinner in the central area suggests that the process of osseointegration, although in an advanced stage of development, is not yet fully completed. The aspect is also suggested by the fact that there are larger or smaller differences from one area to another, regarding the bone structure found at the bone–implant interface.

In periosteal and endosteal zones, the proliferated

bone has a typical secondary bone aspect (lamellar), with parallel arrangement of osseous lamellae. This disposition is very close to the one existing in long bones diaphyses (as is our case), where there are several concentric osseous lamellae, numerous osteocytes, abundant collagen fibers, and reduced matrix.

In the case of the studied bones, the disposition of the lamellae, the number of osteocytes and the density of collagen fibers are comparable to those normally encountered in the diaphyseal bone, but the thickness of the areas occupied by this type of bone is larger than normally. The aspect seems normal if we take into account that the proliferated bone in the bone–implant area has a fan-like appearance, with a significant increase in thickness compared to the normal dimensions of the diaphyseal bone. In this context, all bone areas (periosteal, central, endosteal) are thicker than normal. This thickening of the bone around the implants is physiological and appears as an adaptation to the particular situation faced by the implantation area, starting from the experimental defect

to the end of the osseointegration process of the implants.

The proliferated bone (in the periosteal to the endosteal areas) has mostly the appearance of a secondary bone (lamellar), but there are sometimes large differences from one area to another. These differences are mainly due to the different stages of the bone remodeling process towards a typical compact bone, being more advanced in some areas compared to others. It is certain that three months postoperatively, there is a relatively large number of well-defined osteons (Haversian systems), even if their density is not the same on the entire bone surface near the implants. The osteons are polymorphic, mainly concerning the number of the lamellae existing in each of them, but to some extent, they are also polymorphic in shape (on the section surface). In terms of the density of newly formed osteons, there are relatively large differences from one area to another, with relatively large density in some areas and more rare in others or even disposed at a certain distance. In some areas, the diameter of the osteons and their density are higher than they normally appear in rabbit diaphyseal bone. We consider this aspect as part of the body's effort to strengthen the weakened area following the experimental bone defect.

Goldner's Masson trichrome staining procedure clearly differentiated the woven bone from the residual bone (affected or not), which allows the assessment of the areas where the residual bone has not yet been replaced. Note that in the bone-implant interface area, woven bone predominates, even if the ratio between woven bone and the one to be replaced is slightly different from one area to another. We state that osteocytes are absent in the areas with residual bone and several polymorphic microcavities (regarding their shape and size) are present. The bone density is appropriate in all these areas.

☐ Discussions

The assessment of the osseointegration process of the zirconium implants three months after their insertion into the rabbit femur diaphysis shows that they were very well tolerated by the host organism that did not trigger even discrete rejection processes. Under these conditions, osteolysis and bone proliferation processes were carried out in parallel and ensured, on one hand, the elimination of the large majority of the bone affected by the traumatic surgery and, on the other hand, the proliferation of bone surrounding the implants at this time of the experiment. The implant-bone interface is seen as a key indicator of osseointegration that governs the success and overall survival of implants [19]. By assessing the zirconium implant on a leporine model, we observed a good osseointegration six weeks after the implantation into the femoral bone, with direct bone proliferation at the bone-implant interface. There were no fissures (lacunae), inflammatory infiltration or multinucleated giant cells [20].

While testing the osseointegration of some zirconium implants in comparison with titanium, some authors concluded that there are no significant differences in the bone-implant interface area between the two types of implants [11]. In a similar experiment, other researchers [21] found that two weeks after insertion of the implants in rabbits, the bone covering rate was 54–55% for zirconium and 42–52% for titanium, respectively. After

four weeks, the situation is slightly different; the zirconium implants are covered by a ratio of 62–80% woven bone, and the titanium ones by 68–91%. Other authors inserted zirconium and titanium implants in sheep femur and found that after 12 weeks, the bone-implant interface was covered with 85.5% newly formed bone for zirconium implants and 78.9% for titanium, respectively [22]. Similar results were also obtained by testing the two types of implants in dog [23].

In our experiment, osseointegration was achieved directly with osseous tissue, and not with intermediate tissues (fibrous, cartilaginous), on the whole area of intervention. The newly proliferated bone is arranged in such a way to intimately follow all the uneven surfaces of the implants (of the screw thread) extending over a greater surface than the thickness of the diaphyseal compact bone. This makes the bone proliferated in the implant area have a fan-like aspect, coating the implant on a surface larger than the normal thickness of the diaphyseal bone, which confers a very good fixation and consolidation.

This type of tissue is called non-Haversian fibrous bone, presenting external fundamental systems in the subperiosteal area and internal fundamental systems in the subendosteal area [24].

In other words, repairing bone tissue is a complex and slow process in which affected bone structures are gradually replaced by newly formed bone structures. This makes the area occupied by the affected bone gradually decrease, but until its total replacement with the newly formed bone, a long period of time has to pass. Thus, three months after inducing the experimental defect, residual bone still persists. The positive side is that such areas are reduced in the immediate vicinity of the implants, being better represented and somewhat larger at a certain distance (in depth) from the implant bone interface. The persistence of the areas occupied by the affected bone (absent osteocytes; osteoplasts, in addition to being hollow, appear very polymorphic in shape and size) show that until the repair processes at the diaphyseal bone are completed, a certain amount of time is needed, which cannot be appreciated exactly at this point in the experiment. However, this does not represent such a big problem for the mechanical strength of the bone in its entirety because there are no significant discontinuities, and this unsustainable bone provides a great deal of resistance to the area until its gradual replacement. This bone has lost its cellular component, which would ensure its viability, but not the mineral component that provides its mechanical strength. In other words, the mechanical strength of the area is ensured during the unreeling of the repair processes both by the newly formed bone and the one to be gradually replaced. Considering the fact that the compromised bone was largely replaced by bone structures in advanced stage of remodeling and consolidation, we consider that the process of osseointegration of zirconium implants into the rabbit diaphyseal bone three months after the insertion reaches a consolidation stage sufficiently advanced to support the structures to be built on it.

☐ Conclusions

Three months after the insertion of the zirconium implants, approximately 80% of the compromised bone

(after the experimental defect) was replaced with newly formed bone in advanced stages of remodeling and consolidation. The proliferated bone near the implants gradually acquires a structure comparable to that of the rabbit diaphyseal bone, but the higher density and size of the osteons in the advanced remodeling areas make us believe that it will ultimately have superior resistance. The stage reached by the osseointegration process three months after the insertion of the implants, even if it is not fully completed, ensures a good consolidation of the implants that supports the prosthetic structures, which are to be built on them.

Conflict of interests

The authors declare that they have no conflict of interests.

References

- [1] Walker A, Maltby T. Active ageing: a strategic policy solution to demographic ageing in the European Union. *Int J Soc Welf*, 2012, 21(s1):S117–S130.
- [2] Carr AB, Laney WR. Maximum occlusal force levels in patients with osseointegrated oral implant prostheses and patients with complete dentures. *Int J Oral Maxillofac Implants*, 1987, 2(2): 101–108.
- [3] Bran S, Onişor-Gligor F, Lucaci D, Câmpian RS, Lucaci OP, Dudea D. Sinus lift and dental implant treatment as an option for enhancing the quality of life of our patients. *Rom J Morphol Embryol*, 2013, 54(1):121–124.
- [4] Schliephake H, Hefti T, Schlottig F, Gédet P, Staedt H. Mechanical anchorage and peri-implant bone formation of surface-modified zirconia in minipigs. *J Clin Periodontol*, 2010, 37(9):818–828.
- [5] Ciocan LT, Miculescu F, Miculescu M, Pătraşcu I. Retrieval analysis on dental implants biointegration phases. *Rom J Morphol Embryol*, 2010, 51(1):117–122.
- [6] Comăneanu RM, Barbu HM, Coman C, Miculescu F, Chiuţu L. Correlations between cyto-histopathological tissue changes at the dental implant interface and the degree of surface processing. *Rom J Morphol Embryol*, 2014, 55(2):335–341.
- [7] Albrektsson T, Wennerberg A. The impact of oral implants – past and future, 1966–2042. *J Can Dent Assoc*, 2005, 71(5): 327.
- [8] Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. *Eur Spine J*, 2001, 10(Suppl 2):S96–S101.
- [9] Akagawa Y, Hosokawa R, Sato Y, Kamayama K. Comparison between freestanding and tooth-connected partially stabilized zirconia implants after two years' function in monkeys: a clinical and histologic study. *J Prosthet Dent*, 1998, 80(5):551–558.
- [10] Hashim D, Cionca N, Courvoisier DS, Mombelli A. A systematic review of the clinical survival of zirconia implants. *Clin Oral Investig*, 2016, 20(7):1403–1417.
- [11] Hafezeqorani A, Koodaryan R. Effect of zirconia dental implant surfaces on bone integration: a systematic review and meta-analysis. *BioMed Res Int*, 2017, 2017:9246721.
- [12] Glauser R, Sailer I, Wohlwend A, Studer S, Schibli M, Schärer P. Experimental zirconia abutments for implant-supported single-tooth restorations in esthetically demanding regions: 4-year results of a prospective clinical study. *Int J Prosthodont*, 2004, 17(3):285–290.
- [13] Scarano A, Piattelli M, Caputi S, Favero GA, Piattelli A. Bacterial adhesion on commercially pure titanium and zirconium oxide disks: an *in vivo* human study. *J Periodontol*, 2004, 75(2):292–296.
- [14] Degidi M, Artese L, Scarano A, Perrotti V, Gehrke P, Piattelli A. Inflammatory infiltrate, microvessel density, nitric oxide synthase expression, vascular endothelial growth factor expression, and proliferative activity in peri-implant soft tissues around titanium and zirconium oxide healing caps. *J Periodontol*, 2006, 77(1):73–80.
- [15] Covacci V, Bruzzese N, Maccauro G, Andreassi C, Ricci GA, Piconi C, Marmo E, Burger W, Cittadini A. *In vitro* evaluation of the mutagenic and carcinogenic power of high purity zirconia ceramic. *Biomaterials*, 1999, 20(4):371–376.
- [16] Apratim A, Eachempati P, Krishnappa Salian KK, Singh V, Chhabra S, Shah S. Zirconia in dental implantology: a review. *J Int Soc Prev Community Dent*, 2015, 5(3):147–156.
- [17] An YH, Gruber HE. Introduction to experimental bone and cartilage histology. In: An YH, Martin KL (eds). *Handbook of histology methods for bone and cartilage*. Humana Press, Totowa, 2003, 3–31.
- [18] Mihailović D, Adžuković Z. Histopathological analysis of bone tissue reaction on implanted biomaterials. In: Zivic F, Affatato S, Trajanovic M, Schnabelrauch M, Grujovic N, Choy KL (eds). *Biomaterials in clinical practice: advances in clinical research and medical devices*. Springer International Publishing AG, Switzerland, 2017, 535–537.
- [19] Gahlert M, Roehling S, Sprecher CM, Kniha H, Milz S, Bormann K. *In vivo* performance of zirconia and titanium implants: a histomorphometric study in mini pig maxillae. *Clin Oral Implants Res*, 2012, 23(3):281–286.
- [20] Cavalu S, Ratiu C, Ponta O, Simon V, Rugina D, Miclaus V, Akin I, Goller G. Improving osseointegration of alumina/zirconia ceramic implants by fluoride surface treatment. *Dig J Nanomater Biostruct*, 2014, 9(2):797–808.
- [21] Hoffmann O, Angelov N, Gallez F, Jung RE, Weber FE. The zirconia implant–bone interface: a preliminary histologic evaluation in rabbits. *Int J Oral Maxillofac Implants*, 2008, 23(4):691–695.
- [22] Siddiqi A, Duncan WJ, De Silva RK, Zafar S. One-piece zirconia ceramic *versus* titanium implants in the jaw and femur of a sheep model: a pilot study. *BioMed Res Int*, 2016, 2016:6792972.
- [23] Mihatovic I, Golubovic V, Becker J, Schwarz F. Bone tissue response to experimental zirconia implants. *Clin Oral Investig*, 2017, 21(2):523–532.
- [24] Puşcaşiu D, Puşcaşiu M, Maghiar TT, Miclăuş V. Histologie. Ed. Universităţii din Oradea, 1999, 74.

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