ical implant designs at stability and osseointegration

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Sergio A. Gehrke[1], Carlos P´erez-Albacete Mart´ınez[2], Adriano Piattelli[3], Jamil A. Shibli[4], Aleksa Markovic[5], Jos´e L. Calvo Guirado[6]

[1]Biotecnos Research Center, Santa Maria, Brazil

[2] International Dentistry Research Cathedra, University Catholica of San Antonio de Murcia (UCAM), Murcia, Spain

[3] Oral Pathology and Medicine in the Dental School, University of Chieti-Pescara, Chieti, Italy

[4] Faculty of Dentistry, Universidad de Guarulhos, Guarulhos, Brazil

[5] Department of Clinic of Oral Surgery, Faculty of Stomatology, University of Belgrade, Belgrade, Serbia

[6] *Josè L. Calvo Guirado,* Dentistry Research Cathedra, University Catholica of San Antonio de Murcia (UCAM), Murcia, Spain

Corresponding author:

Sergio Alexandre Gehrke, DDS, PhD

Department of Research

Biotecnos – Technology and Science Rua Dr. Bozano 571

CP 97015-001, Santa Maria, RS, Brazil Tel.: / Fax: +55 55 32227253

e-mail: sergio.gehrke@hotmail.com

Abstract

Objectives: The aim of this study was to perform a histomorphometric and biomechanical comparison of three implants with different designs of the apical area to promote a better bone initial stability and its correlation with the osseointegration.

Material and methods: Fifty-four tapered implants with same length, diameter and surface properties but with three different apical configurations (Group I: MK4: Group II: C1 and Group III: MK7) were inserted in the tibia of rabbits. Implant stability and bone formation were evaluated by resonance frequency analysis measured at 0, 6, 8 and 12 weeks and by histomorphometric analysis performed at 6, 8 and 12 weeks.

Results: Statistical test to compare the stability through the implant stability quotient in the four times showed few differences between the groups and time periods proposed, with significance set at $P < 0.05$. In the bone–implant contact, by comparing the groups in the three times proposed, it was possible concluded that there is a similar behavior among the three implant design ($P < 0.05$).

Conclusion: With the limitations of this animal study, it can be concluded that the design of the apical area influences the implant stability and the bone-to-implant contact.

Key words: bone-to-implant contact, histomorphometric analysis, implant design, resonance frequency analysis

Introduction

Predictability has been recognized for long- term dental implant and restoration success (Simonis et al. 2010*;* Charyeva et al. 2012*)*, which was a great advance in rehabilitation treatments. Initial stability of the implant is, in effect, one of the fundamental criteria for obtaining the osseointegration (Calvo-Guir- ado et al. 2013), while the implant instability results in fibrous encapsulation, thus con- firming previously made clinical observations (Lioubavina-Hack et al. 2006). According to some authors, two effective approaches can be used to reduce time between surgery and prosthetic reconstruction, the reduction in micromotion beneath the critical threshold by means of rigid fixation of loaded implants and the possibility is to optimize the healing period before a safe functional loading can be exerted (Szmukler-Moncler et al. 2000). In addition, animal studies have shown that osseointegration rate is lower in implants with little stability (Sivolella et al. 2012).

Achieving implant stability depends on the implant–bone relation, the surgical technique and the micro-and macroscopic morphology of the implant used (Martinez et al. 2001*;* Buchter et al. 2006*;* Gehrke et al. 2015). The features of the implant system influence the osseointegration mode of implants (Novaes et al. 2010), and the geometric features of an implant influence sufficient initial contact to facilitate primary stability of the implant (Sennerby & Meredith 2008). The macrogeometric features such as the implant body shape and the design, height, density and cut- ting ability of the threads may affect the biomechanics of the implant–bone interlocking, possibly improving implant stability (Chun et al. 2002*;* Chang et al. 2010*)*. On the other hand, important aspects of a fast implant osseointegration include the need to achieve a primary stability between the implant and the bone directly after insertion, the need to insert the implant with minimal surgical trauma and the capability of the implant surface to attach directly to the adjacent bone tissue (Gehrke 2015).

The importance of the implant geometries and surface characteristics, in an effort to achieve better bone anchorage, has been clear for a long time (Albrektsson et al. 1981, 1988; Buser et al. 1991*;* Buchter et al. 2006), and in fact, various implant systems have been introduced over the past several years to achieve a faster bone integration (Buser 1999). So, as described above, several studies have com- pared the macrodesign as the implant length, diameter, forming (conical or cylindrical) and shape of the threads; however, the effect on the apical design of the self-tapping implants and their effect on the osseointegration of the implants were few reports in the literature (Chong et al. 2009*;* Kim & Lim 2011). Then, this study was designed to evaluate the effect of the implant design on the osseointegration at 6, 8 and 12 weeks after the implantation, using biological methods (histology and histomorphometry) as well as biomechanical test of resonance frequency analysis (RFA).

Materials and methods

Implants characteristics

This experimental animal study utilized 54 tapered titanium implants (MIS Implants Models, Bar Lev Industrial Park, Israel), with the same surface characteristics but with different apical configuration (Fig. 1). The 54 conical implants were divided into the following groups: Group 1 (M4®; MIS Implants Models), square threads with progressive depth to the apex and apical portion with a self-tapping system quite pronounced and aggressive; Group 2 (Seven®; MIS Implants Models), dual thread of flat tip, pitch of thread long, progressive thread depth, and apical area presents a medium self-tapping portion; and Group 3 (C1®; MIS Implants Models), triangular threads with flat tips, initially becoming increasingly acute, with increasing depth of the threads from the cervical portion to the apex and the apical area presents a small self-tapping portion.

The implants had sandblasted, large-grit and acid-etched (SLA) surface treatment type throughout the body and internal hex connection. The implant dimension was 3.75 mm in diameter and 10 mm in length (Fig. 1).

Animals and surgical procedure

This experimental protocol was approved by the Ethics Committee of Murcia University,

Spain, which followed the guidelines established by the Council Directive of the European Union for animal care and experimentation. Nine mature New Zealand male rabbits weighing approximately 4 kg were used in this study. The rabbits were anesthetized by intramuscular injection of ketamine (35 mg/kg; Agener Pharmaceutica, Brasilia, Brazil). Then, a muscle relaxant (Rompun 5 mg/kg, Bayer, Sao Paulo, Brazil) and a tranquilizer (Acepran 0.75 mg/kg; Univet, Sao Paulo, Brazil) were injected intra-muscularly. Additionally, 1 ml of local anesthetic (3% prilocaine–felypressin, Astra, Ciudad de Mexico, Mexico) was injected subcutaneously at the site of surgery to improve analgesia and control bleeding. A skin incision with a periosteal flap was used to expose the bone in the proximal medial tibia. The preparation of the bone site was carried out with drills for the conventional drilling sequence recommended by the manufacturer for the 3.75-mm-diameter and 10-mm-length implants: started from the pilot drill (2.4 mm diameter), an intermediate drill (3.0 mm diameter) and then ended with the final drill (3.6 mm maximum diameter provided with each implant), all under copious saline irrigation. Coming to the lateral cortical of the tibia where the apex of the implants was accommodated, generated a bicortical anchorage. One implant of each group was inserted into each tibia, totalizing three implants per tibia. The variation position of the implants was determined by the online program randomization.com. During the insertion, the principal care was that the most part of the apical portion of each implant stayed inserted into the cortical bone, and the stability was measured.

The periosteum and fascia were sutured with catgut and the skin with silk. Postoperatively, a single dose of 600,000 IU Benzetacil was used. After surgery, the animals were placed in individual cages with 12-h cycles of light, controlled temperature (21°C) and *ad libitum* diet normally used by the lab- oratory. No complications or deaths occurred in the postoperative period. All animals were euthanized with an intravenous overdose of ketamine (2 ml) and xylazine (1 ml), being three animals for each time: after 6 weeks (T1), 8 weeks (T2) and 12 weeks (T3). The both tibias were removed (Fig. 2), placed in formalin solution 10% and immediately taken to the laboratory (Biotecnos, Santa Maria, Brazil) for analysis.

Resonance frequency analysis

All rabbits were used for resonance frequency analysis (RFA) to measure the implant stability. A SmartpegTM (Integration Diagnostics AB, Goteborg, Sweden) was screwed into each implant and tightened to approximately 5 N. The transducer probe was aimed at the small magnet at the top of the SmartpegTM at the same time. For comparison between times within the same group, the Friedman test for repeated measures was used followed by the multiple comparison test. For testing the correlations between the time periods and groups, the nonparametric test that generates the Spearman correlation coefficient (Rho, q) was used. All tests were performed at the 5% significance level using the Med- Calc for Windows, version 12.8 (MedCalc Software, Ostend, Belgium).

Results

The surgical procedures were uneventful without inflammation or infection; implants were not lost during the duration of this experiment.

Resonance frequency analysis (RFA)

A mean value was calculated from the me surements performed parallel to the long axis of the rabbit tibia. The mean resonance frequency values (ISQ) for the three investigated implant design are summarized in the Table 1 and evolution illustrated in Figure 4.

Applying the Kruskal-Wallis test, difference was observed between the groups for the times T0 (P = 0.04) and T2 (P = 0.004) and showed no differences between groups for the times T1 (P = 0.31) and T3 (P = 0.23). For the time T0, the multiple comparison test showed only differences between Group 1 and Group 2. In the time T2, the multiple comparison test showed differences only between Group 2 and Group 3.

In the Friedman's test, significant different distance of 2 or 3 mm and held stable during the pulsing until the instrument beeped and displayed the ISQ value. For RFA, the implants were measured immediately after the installation (T0) and during three times of removal (T1, T2 and T3). The implant stability quotient (ISQ) values were measured by OsstellTM Mentor (Integration Diagnostics AB). The ISQ values were measured in two directions proximal to distal and medial to lateral and made an average of each sample (Fig. 3).

Samples treatment for histomorphometric analysis

The samples were dehydrated using an ascending series of alcohols and embedded in glycol methacrylate resin (Technovit 9100 VLC, Kulzer, Germany) to produce undecalcified sections. Undecalcified cut and ground sections that contained the central part of each implant and had a final thickness of 50 lm were produced using a macrocutting and grinding system (Isomet 2000, Buehler, Germany). The sections were stained with toluidine blue and acid fuchsin, and histomorphometric analysis was carried out.

Specimens that had been prepared for the histological analysis of the tissue that sur- rounded the implant were examined using a light microscope (EOS 200; Nikon, Tokyo, Japan). After digitizing the phase of each specimen under light microscope, the linear portions of bone-to-implant contact (BIC) were measured as the percentage of bone in direct contact with implant surface in relation to total perimeter of implant surface inserted into bone, using the program *Image-Tool* version 5.02 for *Microsoft Windows*TM.

Statistics analyses

Statistical analyses were performed using Kruskal-Wallis test followed by multiple comparison test between groups and within Group 2 and Group 3. In the Friedman's test, significant differences between time periods were observed for the Group 1 ($P < 0.0001$), Group 2 ($P < 0.00001$) and Group 3 ($P < 0.00001$). In the multiple comparison test (significance level of 5%), there was no difference between the times T1 and T2 in the Group 1, between the times T2 and T3 in Group 2 and between the times T1 and T2 in Group 3.

Histological observations

At 6 weeks, all groups of implants showed a new bone formation at the bone–implant interface on the cortical bone site of the upper implant. No infiltration of inflammatory cells or proliferation of fibrotic tissue in the interface between the implant surface and the bone had occurred, and adhesion between the new bone and the implant was observed. There was no remarkable difference between the three groups tested (Fig. 5).

In the Group 2, there was some evidence that new bone that had previously adhered to other two groups, probably the explanation for the higher values of ISQ presented. However, the histological findings were similar among the groups (Fig. 6).

On the implant surface of all groups at 12 weeks, the mass of bone was similar to that observed at 8 weeks. However, remark- able bone remodeling had occurred, so that bone marrow was observed at the interface. New bone that had been formed after reabsorption was seen in some cases. However, at many sites, the new bone could not be distinguished from the adjacent preexisting bone, due to the almost complete formation of compact bone (Fig. 7).

Histomorphometric analysis

The mean and standard deviations of the BIC % of the 3 groups at weeks 6, 8 and 12 are presented in the bar graph of Figure 8 and the data summarized in the Table 2. The Friedman test showed significant differences between the proposed time periods for the Group 1 ($P = 0.00147$), Group 2 ($P = 0.01030$) and Group 3 ($P = 0.007$). In the multiple comparison test (significance level of 5%), there was no difference between the times T2 and T3 for the Group 1, between the times T1 and T2 in Group 2 and between the times T1 and T3 in the Group 3. The Kruskal–Wallis test showed no differences between groups for the times T1 ($P = 0.54$), T2 ($P = 0.54$) and T3 ($P = 0.89$).

Correlation between the ISQ and BIC%

Correlations between ISQ and BIC were verified in Group 2 for time T1 ($P = 0.0048$; q = 0.75; CI 95% [0.31–0.92]) and for the Group 1 in the time T2 ($P = 0.0173$; q = 0.66; CI 95% [0.15–0.89]).

Discussion

It has been reported that successful osseointegration of an implant *in vivo* is affected by many factors, such as the surface morphology and geometric shape of the implant, the bone quality and mass around the implant body, and the size and direction of the load during functional occlusion. Furthermore, it was reported that the surface features of an implant, including surface roughness and ultrastructural morphology, have a remark- able influence on osseointegration (Martinez et al. 2001*;* Li et al. 2002*;* Buchter et al.2006*;* Novaes et al. 2010*;* Gehrke et al. 2015). Insights into cellular processes occurring at the implant had been resorbed during the process of bone remodeling. The cervical portion, where contact between implant and cortical bone occurs, showed a slightly accelerated mineralization (observed by the histological coloration) compared with the implant/bone interface have contributed much to an understanding of osseointegration. The understanding of the complex have been used to measure, compare and analyze the responses of the surrounding bone and the amount of new bone that was stimulated by various types of implant, surgical method, site of placement, healing period and conditions to produce extensive data that are clinically applicable (da Silva Neto et al. 2014; Gehrke 2015). In this study, the con- tact ratio between the bone and implant was used.

The histological overview of the bone/im- plant features in the present study demonstrates a congruency between the implant and the surrounding bone tissue. A direct contact between titanium and bone was visible over the whole surface area of the implants during the experimental periods. One underlying reason for the direct bone/ implant contact found in this study may be the three-dimensional geometric relation between the final drill and the implants (in the groups 2 and 3), and the self-tapping properties of these implants. Lightly superiors results presented in groups 2 and 3, especially in the early periods of 6 and 8 weeks after implantations, may be related to the use of end drill provided along with these two models of implants. Previous research has pointed that a region of necrotic bone surrounding the implant exists following surgery and that the extent of this region is influenced by driling speed (Friberg et al. 1995), design *(*Joos et al. 2000*;* Huang et al. 2007*;* Chung et al. 2008*;* Gehrke et al. 2015) and irrigation mode (or absence of irrigation). For most of the research concerning drills and drilling technique variations, the most commonly measured outcome concerns the heat generated at these sites as a function of different variables always referenced by a suitable control group (Calvo-Guirado et al. 2015). bone/implant interactions at different levels will provide an opportunity to evaluate and produce implants with specific and desired biological responses (Davies 2005*;* Natali et al. 2006). Various studies emphasize that the mode of implant osseointegration and stability is dependent to a large extent on the macro- and ultrastructural implant design (Albrektsson & Johansson 2001*;* Davies 2005*;* Buchter et al.2006*;* Vandamme et al. 2007). However, the role of implant geometry and surface structuring in affecting early tissue healing and

implant stability cannot be determined only from histological or biomechanical observations. The dynamics of bone physiology can also not be evaluated several weeks postimplantation after long-term bone remodeling has occurred. Therefore, early bone responses have to be considered when the influence of implant geometry and surface structuring on interface formation is under investigation. Therefore, an implant system provides a range of design, probably designed to increase initial stability and facilitate osseointegration, with the same surface on all models; thus, only the variation of design was evaluated (Gehrke et al. 2015).

Histomorphometric analysis was used for quantitative analysis that is difficult to explain only with findings under a light microscope. There are two indicators of osseointegration: the contact ratio between bone and implant, which measures the degree of contact between bone and implant in the tissue specimen, and the bone surface area, which measures the amount of new bone formation quantitatively within the conchoids of the implant. These methods Excellent adaptation of the host bone to titanium surfaces was observed also on an ultrastructural level in a comparable manner as reported after insertion of self-cutting screws in calvaria bone (Sowden & Schmitz 2002). The results comparing the different types of apical implant designs demonstrated similar results, that is, with suitable bone quantity and quantity. However, the implants with a more aggressive design and highest potential self-tapping showed a slightly inferior performance to the apical implants with smoother design.

Biomechanical evaluation of implant stability can be performed by nondestructive analysis (RFA), used in the present study (Meredith et al. 1997a,b), or destructive (removal torque tests). To visualize the architecture of the tissue/implant interface, the histological techniques (light microscopy) or ultrastructural analysis (electron microscopy) could be used. The different biomechanical and biological approaches, except for the RFA determinations, exclude each other because of the sample preparation. Therefore, a combined association between RFA, previous to the histological preparation, and light microscopy was used in this study to give a better insight into the morphological and functional features of interfacial tissue formation.

The RFA of this study confirms the high primary stability of implants directly after placement. Implant stability after 6, 8 and 12 weeks of osseointegration reached values of the baseline level, indicating high primary implant stability. The range of RFA found in this study corresponds well to the data reported by Meredith et al. (1997a,b; Gehrke & Wiel-Marin 2014). In the present study was found a correlation between the histomorphometric and biomechanical measurements, indicating that the combined histological and biomechanical approaches reflect the biological situation of peri-implant bone. Primary stability is necessary to establish mechanical rest, which is one of the essential factors for the development of osseointegration (Calvo-Guirado et al. 2013).

Conclusion

With the limitations of this animal study, it was concluded that the design of the apical area influences the implant stability and the bone-to-implant contact.

Conflict of interests

The authors have no conflict of interests to declare.

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Figure Legends

Fig. 1. Image showed the design of the implants used in the study. From left to right M4®, Seven®, C1® (MIS Implants M

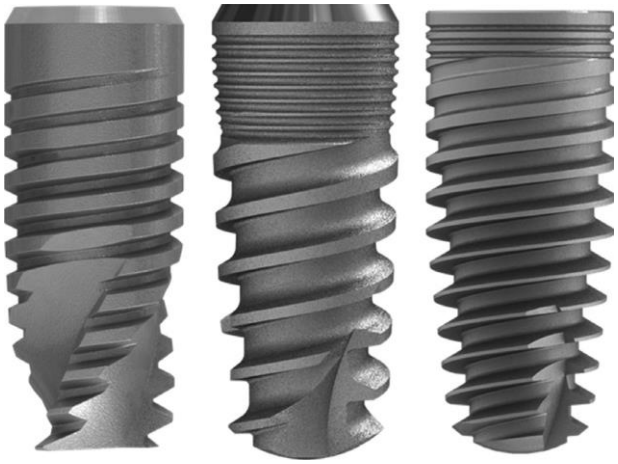


Fig. 2. Both the tibia samples removed of each animal.

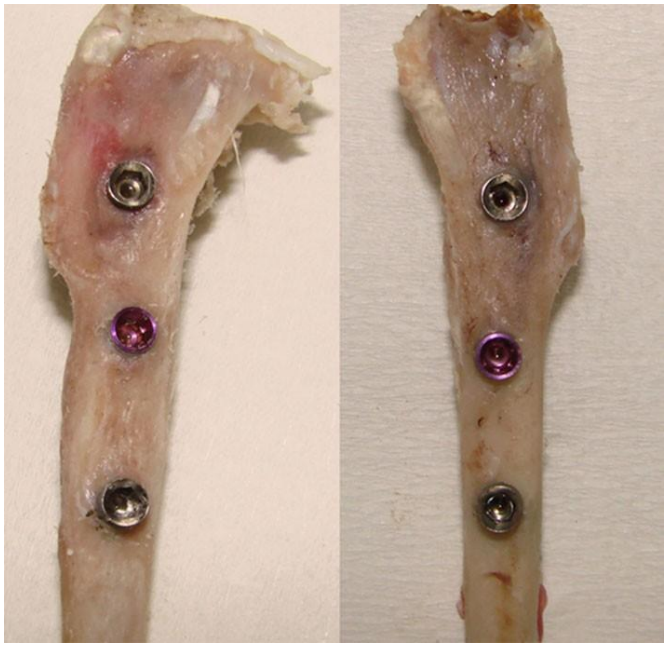


Fig. 3. Scheme showed the directions determined to measure the resonance analysis frequency.

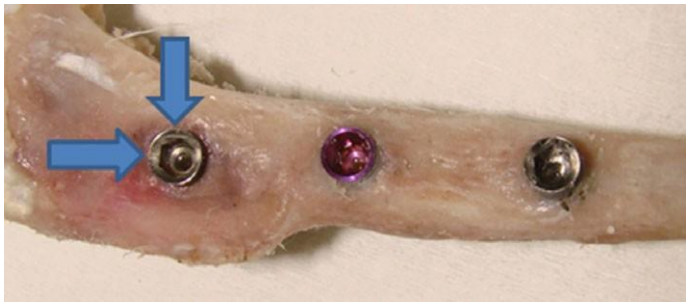


Fig. 4. Bar graph of mean and standard deviation of the resonance frequency values (ISQ) of the groups.

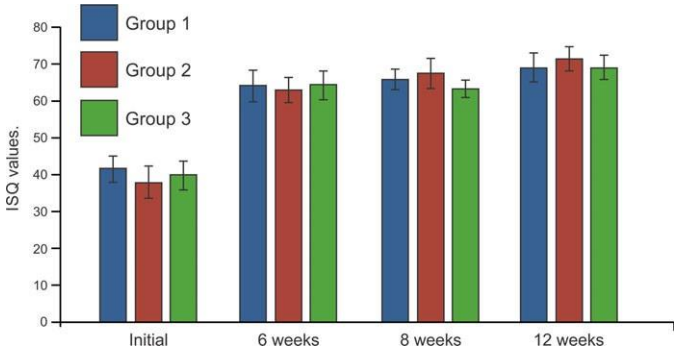


Fig. 5. Histological sections (stained with toluidine blue and acid fuchsin) of the three implant groups with 6 weeks after implantation in rabbit tibiae (original magnification 910). (a) Group 1, (b) Group 2 and (c) Group 3.

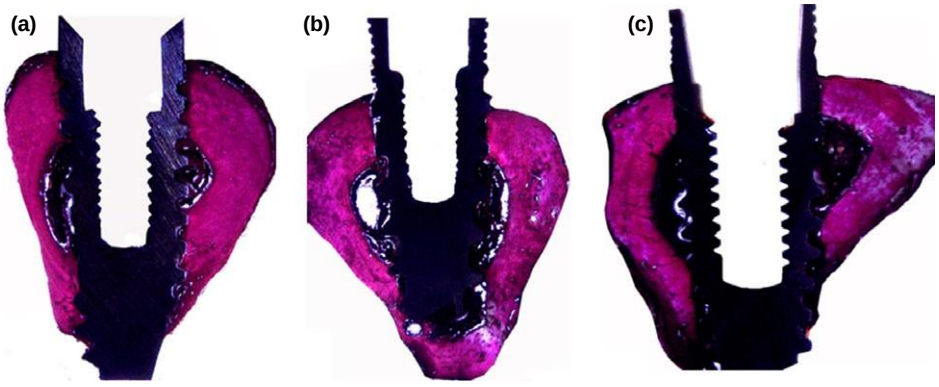


Fig. 6. Histological sections (stained with toluidine blue and acid fuchsin) of the three implant groups with 8 weeks after implantation in rabbit tibiae (original magnification 910). (a) Group 1, (b) Group 2 and (c) Group 3.

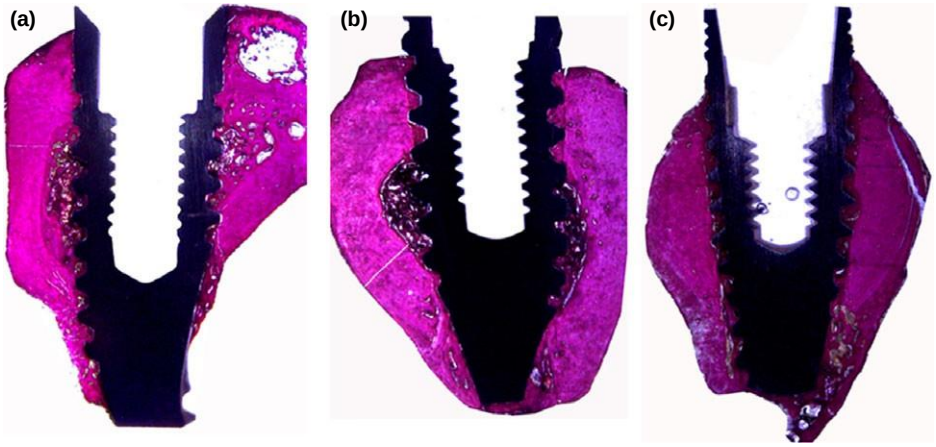


Fig. 7. Histological sections (stained with toluidine blue and acid fuchsin) of the three implant groups with 12 weeks after implantation in rabbit tibiae (original magnification 910). (a) Group 1, (b) Group 2 and (c) Group 3.

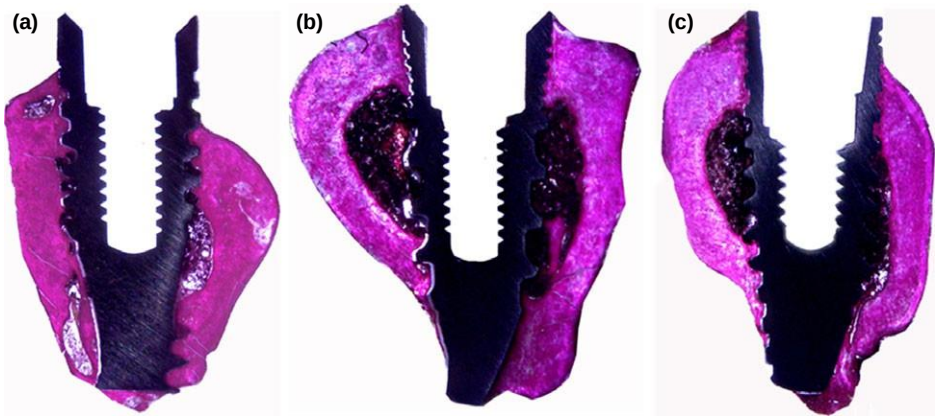
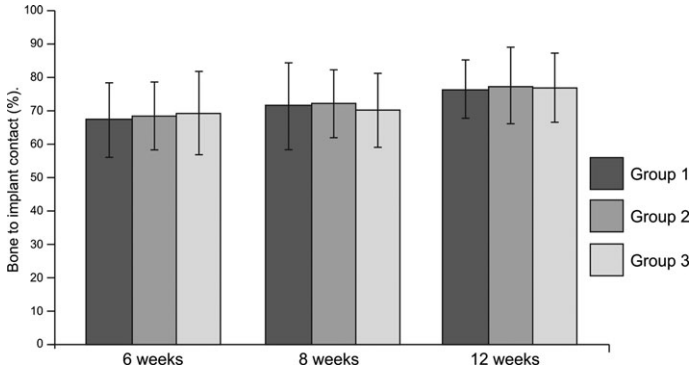


Fig. 8. Bar graph of the bone-to-implant contact values in percentage (%) and standard deviation at each time.



Tables

Table 1. Mean values of ISQ standard deviation (SD) and medians for the groups in each time periods

	Times							
Group	T0		T1		T2		T3	
1	41.3	3.58 (42)	63.9	4.23 (66)	65.5	2.84 (65)	68.8	3.86 (69)
2	37.8	4.24 (37)	62.6	3.42 (62)	67.2	4.02 (68.5)	71.1	3.18 (72)
3	39.8	3.77 (38)	63.9	4.03 (64.5)	62.9	2.98 (63.5)	69.6	3.20 (69.5)

Table 2. Mean values of BIC% standard deviation (SD) and the medians for the groups in each time periods

	Times					
Group	T1		T2		T3	
1	67	7.9% (67.5)	76	8.0% (78.5)	71	8.7% (72)
2	68	8.8% (71)	77	7.4% (79)	72	7.4% (72)
3	69	10.0% (72.5)	76.5	7.1% (79)	70	7.8% (70.5)