The effect of osseodensification drilling for endosteal implants with different surface treatments: A study in sheep

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Received 25 May 2017; revised 18 April 2018; accepted 22 April 2018
Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.b.34154

Abstract: This study investigated the effects of osseodensification drilling on the stability and osseointegration of machine-cut and acid-etched endosteal implants in low-density bone. Twelve sheep received six implants inserted into the ilium, bilaterally (n=36 acid-etched, and n=36 as-machined). Individual animals received three implants of each surface, placed via different surgical techniques: (1) subtractive regular-drilling (R): 2.0 mm pilot, 3.2 and 3.8 mm twist drills; (2) osseodensification clockwise-drilling (CW): Densah Bur (Versah, Jackson, MI) 2.0 mm pilot, 2.8, and 3.8 mm multiluted tapered burs; and (3) osseodensification counterclockwise-drilling (CCW) Densah Bur 2.0 mm pilot, 2.8 mm, and 3.8 mm multiluted tapered burs. Insertion torque was higher in the CCW and CW-drilling compared to the R-drilling (p<0.001). Bone-to-implant contact (BIC) was significantly higher for CW (p=0.024) and CCW-drilling (p=0.006) compared to the R-drilling technique. For CCW-osseodensification-drilling, no statistical difference between the acid-etched and machine-cut implants at both time points was observed for BIC and BAFO (bone-area-fraction-occupancy). Resorbed bone and bone forming precursors, preosteoblasts, were observed at 3-weeks. At 12-weeks, new bone formation was observed in all groups extending to the trabecular region. In low-density bone, endosteal implants inserted via osseodensification-drilling presented higher stability and no osseointegration impairments compared to subtractive regular-drilling technique, regardless of evaluation time or implant surface.

Key Words: implant, bone, insertion torque, osseodensification, histologic

INTRODUCTION
Endosteal implants are used in a variety of medical procedures to facilitate the healing of damaged tissue caused by trauma and pathology.1 One of the prerequisites for clinical success of the implant treatment is the stability of the implant.2 The stability of the implant can be classified as: (1) mechanical stability (primary stability) between implant and bone and (2) biological stability (secondary stability) that occurs as a result of osseointegration.3 Primary stability is obtained as the threads of the implant interlocking with the bone upon insertion, holding the implant in place.4 Factors affecting primary stability include bone quality and quantity surrounding the implant,5 as well as the macro and microgeometric parameters of the implant, which uniquely interlock with the surrounding bone.3 Primary stability is vital to the healing process, as it prevents the implants’ micro-movements during the initial bone remodeling process.

Traditionally, primary stability is obtained by under instrumentation. The difference between the reduced osteotomy dimension and the larger implant diameter commonly leads to increased primary stability of the implant.6 However, extreme under preparation of the implant osteotomy may cause pressure bone necrosis, hindering secondary implant stability or osseointegration.6,7 Since primary stability is purely a mechanical phenomenon, it is at its highest at
the time of implant insertion and decreases over time. This occurs as a result of the wound healing process from the surgical trauma, where first the strain generated releases due to the relaxation effect and due to the microfractures in the bone. Thereafter, the remodeling process stimulates osteoclastic activity that resorbs the surrounding bone structure due to stresses from the implant. Hence, the stability undergoes a transition from being derived from the interlocking between the threads of the implant and the surrounding bone to being derived from successful osseointegration as new bone formation progresses in apposition to the implant, resulting in a secure, long-term stabilization.

A variable in the healing process is the rate at which secondary stability occurs. The rate fluctuates as a result of the rate of the bone remodeling process around the implant. The remodeling rate is the key for the transition from primary to secondary stability and can vary in factors from the health of the patient to the design of the implant system. Taking into account the transition between primary and secondary stability, the overall stability of the implant as a function of time is seen to follow a positive parabolic function, where it initially decreases due to the gradual loss of primary stability, then increases as secondary stability begins to increase due to the induction of new bone formation within the implant threads.

The effects of surface engineering and macrogeometric properties of the implant system on secondary stability of endosteal implants have been described in the literature while the effect of surgical drilling techniques on the magnitude of primary stability is a variable that has been investigated to a lesser degree. However, recent work suggests that certain adjustments during surgery, such as drilling protocol sequence, drill velocity, and design, may accelerate implant osseointegration. This hypothesis is supported by a previous study, which concludes that the utilization of multi stepped drills when preparing osteotomy, as opposed to conical shaped drills, is seen to increase the primary stability of the implant.

Whereas the traditional osteotomy using different step drills is generated by a subtractive method, where bone is drilled away, a unique non excavating drilling sequence has recently been suggested to enhance implant stability via osseodensification drilling. The concept of this technique is to execute a non-subtractive multi-stepped drilling process, which results in the densification of the osteotomy site wall, creating an environment that enhances primary stability due to the presence of residual bone chips. The densification of the osteotomy wall establishes immediate contact between the implant system and bone structure, which creates stability via physical interlocking, and induces osteoblast nucleation on the instrumented bone surrounding the implant, thereby accelerating bone growth.

The conventional drilling process involves a positive rake angle, which is used to extract a small amount of bone as each flute passes to create an osteotomy cleared of bone residue. In contrast, the osseodensification drilling process utilizes a tapered, multifluted bur to create the osteotomy site. This unique bur contains at least four tapered flutes with a negative rake angle, which allows for the creation of a layer of compact, dense bone that surrounds the wall of the osteotomy, and similar to the lamina dura found around teeth. The densifying bur is capable of controlling the expansion process due to the features of the cutting chisel and tapered Shank, which allows the burr to progressively increase in diameter as it drills deeper into the osteotomy site. The expansion process can be operated in both clockwise (CW) and counterclockwise (CCW) drilling directions and is performed at high-drilling speeds (+1000 rpm). The counterclockwise drilling direction is utilized in bone with low-density, and it is not only more efficient, but also optimizes the cooling since the irrigation reaches the tip of the bur more easily, while the clockwise drilling direction is better suited for higher-density bone. Although the osseodensification surgical technique has been demonstrated in bench top in vitro and in vivo animal studies, it is crucial to quantify the techniques from a biomechanical and biological basis in a translational, large animal model.

The objective of this study was to evaluate the effect of osseodensification on the initial stability and progression of osseointegration of endosteal implants that possessed either machine cut (M) or acid etched (A) surfaces. The hypotheses tested included: (1) the implant would present higher insertion torque values when placed into osseodensification drilling sites regardless of surface treatments; (2) no osseointegration impairment would be observed for both acid etched and machine cut implants placed in osseodensification drilled osteotomies compared to the control subtractive drilling, and; (3) no osseointegration decreases would be observed for implants at the 12-week time point when compared to the 3-week time point.

MATERIALS AND METHODS
This study utilized 72 Ti-6Al-4V implants (4 mm diameter by 10 mm length) (D3, Emflis, Itú, SP, Brazil). The implant surfaces provided were textured (grit-blasted/acid-etched, discussed as acid-etched throughout the manuscript, n = 36, properties reported elsewhere), and as-machined (n = 36).

Preclinical in vivo model
A translational, large preclinical animal model was used in this study. The iliac crest of the sheep hip model was used due to its size and low bone density, allowing the placement of all experimental groups nested within each subject to maximize statistical power, while minimizing the number of animals. The study was conducted according to the ethical approval from the Institutional Animal Care and Use Committee of the Ecole Veterinaire d’Alfort under ARRIVE guidelines.

Six male sheep (~60 kg) were used in this study. Six implants were inserted into the ilium of each animal bilaterally, resulting in a total of 72 implants placed. The right side provided samples that remained in vivo for 12 weeks and the left side samples that remained in vivo for 3 weeks. Three implants of each surface type were placed in each
animal, into bone sites, which were prepared via 3 different surgical techniques: (1) subtractive regular drilling (R) in a 3 step series of a 2.0 mm pilot, 3.2 and 3.8 mm twist drills, (2) clockwise drilling (CW) with Densah Bur (Versah, Jackson, MI) 2.0 mm pilot, 2.8, and 3.8 mm multi-fluted tapered burs, and (3) osseodensification counterclockwise drilling (CCW) with Densah Bur (Versah, Jackson, MI) 2.0 mm pilot, 2.8, and 3.8 mm multi-fluted tapered burs. Drilling was performed at 1100 rpm and complimented by saline irrigation. To minimize bias from the different implantation sites (1–6), the implant surgical technique (R, CW, and CCW) and implant surface (as-machined and acid-etched) distribution was interopolated as a function of the animal subject allowing the final comparison of the same number of as-machined and acid-etched implants placed in sites 1 through 6 by R, CW, and CCW surgical drilling at both 3 and 12 week time points. Insertion torque was recorded when the implant neck reached the level of the outer bone using a calibrated digital torque meter.

Prior to surgery, anesthesia was induced with sodium pentothal (15–20 mg/kg) in Normasol solution into the jugular vein of the animal and maintained with isoflurane (1.5–3%) in O₂/N₂O (50/50). Animals were monitored using ECG, SpO₂, end tidal CO₂, and body temperature with a circulating hot water blanket being used for regulation. The surgical site was shaved and treated with iodine solution prior to the surgery. A ~10 cm incision was made in the anteroposterior direction over the ilium, followed by dissections of fat tissue until muscular tissue was reached. The ilium was exposed following a blunt direction of the muscular plane and the application of a periosteal elevator. The insertion torque of each implant was performed to the cortical level and the values were measured and recorded using a digital torque meter (Tohnichi STC®-G, Tohnichi, Japan). Layered closure with nylon 2–0 for skin and Vicryl 2–0 for muscle was performed. Cefazolin (500 mg) was intravenously administered pre-operatively and post-operatively. Food and water ad libitum was offered to the animals postoperatively. The animals were sacrificed by anesthesia overdose and samples were retrieved by sharp dissection.

Histologic procedures and histomorphometric analysis
Following euthanasia, the hips were reduced to small implants in bone blocks and fixed in formaldehyde (10% for 24 h) followed by ethanol (70% for 6 days) prior to transport to histologic processing. Biological samples were shipped from France to the United States of America in accordance to regulations relative to international air transport of biological samples, in this case UN 3373 for transportation of category B substances including 49 CFR, Part 173.199 or IATA Packing Instruction 650.23,24

Experimental groups were processed for histological and histomorphometric analyses using step-by-step dehydration in ethanol and methyl salicylate, followed by a final embedding in methylmethacrylate (MMA). Non-decalcified histological sections were prepared, as previously reported.20 A slow-speed precision diamond saw (Isomet 2000, Buehler Ltd. Lake Bluff, IL) was used to section the samples along the long axis of the implant to a thickness of ~300 μm. Each section of the tissue was then glued to an acrylic plate using a photolabile acrylate-based adhesive (Technovit 7210 VLC adhesive, Heraeus Kulzer GMBH, Wehrheim, Germany). This was followed by the grinding and polishing of each sample under copious water irrigation with increasingly finer grit silicon carbide (SiC) abrasive papers (600, 800, and 1200) (Metaserv 3000, Buehler Ltd., Lake Bluff, IL) to a thickness of ~100 μm. The given samples were stained with Stevenel’s Blue and Van Gieson’s Picro Fuschin (SVG) stains. Histological images were obtained via an automated slide scanning system and specialized computer software (Aperio Technologies, Vista, CA). For histomorphometric analysis an image processing and analysis software (ImageJ, NIH, Bethesda, MD) was used. To quantify and evaluate the osteogenic parameters around the peri-implant surface, bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO) parameters were utilized. BIC was selected since it quantifies the degree of osseointegration derived from primary stability and by measuring the percentage of bone in contact with the implant surface perimeter. BAFO quantifies the degree of osseointegration derived from secondary stability and is recorded by measuring the percentage of bone (newly formed and non-vital autografted/native bone due to instrumentation) within the implant threads compared to the area of the threads. The analyses were performed by one person (B.L.) in a blind fashion.

Statistical analysis
The histomorphometric and biomechanical testing data are presented as mean values with corresponding interval values that represent 95% confidence (mean ± 95% CI), % BIC, %BAFO, and Insertion torque data were collected and aligned along a linear mixed model with fixed factors of implant surface treatment (M and A) and surgical drilling technique (R, CW, CCW) and a random intercept. After administering a significant omnibus test, post-hoc comparison of the three drilling method means was gathered using a pooled estimate of the standard error. Preliminary analyses have shown indistinguishable variances in the study of all three dependent variables (Levene test, all p > 0.25). The analysis was accomplished using IBM SPSS (v23, IBM Corp., Armonk, NY).

RESULTS
The recorded insertion torque value for the R surgical technique was approximately 10N·cm, and the values significantly increased above 50N·cm for the CW drilling direction and roughly 80N·cm for the CCW drilling direction [Figure 1(A)]. The statistical analysis of insertion torque as a function of surgical technique indicated that both osseodensification drilling techniques presented higher insertion torque values compared to the R surgical technique (p < 0.001), with the same degrees of osteotomy under preparation [Figure 1(A)]. After evaluating the insertion torque as a function of implant surface treatment collapsed over surgical technique and time, statistically homogenous insertion torque levels were
observed between acid etched (A) surface treated and machine cut (M) implants ($p > 0.6$) [Figure 1(B)].

BIC values were significantly lower for the R surgical technique relative to the CCW and CW surgical techniques ($p < 0.024$) [Figure 2(A)]. Statistical analysis also revealed that the acid etched surface treatment yields a significantly higher %BIC than the machine cut implants ($p < 0.001$) [-Figure 2(B)]. There was no statistical difference in the BIC as a function of time between the 3-week and 12-week time points ($p > 0.5$) [Figure 2(C)].

When BIC was collapsed over time in vivo, statistical analyses showed no significant difference between the surgical techniques for the acid etched implants, and that osseodensification surgical technique (CW and CCW) yielded a significantly higher BIC than the R surgical technique for the machine cut implants ($p < 0.02$) [Figure 3(A)]. When BIC was collapsed over surface, osseodensification drilling techniques presented higher BIC values ($p < 0.04$) at 3 weeks in vivo and 12 weeks in vivo (significant between CW and R, $p < 0.03$ with CW presenting intermediate values between CW and R) [Figure 3(B)]. When BIC values were collapsed over surgical technique, significantly higher values were observed for acid-etched surfaces relative to machined surfaces ($p < 0.01$) [Figure 3(C)].

While evaluating the time, surface treatment, and the surgical technique altogether, it is also seen that there is no statistical difference between surgical techniques for the A implants at the 3-week time point ($p > 0.2$) [Figure 4(A)]. However, the values for the surgical techniques of the M implants at this time point increase significantly ($p < 0.001$) from the regular drilling technique to the osseodensification techniques (CW and CCW), where the CW drilling technique lies at an intermediate value that is significantly different from both the lower R drilling technique and the higher CCW drilling technique [Figure 4(A)]. At the 12-week time point for both implant surfaces, the CW drilling technique yielded significantly higher BIC than the R drilling technique ($p < 0.05$), with the CCW drilling technique presenting an intermediate value [Figure 4(A)].

Osseodensification drilling techniques (CW and CCW) presented significantly higher BAFO values than the R drilling technique ($p = 0.001$) [Figure 5(A)]. There is no statistical difference in BAFO as a function of implant surface treatment [collapsed over surgical technique and time point, Figure 5(B)] ($p > 0.2$). However when collapsed over surface treatment and drilling technique, a significant increase was observed for BAFO analyzed as a function of time ($p < 0.02$) [Figure 5(C)].

When evaluating BAFO as a function of both surgical technique and implant surface treatment, it was observed that osseodensification drilling techniques (CW and CCW) yield significantly higher BAFO% than the R drilling technique for the acid etched implants ($p < 0.01$), while in the machine cut implant, the CCW drilling technique yielded a significantly higher BAFO than the R drilling technique ($p < 0.01$) [Figure 6(A)]. Analysis of BAFO as a function of both surgical technique and time reveals that for both time points, osseodensification drilling techniques (CW and CCW) present significantly higher values of BAFO relative to the R drilling technique ($p < 0.03$) [Figure 6(B)]. While significantly higher degrees of BAFO were observed for the acid-etched implants relative to the machined implants at 3 weeks ($p < 0.03$), no significant difference in BAFO was detected at 12 weeks ($p > 0.8$) [Figure 6(C)].

Comparison of the BAFO at the different time points while considering both surface treatment and surgical technique reveals that BAFO for the osseodensification drilling techniques (CW and CCW) were significantly higher than the R drilling techniques both surface treatments and time points ($p < 0.03$), with the exception of the machine cut implant at the 12 week time point that yields no statistical difference ($p > 0.7$) [Figure 4(B)].

Histological evaluations indicate osseointegration of all implants. Regardless of the implant surface treatment and surgical technique, the pattern of osseointegration presented extensive remodeling around the cortical shell, as sites of bone resorption and new bone formation were observed in close proximity to the implant surface. Qualitative analysis of the R drilling technique revealed new bone growth in both the cortical and trabecular regions with a notable lack of bone fragments present, while histological images of the osseodensification drilling techniques (CW and CCW)

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**FIGURE 1.** Statistical summary for (A) insertion torque as a function of surgical technique, and (B) insertion torque as a function of implant surface. Same letters represent statistically homogenous groups, data presented as mean ± 95%CI.
revealed a presence of bone chips to a lower (CW) and higher (CCW) extent (Figures 7 and 8). The presence of bone chips was more notable among the CCW surgical technique samples, as these bone chips were present along the length and within the threaded regions of both types of surface treatments. Regardless of the surface treatment or the cutting direction of the surgical technique employed and the measure of bone chips present at the area of initial contact, the bone chips resulted in the formation of new bone on the implant surface.

FIGURE 2. Statistical summary for BIC as a function of (A) surgical technique, (B) implant surface, and (C) time in vivo. Same letters represent statistically homogenous groups, data presented as mean ± 95% CI.

FIGURE 3. BIC statistical summary for (A) surgical technique and implant surface collapsed over time, (B) surgical technique and time in vivo (collapsed over implant surface), and (C) implant surface and time in vivo (collapsed over surgical technique). Same letters represent statistically homogenous groups, data presented as mean ± 95% CI.
DISCUSSION

The purpose of this study was to qualitatively and quantitatively evaluate the effect of the osseodensification surgical technique as a function of time and implant surface treatment. The sheep hip model was an appropriate model to test this procedure due to its low-density environment.12,25,26 The results demonstrated that osseodensification directly influenced insertion torque values, which served as a factor in this study to gauge the primary stability of the device. Analysis after both 3 and 12 weeks in vivo indicated that the surgical technique of the experimental group positively influenced the osseointegration of the device, regardless of the osseodensification drilling direction (CW or CCW) utilized. The implant system used in this study presents a hybrid healing osseointegration pathway, which allowed the observation of the effect of osseodensification both between implant outer threads and the osteotomy walls (interfacial remodeling), as well as direct bone formation at healing chambers (intramembranous-like) as a function of different surgical instrumentation techniques.1,6,27

No differences were observed when the bone-to-implant contact percentages (BIC) were evaluated between the 3-week and 12-week time points (collapsed over surface treatment and surgical technique). These results can be attributed to the osteotomy, which measured as slightly smaller than the implant, causing the implant to have immediate, intimate interface with the surrounding bone. As a result, a high degree of BIC at the 3-week time point is inevitable and not a result of bone growth, meaning that a lower measurement at the 12-week time would only be brought on as a result of a negative bone response to the implant system. A significant increase in BIC was observed in the osseodensification drilling techniques (CW and CCW) when compared to the R drilling technique, suggesting that osseodensification is influential at the intimate interface between implant and surrounding bone, represented by the increase in insertion torque values in said test groups.20,25,26

A significant difference was observed when bone area fraction occupancy percentage (%BAFO) was analyzed as a function of drilling technique (collapsed over time and surface treatment) and as a function of time (collapsed over surface treatment and drilling technique), but no statistical difference was present when BAFO was evaluated as a function of surface treatment (collapsed over time and drilling technique). The histological observation revealed that the compacted bone chips that allowed for the higher degrees of insertion torque in the osseodensification groups were also responsible for the larger degree of bone formation, which resulted from compacted bone acting as a nucleating surface to facilitate the bridging of implant and bone.20 Data shows that BAFO was significantly affected by time, which is due to the higher degree of healing that occurs over a longer period of time. The most notable result is observed when analyzing the BAFO with respect to time, surface treatment, and drilling technique, where there lacks a statistical difference between the acid etched and machine cut implants at both time points for the counterclockwise (CCW) osseodensification drilling technique. Surface treated implants have gained popularity over machine cut implants during the last decades purely based on their superior performance in areas of low bone density when compared to machine cut implants.28 In the present study, the lack of a statistical difference between the acid etched and machine cut implants at both time points for the counterclockwise (CCW) osseodensification drilling technique suggests that despite the osseodensification drilling technique. Since the role of the microtexturing is mainly to enhance bone apposition to the implant during the early stages of healing,27 the clinical
performance of smoother surfaces combined with alternative surgical instrumentation techniques, such as osseodensification, remains to be investigated.

The improvement of quality/quantity of bone surrounding the implant to increase primary stability has been a theory that has been previously explored, but it mainly focused on improving primary stability where sinus elevation is necessary in the site.\textsuperscript{31,32} The osteotome technique involves the compression of the surrounding bone through an impaction, where improved primary stability is perceived by clinicians.
via increased insertion torque values. Currently, there is insufficient evidence supporting this surgical technique as a superior surgical approach compared to other techniques currently available.

Histomorphologic and histometric results from this study suggested the lack of negative biologic responses in any of the studies groups. Not only does the osseodensification drilling improved primary stability and bone contact through the reversed compression exerted due to the elastic bone spring-back effect, but also as a result of the densification of the osteotomy site, which caused instrumentation related autografting owing to the drill design. It is notable that the CW surgical technique also presented autograft particles surrounding the implant that act as a nucleating site for new bone growth, although to a lesser extent than that seen in the CCW surgical technique. This biologic phenomenon was evident in the histologic micrographs showing that bone remodeling was active at the time of 3 weeks of healing in vivo. No pathologic bone breakdown was observed further at 12 weeks in vivo suggesting that all surgical techniques tested in the current study were within the range of compression that biology will compensate without provoking negative responses.

The postulated hypotheses that: (1) implants would present higher insertion torque values when placed into osseodensification drilling sites regardless of surface treatment, (2) no osseointegration impairment would be observed for both acid etch and machine cut implants placed in osseodensification drilled osteotomies compared to the control subtractive drilling, and (3) osseointegration would not decrease for implants...
at the 12-week time point when compared to the 3-week time point were accepted. The present study indicates that regardless of the time point or implant surface treatment, the osseodensification surgical techniques have presented improvements in primary stability and osseointegration indicators partly owing to the compacted autograft composed of densified, autologous bone chips. Future studies comprising an intermediate time point between the short and long term in vivo time points are warranted to further characterize the osseointegration pathway taken as a result of the osseodensification surgical technique.

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