Osseodensification Versus Subtractive Drilling Techniques in Bone Healing and Implant Osseointegration: Ex Vivo Histomorphologic/Histomorphometric Analysis in a Low-Density Bone Ovine Model

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Purpose: The aim of this study was to qualitatively and quantitatively assess the effect of osteotomy preparation by conventional, subtractive, or osseodensification instrumentation on osteotomies, treated with or without endosteal implants, and healing capacity. ***Materials and Methods:*** Seven sheep were used, and 56 osteotomies were made in the left and right ilium of the sheep (n = 8/sheep [4 per side/time point (3 and 6 weeks)]). Two different instrumentation techniques were used: *(1)* conventional/regular drilling in a three-step series of a 2-mm pilot and 3.2-mm and 3.8-mm twist drills and *(2)* osseodensification drilling with a Densah Bur 2.0-mm pilot and 2.8-mm and 3.8-mm multi-fluted tapered burs. Drilling was performed at 1,100 rpm with saline irrigation. ***Results:*** Qualitative histomorphometric evaluation of the osteotomies after 3 and 6 weeks did not indicate any healing impairment due to the instrumentation. In all samples, histologic examination suggested bone remodeling and growth (empty and treated with an implant), irrespective of preparation technique. Osteotomies prepared using the osseodensification instrumentation showed the existence of bone chips autografted into the trabecular spaces along the length of the osteotomy wall. ***Conclusion:*** The osseodensification group yielded higher osseointegration rates, as distinguished through qualitative assessment, bone-to-implant contact, and bone-area-fraction occupancy, indicating an increased osteogenic potential in osteotomies prepared using the osseodensification technique. *Int J Oral Maxillofac Implants 2021;36:903–909. doi: 10.11607/jomi.8828*

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Endosteal implants have been widely used in dentistry due to their predictability and reliability for oral rehabilitation. The ultimate success of a surgically placed implant follows from a sequence of bone modeling-remodeling processes on the surface titanium device with respect to time. The bone healing process proceeds through three distinct phenomena: osteoinduction, the stimulation of pluripotent cells to differentiate into preosteoblasts[1]; osteoconduction, a process by which bone regenerates onto the surface and into healing chambers of an implanted device; and osseointegration, where newly generated bone is in direct contact with the implant surface without any intermediate soft tissue component.[2]

Development and refinement of techniques to improve osseointegration have been ongoing and have been studied for more than a half a century.[3] Although high survival rates are reported, implant failure does occur, especially in areas with low-quality bone. One of the primary reasons for failure under these conditions is a result of deficient primary stability, which is the stability achieved by the direct interface between the endosteal device and bone.[4] Primary stability depends principally on the type of bone, implant design, and drilling technique. Relative to the numerous studies conducted on improving primary stability via implant design modifications, there are only a few focusing on modification of the novel drilling techniques.

The most common osteotomy instrumentation utilized by clinicians has been conventional, subtractive drilling. While the majority of the literature pertaining to implant placement through conventional drilling is positive, it does have disadvantages, such as "excavating" bone, a condition that leads to increased remodeling time and wastes viable bone fragments that could bridge the gap between the osteotomy and the implant surface.[5–9] In an effort to enhance primary stability, an undersized osteotomy can be used with caution, as the increase in forces at the bone-implant interface results in microfracture formation and necrosis of bone, conditions that may delay secondary stability/osseointegration.

To address the shortcomings of subtractive instrumentation, an additive drilling methodology, commonly referred to as "osseodensification," has been explored as a suitable alternative.[9–12] This additive (noncutting) drilling technique has been shown to provide two distinct advantages: *(1)* promotion of primary stability by laterally compacting bone into the walls of the osteotomy chamber being formed, hence increasing bone density; and *(2)* preserving bone-chip autografts to act as nucleating surfaces at the bone-implant interface, facilitating osseointegration and secondary stability.[10–13] The osseodensification instrumentation direction is done in a counterclockwise manner using a negative rake angle bur.[9]

Previous studies have illustrated that osseodensification osteotomies yielded superior results, in terms of bone regeneration, in comparison to conventional, subtractive instrumentation, with respect to primary stability and histomorphometric parameters.[13] Building upon the present authors' previous results, the objective of the present study was to qualitatively and quantitatively evaluate a bone regeneration pattern in empty (control) osteotomy sites and the bone regeneration and osseointegration around dental implants placed using the osseodensification and conventional drilling techniques to analyze the autograft potential of this additive drilling methodology to assist in the bone healing process.

MATERIALS AND METHODS

Upon approval from École Nationale Vétérinaire d'Alfort (Maisons-Alfort) Animal Care and Use Committee, seven (n = 7) adult sheep were obtained and allowed to acclimate for approximately 1 week. All surgical procedures were carried out under a total aseptic environment and general anesthesia as follows: each animal was injected with sodium pentathol (15 to 20 mg/kg) in Normasol solution in the jugular vein. Anesthesia was maintained with isoflurane (1.5% to 3%) in O_2/N_2O (50/50). Concurrently, ECG, SpO_2, and final tidal CO_2 were used to track vital signs. The right ilium served as the 6-week group; prior to surgery, the site was shaved and prepared with iodine solution. An incision of ~10 cm was made in the anteroposterior direction over the iliac crest bilaterally. Both iliac bones were exposed subperiosteally, and four osteotomies (two per instrumentation) were prepared in each ilium using two different instrumentation techniques: two were conducted using conventional drilling, as suggested by the manufacturer (Emfils) by using a three-step series of 2-mm pilot and 3.2-mm and 3.8-mm twist drills; and two were prepared using osseodensification counterclockwise drilling with Densah Bur (Versah LLC) with 2.0-mm pilot and 2.8-mm and 3.8-mm multifluted tapered burs. Drilling was performed at 1,100 rpm with continuous saline irrigation. Two osteotomies, one for each instrumentation method, received an implant (standard implant 4.0-mm diameter × 10-mm length; Emfils, Itu), while the remaining two were left empty. Surgical sites were sutured using vicryl 2-0 for muscle and nylon 2-0 for skin. Cefazolin (500 mg) was administered preoperatively and postoperatively via intravenous injection to reduce the appearance of postoperative complications. Three weeks after the first surgical intervention, the left ilium was operated in an identical fashion and served as the 3-week healing group. Postoperative care included food and water ad libitum.

Animals were sacrificed 6 weeks after the first surgical intervention by an anesthesia overdose. Samples were retrieved by removing the left and right ilium of each sheep en bloc. Specimens were processed first by dehydrating them in a series of increasing ethanol, 70% to 100%, solutions, and then they were embedded in methyl methacrylate (MMA). Following polymerization, the blocks were sectioned into slices of ~300-μm thickness (IsoMet Low Speed precision cutter), and thereafter glued to histologic slides (Exakt Technologies) using Loctite 408 instant adhesive (Henkel). Slides were ground and polished under continuous water irrigation using a series of silicon carbide (SiC) abrasive papers (Buehler) to a thickness of approximately 100 μm (Buehler Metaserv 3000). Stevenel's blue and Van Gieson fuchsin (SVG) were used to stain the slides to distinguish soft and mineralized tissue.

Stained slides were scanned (Aperio Technologies) for histomorphometric analysis using the ImageJ software (NIH). Histologic slides were qualitatively evaluated and quantitatively analyzed. Quantitative assessment of bone-to-implant contact and bone-area-fraction occupancy was completed on samples that had an implant, and the samples were scanned and exported as digital images. The empty osteotomies were evaluated for bone-area-fraction occupancy as a

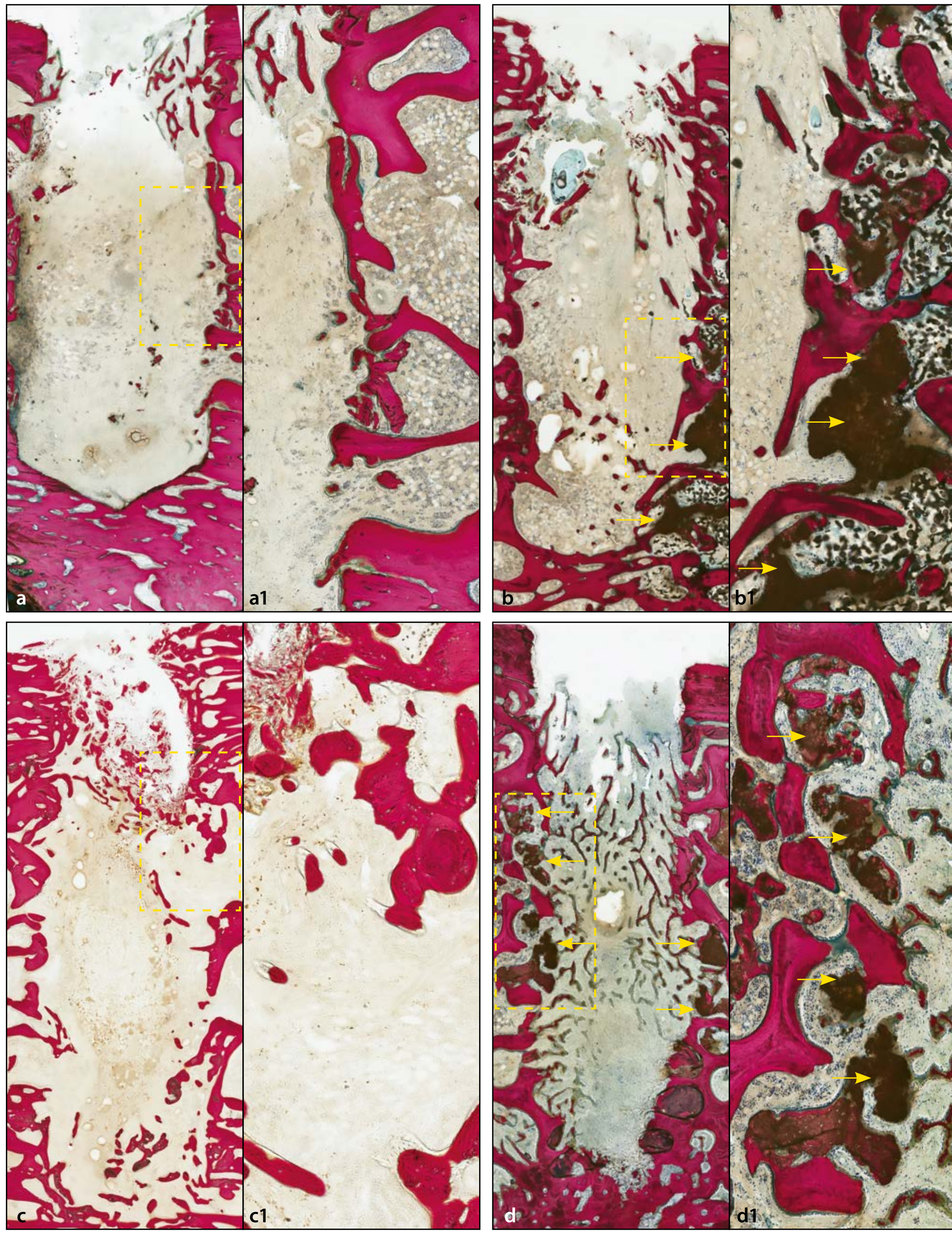

Fig 1 Histologic image showing conventional and osseodensification techniques. Overview of the osteotomy generated at *(a)* 3 weeks—regular and *(b)* 3 weeks—osseodensification. Higher magnifications of *(a1)* 3 weeks—conventional and *(b1)* 3 weeks—osseodensification, with the latter depicting the formation of an autograft bone in the trabecular space around the perimeter of the osteotomy. At 6 weeks, *(c)* conventional and *(d)* osseodensification represent new bone formation occurring from the outer perimeter of the osteotomy to the center of the defect. High-resolution insets at 6 weeks for *(c1)* conventional and *(d1)* osseodensification focus in on the bone chips. (The arrows show a remaining bone chip. Samples stained with Van Geison's fuchsin and Stevenel's blue.)

function of area. All analyses were completed by a single, blinded investigator (O.M.).

Statistical Analyses

Histomorphometric test results are provided as mean values with corresponding 95% confidence (mean ± 95% CI) interval values. Bone-to-implant-contact, % bone-area-fraction occupancy, and insertion torque data were collected and subjected to a linear mixed model with the significance level set at .05 ($\alpha = .05$) and fixed factors of surgical instrumentation method, control (conventional) and experimental instrumentation (osseodensification), and time in vivo (3 vs 6 weeks). The analysis was accomplished using IBM SPSS (v23, IBM).

RESULTS

No surgical site revealed any sign of inflammation or infection over the immediate postoperative exploration, as well as no proof of implant failure at the time of necropsy.

Negative Osteotomies

Histologic Analysis (Qualitative and Quantitative). Histologic micrographs of the empty, negative osteotomies prepared with conventional (subtractive) and osseodensification (additive) instrumentation were evaluated after 3 and 6 weeks (Figs 1a to 1d). The initial qualitative evaluation of the histologic micrographs did not indicate any healing impairment due to respective

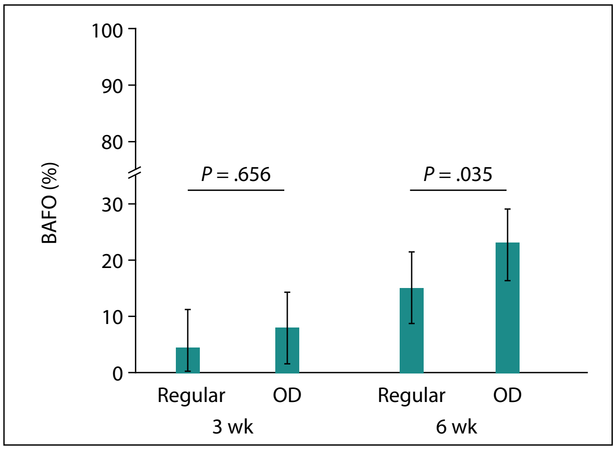

Fig 2 Bar graph comparing bone-area-fraction occupancy (BAFO) between the two drilling techniques (regular and osseodensification) at 3 and 6 weeks (mean ± 95% confidence interval).

instrumentation techniques. At the early time point, 3 weeks, both groups, conventional and osseodensification, did not depict bone regeneration at the edges and centripetally (Figs 1a and 1b). Although minimal healing was observed at the early time, osseodensification histologic micrographs did depict the presence of bone "chips" fragments in the trabecular space (Figs 1b and 1b1), which were not present in the conventional group. At the later time point, 6 weeks, there was a more pronounced bone regeneration present toward the center of the osteotomy (Figs 1c and 1d) for both instrumentation techniques, conventional and osseodensification, in comparison to the 3-week time point. Similar to the 3-week time point, bone fragments were found integrated at the peripheral edges of the osteotomy of the osseodensification group (Figs 1d and 1d1). While all the empty, negative osteotomies, independent of instrumentation, depicted centripetal bone growth, the qualitative analysis did indicate that osseodensification yielded superior bone regeneration. Quantitative evaluation as a function of bone-area-fraction occupancy, the area of the osteotomy, yielded ~5% and ~20% bone regeneration, independent of the instrumentation technique at 3 and 6 weeks, respectively. Statistical analysis as a function of the instrumentation technique, conventional vs osseodensification, was completed at both times in vivo, 3 and 6 weeks. At the earlier time, no statistical differences were noted between conventional (4.5% ± 6.5%) and osseodensification (7.8% ± 6.5%; Fig 2). At the 6-week healing time, both techniques showed significant increases in comparison to the 3-week healing time, with osseodensification (22.8% ± 6.5%) yielding the statistically superior results compared with conventional (14.9% ± 6.5%; $P = .035$; Fig 2).

Osteotomies with Implant

Histologic Analysis (Qualitative and Quantitative). Qualitative histologic assessment showed that in the peri-implant area, all implants presented favorable bone formation (Figs 3a to 3d). Compared with samples instrumented via conventional drilling (Figs 3a and 3b), independent of time in vivo, the bone volume in samples prepared with osseodensification is more notable (Figs 3c and 3d). The osteotomies prepared with the osseodensification instrumentation reveal bone chips in the proximity (Figs 3c and 3d), whereas, in conventional samples, the presence of these chips is seldom seen (Figs 3a and 3b), suggesting that bone chips compacted into the trabecular space wall of the osteotomy at the time of osseodensification instrumentation increased osteogenesis by acting as nucleating sites.

Quantitative evaluation of integration of the implants as a function of time in vivo, independent of the instrumentation technique, showed a significantly ($P < .005$) higher % bone-to-implant contact at 6 weeks (52.6% ± 6.9%) compared with % bone-to-implant contact at 3 weeks (22.5% ± 6.9%). Analyzing bone-to-implant contact percentages as a function of drilling technique and time in vivo, no statistical differences were observed between the conventional (22.14% ± 11.9%) and osseodensification (28.18% ± 11.9%) groups (Fig 4a) at 3 weeks. However, at the 6-week healing time, both techniques showed greater values in comparison to the 3-week healing, with osseodensification (60.2% ± 11.9%) yielding statistically superior results to conventional (43.0% ± 11.9%; $P = .032$; Fig 4a). Further evaluation of % bone-area-fraction occupancy, independent of the instrumentation technique, yielded statistically homogenous values between 3 weeks (44.2% ± 4.9%) and 6 weeks (46.4% ± 4.9%; $P > .05$; Fig 4b).

Analysis of BAFO as a function of instrumentation technique (conventional vs osseodensification), and time in vivo (3 and 6 weeks) showed statistical differences at the early time in vivo, 3 weeks, ($P = .01$), with the osseodensification instrumentation (48.3% ± 7.4%) yielding higher values in comparison to conventional (33.4% ± 7.4%; Fig 4b); the same outcome was observed at 6 weeks with % bone-area-fraction occupancy of the osseodensification group (51.23% ± 7.4%), which was statistically higher compared with the conventional group (41.49 ± 7.4; $P = .041$; Fig 4b).

DISCUSSION

Osseodensification has been identified as a promising and useful technique for various types of bone.[11,13–15] The justification for using this technique is that bone densification not only results in higher primary stability,

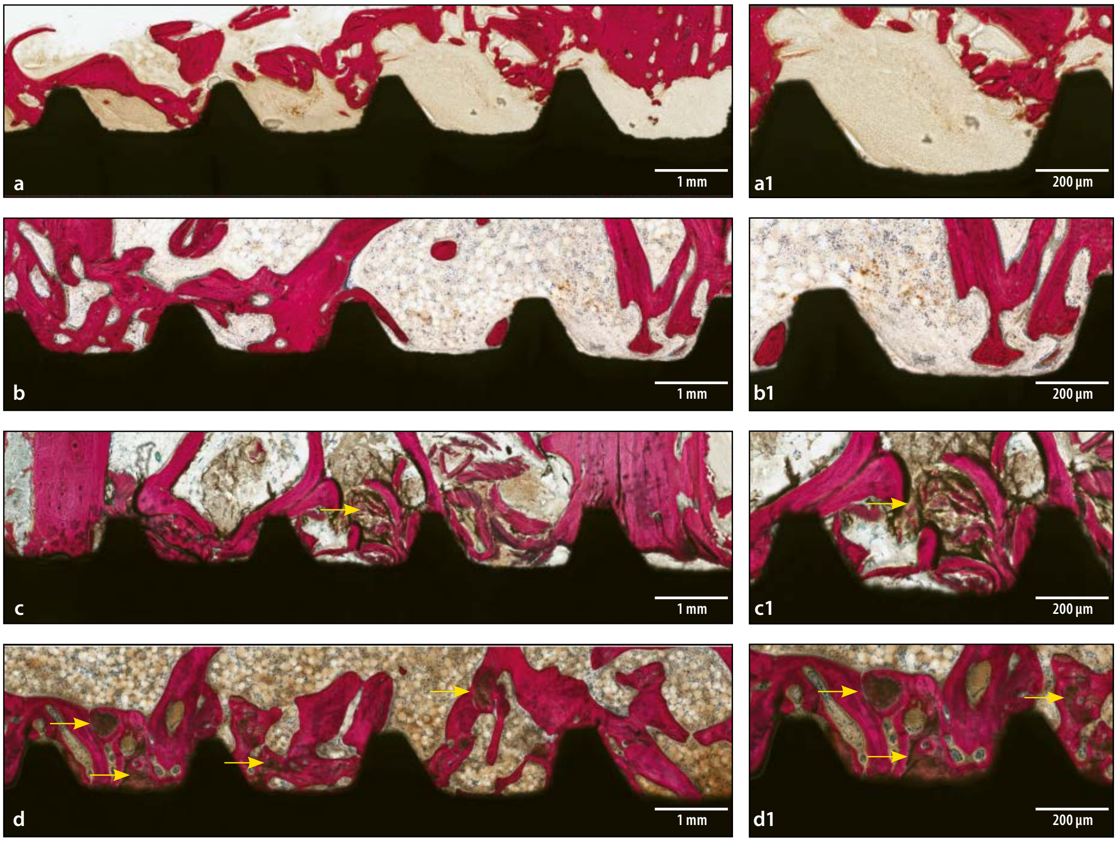

Fig 3 Survey histologic micrographs for regular at *(a)* 3 and *(b)* 6 weeks. *(c and d)* Osseodensification at 3 and 6 weeks, respectively. Highly correlating high-magnification histologic micrographs at *(a1)* 3 weeks—conventional, *(b1)* 6 weeks—conventional, *(c1)* 3 weeks—osseodensification, and *(d1)* 6 weeks—osseodensification.

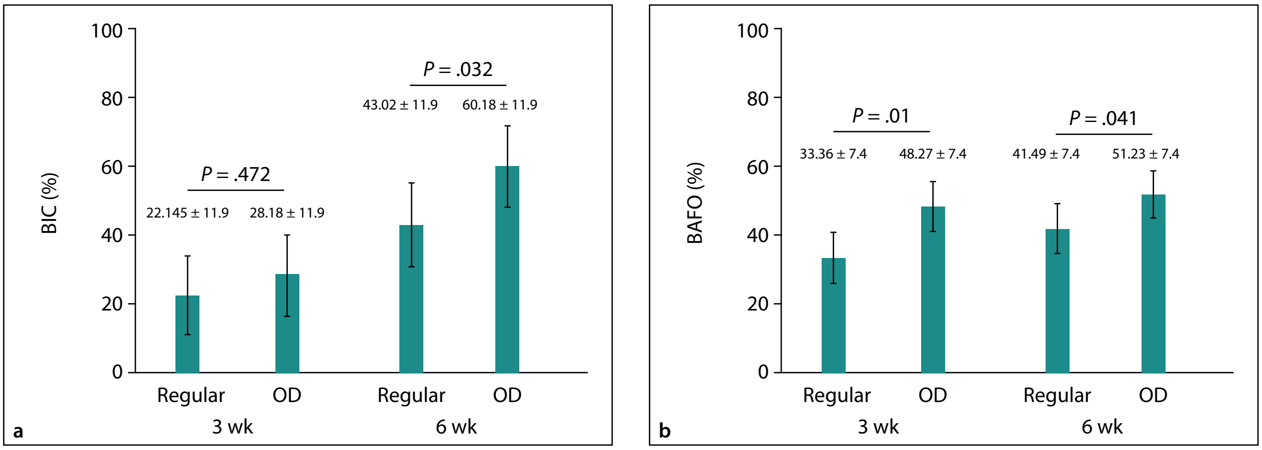

Fig 4 Histomorphometric data (mean ± 95% confidence interval): *(a)* bone-to-implant contact and *(b)* bone-area-fraction occupancy as a function of surgical technique (conventional vs osseodensification) as a function of time, with corresponding *P* values.

due to increased physical interlocking between the bone and the implant, but it expedites secondary stability through the apposition of particulate bone autografts in the osteotomy wall trabecular spaces, creating healing chambers, acting as nucleation sites of osteogenesis close to the implant.[16] Osseodensification can also avoid the practice of undersized osteotomies, a strategy adopted to increase primary stability, which has been known to result in bone necrosis and implant failure due to excessive bone pressure and microcrack formation.[17]

The aim of this study was to qualitatively and quantitatively evaluate bone healing patterns in empty osteotomies (control) and compare them to the bone healing and osseointegration around dental implants placed using either the osseodensification or conventional drilling technique in low-density trabecular-type bone. The iliac crest of the sheep was a favorable model to utilize due to its low-density characteristics, allowing for the evaluation of the healing capacity. Also, the relatively large size of the ilium allowed the nesting of all experimental groups within each animal, reducing the number of animals and maximizing the power of the study. Furthermore, sheep are considered a suitable translational model due to the comparable size and bone metabolism rate with humans.

The qualitative results of this study showed no evidence of impaired bone healing in the assessment of empty osteotomies at any time points. These results are consistent with the results of Witek et al,[12] who compared osseodensification clockwise and counterclockwise, drilling with conventional subtractive drilling in the hip of an ovine model, and found that at 6 weeks, the osseodensification groups had more pronounced autografting along the osteotomy walls compared with the conventional group, and the osseodensification counterclockwise group had a healing pattern that was identical to its equivalent counterpart (osseodensification clockwise), but increased evidence of residual bone chips compared with the osseodensification clockwise. Relative to the aforementioned study, the present study included an earlier time in vivo of 3 weeks and analyzed the nucleation potential of bone chips within the osteotomy, the autograft. Nevertheless, no differences in the healing pattern were observed between conventional and osseodensification drilling, but the presence of bone chips was again observed in the osseodensification group at 3 weeks and then more integrated at 6 weeks, showing the viability of the autografted bone and its ability to integrate into the osteotomy wall. From a quantitative point of view, in the previous study, Witek el al[12] did not show any differences in bone-area-fraction occupancy percentages of empty osteotomies at 6 weeks. However, this study showed greater bone-area-fraction occupancy values at 6 weeks in the osseodensification group compared with the conventional group, a circumstance that was probably also due to the autograft effect of integrated bone chips within the osteotomy trabecular space wall.

Regarding the healing pattern around dental implants, histomorphometric results from previous studies comparing osseodensification and conventional drilling techniques showed higher bone-to-implant contact and bone-area-fraction occupancy values in osseodensification groups at longer times in vivo, regardless of the implant's characteristics. Alifarag et al[14] evaluated the relationship between drilling technique and two different implant macrogeometry designs, indicating higher bone-to-implant contact and bone-area-fraction occupancy values in the osseodensification technique compared with conventional drilling at 3 weeks regardless of the implant system used. Oliveira et al,[15] on the other hand, evaluated drilling technique and implant surface treatment, concluding that the combination of osseodensification with machined implants resulted in similar bone-to-implant contact and bone-area-fraction occupancy values obtained with rougher implant microgeometry placed using the conventional technique, suggesting that the drilling technique greatly improves the early osseointegration of machined devices to degrees comparable to rough-surfaced devices at 3 and 6 weeks. Both studies agreed on attributing the improved bone-to-implant contact and bone-area-fraction occupancy to the compacted bone chips acting as nucleating surfaces that bridged the implant and surrounding bone.

The results of this study yielded no differences in terms of bone-to-implant contact between osteotomies that had been instrumented with either the conventional or the osseodensification technique at 3 weeks. This result is consistent with Lahens et al,[13] a similar study, and is attributable to the size of the osteotomy, which was, both in the present authors' previous study and the present study, slightly smaller than the implant diameter, causing the implant to have an instant and close contact with the surrounding bone. However, in the present study, at 6 weeks, significantly greater values of bone-to-implant contact were observed for the osseodensification instrumentation compared with the conventional technique, while regarding bone-area-fraction occupancy for the osseodensification instrumentation, significantly higher values than the conventional were recorded at both the 3-week and 6-week time points ($P < .05$). These observations, concordant with reports in the literature where a significant difference was recorded in bone-area-fraction occupancy between conventional and osseodensification instrumentation as early as 3 weeks,[13] can be explained by the interfacial bone healing process that takes place between primary and secondary stability, characterized by bone remodeling processes. Remodeling sites usually occur in the proximity of microcracks, as can be seen in implants placed using the conventional technique, with all potential nonviable or "damaged" bone being the first resorbed.[18,19] Notably, osseodensification allows achievement of high primary stability degrees without inducing excessive strain in the bone upon insertion while providing viable bone, in the form of bone particulate autograft in both the trabecular space and in immediate contact with the implant.

The increased bone-area-fraction occupancy at all time points in the osseodensification group can be unequivocally attributed to the bone chips that could be seen in the empty osteotomies, which are also included in the healing chambers and can be considered as a viable autograft. These additive bone chips expedite the remodeling process over time and act as a bridge between the preexisting bone and the healing chambers, ultimately generating optimal conditions, significantly increasing the bone-area-fraction occupancy percentages at both times in vivo. This unique additive process takes advantage of the bone chips, which are otherwise discarded in the conventional subtractive drilling process.

This proposed mechanism of action to explain the greater performance of osseodensification compared with the conventional technique is also supported by the findings of Choi et al,[20] who demonstrated that contact osteogenesis, although also relying on signals produced in distance osteogenesis, is mainly dependent on triggering factors (mainly BMP2) originated from the native bone. In other words, bone growth around endosteal implants is mainly influenced by the surrounding host's osteotomy bone walls, utilizing the proteins and bone chips expelled during drilling. Therefore, it is justified that the osseodensification method, a technique that utilizes the existing bone debris and their intra/extracellular proteins, would positively influence the natural wound healing response in comparison to the conventional technique. It is recommended that future studies must be conducted in order to fully understand the signaling cascade process at a molecular level, as well as the full mechanisms of action through which osseodensification exerts its influence on the osseointegration of endosteal implants.

CONCLUSIONS

Despite the limitations of this study, it can be established that the osseodensification drilling technique does not impair bone defect healing and is associated with greater degrees of implant osseointegration both at 3 and 6 weeks compared with the conventional instrumentation.

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