Original Article

Comparative Histological Assessment of Osseodensification Implant Site Preparation Versus Conventional Drilling Technique

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ABSTRACT

Purpose: is to analyze histologically in a dog model how osseodensification (OD) implant site preparation method influence the bone area fraction (BAF), the bone implant contact (BIC) and bone mineral density (BMD) around dental implants compared to the conventional drilling method.

Materials and Methods: this experimental study was conducted on four dogs using split mouth fashion so that each side received two implants with two different implant-site preparation technique. In the study group, Densah Burs were used to osseodensify the osteotomy site where conventional cutting drills were used in the control group. Light Microscope (LM) examination of HE-stained sections was used to evaluate BAF, while Scanning Electron Microscope (SEM) was used to evaluate BIC and BMD.

Results: osseointegration was almost similar in the control and experimental groups examined by both LM and SEM. There was no statistically significant difference between both groups in BAF, BIC and BMD.

Conclusion: it can be concluded that OD technique using Densah Bur didn't show any significant difference in the BAF, BIC or BMD than the conventional drilling methods in the experimental duration used.

Key Words: Dental Implant, Osseodensification, BIC, Densah Bur.

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INTRODUCTION

Primary implant stability is affected by both the quality and the quantity of bone of the osteotomy site. Hence, a precise evaluation of bone structure is essential before implant placement. [1, 2,3] The term bone quality depends on bone density, bone vascularity, bone metabolism and other factors that may affect implant outcome. Many authors describe bone density as being equivalent to bone quality. This includes physiological and structural parts and the degree of bone tissue mineralization.

Clinical studies have reported that dental implants in the mandible have higher survival rates compared to those in the maxilla, having thinner cortical bone combined with thicker trabecular bone compared to the mandible. [1,4,5]In contrast to the previous studies, additional studies in the posterior mandible showed high failure rates due to the poor bone quality as well as other additional factors. [6,7]

The osseointegration process leads to new bone apposition on the implant surface and allows reaching the implant secondary stability that is the functional contact between living bone and titanium dental implant. In case of poor bone density, such as upper human jaw, the insufficient bone amount around the implants could negatively influence the histomorphometric parameters (such as BIC% and bone volume percentage [BV%]) and, consequently, both primary and secondary implant stabilities. Undersized implant site preparation [8,9] and the use of osteotomes to condense bone [10,11] are surgical techniques proposed to increase primary implant stability and BIC% in poor density bone.

Osseodensification (OD), a non-extraction technique with specially-designed burs, was developed by Huwais in 2013. [12] The bur geometry, rotating in reverse mode (anti-clockwise direction) at a rotating speed of 800 to 1500 rpm with profuse saline solution irrigation to prevent bone overheating, allows compacting the bone along the inner surface of the implant osteotomy site without cutting. Furthermore, the compacting of residual bone remnants, which act as nucleating surfaces for osteoblasts

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around the implant, function as an autograft facilitating osseointegration [13-16] The bouncing motion (in and out movement) is helpful to create a rate- dependent stress to produce a rate dependent strain, and allows saline solution pumping to gently pressurize the bone walls. This combination facilitates an increased bone plasticity and bone expansion. [17,13] These burs combine advantages of osteotomes with the speed and tactile control of the drilling procedures.

The new burs allow bone preservation and condensation through compaction autografting during osteotomy preparation, increasing the peri-implant bone density (%BV), and the implant mechanical stability was reported by in vitro testing. [18] Osseous densification was shown to increase the insertion and removal torques of the implants compared to standard drilling and extraction drilling. This demonstrates increased implant primary biomechanical stability. [19]

In 2019, Neelam Das et al. [20] explained osseodensfication drilling concept of hydrodynamic bone preparation which is characterized by low plastic deformation of bone that is created by rolling and sliding contact using a densifying bur (Densah™ burs) that is fluted such that it densifies the bone with minimal heat elevation through a non-extraction technique, with specially designed burs to increase bone density by expanding an osteotomy site [21].

In 2018, Ali Farag et al. [22] reported that clinical complications rate for orthopedic cases are around 32-35% when dental implant failure rate was about 6-10%. [23,24] The 16% Of the orthopedic failure rate were associated with screw loosening during the healing process which resulted from either disability of achievement of primary stability within the preparation method or failure of osseointegration within the healing process. Therefore, it was predicted that osseodensification method could minimize the potential complications because it helps to achieve higher insertion torque results, initial stability, and increase of osseointegration success rate.

New bone formation around dental implants could be detected with all histological staining methods. Examination of histologically-stained sections and applied histomorphometric measurements as BIC and bone-area fraction are key indicators of dental implants success [25]. Electron microscopic methods, either scanning or transmission were used successfully to observe regions of new bone formation and bone implant contact areas. Imaging of the sections with SEM enabled qualitative and/or quantitative assessment of the bone-implant contact [26]. Energy-dispersive X-ray spectroscopy (EDXA) is used, with scanning electron microscopy to determine the elemental composition of a sample of interest [27].

This study aimed to evaluate the effect of osseodensification technique on BAF, BIC and BMD in comparison to the conventional drilling technique.

MATERIALS AND METHOD

The study was conducted on four mature mongrel dogs of comparable weight (10 to 15 Kg) with age range from nine months to one year. The dogs were kept in the animal house of the faculty of veterinary medicine Cairo University for 6 months. This study was revised by the Faculty of Dentistry of Ain Shams University Research Ethical Committee and got ethical committee approval (FDASU-RecIM011740).

Figure 1: The left Photograph showing Densah burs used for placing the implants and the right photograph showing the Neobiotech drills

Figure 2: A photograph reveals the drilling of an osteotomy site using Densah bur via anti-clock wise technique under copious irrigation

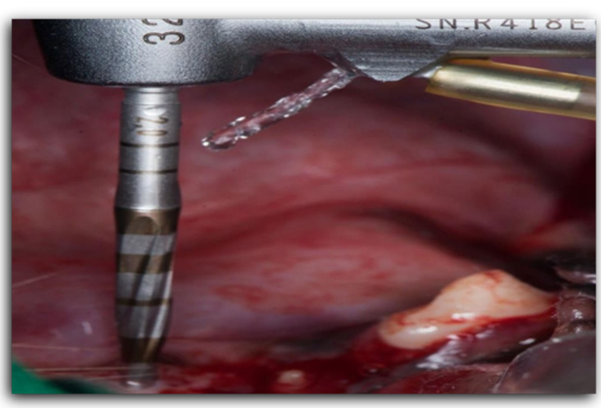
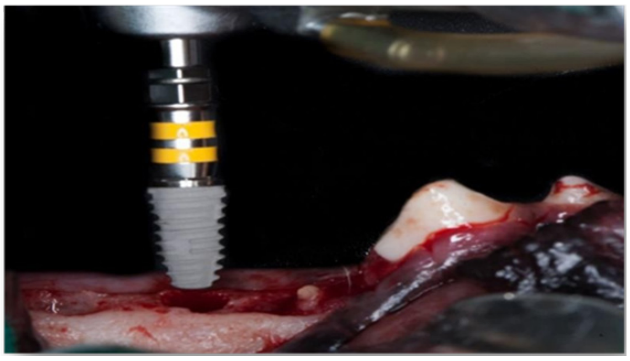


Figure 3: A photograph of implant insertion in control group



Three months prior to implant placement, each dog received bilateral extraction of four mandibular premolar teeth, two teeth from each side, in the operating room under IV general anesthesia using Ketamine Hydrochloride (7 mg / kg body weight) and Xylazine Hydrochloride (1 mg / kg body weight) that were slowly injected via intravenous cannula. Level of anesthesia was maintained by a solution of 2.5% Thiopental Sodium (20-30 mg/kg body weight) in addition to para-periosteal local injection 2% of Mepivacaine hydrochloride as local anesthesia. Each dog jaw was splitted so that it included two implants on each side that had been placed with two different implant site preparation methods. Control side, the left side, in each dog included two implants placed with the cutting implant site preparation drills supplied by the implant manufacturer, while the study side, the right side, included two implants placed with the Osseodensification implant site preparation Densah drills. The four dogs were sacrificed 12 weeks postoperatively by giving them an overdose of thiopentone sodium intravenously. [28] Dogs were burnt in the faculty of veterinary medicine Cairo University Medical Incinerators.

Mandibular segments containing the implants and the adjacent bone were retrieved to be sectioned. Sectioning was achieved by using a diamond blade on a microtome using low speed saw (Isomet®: Cutting edge is Isomet Disk no 114246 Buehler) under water cooling at speed 2500 rpm at Dr.Emad AbdelFattah dental research center. Two central sections of each specimen's block were selected for light microscope examination, SEM and EDXA at different magnifications.

Specimen preparation for SEM examination:

One of the calcified central sections of each specimen was sputter□coated with a thin layer of gold and examined with Quanta®250: scanning high resolution field emission at different magnifications. [29] Scanning electron photomicrographs were captured and BIC was measured and analysed by image analysis software (Image J v. 1.43u—National Institutes of Health, Bethesda, MD, USA).

Energy-Dispersive X-ray Analysis was performed by SEM-attached EDXA unit on control and test samples to determine Bone Mineral density. Elements selected for EDXA assessment were Calcium, Phosphorus, Carbon and Oxygen. Calcium and phosphorus were selected as they are major components of bone inorganic content as elements of bone hydroxyapatite crystals, while carbon and oxygen are major components of the organic content of the bone soft tissue. The assessment and comparison of those elements' percentage in both control and experimental groups would give evidence of the degree of maturation and compaction of bone around the dental implant. Similar or close percentages of inorganic and organic elements in both groups indicate almost similar degree of bone maturity and hence could be indicative of implant stability.

Specimen preparation for HE staining for LM examination:

One central section from each specimen was fixed, processed and stained with both hematoxylin and eosin [30] Histological analysis was performed by LM (Olympus model: BX60F5 – Olympus optical company. Limited – Japan) at different magnifications. BAF indicates the area occupied by the mineralized bone matrix in relation to the total field area expressed as percentage. Five different photomicrographs of the peri-implant bony areas were taken for each specimen and the histomorphometrical data were analyzed by image analysis software (Image J v. 1.43u—National Institutes of Health, Bethesda, MD, USA). [31] BAF was measured according to the equation: BAF=Bone area/[Bone area + marrow spaces area] % [32]

Statistical methodology:

Statistical analysis of collected data of BAF, BIC % and EDXA bone mineral density was performed with IBM® SPSS® Statistics Version 20 for Windows. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed parametric (normal) distribution. Paired sample t-test was used to compare between two groups in related samples. The significance level was set at $P \leq 0.05$.

RESULTS

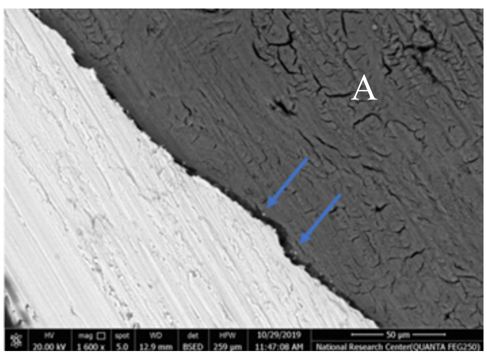
I. Scanning electron microscope Results:
1. Control group:

Examination of scanning electron micrograph of control group showed that some samples exhibited almost complete BIC around all the implant surface . Further magnification of samples with good BIC revealed very close contact between implant surface and bone (Fig. 4a). Other samples presented areas of soft tissues in direct contact with implant surface, so that BIC was not achieved all around the implant surface (Fig. 4b). More areas of bone marrow and less bone compaction were observed. With further magnification, soft tissue fibers could be seen attached to implant surface (Figure 4b).

2. Experimental group:

Examination of scanning electron micrographs of experimental group presented that most of samples exhibited almost complete BIC around all implant surface (Figure 5a). Few samples presented areas of soft tissue contact around the implant surface. Further magnification of these micrographs revealed that most of implant surface showed good BIC (Figure 5b). Even what looked like areas of separation between bone and implant surface, presented metal particles attached to bone surface or vice versa (i.e., bony structures separated from bone and attached to implant surface.

Figre 4a: A scanning electron micrograph of the control group showing very close contact between implant surface and bone (X1600). **Figure 4b**: A scanning electron micrograph of control sample revealing areas of soft tissues intervening between implnat surface and bone (arrows) (X130)



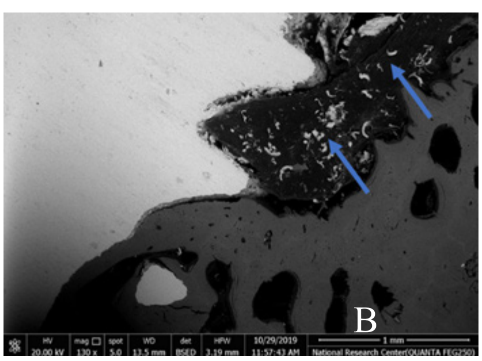
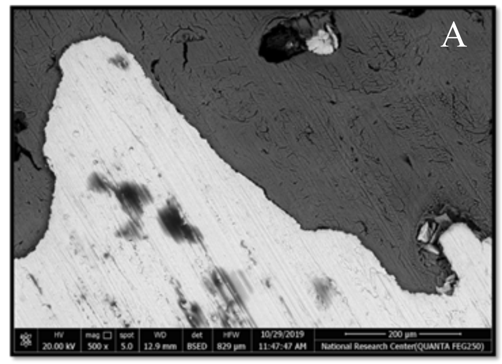
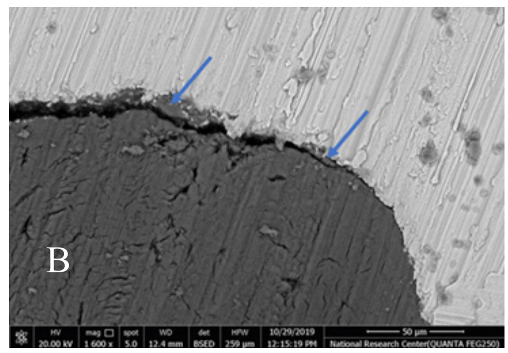


Figure 5a: A scanning electron micrograph of the experimental group showing very close BIC (X500). **Figure 5b**: Further magnification of the experimental group showing metal particles attached to bone surface (arrows) (X1600).





Light Microscopic examination of HE-stained sections

Examination of HE-stained sections of control group by LM showed trabecular bone in close vicinity to the implant space followed by compact bone with regularly formed osteons (Haversian Systems) presenting well distributed Haversian canals and Volkmann's canals. Examination of the peri-implant bone area revealed the structure of the spongy bone with regular trabecular pattern, fully mineralized matrix and apparent increase in osteocytic count than the nearby cortical bone. Osteocytes appeared within their lacunae with normal cellular and nuclear morphology. Resting lines and reversal lines in the peri-implant bone area were noted with no signs of inflammation or bone resorption (Figure 6). Examination of study group disclosed cancellous bone in close vicinity of the implant space followed by compact bone with regularly formed osteons and evenly distributed Haversian and Volkmann's canals. The histological picture was almost similar to the control group. The adjacent compact bone and also cancellous bone appeared with close resemblance to the control group, with resting and reversal lines were evident normally in HE-stained sections (Figure 7).

Figure 6: A photomicrograph of the perimplant bone area of the control group showing the trabecular bone in close vicinity of the implant surface (arrow head) followed by compact bone with well organized structure , osteocytes revealed normal cellular and nuclear morphology (arrow) (X100) (H&E)

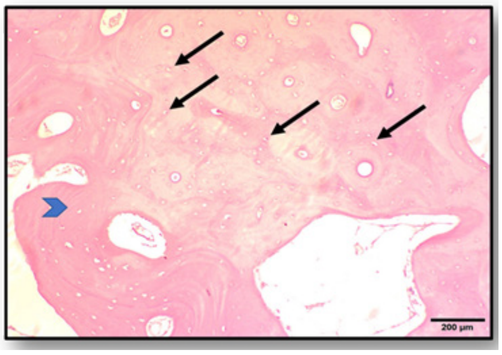
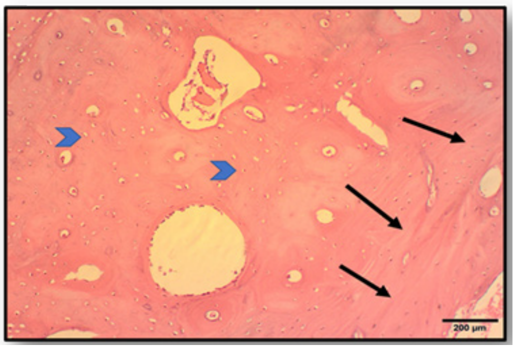


Figure 7: A photomicrograph of the peri- implant bone area of the experimental group presented close resemblance to the control group . Trabecular bone in close vicinity of the implant surface (arrows) and compact bone next to it (arrow heads) showed well organized structure and osteocytes with normal cellular and nuclear morphology (X100) (H&E)



Statistical Results of Scanning Electron Microscope

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests, data showed parametric (normal) distribution. Paired sample t-test was used to compare between two groups in related samples. The significance level was set at $P \leq 0.05$.

1. BIC%:

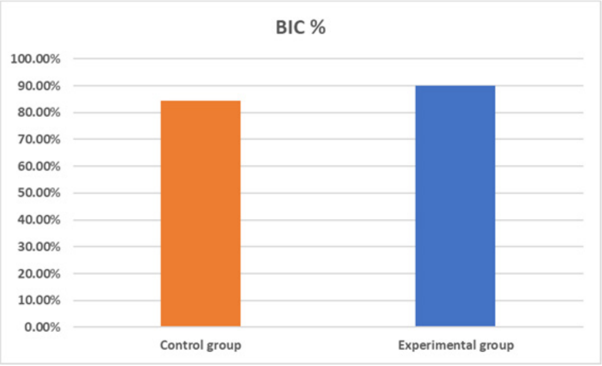
There was no statistically significant difference between (Control) and (study) groups where (p=0.245). The highest mean percentage was found in (study) group (=89.88%) and Standard Deviation (= 8.54), while the lowest mean percentage was found in (Control) group (=84.37%) and Standard Deviation (=10.18). (Figure 8 table 1)

Table (1): The mean, standard deviation (SD) values of BIC % of different groups

Variables	BIC %	
	Mean	SD
Control group	84.37%	10.18
Study group	89.88%	8.54
p-value	0.245 ns	

ns; non-significant (p>0.05)

Figure 8: Bar chart representing BIC % for different groups



2. BMD:

Experimental group presented higher levels of calcium, phosphorus and oxygen and lower level of carbon. However, there were no statistically significant difference between the selected measured elements; calcium, phosphorus, carbon and oxygen between the control and experimental groups. (Table 2)

	Calcium	Phosphorus	Carbon	Oxygen
Control group	20.28	9.43	29.08	37.14
Experimental group	20.42	9.56	23.94	39.27

Statistical results of histological analysis of BAF%

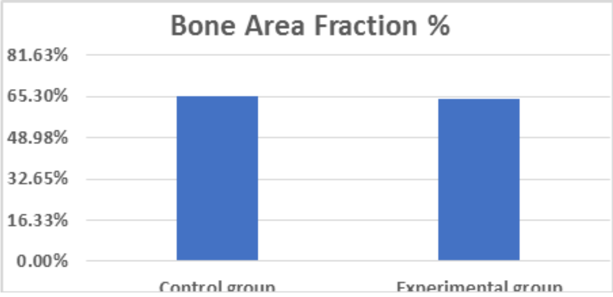
There was no statistically significant difference in BAF% between (Control) and (study) groups where (p=0.796). The highest mean percentage was found in (Control) group (65.24%) and a Standard Deviation (4.72), while the lowest mean percentage was found in (study) group (64.46%) and a Standard deviation (5.03).
(Figure 9 table 3)

Table (3): The mean, standard deviation (SD) values of Bone Area Fraction measurements (BAF %) of different groups

Variables	BAF %	
	Mean	SD
Control group	65.24%	4.72
Study group	64.46%	5.03
p-value	0.796 ns	

ns; non-significant (p>0.05)

Figure 9: Bar chart representing Bone Area Fraction percentage for different groups



DISCUSSION

In the present study a split mouth design was followed to facilitate comparison between the types of drilling burs within each sample providing a similar healing potential with similar immunological and microbiological conditions. [33]

The drills of the study group were designed to be applied in an anti-clock wise direction that allows the condensation and auto-grafting of bone alongside the walls and at the apical end of the osteotomy site that led to an increase in the contact surface area between the fixture and the bony walls. [13-15] On the contrary, the drills of the control group were designed to extract bone along osteotomy walls in purpose of providing a sufficient room for the implant which in sequence will lead to decrease in the amount and quality of the bone engaged to the fixture. [34,13-15]

The dog model is commonly used in dental studies [35] because of many advantages including bone size, body weight, and bone quality when compared to humans. [36] Moreover, they are similar to human beings in terms of formation of secondary osteons, epiphyseal fusion after maturity, comparable intra-cortical remodeling activity and age-associated bone loss. [37,38]

The experimental group showed that some samples presented a good contact between implant surface and peri-implant bone in almost all the implant surface. Other samples presented some areas of contact of implant surface with peri-implant bone, while other areas showed implant surface contact with fibrous unmineralized tissue. The good contact between implant surface and peri-implant bone in the experimental group compared to the control group, was confirmed by the histomorphometric analysis of the BIC% in the SE micrographs of both groups. It showed higher BIC% in the experimental group than the control group, but the difference was statistically insignificant. A significant increase in BIC% in OD drilling technique than the regular drilling method in sheep cervical vertebrae model in a previous study was explained by the production of autologous bone chips in the OD group with greater frequency relative to the control, which acted as nucleating surfaces promoting new bone formation around the implants, providing higher stability and greater bone density. [16] It was proposed that OD drilling technique was designed to allow for additive drilling. Bone fragments created during drilling are displaced laterally and result in densification of the osteotomy wall via osteocompaction. The bone fragments have shown to significantly increase primary stability, while simultaneously functioning to bridge the gap created between the implant surface and osteotomy wall. [39,40] Although it was reported that BIC% was insignificantly higher in conventional drilling technique than OD method, it was also hypothesized that the instrumentation when using the OD technique promotes a wider implant bed in low-density bone, enabling primary stability for dental implants. [41]

Statistical analysis of BMD through EDXA results demonstrated non-significant differences in selected elements (calcium, phosphorus, carbon and oxygen) between study and control group in the peri-implant bone area. These results implied almost equal amounts of minerals (Calcium & Phosphorus) in the study and control groups as essential components of the hydroxyapatite crystals; the building units of the living hard tissues as bone. The comparable results between control and study groups were also evident in the organic elements (Carbon & Oxygen) as fundamental components of living soft tissues. Furthermore, examination of SE micrographs of experimental and control groups exhibited presence of bony particles attached to implant surface and vice versa (i.e., metallic particles attached to bone front against the implant surface). These results further confirmed the osseointegration occurrence between the implant surface and the peri-implant bone. These results were in agreement with MelloMachado et al. in 2021, where their histological examination of the implant-bone interface didn't detect fibrous tissue formation. [41]

In the present study, examination of HE stained sections of the study group demonstrated well-formed cancellous bone at the bone-implant interface followed by regularly formed osteons of compact bone.

Remodeling was much affirmed by the presence of cement lines. The cement line, reflecting secondary osteon formation and lamellar bone deposition. Resting lines representing rhythmic deposition of new bone and reversal lines, interfaces between the old bone and new bone, were also observed. Statistical analysis of BAF revealed non-significant difference between control and study group. These results were in accordance with MelloMachado et al., in 2021, who reported that BAF was similar in both drilling techniques; OD and the regular one. [41]

On the other hand, Trisi et al. , in 2016, [15] reported significant increase of ridge width and bone volume percentage (%BV) with OD technique than the regular method in sheep iliac crests. Also, Lopez et al. in 2017, [42] who reported a final significant increase in BAF in OD group than the regular drilling technique group in sheep cervical vertebral bodies. Same results were confirmed by Witek et al. in 2019, reported a significantly higher BAF in OD group in iliac crests of female sheep. [39] The difference in results significance may be related to the difference of experimental animal model used (dogs or sheep), bone implanted (jaw or iliac crest) or duration of experiment.

A systematic review carried out by Pai et al. in 2018, [43] on articles concerned with OD drilling technique concluded that this method resulted in undersized osteotomy compared to conventional drills. It also resulted in improved bone density and increase in percentage bone volume and bonetoimplant contact, thereby improving implant stability. It was suggested that OD resulted in production of bone fragments that acted as nucleating surfaces promoting new osteogenesis around the implants and providing greater bone density and better stability. In the same context, a systematic review conducted on articles published about OD by Densah bur, by Padhye et al. in 2020, inferred that osseodensification is an efficient way to enhance primary stability of implants in low density bone in an animal model. The purpose is to create a condensed layer of autografted bone along the periphery and apex of the implant. This would, in turn, increases the bone-implant contact enhancing the insertion torque values, and thus, implant primary stability. They suggested that long term clinical success should be ascertained by further clinical results. [44]

Hindi & Bede, in 2020, [45] conducted CBCT and periotest on OD-prepared implants in clinical study. They concluded that osseodensification resulted in high primary stability and increased peri-implant bone density but it did not prevent the implant stability drop during the first 6 weeks after insertion of implants. They suggested that the drop in implant stability is associated with resorption of bone in contact with the implant surface during the first weeks of healing. The resorbed bone is replaced with newly formed viable bone which represents the transition of the implant stability from mechanical anchorage responsible for primary stability to biological attachment responsible for secondary stability. Sultana et al. in 2020, in a clinical study, found no statistically significant difference between OD and regular drilling methods in implant stability and crestal bone levels in spite of the small sample size and short period of investigation. [46]

On the other hand, Almutairi et al. in 2019, concluded that implants placed in regular drilling osteotomies had a significantly better primary stability than the implants placed in OD osteotomies in thick cancellous bone slices obtained from the head of Cow femur bone. [47]

In the present work, BIC%, BMD and BAF statistical analysis revealed insignificant differences between conventional drilling technique and OD method. This could be referred to limitations of this study including small sample size. This could be also attributed to time lapse between surgical procedure and time of scarification of experimental animals. This time lapse could have allowed for proper formation of new bone and remodeling of bone to reach final bone structure in both groups. Although the comparable results between the two methods confirmed the safety of OD technique, it didn't assess the histological effect of the OD method in the early phases of the healing period. Short term histological analysis is recommended to assess primary BIC%, BMD and BAF early after surgical procedures.

The difference in results between the present work and other studies performed on OD method, could be caused by using different experimental animal models, different durations, different assessment methods and different tissues selected for performing the studies. Type of bone selected for the experiment, whether compact, spongy, low density, high density, old or new extraction socket, could all have different effects on the experimental results.

A literature review by Inchingolo et al. in 2021, has concluded that literature is deficient in studies concerning the osseodensification and limited to papers on animals and clinical cases with short-term follow up, which do not help researchers to perform an objective assessment of the advantages of the technique treated; one of the causes is surely the innovativeness of the drills for osseodensification, which still today are not part of the standard implant clinical practice. This technique seems to be promising in the case in which the autologous bone is poor in quality (i.e., cases in which the missing dental element lasted up to provoke the atrophy of the autologous bone of the patient, or very hard areas for the implant primary stability by respecting of the noble anatomic areas), as it "compacts" and "respects" the bone that is directly adjacent to the graft site of the implant. Further studies would turn to the use of drills in cases in which a maxillary sinus lift would be necessary. [40]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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