 DOI: 10.21608/adjalexu.2024.241416.1419.

ASSESSMENT OF BONE CELL VIABILITY IN BONE HARVESTED UNDER TWO DIFFERENT DRILLING SPEEDS DURING IMPLANT BED PREPARATION IN THE MANDIBULAR POSTERIOR SITES (A RANDOMIZED CONTROLLED CLINICAL TRIAL)

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ABSTRACT

BACKGROUND: Viability of the harvested bone during osteotomy preparation is affected by the generated heat, which in turn is related to drilling speed and time; this bone viability is considered an indicator of the quality of the harvested autograft.

STUDY OBJECTIVE: The aim of this study was to assess the effect of two different drilling speeds on bone cell viability of bone harvested during osteotomy preparation.

MATERIALS AND METHODS: A split mouth randomized controlled clinical trial was carried out on 8 patients, 16 mandibular premolar /molar edentulous ridge sites, using a trephine bur, 8 osteotomies were drilled using a speed of 1000 rpm (Control Group) and the other 8 using 400 rpm (Test Group), implants were placed, the harvested bone viability was evaluated histologically in both groups.

RESULTS: The histological assessment revealed better viability features in samples harvested using 1000 rpm (Control Group).

CONCLUSION: Drilling at 1000 rpm seems to yield stronger autologous bone viability than drilling at 400 rpm.

KEYWORDS: Bone viability, Drilling speed, Implant bed, Autograft harvesting.

RUNNING TITLE: Viability of bone cells under two drilling speeds.

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INTRODUCTION

Due to recent innovative progress in biodental materials and bone tissue engineering, dental implants became one of the most successful procedures used for replacing lost tooth/teeth, with a success rate reaching 95% (1).

It has been approved that exceeding temperature of 47°c for 1 minute of drilling causes bone necrosis (2).

Tehemar et al (3) stated that achievement of the atraumatic surgical preparation for osteotomy site is correlated to many elements, one of which is avoidance of bone overheating. This overheating process is influenced by various parameters, which have been categorized as follows: factors related to operator, including drilling speed, drilling motion, drilling pressure, and drilling time; factors related to manufacturer, including irrigation system, drill design, drill cutting efficiency, and implant system; factors related to surgery, including thickness of the cortical bone, condition of the site, depth of drilling;

and factors related to patients, including age and bone density.

After losing a tooth/teeth horizontal and vertical bone loss occurs in both mandible and maxilla, bone resorption may reach 50% in the first year, 30% of which occurs in the first three months (4,5). Therefore, implant placement in the proper prosthetic position may turn into a challenging procedure (6).

After planning to replace a missing tooth with an implant, and if a minor grafting procedure is required, a second surgical site could be avoided with the use of a trephine bur, it can harvest an adequate amount of graft from the osteotomy site and augment the existing bone defect at the same procedure (7).

Many bone graft substitutes have been introduced commercially to correct bone defects, e.g. allografts, xenografts, and alloplastes (8).

In spite of the comprehensive research, no material could completely replace the autografts as it is osteogenic, osteoinductive, osteoconductive and contains viable bone cells with its bone morphogenetic proteins, these Viable cells possess the ability to modulate both bone resorption and neoformation, during its viable status and even after their death, therefore, autografts are still acknowledged as the gold standard of graft materials (9).

The crucial factor for the success of autografts is to harvest them with the largest possible amount of viable bone forming cells i.e. osteoblasts and osteocytes (10).

There are many ways to assess the viability of these bone cells, one of the accurate ways is direct microscopic examination (11).

As the drilling speed and bone damage are directly proportional to the heat generated during drilling (3,12), it is rational that reducing the drilling speed will preserve the viable osteocytes, thus, maintaining the potential for bone regeneration and successful osseointegration (13,14).

On the other hand, many studies reported that raising the drilling speed to a certain level will reduce the cutting time, resulting in less bone destruction and more bone viability (15,16).

The controversy in this point emerged early in the research field, historically, *Matthews and Hirsch in 1972* (17), found a positive correlation between drilling speed and heat generation within the speed range of 345 to 2,900 rpm.

There is a shortage of evidence regarding histomorphometric assessment of bone viability in humans, particularly in relation to different depths and speeds of implant osteotomy drilling. Yet, this subject remains controversial, encompassing a spectrum ranging from excellently preserved bone cells to 100% loss of osteocytes and bone viability (18,19).

The year 2015 witnessed a systematic review of heat generation during the preparation of implant sites. It revealed that majority of the studies were carried out (in vitro), on non-living bone specimens. The primary distinctions between viable and non-viable bone tissue lie in absence of hydrodynamic blood system, lower bone density, and absence of viable cells in necrotic bone (20).

Many researchers investigated the viability of bone cells by using different drilling speeds on experimental animals (16,21,22). However, few studies assessed it clinically (9,23).

The aim of the present study was to evaluate and compare histologically the viability of the bone tissues collected by using a trephine bur under two different drilling speeds.

The null hypothesis of this study was that harvesting implant bed sites using a high drilling speed as 1000 rpm would show histologically the same levels of viable bone cells as the bone harvested using a low speed drilling at 400 rpm.

MATERIALS AND METHODS

Study design: This study was a split mouth randomized controlled clinical trial.

The PICO question was: (P) Patients having two or more healed bony sites at mandibular Premolar/Molar area indicated for implant placement. (I) Intervention, bone collection using a trephine bur at 400 rpm speed (9), during the osteotomy site preparation (C) compared to the bone harvested at 1000 rpm drilling speed (16); (O) to compare histologically the bone cells viability in both groups.

The study was accepted by the Research Ethics Committee of the Faculty of Dentistry, Alexandria University (IRB NO:00010556 -IORG 0008839). Registration of the study was done at U.S National Institutes of Health Clinical Trials Registry (NCT05222737). It also followed the principles of modified Helsinki Code for Human Clinical Studies (2013) (24) and CONSORT 2010 guidelines for reporting randomized clinical trials (25).

Sample size: Sample size was based on 95% confidence level to detect differences in bone cell viability in autografts between two different drilling speeds. Tabassum et al (9), reported mean ± SD cell viability= 97.6 ± 1.20 after standard drilling speed, and 97.4 ± 1.20 after the low drilling speed protocol. The calculated mean ± SD difference in bone cell viability= 0.02 ± 1.20 and 95% confidence interval= -0.93, 1.33. The required sample size was calculated to be 7 participants (14 sites), increased to 8 participants (16 sites) to make up for the loss to follow-up (26).

Software: MedCalc® Statistical Software version 20.013 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

Study setting: A total number of 8 patients with 16 missing mandibular premolar/molars, indicated for implant placement was included in this study. The patients were recruited from the outpatient clinic of the Department of Oral Medicine, Periodontology, Oral Diagnosis, and Oral Radiology, Faculty of Dentistry, Alexandria University.

Histological procedure was carried out in Oral Biology Department laboratory. Faculty of Dentistry, Alexandria University.

Inclusion criteria: Systemically healthy male and female patients, aged 21 to 60 years old, indicated for implant placement of two or more missing premolars and/or molars of the mandibular arch. All patients had adequate bone volume, and sufficient interarch space as well as adequate oral hygiene.

Exclusion criteria: Patients with a medical or psychological condition that contraindicates implant osseointegration or surgical procedures, pathology at the site of intervention, poor patient compliance, parafunctional habits such as bruxism and clenching, heavy smoking, and alcoholism.

Materials

Three trephine drills with an inner diameter of 2.3 mm and an outer diameter of 3.00 mm were used (MEGAGEN™ Implant System, Seoul, Korea), one drill for every 5 or 6 osteotomies to avoid drill dullness.

-KISplant root form implant system & its surgical kit were used (KISplant, KUWOTECH IMPLANT SYSTEM CO, Ltd. South Korea).

- Cone Beam Computerized Tomography (Scanora 3DX Soredex, Helsinki, Finland).

Grouping and Randomization

Control group: Eight mandibular edentulous ridges were prepared for osteotomy using 1000 rpm drilling speed.

Test group: Eight mandibular edentulous ridges were prepared for osteotomy using 400 rpm drilling speed.

Randomization: To limit the potential of confounding, random allocation was done, by employing a computer-generated random sequence of numbers to assign treatment status (27). The process of randomization has been performed in the following manner:

1-Each edentulous ridge in this study was given a number from 1 to 16.

2-Using computer-assisted software (http://www.randomizer.org), a number of 8 sites were selected in each group.

II- Methods

A. Pre-surgical phase: A comprehensive medical and dental history was obtained.

- Phase I therapy was initiated. Patients were advised to keep optimal oral hygiene.
- Proper intraoral examination of the surgical site was performed to evaluate relation of the edentulous ridge to adjacent teeth and the estimated available mesiodistal, buccolingual, and occluso-gingival space.
- Radiographic evaluation was performed by obtaining a cone-beam computed tomography (CBCT), bone density was recorded using computer-assisted software (OnDemand 3D software, cybermed, Inc, Seoul, Korea), and. The preoperative radiographs were used to confirm the diagnosis and to estimate the implant size.
- Surgical procedures were explained to all participants in a simple way, then their informed consent was obtained.

Surgical phase

- All surgeries have been performed by the same operator.
- Patients were instructed to rinse with an antiseptic mouthwash. (Chlorhexidine 0.12%) (Hexitol; under license of The Arab Drug Company (ADCO)-Egypt) before the surgery.
- A loading dose of 875 mg of Amoxicillin and 125 mg of Clavulanic acid (1 g Amoxicillin Clavulanate capsule) (Augmentin 1gm Tablets, Medical Union Pharmaceuticals (MUP), GlaxoSmithKline (gsk) Cairo, Egypt) was given orally to the patient one hour before the surgery.
- All procedures were accomplished under local anesthesia (4% Articaine with 1/200 000 adrenaline Solution) (Ubistesin TMforte (ArticaineHCLwithepinephrine1:100,000, 3M ESPE, Seefeld, Germany), using inferior alveolar nerve block technique. Then a Crestal flap was reflected at the intended sites using a 15 C scalpel & periosteal elevator.

Control Group: Osteotomy was prepared using a trephine drill with size 3.5 mm outer and 2.5 mm inner diameter on a 1000 rpm speed, and a 7mm depth. (Figure 1.A)

The osteotomy was completed to the final length and diameter of the decided implant using the conventional drill/drills of (KISplant) implant system at a speed of 1000 rpm either, and then implants of the same system were placed.

Test Group: Osteotomy was prepared using a trephine drill with the same size and depth as group I but at 400 rpm speed, the osteotomy was finalized at 1000 rpm speed using the conventional drill/drills of (KISplant) implant system, implants of the same system were inserted.

- External saline irrigation was set up at 20 ml/min in all surgeries
- The bone was collected from the trephine drill, preserved in 10% neutral formaldehyde and sent for histological assessment. (Figure 1.B)

- The flap was repositioned and sutured with a 4-0 silk suture (Ethicon silk suture, Johnson & Johnson, Somerville, NJ). In an interrupted manner after implant placement in both groups.

Post surgical care

-Suitable postoperative oral management was performed.

-Patients received convenient post operative medications and instructions.

-After three months, the implants were exposed and steps of fixed prosthetic construction were performed accordingly.

Histological procedure

Tissue preparation for Histological procedure.

The obtained bone particles were left in 10% neutral buffered formalin for 5 hours. Specimens were washed in running water to remove the fixative solution. then placed in ascending concentrations of ethyl alcohol (50%, 70%, 90%, and 2 changes of absolute concentration), finally placed in xylene to remove the alcohol from tissues.

For infiltration, Specimens were placed in a dish of molten paraffin wax in a constant-temperature oven, regulated to about 60 degrees centigrade for about 2 hours.

After infiltration, specimens were embedded, each in the center of a paraffin wax block. Then it was cut using a microtome to obtain serial 5 µm thick sections which were mounted and stained with Hematoxylin and Eosin (H&E) (28), Slides were examined by Optika microscope (OptikamB5, C-B5) for evaluation of the observations.

Morphometric analysis

From each wax block, 3 sections from 3 specific depths were obtained, mounted on glass slides, then examined in the light microscopy. The best of the three sections was labeled to be used for quantifications of the number of viable osteocytes, those filling the lacunae or peripherally dislocated versus the number of empty lacunae (29).

Images were captured with magnification x40 from each section to get a total of 24 readings for each group for either viable or non-viable cells.

Statistical methodology

Data were collected and analyzed using Statistical Package for Social Science (SPSS) program (ver 25) (30) Data were described using minimum, maximum, median, and 95% CI of the median, 25^{th}-75^{th} percentile (31). Comparisons were carried out between two studied independent not-normally distributed subgroups using Mann-Whitney U test (32). Kendall's tau correlation coefficient was used (33).

During sample size calculation, beta error accepted up to 20% with a power of study of 80%. An alpha level was set to 5% with a significance level of 95%. Statistical significance was tested at p value <.05 (34).

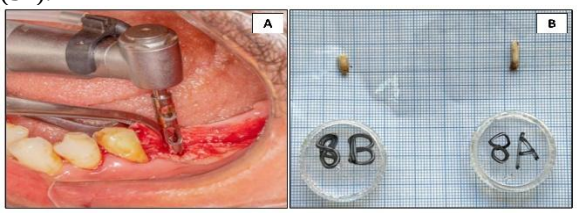

Figure 1: (A) showing the trephine bur used for harvesting bone samples, **(B)** showing bone samples harvested for the control group (1000 rpm-sample A) & test group (400 rpm sample B), both were collected from same patient (No. 8).

RESULTS

Histological results

Examination of bone specimens obtained with high speed revealed considerable differences, from those obtained with low speed. These differences included the following:

The outline and borders.

Surface structure and homogeneity.

Osteoblasts on the internal or external border of the specimen or particles comprising it.

The accommodated osteocytes as regarding the following:

a-Healthy and sizable.

b-Pyknotic and small in size.

c- Absent from empty lacunae.

Control Group [High speed group (1000 rpm)]

Most of the obtained specimens of this group had regular outlines and intact borders with considerable surface area and contained normal density of bone marrow between its parts, (Figure 2.A)

At high power of examination, the specimens or their different parts had regular and homogenous surfaces and contained considerable density of osteocytes, most of them appeared healthy, sizable, and occupied the greater width of the lacunae, (Figure 2.B)

Other cells appeared smaller and peripherally displaced. However, empty lacunae devoid of osteocytes were traced either among the healthy ones or among the displaced cells. (Figure 3.A)

In most of the specimens of this group, well organized voluminous osteoblasts were traced on the outer border or lining of the internal margins of these specimens. (Figure 3.B)

Test Group [low speed group (400 rpm)]

Five of these specimens had disorganized appearance with irregular outlines and shredded margins, and even three of them exhibited macerated edges with subsequent decrease of the surface area than in Control Group. (Figure 4.A)

The quality of the surface of these specimens and their structural details were disorganized with loss of their homogeneity. (Figure 4.B)

Necrotic bone trabeculae were observed in superficial sections of some specimens. However, their central regions (distant from the effect of the drilling heat) had better appearance and more organized structure and accommodated osteocytes of moderate healthy appearance besides pyknotic ones or those lost from the lacunae. (Figure 5.A)

On the outer border of the specimens or their different parts adjacent to bone marrow, osteoblasts exhibited moderate features of activity regarding size and shape. (Figure 5.B)

Statistical Results

Eight patients requiring implant placement were included in a split mouth clinical trial, aligned into two groups. Control Group included eight mandibular edentulous ridges that were prepared for osteotomy using 1000 rpm drilling speed, Test Group included eight mandibular edentulous ridges that were prepared for osteotomy using 400 rpm drilling speed.

Data are presented as median [25th–75th percentile] and 95% Confidence Interval (CI) of the median.

For the 1000 rpm group (n=8), the Average Number of viable osteocytes ranged from 37.67 to 67.33, with a median of 58.33, 95% CI of the median of 43.67-65.33 and [25th–75th percentile] of [49.00-64.50].

For the 400 rpm group (n=8), the Average Number of viable osteocytes ranged from 22.00 to 32.33, with a median of 28.67, 95% CI of the median of 24.00-32.33 and [25th–75th percentile] of [23.50-30.33]. There was a statistically significant higher number of the Average Number of viable osteocytes in the 1000 rpm group compared with the 400 rpm group (p=.400). (Table 1) (Figure 6.A)

In the 1000 rpm group (n=8), the Average Number of non-viable osteocytes ranged from 8.00 to 11.67, with a median of 9.17, 95% CI of the median of 8.33-10.33 and [25th–75th percentile] of [8.50-10.00]. In the 400 rpm group (n=8), the Average Number of non-viable osteocytes ranged from 16.67 to 42.33, with a median of 35.33, 95% CI of the median of 32.67-38.33 and [25th–75th percentile] of [32.83-37.83]. There was a statistically significant higher number of the Average Number of non-viable osteocytes in the 400 rpm group compared with the 1000 rpm group (p=.400). (Table 2) (Figure 6.B)

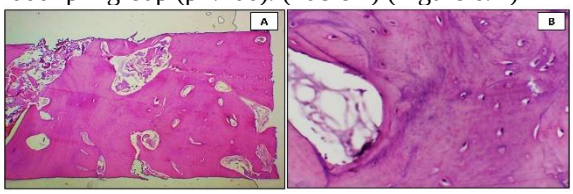

Figure 2:(A) [(photomicrograph of decalcified section (DS), H&E), control group] showing regular outline and organization of a whole specimen with perfect quality of the bone constituting its parts and normal density of the bone marrow and soft tissue matrix around its different parts, X: 40, **(B)** [DS, H&E, control group] showing high power view of a part of the specimen seen in fig.2.A revealing the density of healthy, viable and sizable osteocytes, X: 400.

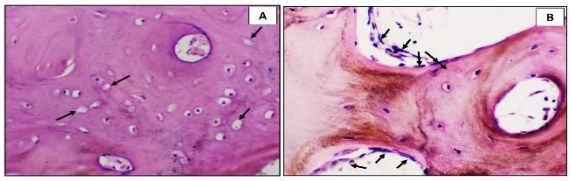

Figure 3: (A) [photomicrograph of DS, H&E, control group] showing high power view of slightly displaced osteocytes to the periphery of the lacunae and some lacunae appear empty (arrows), X: 400. **(B)** [DS, H&E, control group] showing well organized voluminous osteoblasts on the outer border or lining the internal margins of the specimens, X: 400.

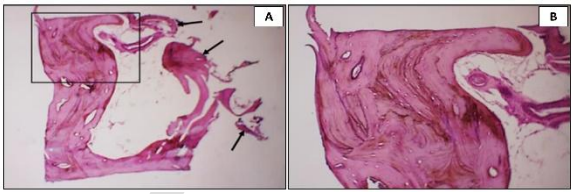

Figure 4: (A) [photomicrograph of DS, H&E, test group] showing a specimen with disorganized appearance, irregular outline and shredded margins (arrows), X: 40. **(B)** higher magnification of the inset in the previous figure illustrating predominance of empty lacunae with few containing pyknotic osteocytes. The surface of the specimen exhibits irregularities with loss of normal homogeneity. X:100.

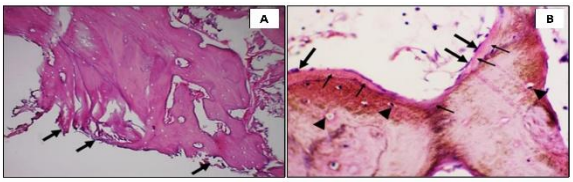

Figure 5: (A) [photomicrograph of (DS, H&E, test group] showing macerated and necrotic edges of the trabeculae (arrows) at the margins of the specimen. Prevailing empty osteocytes' lacunae are seen in specimen parts adjacent to these necrotic trabeculae while the deeper ones appear viable, X: 100. **(B)** [DS, H&E, test group] showing flattened osteoblasts (thick arrows) on the border of one of the bone specimens adjacent to thin ribbon of osteoid (thin arrows). Note the few pyknotic osteocytes (arrow heads) and the empty ones, X:400.

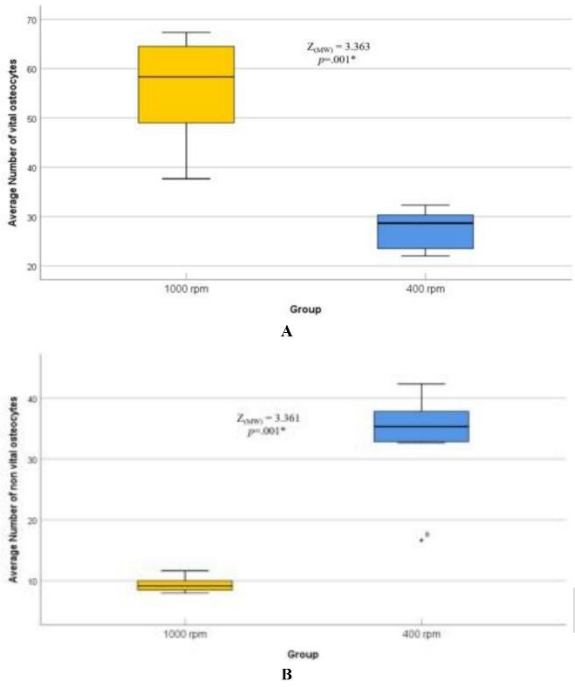

Figure 6:(A) Box and whisker graph of Average number of viable osteocytes in the studied groups, the thick line in the middle of the box represents the median, the box represents the inter-quartile range (from 25th to 75th percentiles), the whiskers represent the minimum and maximum. **(B)** Box and whisker graph of Average number of non viable osteocytes in the studied groups, the thick line in the middle of the box represents the median, the box represents the inter-quartile range (from 25th to 75th percentiles), the whiskers represent the minimum and maximum after excluding extremes (black asterisks) (numbers indicate the serial number of the patients).

Table (1): Comparison of the Average Number of viable osteocytes between the two studied groups

Average number of viable osteocytes	Group		Test of significance *p*-value
	1000 rpm (n=8)	400 rpm (n=8)	
Min-Max	37.67-67.33	22.00-32.33	$Z_{(MW)}$=3.363 *p*=.001*
Median	58.33	28.67	
95% CI of the median	43.67-65.33	24.00-32.33	
25th Percentile – 75th Percentile	49.00-64.50	23.50-30.33	

n: number of patients
Min-Max: Minimum to Maximum
CI: Confidence interval
$Z_{(MW)}$: Z of Mann-Whiteny U test
*: Statistically significant (p<.05)

Table (2): Comparison of the Average Number of non-viable osteocytes between the two studied groups

Average Number of non-viable osteocytes	**Group**		**Test of significance *p*-value**
	1000 rpm (n=8)	400 rpm (n=8)	
Min-Max	8.00-11.67	16.67-42.33	$Z_{(MW)}$=3.361 *p*=.001*
Median	9.17	35.33	
95% CI of the median	8.33-10.33	32.67-38.33	
25th Percentile – 75th Percentile	8.50-10.00	32.83-37.83	

n: number of patients
Min-Max: Minimum to Maximum
CI: Confidence interval
$Z_{(MW)}$: Z of Mann-Whiteny U test
*: Statistically significant (p<.05)

DISCUSSION

The viability of bone cells can be compromised by high-speed rotating instruments due to heat and mechanical injury they generate (10,15). Preservation of cell viability of the autograft is of utmost importance in its success, so, bone viability assessment may provide a precise approach to measure the degree of cellular damage (23).

Recent reviews emphasized the significance of osteocytes and labeled it as a fundamental requirement for the success of autografts. Osteocytes are key to the recruitment and proliferation of new osteoblasts. Therefore, presence of viable osteocytes is of ultimate value (35,36).

The current study attempted to histologically investigate the viability of bone tissues under two different drilling speeds (1000 and 400 rpm).

In the present study, trephine bur was used for harvesting bone samples, it allows exploration of the effect of drilling speed exclusively on the bone cells located on the outer surface of bone. This is supported by the observations of *Miron et al* (10) who noticed that bone particles collected using trephine bur and bone scraper exhibited a greater cell content and an enhanced capacity to differentiate and generate mineralized tissue, along with a more pronounced expression of osteoinductive proteins.

Drill wear was put in concern in this study, 3 new trephine drills were used without exceeding 6 osteotomy preparations for each. This was justified by former research which reported increasing in temperature production with frequent use of the cutting instrument in implant bed preparation (37).

Histological examination of bone samples in the present study revealed considerable differences between the two groups. The control group (1000 rpm)

samples had regular outlines and intact borders with considerable surface area. The specimens contained a considerable density of osteocytes, most of them appearing healthy, sizable, and occupying the greater width of the lacunae. Moreover, the test group had a disorganized appearance with irregular outlines and shredded margins. Necrotic bone trabeculae were observed.

These histological observations are in line with a study conducted in 2017 by *Tabrizi et a*l (23) which investigated the impact of 1000 and 1500 rpm drilling speeds, in 10 and 13 mm depths on bone viability, they observed a well-conserved bone structure and a substantial number of viable osteocytes, however, they used a bone collector drill other than trephine bur and did not compare these results to low drilling speed samples.

The histomorphometric analysis of the current study revealed a statistically significant higher number of viable osteocytes with a median of 58.33 in the control group (1000 rpm) compared to 28.67 in the test group (400 rpm) (p=.400). And a statistically significant higher number of non-viable osteocytes with a median of 35.33 in the test group compared to 9.17 in the control group (p=.400), revealing superior viability features in the control group.

These findings are in accordance with *Marzook et al (2020)* (16), who investigated the impact of three different drilling speeds (1000, 1500, and 2000 rpm) on dense bone of rabbit's femur, and found that higher drilling speed resulted in the least amount of non viable osteocytes close to the cut surface.

The results of the current study are also supported by a study conducted by Aldabagh et al (21), who utilized (1250, 2000, and 2500 rpm) on bovine femoral cortical bone, for 7 mm depth, and reported that 2500 rpm speed minimized the risk of bone damage, therefore the initial healing of dental implants, they concluded that decrease in rotational speed necessitates longer drilling duration and generates greater frictional heat, thereby increasing likelihood of bone destruction.

Contrary to the findings of the present study, in 2020, *Tabassum et al* (9), investigated the viability of bone particles collected from flutes of the drilling burs, they reported that drilling at a low speed (less than 200 rpm) with no irrigation led to a significant increase in cell multiplication (DNA content) and differentiation in autogenous bone fragments, as compared to higher drilling speeds (400 to 600 rpm), thus, they concluded that efficiency of autogenous bone particles harvested by low speed drilling was superior.

Reingewirtz et al (12), reported a significant positive association between the increase in temperature and rotational speed during the process of cutting bovine dense bone, utilization of low speed drilling (400-800) rpm resulted in a decrease in heat generation compared to high drilling speed (24000-40000) rpm, nevertheless, this was an extremely high speed in comparison to the speed range investigated in the present study.

However, a multitude of factors can contribute to these contradictory outcomes, including the study models, conditions of the osteotomy site, characteristics of the drill, and techniques of observation (7,20).

Temperature rise during sustained drilling could be explicated by lack of cooling fluid in the deep part of the osteotomy and the blockage caused by bone residue on the drill's cutting edge. Using slower speeds exaggerates this blockage and reduces the drill's cutting efficiency, resulting in more time required to prepare the bone bed and further heat generation (38), which in turn affects the bone tissue viability as proposed in the current study.

The present study revealed more viable osteocytes in samples of the control group (1000), indicating superior viability features. However, the exerted force during drilling was not precisely recorded for each case, which was a concerning limitation. Further studies are required to detect the ultimate speed for harvesting autogenous bone.

CONCLUSIONS

Regarding the current study, it is feasible to conclude that using 1000 rpm speed in dense bone in the presence of coolant generates less heat and preserves more bone viability, so it is reliable to use the 1000 rpm during osteotomy preparation in cases that need harvesting small amounts of autogenous bone grafts.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING STATEMENT

The authors received no specific funding for this work.

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