Primary Stability of Turned and Acid-Etched Screw-Type Implants: A Removal Torque and Histomorphometric Study in Rabbits

Eduardo de Lima Fernandes, DDS, MSc[1]/Ieda Levenzon Unikowski, DDS, MS[2]/
Eduardo Rolim Teixeira, DDS, PhD[3]/Nilza Pereira da Costa, DDS, MSc, PhD[4]/
Rosemary Sadami Shinkai, DDS, MSc, PhD[5]

Purpose: *This study evaluated the effect of primary stability on the osseointegration of turned and acid-etched screw implants in a rabbit model.* ***Materials and Methods:*** *One pair of turned and acid-etched implants (3.75 in diameter, 5.5 mm long) was placed in each tibia of 15 rabbits. In the right tibial metaphysis the implants were inserted according to a standard surgical protocol. In the left tibia, the osteotomy sites were enlarged using a sequence of drills, and 2 implants were placed with reduction of primary stability. Animals were sacrificed 9 weeks after surgery. Histomorphometric and removal torque analyses were performed to evaluate bone-implant contact and strength of osseointegration.* ***Results:*** *Surface texture had a significant effect on percentage of bone-implant contact (P = .001). Acid-etched implants with high primary stability showed the highest percentage of bone-implant contact (77%), followed by acid-etched implants with low primary stability (61%), turned implants with low primary stability (56%), and turned implants with high primary stability (46%). For removal torque, acid-etched implants had higher peak mean values than turned implant groups (P < .001). Reduction of primary stability was not significant to either percentage of bone-implant contact (P = .645) or removal torque values (P = .214).* ***Conclusion:*** *Acid-etched implants had higher bone response and implant fixation than turned implants, regardless of primary stability.* INT J ORAL MAXILLOFAC IMPLANTS 2007;22:886–892

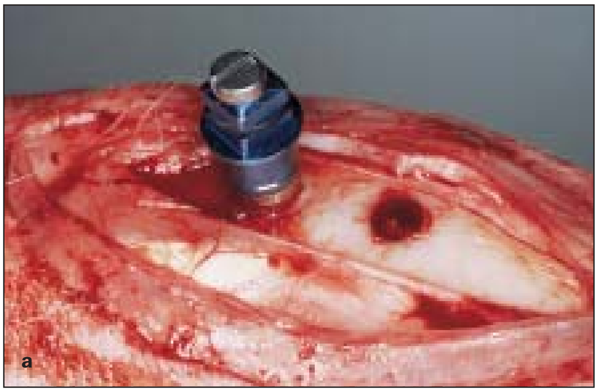
Key words: *dental implant, osseointegration, primary stability, surface treatment*

Primary stability during implant surgery may help optimize early osseointegration of screw-shaped titanium implants.[1,2] Implants with deficient initial stability are susceptible to micromotion at the bone-implant interface, which may affect the bone healing process and result in fibrous encapsulation.[3] Achievement of primary stability depends on adequate preparation of the bone site to receive the implant and demands strict adherence to surgical protocols and use of specific instruments for each implant system.[4] However, the role of primary stability in bone healing around different types of implants still is unclear, as some studies suggested that primary stability may not be necessary for successful osseointegration in type 4 bone[5] or in cortical- or trabecular-like bone in rabbits.[6]

Several methods have been used to modify the implant surface topography, such as the creation of an isotropic surface (ie, one with randomly distributed asperities) or an anisotropic surface (ie, one with irregularities with a directional pattern), to

[1]Associate Professor, Department of Prosthodontics, Lutheran University of Brazil, Canoas, RS, Brazil.
[2]Private Practice, Porto Alegre, RS, Brazil.
[3]Associate Professor, Department of Prosthodontics, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.
[4]Professor, Department of Oral Surgery, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.
[5]Associate Professor, Department of Prosthodontics, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Correspondence to: Dr Eduardo de Lima Fernandes, Rua Arthur Rocha, 389 - apt 202. 90450-171, Porto Alegre, RS, Brazil. Fax: +55 51 33326188. E-mail: edu2f@terra.com.br



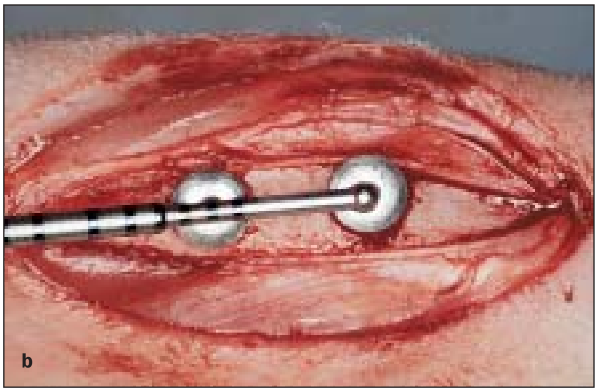


Fig 1 *(a)* Implant with mount inserted into the osteotomy site. *(b)* Implants placed in the tibiae metaphysis approximately 10 mm apart.

speed up the healing process and allow earlier loading. However, there is no clear scientific clinical evidence supporting the benefits of specific surface characteristics.[4,7] Some in vivo studies have shown that surface treatment of titanium implants seems to affect the osseointegration process and increases bone-implant contact ratios in short-term periods compared to machined implants, even in bone of poor quality.[8–16] Osteoblastic cells in contact with rough implant surfaces express their phenotype differently in terms of speed and amount of bone protein production compared to cells in contact with smoother surfaces.[17–21] Regarding surface roughness, moderately rough surfaces (Ra/Sa between 1.0 and 2.0 μm) seem to display stronger bone response (ie, more interfacial bone than smoother or rougher surfaces), but the differences often are small and insignificant.[22] However, a recent systematic review of experimental animal studies reported a positive relationship between surface roughness (Ra/Sa from 0.5 to 8.5 μm) and implant fixation on bone response after 12 weeks of implantation.[23]

Although primary stability and surface treatment may individually affect the osseointegration process, the combined effect of both variables in vivo is unknown. A recent literature review by Jokstad et al[4] reported the lack of a definitive protocol for implant use with respect to implant geometry and surface topography in cases of low bone density. Animal studies and long-term clinical data also are not conclusive regarding the potential to achieve osseointegration in low-density bone with varying surface textures. The aim of this study was to compare the osseointegration of turned and acid-etched implants placed with low primary stability in rabbit tibia using mechanical and histologic methods. The a priori hypothesis was that acid-etched implants with good primary stability would have higher bone-implant contact and strength of osseointegration than other surface texture/primary stability combinations.

MATERIALS AND METHODS

Sixty screw-shaped, external-hex commercially pure titanium (ASTM grade 4) implants, 5.5 mm in length and 3.75 mm in diameter, were used in this study. Thirty implants had a l machined turned surface (Master Screw; Conexão Sistemas de Prótese, São Paulo, Brazil), and 30 implants had an acid-etched surface (Master Porous; Conexão Sistemas de Prótese). The surface roughness (Ra), according to the manufacturer, was 0.65 ± 0.11 μm for the turned implants and 0.51 ± 0.10 μm for the acid-etched implants. The implants were specifically manufactured for this study with a length of 5.5 mm to avoid bicorticalization during implant placement.

Twenty female adult New Zealand White rabbits (*Oryctolagus cuniculus*) weighing 3.5 to 4.5 kg were used in the study, which was approved by the university ethics committee. For surgery, anesthesia was induced by intramuscular injections of xylazine hydrochloride (Rompun; Bayer, São Paulo, Brazil; 1 mL/3 kg of body weight), ketamine-xylazine (Dopalen; Agribrands do Brazil, São Paulo, Brazil; 20 mg/kg of body weight), and acepromazine (Acepran 1%; Univet, São Paulo, Brazil; 1 mL/3 kg of body weight).

The tibial metaphysis was chosen as the experimental site. The surgical area was shaved and cleaned before skin incision and tissue dissection down to periosteum. Periosteal flaps were raised, and 2 implant sites per tibia were prepared with a standardized drilling procedure. First, the implant sites were marked 10 mm apart with a guide drill. They were then sequentially enlarged by a 2.0-mm twist drill, a pilot drill, and a 2.8-mm twist drill. In the left tibia, the bone perforations were further enlarged using a 3.15-mm twist drill, and the osteotomy sites were pretapped at 35 rpm, so that the implants placed into these osteotomy sites would be stabilized at the upper cortical bone but not at the lower cortical bone (ie, to prevent bicorticalization; Fig 1).

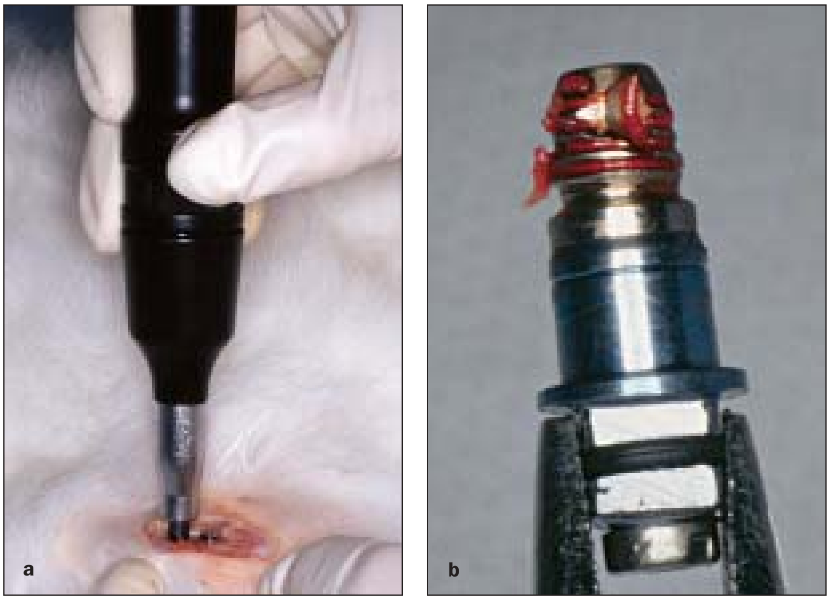


Fig 2 *(a)* Removal torque test. *(b)* Acid-etched implant after removal torque test. Some bone chips were attached to the implant surface.

Two implants were placed in each tibia: one turned and one acid-etched implant. In the right tibia, primary stability was achieved with both implants, as recommended by the optimal surgical protocol (high primary stability: control groups). Initial stability/instability of the implants was assessed as in previous studies.[6,24] In the left tibia, 1 turned and 1 acid-etched implant were inserted with low primary stability. Rotational mobility was observed inside the osteotomy site at finger drive pressure (low primary stability: test groups). No lateral and/or apical movement was clinically observed in either the control or test groups. All implants were gently screwed into place until the implant platform was level with the bone surface; bicorticalization was avoided. Titanium cover screws were placed on the implants, and fascia and skin flaps were closed in separate layers using single resorbable sutures (Categut 4.0; Johnson & Johnson/Ethicon, Somerville, NJ). Postoperatively, each animal received oxytetracycline hydrochloride antibiotic (Terramicina/LA, Laboratórios Pfizer, São Paulo, Brazil; 1 mL/3 kg of body weight) for 10 days. Five animals died within 2 weeks postsurgery due to systemic problems; therefore, the final sample comprised 15 animals.

Removal Torque Measurement

Nine weeks after surgery, 10 animals were randomly selected for the mechanical test, anesthetized as previously described, and sacrificed. The implant sites were surgically exposed, the cover screws were removed, and an implant mount was secured on each implant. Implant osseointegration was evaluated at this stage for mobility, bone loss, or any clinical sign of infection at the surgical sites. For implants with osseointegration, a reverse torque rotation force was applied individually to each implant using a torque gauge (TSD150 Torqueleader, MHH Engineering, Bramley, Guilford, Surrey, United Kingdom) until rotation was detected (Fig 2). The removal torque equipment was calibrated and certified in compliance with the requirements of ISO 6789.1992 and BSEN 26789/1994. Peak values of resistance to reverse torque rotation were recorded in Ncm.

Histomorphometric Analysis

The remaining 5 animals were sacrificed, and implants were removed in block with the surrounding hard and soft tissues. The implant-bone blocks were dehydrated in a graded series of alcohols and embedded in glycol methacrylate media (Technovit 7100 GMA; Kulzer, Wehrheim, Germany) to be sectioned longitudinally to the implant axis using a diamond disk mounted in a electric saw (Labcut 1010; Extec Corp, Enfield, England). From each block, 3 to 4 slices approximately 0.5 mm thick were obtained; ground to a thickness of 0.1 mm with 600-, 800-, and 1200-grit silicon carbide paper under water refrigeration; and stained with 1% toluidine blue. The histomorphometric analysis was performed using a surgical microscope (DF Vasconcellos MC-M31; DF Vasconcellos, São Paulo, SP, Brazil) connected to a digital camera (Samsung CCS-212; Samsung Opto-Electronics America, Secaucus, NJ). The digital images (16× and 25× magnification) were analyzed using the software UTHSCSA Image Tool version 3.0 (The University of Texas Health Science Center at San Antonio; download available at http://ddsdx.uth-

Table 1 Removal Torque (Ncm) 9 Weeks After Implant Placement in the Rabbit Tibia (n = 10 rabbits)

Primary stability/ Surface texture	Mean*	SD	N
Low			
Turned	29.4 a	4.2	7
Acid-etched	52.9 b	13.3	9
Total	42.6 A	15.7	16
High			
Turned	33.1 a	4.3	9
Acid-etched	47.4 b	9.5	10
Total	40.6 A	10.3	19
Total			
Turned	31.5 A	4.5	16
Acid-etched	50.0 B	11.4	19
Total	41.5	12.9	35

*Means followed by different letters are statistically different at the .05 level (ANOVA for repeated measures and adjustment for multiple comparisons with the Bonferroni correction).

Table 2 Histomorphometric Evaluation Using 100-μm-thick Slices: Percentage of Bone-Implant Contact for Each Implant Group 9 Weeks After Implant Placement in the Rabbit Tibia (n = 5 rabbits)

Primary stability/ Surface texture	Mean*	SD	N
Low			
Turned	55.6 a	12.2	5
Acid-etched	61.4 ab	14.6	5
Total	58.5 A	13.1	10
High			
Turned	45.9 a	7.3	5
Acid-etched	77.0 b	9.2	5
Total	61.5 A	18.2	10
Total			
Turned	50.7 A	10.8	10
Acid-etched	69.2 B	14.2	10
Total	60.0	15.5	20

*Means followed by different letters are statistically different at the .05 level (ANOVA for repeated measures and adjustment for multiple comparisons with the Bonferroni correction).

scsa.edu) to measure the percentage of direct bone-implant contact in relation to the overall implant surface. Spatial calibration for linear measurement was performed for each implant image using a standard grid that was digitized with the specimen.

Statistical Analysis

The outcome measures peak removal torque and percentage of bone-implant contact were analyzed by analysis of variance (ANOVA) for repeated measures with primary stability (high versus low) as the between-subjects effect and surface texture (turned versus acid-etched) as the within-subject effect. Multiple comparisons of estimated means were adjusted with the Bonferroni correction. The level of significance was set at 5% for all analyses.

RESULTS

Table 1 displays the group means of peak removal torque. Five of 40 implants did not show evidence of osseointegration according to the clinical criteria used: 1 turned implant with high primary stability, 3 turned implants with low primary stability, and 1 acid-etched implant with low primary stability. Preliminary tests indicated no significant difference due to the number of implants per group; therefore only osseointegrated implants were considered for analysis. Surface texture had a significant effect on removal torque ($P < .001$); turned implants had lower removal torque values than acid-etched implants. Level of primary stability had no significant effect on removal torque ($P = .214$), but the interaction of surface texture and primary stability was significant ($P = .012$).

All implants submitted to histologic evaluation showed direct contact between the bone and the implant surface (Fig 3). The highest mean percentage of bone-implant contact was measured for the acid-etched implants with high primary stability (77%), followed by the acid-etched implants with low primary stability (61%), turned implants with low primary stability (56%), and turned implants with high primary stability (46%) (Table 2). Surface texture had a significant effect on percentage of bone-implant contact ($P = .001$), but no significant difference was found as a function of primary stability ($P = .645$). The interaction of level of primary stability and surface texture was significant ($P = .006$).

DISCUSSION

This study showed that primary stability did not affect bone-implant interaction of nonloaded turned or acid-etched implants in rabbit tibiae 9 weeks after surgery as measured by removal torque and histomorphometric analyses. However, acid-etched implants had higher bone-implant contact and peak removal torque than turned implants, suggesting that surface treatment is more important for osseointegration than initial implant stability. Recent reviews have highlighted the lack of clear evidence of the relative influence of implant geometry, material, surface topography, and chemistry.[4,22] The turned and acid-etched screw implants used in the present

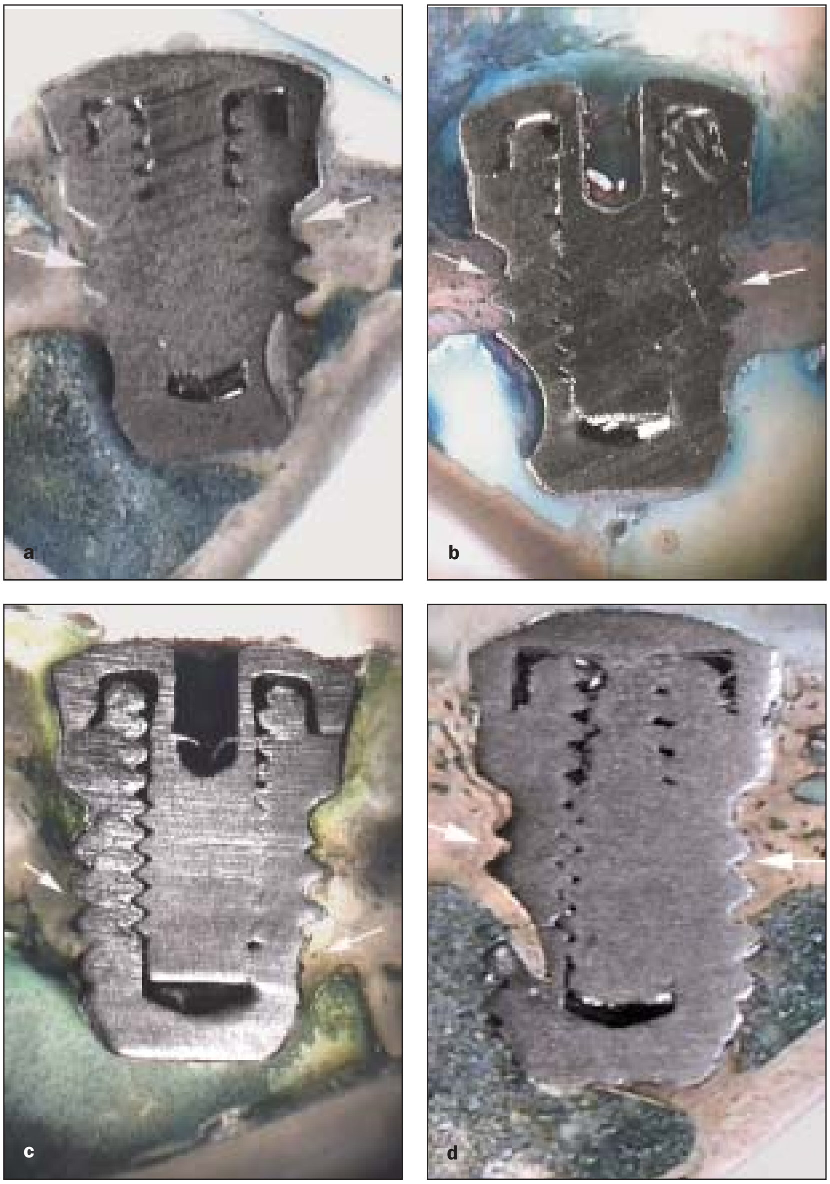


Fig 3 Histological specimens (100-μm-thick slices). Arrows indicate bone-implant contact. *(a)* Acid-etched implant with high primary stability. *(b)* Acid-etched implant with low primary stability. *(c)* Turned implant with high primary stability. *(d)* Turned implant with low primary stability (for all images, original magnification ×16).

study had similar shapes and Ra values (0.65 ± 0.11 μm and 0.51 ± 0.10 μm, respectively) and could have been classified as "minimally rough" (Ra or Sa range of 0.5 to 1.0 μm).[22] The main difference between the 2 types of implants was the surface texture. The turned machined implant had an anisotropic surface characterized by cutting marks with a directional pattern, while the acid-etched implant had an isotropic surface with randomly distributed surface asperities.[4] Previous studies with acid-etched implants with similar Ra values also showed higher bone-implant contact and removal torque values for acid-etched and turned surfaces.[10,12]

All implants submitted to histomorphometric analysis showed direct bone formation, but acid-etched implants had higher percentages of bone-implant contact than turned implants. Previous studies have shown that implants with surface treatment exhibit more bone-implant contact in the early stages of osseointegration.[9,12,15,25] Implant surface treatment seems to strongly influence the phenotype expression of osteoblast-like cells: extracellular matrix production, cell alignment, orientation, attachment, and cell activity.[17,20,21,26] In rough-surface implants, the bone formation follows a 3-dimensional pattern, with cellular extensions inside the surface irregularities, while in turned implants, bone deposition occurs in more uniform layers along the microgrooves produced by the machining process. The mineralization stage of the bone matrix may not have affected the osseointegration of the experimental groups at 9 weeks postoperatively, as some studies have reported no differences in implant removal torque after 2 months of load-free bone healing in rabbits.[27,28] Sennerby et al[27] found no difference in removal torque values for screw-shaped implants inserted in rabbit tibiae for 6 weeks, 3 months, and 6 months; in cancellous bone (femoral intra-articular implants), there was no difference between removal torque values at 3 and 6 months. Furthermore, Meredith et al[28] found little change of resonance frequency 40 days after the insertion of titanium implants in rabbit tibia.

Turned and acid-etched implants with low primary stability did not differ significantly with respect to bone-implant contact (ie, the histomorphometric analysis), but the mechanical strength of the bone-implant interface was significantly higher for the acid-etched implants. Also, some acid-etched implants showed pieces of bone remaining on the implant surface after removal torque testing. This could indicate better mechanical interlocking between bone and acid-etched implants and explain the higher removal torque values compared to the turned counterparts. All turned implants had rupture at the implant-bone interface after removal torque testing. Sennerby et al[27] also found that machined implants unscrewed after 12 months had rupture between the implant surface and the calcified bone in rabbit tibia. Removal torque measurements are invasive biomechanical tests that provide information on the rigidity of the implant in the bone.[29] As removal torque testing measures the shear forces of the interface between bone and implant, the results do not always show a direct relation with bone response or surface roughness. Thus, it is also necessary to measure bone-implant contact.[23] Measurement of removal torque and percentage of bone-implant contact require destruction of the study specimens; therefore, it is not possible to make direct inference of a possible threshold value to clinical success or survival rates. However, according to the report of a recent consensus conference, a minimum of 35 to 40 Ncm of insertion torque (primary stability) is believed necessary for immediate loading of implant-supported prostheses in the edentulous mandible.[30] The mean removal torque values found in the present study (31 Ncm for turned implants and 50 Ncm for acid-etched implants; Table 1) suggest that nonloaded acid-etched implants had acceptable biomechanical performance in rabbit tibiae 9 weeks after surgery, even in the absence of primary stability.

The present study aimed to evaluate nonloaded implants in a conventional 2-stage surgery. It was assumed that in implants with surgically-created low primary stability, micromovement at the bone-implant interface during the healing phase could result in the formation of fibrous tissue around some implants. Lioubavina-Hack et al[3] showed no bone-to-implant contact and fibrous encapsulation of implants without primary stability after 1, 3, 6, and 9 months in a rat model. This was not observed in the present study, possibly because of the implant design used: the cervical portion of the implants was larger than the apical part, which provided vertical stability at the crestal cortical bone. Also, the initial low primary stability of implants was obtained by using a sequence of large-diameter surgical drills to allow a limited amount of rotation (ie, rotation of implants inside the osteotomy site at finger drive pressure). Ivanoff et al[6] found that initial rotational mobility did not reduce the bone formation around unloaded machined implants in cortical or trabecular bone of rabbits after 12 weeks. However, it is possible that some loading occurred through the rabbit skin and influenced the findings of the present study. Although all histologic specimens showed bone formation, in the rabbits selected for the removal torque testing 3 of 10 turned implants with low primary stability did not osseointegrate.

Within the limitations of this study, the results suggest that surface texture is more important than primary stability for bone response and implant fixation in the tested model of nonloaded implants in a conventional 2-stage surgery. Further studies are needed to investigate specific regulation mechanisms of cell activity in different stages of bone maturation around implants with different surface topographies and initial stability in cases of immediate, early, or conventional functional loading. Also, clinical studies will help clarify the affect of these variables on success rates when bone of poor quality or systemic diseases affecting bone metabolism are present.

CONCLUSION

Acid-etched implants had higher bone-implant contact and removal torque values than turned implants regardless of primary stability in rabbit tibiae 9 weeks after surgery. Rotational mobility of implants did not affect osseointegration in this model.

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