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Analysis of the Early Stages of Osseointegration in Two Surfaces Dental Implants: Pilot Study

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Abstract

Objective: The aim of this article is to compare two surface treatment types of the dental implants inserted in tibiae of rabbits on the early stages of the bone repair considering the pattern of maturation of collagen fibers and the osseointegration process.

Materials and methods: 10 modSAE (sandblast and acid etched modified) and 10 AO (anodic oxidation) implants were evaluated such as bone-implant contact (BIC), and collagen fiber orientation verified by circular polarized light (BICCPL). The results were measured at 7, 21 and 42 postoperative days.

Results: The modSAE implants induced greater BIC at all times observed. The BICCPL analysis revealed the both implants showed a maturation pattern collagen fiber formation. However, BICCPL values of modSAE were higher than AO values at 21 days.

Conclusions: In conclusion, the topography and surface characteristic of modSAE implant showed a tendency to improve bone tissue integration and also contributes to maturation of collagen fibers. This fact was evidenced at 21 days. The study shows the influence of topography and surface treatment of titanium dental implants in different stages of bone repair.

Keywords: Collagen fibers; Histomorphometric analysis; Implant surface; Osseointegration; Bone repair

Introduction

A major advance in dentistry has been the successful replacement of lost natural teeth by osseointegrated implants. Osseointegration is a phenomenom where intimate contact between bone and biomaterials occurs at the optical microscopy level, enabling dental implants to replace load-bearing tooth organs restore their form and intraoral function [1]. The most recent generation of dental implant surfaces have been modified by chemical/physical treatments and nanotechnology in order to further improve the bone integration process. Different technologies, including grit-blasting, acid-etching, anodic oxidation, coating or combinations of techniques have been used to change the surface topography in an attempt to improve implant fixation with bone [2].

Based on this information, several authors have recommended the immediate use of surface treated implants [3-7]. However, there is still no consensus about the safety of using them before the osseointegration process has fully developed [8]. The preferred orientation of collagen fibers in bone is one such feature. Investigations of collagen fiber orientation in bone by circularly polarized light microscopy provide important insight on the biomechanical efficacy of the skeleton, particulary with respect to studies of functional morphology [9]. In osseointegration of dental implants, studies have demonstrated the use of the method of circular polarized light as an important criterion for research in pattern of collagen fiber orientation since this pattern is closely related to bone repair. The orientation of collagen fibers is strictly dependent on the shape of the implants [10]. Experimental studies have compared different types of implant surfaces and geometries [11-13]. However, few studies have compared chemically treated sandblast and acid etched, which is hydrophilic (modified), with anodic oxidized surface [11]. The purpose of the present study was to compare the both cylindrical implants modSAE (chemically treated sand-blast and acid etched modified) and AO (anodic oxidation surface) to evaluate aspects such as bone-implant contact (BIC) and collagen fiber orientation verified by means of circular polarized light (BICCPL) on early stages of osseointegration.

Materials and Methods

This study was submitted to the Research Ethics Committee of the São Paulo Hospital, Federal University of São Paulo School of Medicine, and was approved under Protocol No. 1912/08.

10 male rabbits of the New Zealand strain were used in this experiment. Each tibia received 2 implants, totaling 20 implants, being 10 implants with an oxidized surface (Branemark System MKIII TiUnite, Nobel Biocare AB, Gothenburg, Sweden; AO) 4.0 mm in diameter and 8.5 mm long, and 10 implants with a hydrophilic sand-blasted and acid etched surface (Bone Level SLActive, Institute Straumann AG, Basel, Switzerland; SAE), 4.1 mm in diameter and 8.0 mm long.

Operative procedure

The animals were anesthetized and after that were made two bone perforations in each tibia with 10 mm distance between them. Two

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implants were inserted alternatively in each tibia: 1 modSAE and 1 AO type, at the proximal and distal positions. The primary stability was 30 N/cm.

The animals of each group were subjected to euthanasia on the 7th, 21st and 42nd days.

Sample preparation and histomorphometric analysis

The tibias were sectioned at the extremities using micro motor, and were kept 10% buffered formaldehyde. The samples were processed in the Hard Tissue Research Unit, Department of Biomaterials of New York University College of Dentistry, USA.

The fixated bone–implant biopsies were dehydrated in a graded series of alcohol and then embedded in a light-curing plastic resin. The plastic blocks were sectioned using a sawing and grinding technique (Exakt 300 CP Band System (Exakt Technologies, Oklahoma City, OK, USA). Sections were stained with Stevenell Blue-Van Gieson. High-resolution image montages were acquired with an Aperio GL ScanScope Slide Scanner (Vista, CA, US) using an Olympus UPlanSApo. The circularly polarized light images were acquired with a Leica DMRX/E Universal Microscope fitted with a Leica PL Fluotar (Wetzlar, Germany).

The histomorphometric measurements and analysis of collagen fiber maturation were obtained using software Axio Vision 4.7.

Results

BIC and BICCPL values measurements

Table 1 provides the values of BIC, BICCPL for the different groups at the 3 time intervals. When the modSAE implant was used, the BIC values were higher than those of the AO implants at all of the time intervals observed. At 21 days the BICCPL results were coherent with those obtained in BIC, and modSAE implants showed greatest value of collagen fibers orientation adjacent implant.

Histology

The histological examination revealed that the threads of AO and modSAE implants were occupied by new bone tissue at 21 days, respectively (Figures 1 and 2). At the same period, were noted the formation of a disorganized bone tissue in contact with the implants and, adjacent to this, a bone tissue with a defined orientation patterns, as showed at AO (Figure 3) and modSAE implants (Figure 4). In addition, the images showed the presence of osteoblasts in the osteoid matrix and osteocytes in the bone matrix (Figures 5 and 6). However, at 42 days, the formation of organized bone tissue was detected close to the implants surfaces AO and mod SAE, respectively (Figures 7 and 8).

Circularly polarized light

Observations under circularly polarized light revealed the defined orientation patterns of collagen fibers. This pattern was observed close to the surfaces of modSAE and AO implants, respectively (Figures 9 and 10). Dark and bright regions represent collagen fibers that are able to resist to compressive and tensile forces, respectively. This is an indication that the maturation of collagen fiber pattern occured at 21 days.

Discussion

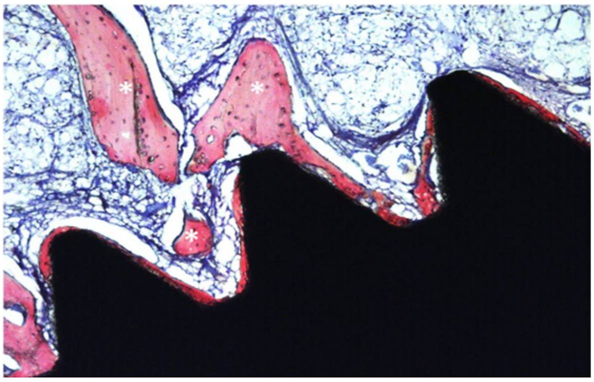
There are several surface treatments of dental implants in order to promote the adhesion of osteoblasts to titanium [11]. Thus, experimental studies comparing different types of surface and geometry of implants has been performed [6,10-13]. However, few studies have compared the implants with hydrophilic sandblasted and acid etched surface with anodic oxidation surface in the early stages of bone repair [11]. In this work, the purpose was to compare these 2 implants, modSAE and AO, evaluating aspects such as bone-to-implant contact (BIC) and orientation of collagen fibers (BICCLP) on early stages of bone repair.

The results, obtained by descriptive analysis of BIC, showed that when modSAE implant was used, the amount of new bone tissue formed around the implant was higher than that obtained for AO implant, indicating that the surface of modSAE is more suitable for stimulating bone formation. These results are similar by Bornstein et al. [14] findings, that showed the greater amount of bone formed (BIC) for implants with hydrophilic sandblasted and acid etched surface in

Groups	Days	Mean BIC % (min. to max.)	Mean BICCPL % (SD)
AO	7	25.3 (23.4 to 27.3)	18.1 (±2)
	21	39.2 (35.4 to 43)	34.1 (±2)
	42	27.2 (22.4 to 32.1)	25.1 (±9.3)
Modified SAE	7	45.1 (41.6 to 48.5)	16.9 (±3.8)
	21	51.9 (34.5 to 69.2)	46.9 (±8.5)
	42	51.5 (43.6 to 59.5)	30.9 (±7)

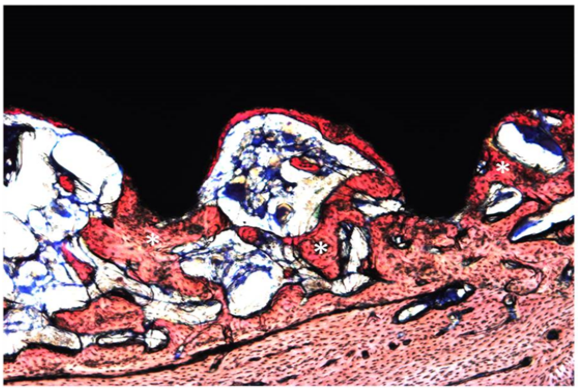
SD= Standard Deviation

Table 1: Mean BIC, BICCPL (BIC circularly polarized light).



* New bone tissue formation within and outside the threads

Figure 1: AO implant 21 days. FW = 2610 μm.



* represent areas with new bone tissue formation adjacent to implant.

Figure 2: Modified SAE implant 21 days. FW = 2610 μm.

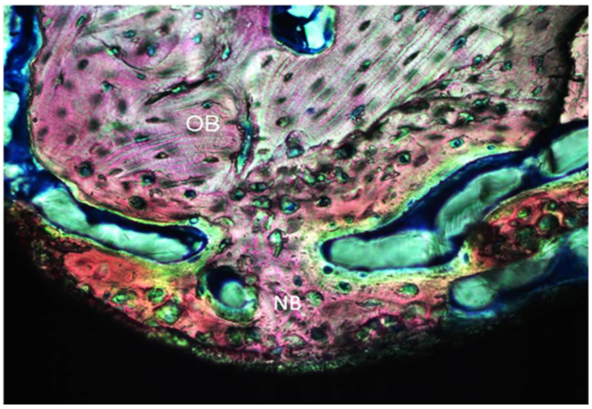


Figure 3: AO implant 21 days. New bone tissue formation adjacent to implant (NB) and old bone (OB). FW=400 μm.

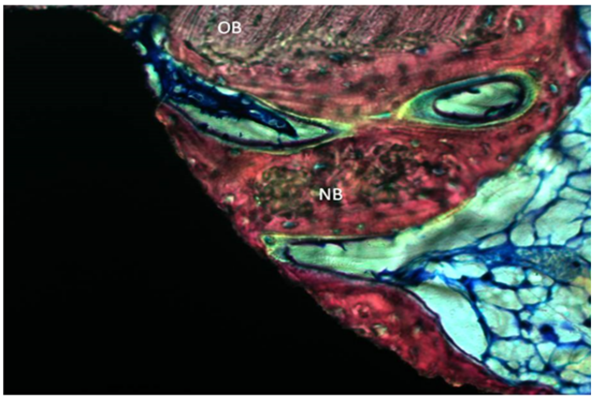
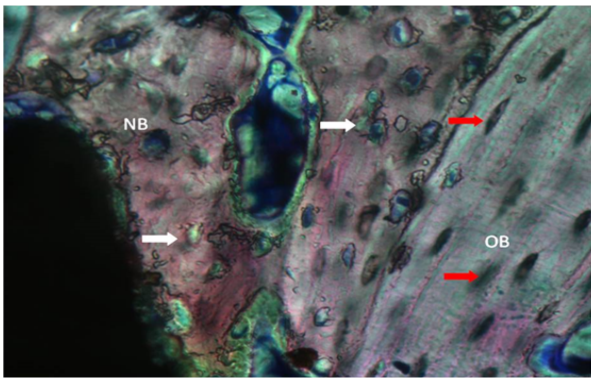


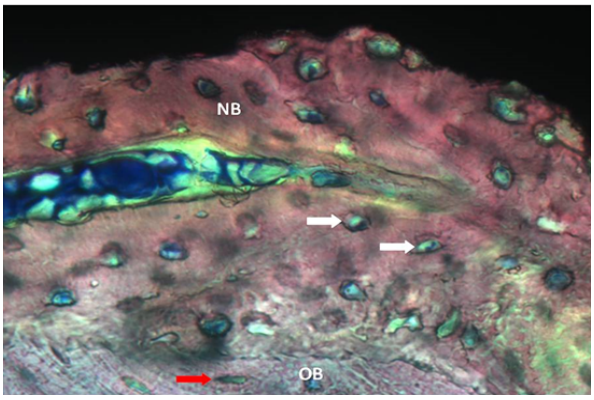
Figure 4: Modified SAE implant 21 days. New bone tissue formation adjacent to implant (NB) and pre-existent bone (OB). FW = 400 μm.



NB=new bone and OB=old bone; Organized tissue with presence of osteocytes (red arrows) and osteoblasts (white arrows).

Figure 5: AO implant 21 days. Presence of high cellularity close to implant, represented by developing and adjacent bone tissue. FW = 200 μm.

the mandible of dogs after 2 weeks of loading. The hydrophilic property of the implant modSAE can stimulate the migration of osteoblasts in the early stages of bone formation [15,16], which justifies the results observed. Recently, Gottlow et al. [11] carried out a comparative study to evaluate the osseointegration of implants with hydrophilic sandblasted and acid etched surface and oxidized surface (OX). In agreement with present study, HSBA (modSAE equivalent) had high value of BIC than OX (AO equivalent) on the first days after insertion. In the analysis in 21 days after insertion, the implants HSBA and OX had the same value of BIC, unlike the present study has demonstrated



New bone tissue adjacent to modified SAE implant (NB) with osteoblasts (white arrows) in osteoid matrix delimited by bone tissue with defined pattern (OB) and osteocytes (red arrow).

Figure 6: Modified SAE implant 21 days. FW = 200 μm.

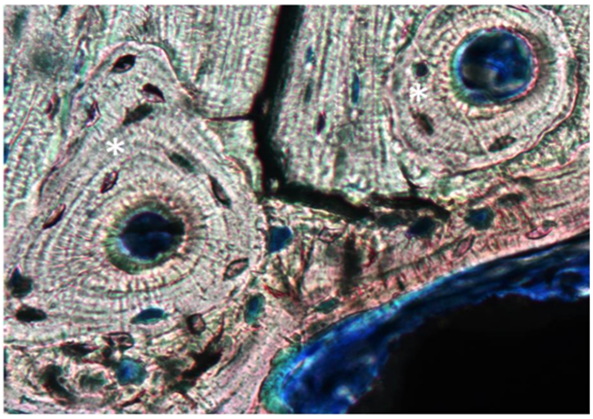


Figure 7: AO implant 42 days. Mature bone tissue with osteons involving the Harvesian system (*). FW= 400 μm.

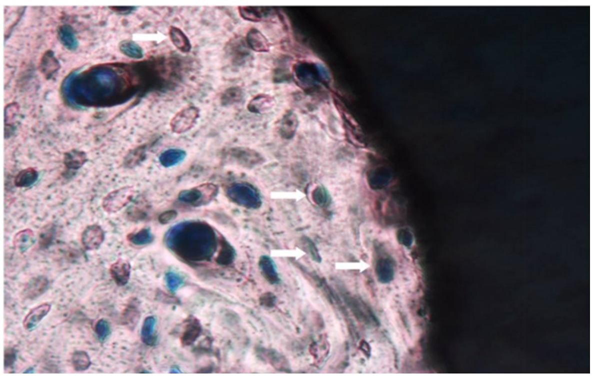
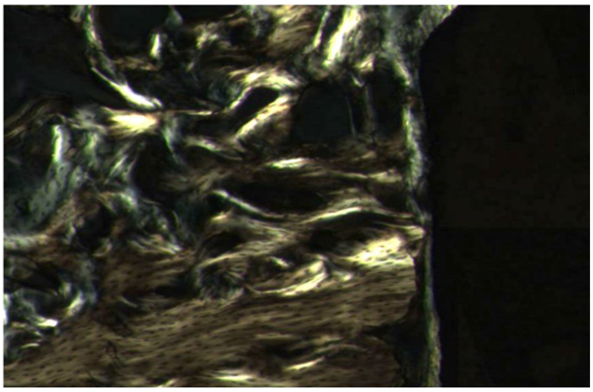


Figure 8: Modified SAE implant 42 days. Calcified bone matrix with osteocytes close to the modified SAE implant (white arrows). FW=200 μm.



The images show bone tissue with transverse or alternated collagen fibers. Note the predominance of dark collagen fibers in this image, denoting that the bulk of fibers perpendicular to the axial plane of the implant are best oriented to resist compression.

Figure 9: Modified SAE implant 21 days imaged with CPL. FW = 1305 µm.

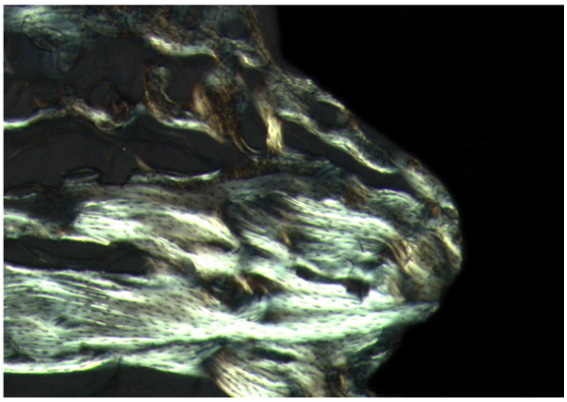


Figure 10: AO implant 21 days imaged with CPL. Note the predominance of dark collagen fibers at the top of the image and bright fibers in the lower half of the image. FW = 1305 µm.

higher BIC for modSAE. Furthermore, in the last analysis after 42 days, unlike the findings of Gottlow et al. [11] where OX showed higher BIC values than HSBA, modSAE showed BIC values higher than the AO. This difference may be due different geometry of the implants used in our work (modSAE and AO, both cylindrical) in relation to the model compared by the author (OX and HSBA, conical and cylindrical, respectively). In addition, the implant modSAE presents the greatest number of treads. This characteristic might have influenced the increase in the formation of bone tissue and consequently resulted in a higher BIC value. This fact has been supported by Ivanoff et al. and Brunski et al. [17,18], who mentioned that the implant treads maximize initial contact, improve initial stability, enlarge implant surface area and favor dissipation of interfacial stress.

Determination of the orientation of collagen fibers in human tissues, such as bones, is indispensable when studying the relationship between the physical properties and the structures [19]. Several studies relate the analysis of polarized light and maturation pattern of collagen fibers. However, there is a scarcity of data in the literature regarding the early loading analysis. A comparative study of Traini et al. [10] in human bone, observed the higher percentage a of transverse collagen fiber formation, 32.96% (bright), while only 19.70% (dark) was composed of longitudinal collagen fibers around implants that received immediate function. However, this study evaluated the samples 6 months after loading, not being possible to detect, in this period which the collagen fibers begin their maturation. In studies of BICCLP, the results of the present study demonstrated that collagen fibers appeared with bright appearance in its majority in the bone tissue cortical and slightly spongy in all periods (7, 21 and 42 days). At 7 days the AO implant presented a higher BICCLP mean in comparison with the modSAE type, however, at 21 and 42 days, the modSAE implant exceeded the AO type in BICCLP measures. This result suggests that the surface of modSAE promoted an acceleration of maturation of collagen fibers after the seventh day contributing to greater osseointegration.

A highlight of this work was to compare modSAE and AO implants taking to consideration the bone tissue formation, stability and the pattern of collagen fibers orientation on the initial stages of the bone repair.

The set of results obtained from the present study add to the literature and reinforce the importance of topography and surface treatment of titanium dental implants in different stages of bone repair.

Conclusion

In conclusion, the topography and surface characteristic of modSAE implant showed a tendency to improve bone tissue integration and also contributes to maturation of collagen fibers. This fact was evidenced at 21 days. The study shows the influence of topography and surface treatment of titanium dental implants in different stages of bone repair.

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