Evaluation of Bone Heating, Immediate Bone Cell Viability, and Wear of High-Resistance Drills After the Creation of Implant Osteotomies in Rabbit Tibias

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Purpose: *The purpose of this study was to evaluate the influence of reusing high-resistance drills on bone heating, immediate bone cell viability, and drill wear after performing implant osteotomies in rabbit tibias.* ***Materials and Methods:*** *Two hundred sequential implant osteotomies were created in the superior tibial cortex of 12 White male rabbits. Six groups were established (G1 to G6) according to the number of osteotomies performed with each drill (0, 10, 20, 30, 40, and 50). Drilling began with a spear drill, followed by 2.0-mm, 2.8-mm, 3.0-mm, and 3.15-mm helical drills. The receptor beds were collected for immunohistochemical analysis, thermal changes were quantified, and the drills were subjected to scanning electron microscopy analysis.* ***Results:*** *A high degree of correlation between drill wear and number of osteotomies was observed (Pearson correlation coefficient, r = 0.984). Spear drills underwent twice as much deformation as helical drills. The bone heating analysis concluded that there was no statistically significant relationship between the number of osteotomies and bone heating (P > .05), but there were greater thermal changes during drilling with the spear drill than during drilling with helical drills (ratio 3:1). Immunohistochemical analysis showed a physiologic balance of osteoprotegerin and RANKL (receptor activator of nuclear factor κB ligand) immunolabeling in all groups; however, there was greater immunolabeling of all proteins in group G6 (50 osteotomies).* ***Conclusions:*** *The tested drills did not cause significant bone heating after being reused 50 times; however, they caused more tissue trauma in the 50th osteotomy. Worn drills that are reused may be expected to cause excessive damage to the bone tissue and could adversely affect the osseointegration process.* Int J Oral Maxillofac Implants 2011;26:1193–1201

Key words: *cell survival, dental implants, immunohistochemistry, osteotomy, scanning electron microscopy*

Oral rehabilitation with osseointegrated implants has been performed extensively worldwide in diverse clinical situations, with high success rates and excellent predictability.[1,2] Primary bone repair of the receptor bed plays a fundamental role in osseointegration.[3] Excessive trauma during surgery may negatively affect tissue maturation at the bone-to-implant interface, diminishing the predictability of osseointegration.[4] Therefore, to preserve tissue viability at the time of implant placement, it is necessary to perform adequate preparation of the surgical bed.[5,6]

After implant osteotomy, a thin layer of necrotic tissue at the preparation margin will always form, even when appropriate care is taken with the surgical technique.[7] This necrotic tissue will be resorbed and replaced by viable tissue during the repair process. Nevertheless, the extent of this necrotic zone depends on various factors, such as temperature; pressure; shape, size, and cutting edge of the drill; type of osteotomy (continuous or intermittent, sequential or in a single step); irrigation; rotational speed; duration of bone heating; and density of the osteotomized bone.[6,8–10]

Bone tissue is very susceptible to thermal injury, and the temperature threshold for tissue survival during osteotomy is 47°C when drilling is maintained for more than 1 minute.[11,12] Heating in excess of this limit could

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lead to primary failure to achieve osseointegration. The repeated use of drills during preparation for implant placement may increase their deformation and cutting efficiency, with consequent increases in frictional heat.[4]

At present, many implant companies do not indicate how many times a drill should be used, thereby hindering the understanding of dentists about the optimal frequency of drill replacement. This could result in greater tissue trauma to the surgical bed, leading to higher rates of implant loss.[4]

The first step in the bone repair process largely depends on the cellular and vascular elements of the tissue.[9] Osteocytes are multifunctional cells that actively participate in cell turnover, and they are very sensitive in regard to translating aggravations to the tissues into biochemical signals.[13] In this context, extracellular matrix proteins play an important role in the ossification process. They contribute to increasing cell activity around implants and consequent osseointegration.[14,15] Among these proteins, osteoprotegerin (OPG), which is secreted mainly by osteoblasts, is considered a physiologic regulator of bone resorption, acting directly on tissue remodeling.[16–18] Another protein that participates in bone tissue dynamics is the activator receptor ligand of nuclear factor κB (RANKL). Some cells, such as osteoblasts, bone stromal cells, endothelial cells, and activated T lymphocytes, express this protein.[19,20] Their function is to link to the RANK protein present in the cell membrane of osteoclasts, thereby activating these cells. OPG and RANKL expression regulate osteoclastic activity, preventing the activation and maturation of osteoclasts and consequent bone resorption.[21,22]

Osteocalcin, the most abundant noncollagenous protein in bone, is produced by osteoblasts and plays an important role in the tissue mineralization process. It has been suggested that its action occurs during the initial stages of bone repair, and it is essential to the regulation of osteoblast activity.[23–25]

With respect to the harmful effects of bone heating on the tissue repair process and consequent osseointegration of implants,[26] the overuse of implant drills could cause excessive deformation, negatively influencing bone repair. Therefore, the purpose of this study was to evaluate the effect of reusing drills specifically designed to resist deformation on bone heating, immediate cell viability, and drill deformation while creating implant osteotomies in rabbit tibias.

MATERIALS AND METHODS

This study used 12 White male rabbits (*Oryctolagus cunilicus*, New Zealand) with body weights of 3 to 4 kg. The protocol was submitted to and approved by the Ethics Committee on Animal Experimentation of the Universidade Estadual Paulista (UNESP), Araçatuba Dental School (protocol no. 2008-005051).

The animals were anesthetized by intramuscular administration of a combination of 50 mg/kg of ketamine (Vetaset, Fort Dodge Saúde Animal) and 5 mg/kg of xylazine hydrochloride (Dopaser, Laboratório Calier do Brasil). A total of 30 drills were used; these were divided into six groups (G1 to G6) that corresponded to the number of times each drill was used (0, 10, 20, 30, 40, and 50 times).

Surgery

To initiate surgery, an incision was made in the medial portion of the right and left tibias of each rabbit, followed by reflection of the soft tissue and detachment of the periosteum to perform the osteotomies. A 1,200-rpm electric motor connected to a 1:16 reduction contra-angle (Kavo) was used to perform the osteotomies. The system used (Conexão Sistema de Próteses) was fitted with drills made of M340 steel (Bholer), which feature high resistance to deformation, according to the manufacturer. Preparation of the defects began with a spear drill to delimit the location of the perforations and rupture the cortical bone. Next, helical drills with diameters of 2.0 mm, 2.8 mm, 3.0 mm, and 3.15 mm were sequentially used at a constant depth of 4 mm (Fig 1). Therefore, only the superior cortex was osteotomized, which corresponds to type 1 bone.[27] During drilling, abundant external irrigation was supplied by a 0.9% sodium chloride solution (Darrow).

Each animal received 10 implant osteotomies in each tibia, and the tissues containing the surgical bed at the 10th, 20th, 30th, 40th, and 50th drilling operations were collected for immunohistochemical analysis.

The groups were divided according to the number of perforations made with each set of drills. Thus, the first group corresponded to the drills that were not used (G1 with no osteotomy), such that only scanning electron microscopic (SEM) analysis of the drills was performed. The second group corresponded to the drills that had been used 10 times (G2 with 10 osteotomies), and one rabbit tibia for each sample was required. The third group corresponded to the drills used to create 20 osteotomies (G3 with 20 osteotomies), and two tibiae were required for each sample (10 osteotomies in each tibia). Subsequently, the fourth group corresponded to the drills used to create 30 osteotomies (G4) in three tibiae, the fifth group corresponded to the drills used to create 40 osteotomies (G5) in four tibiae, and the sixth group corresponded to the drills used to create 50 osteotomies (G6) in five tibiae.

The pressure exerted on the bone tissue during the osteotomies was not measured. However, each surgical stage was performed by the same operator, who took care to exert little pressure and perform drilling

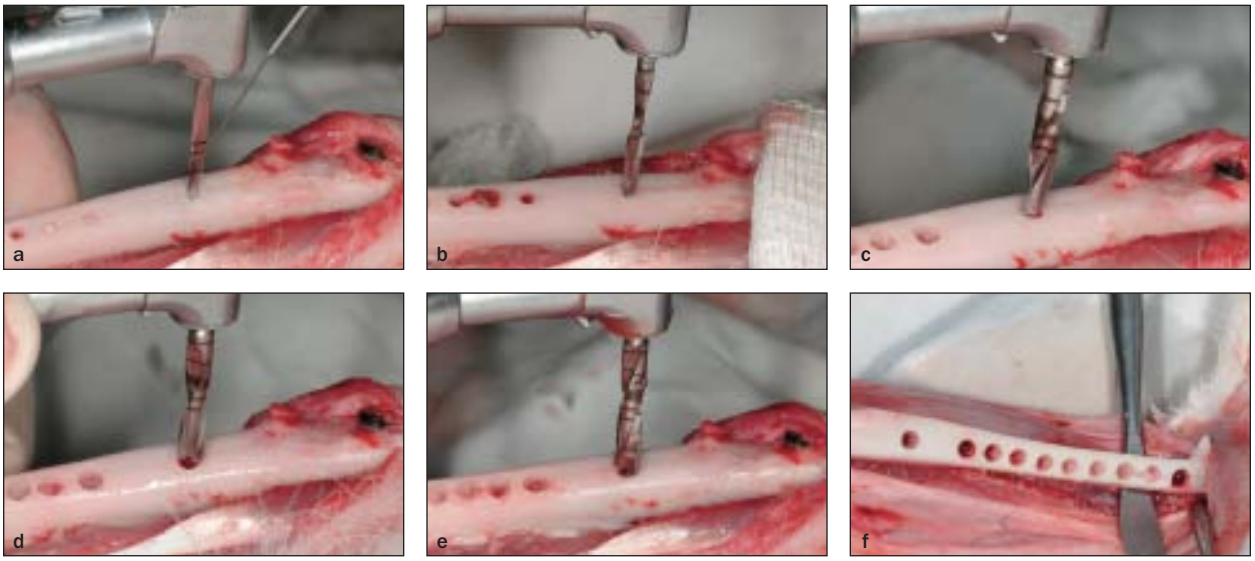


Fig 1 Sequence of osteotomies performed in rabbit tibias. *(a)* Spear drill; *(b)* 2.0-mm helical drill; *(c)* 2.8-mm helical drill. *(d)* 3.0-mm helical drill; *(e)* 3.15-mm helical drill; *(f)* rabbit tibia after the completion of implant osteotomies.

intermittently during a time interval of less than 1 minute to allow the bone to escape through the perforation and the irrigation solution to cool the site.

The animals were killed immediately postoperatively by intramuscular administration of a lethal dose of 30% chloral hydrate (2 mL/kg). The tissues were removed, and excess soft tissue was eliminated. The tissues were fixed in 10% formaldehyde solution for 48 hours.

Bone Heating Analysis

Thermal quantification was performed with the use of a digital thermometer (Salvterm 700C, sensor type J, Salgas Indústria e Comércio), which captured the bone surface temperature before each drilling operation (Initial Temperature) and was introduced into the studied osteotomies immediately after each drilling operation (final temperature). Thus the maximum change in temperature was calculated as: final temperature − initial temperature. To serve as a negative control of thermal analysis, a sequence of drilling without irrigation was performed.

Immunohistochemical Analysis

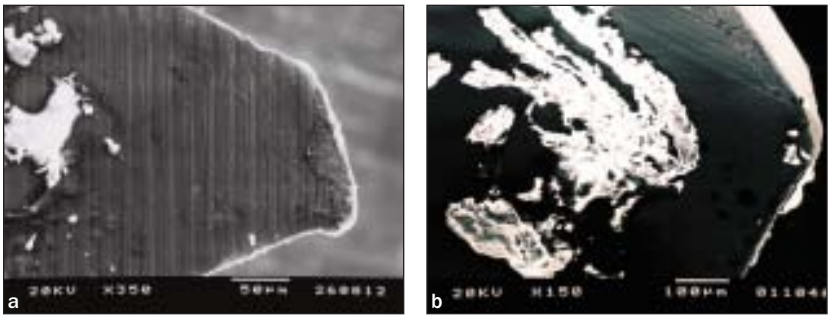
The tissue samples collected were decalcified in 5% ethylenediaminetetraacetic acid for 3 months. After this, they were embedded in paraffin and cut transversely on a microtome into 16-µm-thick sections.

For immunohistochemical processing, OPG (goat anti-OPG, Santa Cruz Biotechnology, SC21038), RANKL (goat anti-RANKL, Santa Cruz Biotechnology, SC7627), and osteocalcin (goat anti-OC, Santa Cruz Biotechnology, SC18319) were used as primary antibodies. Detection was performed by the immunoperoxidase method using anti-goat biotinylated secondary antibody (Pierce Biotechnology). Streptavidin/biotin (Dako) was used to amplify the signal. The reactions were revealed with diaminobenzidine (Dako) chromogen. After this, the specimens were counterstained with Harris hematoxylin, dehydrated in stages, and mounted on slides with Permount.

Control procedures were performed by omitting the primary antibodies (negative control). The inferior cortices of the rabbit tibias were evaluated as positive controls to verify the effectiveness of the antibodies used in a region other than the studied region. The cells that showed positivity for the analyzed proteins were qualitatively evaluated by means of an optical microscope with 16× and 20× magnification objectives (Leica Aristoplan Microsystems, Leitz) coupled to an image-capturing camera (AxioCam MRc 5, Carl Zeiss) and connected to a Pentium III microcomputer with digitized image analysis software (AxioVision 4, version 4.5.0.0, Carl Zeiss). Portions of the sectioned tissue samples were stained with hematoxylin and eosin (Merck) to serve as references for the cytoarchitecture of the tissue samples processed by immunohistochemistry.

Analysis by SEM

All 30 drills used to create the osteotomies were submitted to evaluation by SEM (JSM T220A, Jeol). Two photomicrographs (front and back) were obtained of the active tip of each drill before and after use in 10, 20, 30, 40, and 50 osteotomies at 350× magnification. These were used to quantify the regions of deformation, such as steel melting, loss of substance, condensation of steel detached from the active tip of drills, and cavity-shaped



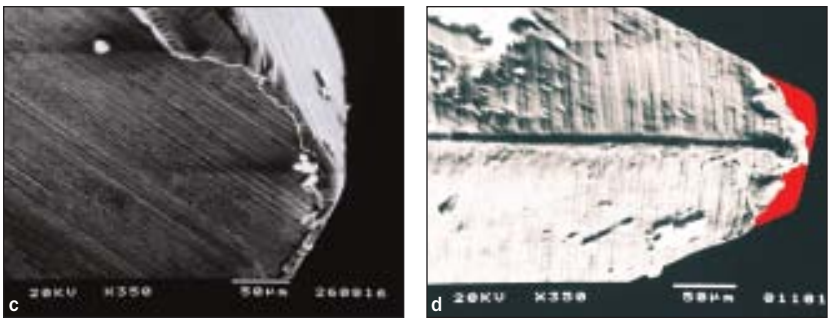


Fig 2 Photomicrographs of reused implant drills obtained with SEM at 350× magnification. *(a)* Material melting at the active tip of the drill; *(b)* deposition of steel detached from the drill at its active point; *(c)* cavity-shaped defects; *(d)* image superimposed with the red template to help visualization of loss of substance from the active tip of the drill.

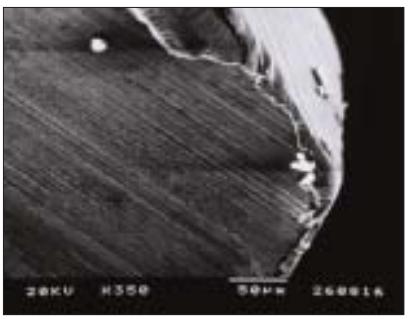


Fig 3 Sample observed with Imagelab 2000 software for quantification of drill defects.

defects (Fig 2). To facilitate the quantification of the areas with loss of substance, a template of the initial spear drill was constructed, and it was superimposed on the other images of spear drills of the evaluated groups.

Imagelab 2000 software was used to calculate image areas. With the tools *select region*, *calculation of regions,* and *calculation spreadsheet*, regions of deformation were demarcated, calculated in pixels, and totaled (Fig 3). Then the percentage of the area of deformation with respect to the entire photomicrograph of each drill was calculated.

Statistical Analysis

The Kruskal-Wallis test was used for thermal analysis and the Pearson coefficient of correlation (*r*) was used for SEM analysis. For the immunohistochemical analysis, the following scores were given to specimens according to the labeling of cells located in certain areas: negative (–), positive (+), superpositive (++), and hyperpositive (+++). For statistical analysis, the scores attributed were converted into percentile average frequencies of 0%, 20%, 60%, and 90%, according to the methodology previously described in the literature,[28] for the expression of each evaluated protein. The results were submitted to the nonparametric Kruskal-Wallis, Dunn, and Mann-Whitney tests ($\alpha = 5\%$).

RESULTS

In this study, a total of 1,000 drillings were performed in 24 tibias of 12 rabbits. During the surgical procedure there were no complications.

Bone Heating Analysis

The mean initial bone temperature during drilling was 26.8°C. The maximum temperature attained was 30.5°C, and the maximum change was an increase of 5.1°C from the initial temperature. There was no statistically significant correlation between the increase in the number

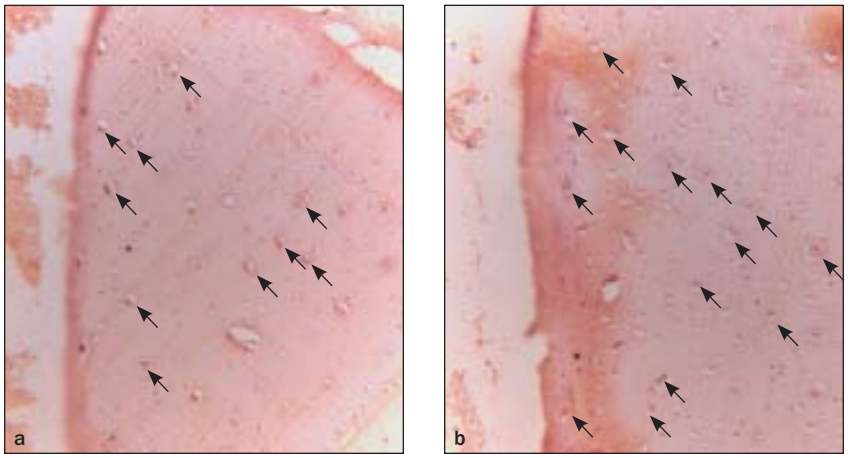


Fig 4 Immunohistochemical images of the osteotomized beds (magnification ×200). *(a)* Expression of OPG in G4. Brownish immunolabeling is apparent in the cytoplasm *(arrows)*. *(b)* RANKL expression in G4. Note the immunolabeling of osteocytes in the cytoplasm *(arrows)*.

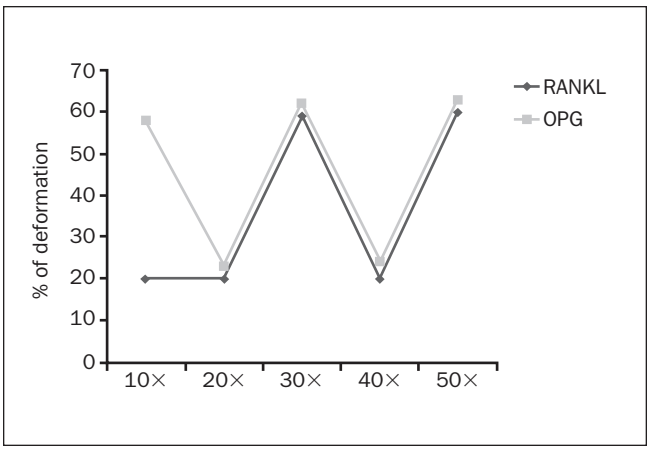


Fig 5 Comparison of the relationships between RANKL and OPG expression and reuse of drills.

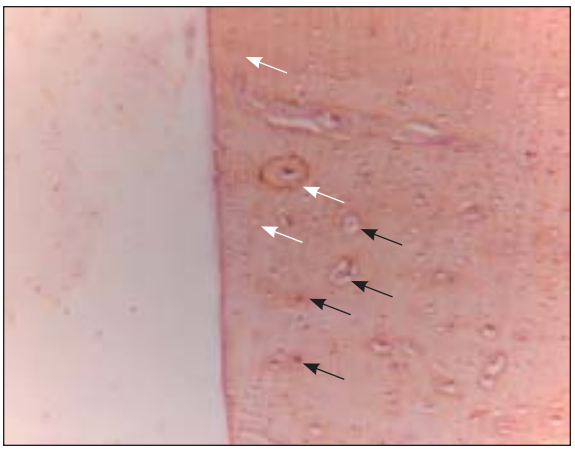


Fig 6 Expression of osteocalcin in G6. Black arrows = immunolabeling in cytoplasm; white arrows = background immunolabeling.

of drillings and the temperature increase ($P > .05$). Nevertheless, it was observed that the mean temperature change during the use of the spear drill was 2.5°C, whereas with the helical drills it was 0.76°C. These measurements suggest that, during initial osteotomy, the heat generated in the receptor bed was higher than it was in the subsequent drillings, in a ratio of approximately 3:1. In the negative control group (no irrigation), the maximum temperature attained was 38°C, with mean thermal changes of 5.2°C and a maximum change of 10°C.

Immunohistochemical Findings

The expression of the evaluated proteins was observed in the superior cortices of rabbit tibias at the osteotomy margins. The values related to the expression of OPG and RANKL were compared between each defect by the Mann-Whitney test, and no statistically significant difference was found ($P = .690$). This confirmed the equilibrium in the expression of these proteins observed in the study, indicating that even in the last groups there was physiologic equilibrium in the production of these proteins (Figs 4 and 5).

Osteocalcin was expressed in the superior cortex in all analyzed groups, without qualitative changes in the dynamics of tissue expression. The background marking characteristic of this protein was observed in the superior cortex, proving that the bone remained viable, even in the last groups (Fig 6).

To evaluate the differences in protein expression with respect to the number of drillings, the Kruskal-Wallis test was used. Statistically significant differences were observed among the studied groups ($P = .005$). After this, the Dunn test was performed to identify the groups that showed significant differences between them. The result showed that there was a statistically significant difference only for G6 ($P < .05$). This confirmed the observation that this group showed exacerbated expression of all the proteins in comparison with the other groups (Fig 7). This suggests that the tissue suffered greater tissue damage after the 50th osteotomy.

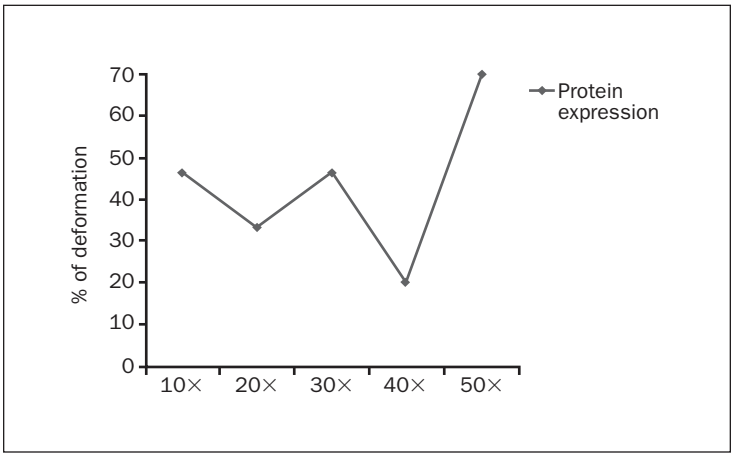


Fig 7 Oscillation curve of protein expression according to the reuse of drills.

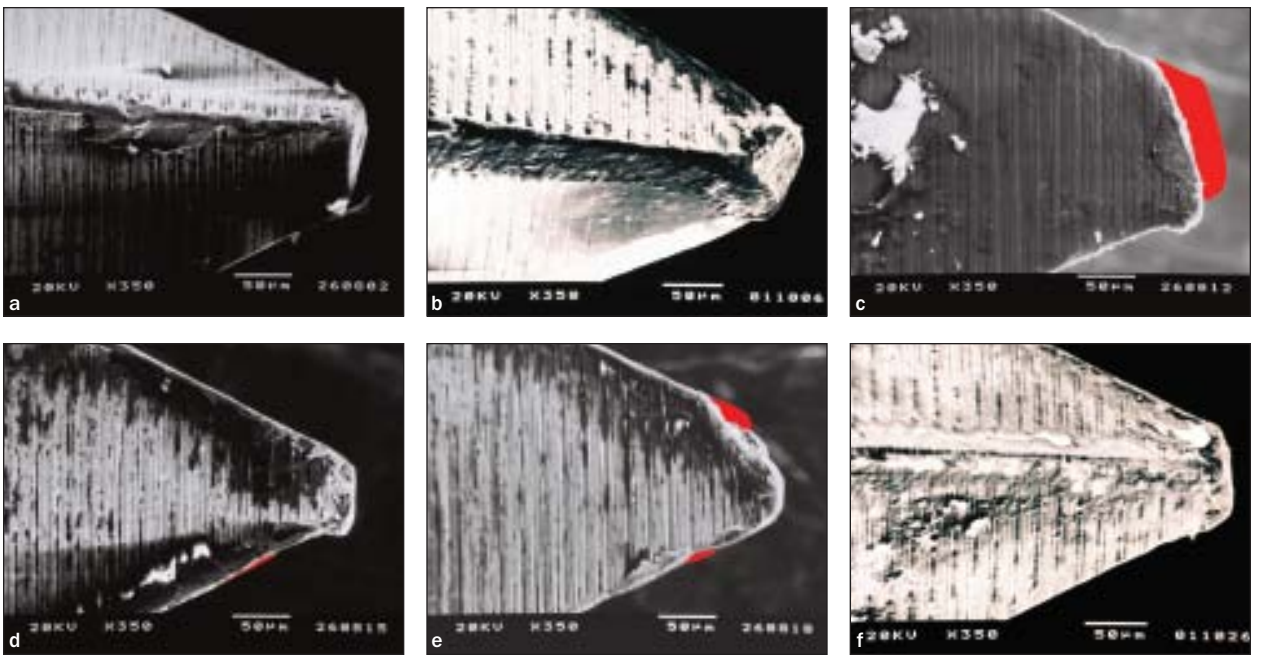


Fig 8 SEM images showing wear. *(a)* G1, unused drill; *(b)* G2, drill reused 10 times; *(c)* G3, drill reused 20 times. *(d)* G4, drill reused 30 times; *ea*) G5, drill reused 40 times; *(f)* G6, drill reused 50 times.

In the negative control, the blades did not react and there was no marking, confirming the specificity of the immunohistochemistry processing. In the positive control, performed in the inferior cortex that was not drilled, cell marking was observed in osteocytes for each evaluated protein, confirming the effectiveness of the administered antibodies.

In the hematoxylin-eosin–stained histologic controls, the osteocytes presented intense hematoxylin staining along the entire superior and inferior cortices analyzed, without major morphologic tissue alterations.

SEM Observations

Different deformation patterns were seen on the drill surfaces. Images suggesting loss of substance, steel melting, condensation of steel detached from the active point of drills, and cavity-shaped defects were observed.

In G1, there were some factory defects, which were considered and quantified in the analysis as initial defects. The defect showed a mean deformation of 9% at the active tip of each drill. Thus, this group was used as the control group for the analysis in question. Continuing with G2, G3, G4, G5, and G6, a gradual increase in deformation was observed; however, at all times these differences were small, with means that ranged from 3% to 6% between each group. It was also observed that, even in conformity with this trend of an increase in deformation as the number of times the drill was used increased, the mean percentages of deformation found in the evaluated groups were low (Fig 8). Thus, a

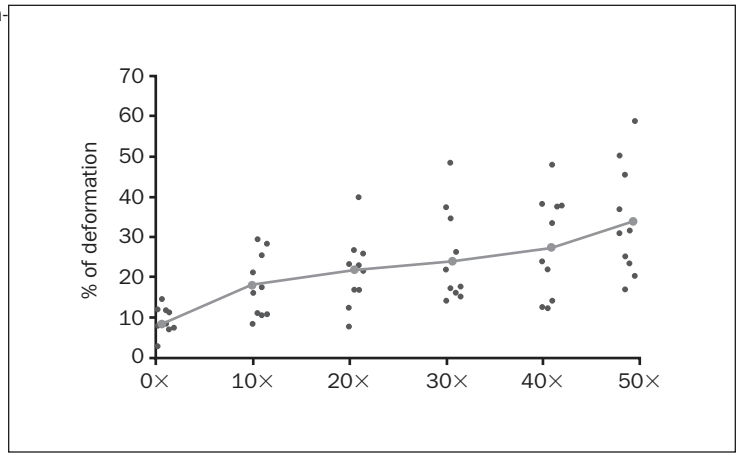


Fig 9 Dispersion graph with mean drill deformation values united linearly.

dispersion graph was constructed to analyze the variables *percentage of deformation* and *number of osteotomies*. This made it possible to observe the gradual increase in deformation, which varied in direct proportion to the increase in the number of times the drills were used. The mean deformation values (Table 1) describe the trend of linear growth in the direction of the increase in the number of drillings (Fig 9). Quantification of the areas of deformation also helped verify the strong directly proportional correlation between the variables *deformation* and *number of times a drill was used* (Pearson coefficient of correlation, r = 0.984).

The majority of the time, the spear-type drills showed greater deformation than the other drills, representing the upper limit of the standard deviations in the dispersion graph. On average, these drills showed 33.88% deformation, while the other drills underwent a mean deformation of 22.79%. If the initial deformation values were diminished (9% of these values), one would find that after the use of these spear drills, there was deformation in an approximate ratio of 2:1 in comparison with the helical drills.

Table 1 Mean Drill Deformation Values (%) as Detected by SEM

No. of uses	Mean wear (%)
Not used (G1)	9.26
10 times (G2)	17.86
20 times (G3)	21.35
30 times (G4)	24.84
40 times (G5)	27.95
50 times (G6)	33.97

DISCUSSION

Bone heating and its consequent influence on the viability of the osteotomized implant bed has previously been studied by several authors,[29–36] with the rabbit tibia being the most common model used to evaluate the osseointegration of different types of implants.[37–40] Nevertheless, with the subsequent appearance of various drilling systems on the market, this topic remains relevant and continues to generate discussion, particularly regarding the longevity of implant drills.

According to Harris and Kohles,[34] repeated autoclave sterilization cycles cause a reduction in the cutting power of drills. However, Jochum and Reichart[41] found no statistically significant difference in bone heating between drills that were reused after washing and sterilization and drills that were used after washing only. In this study, only the direct effect of reusing the drills on bone heating, cell viability, and drill deformation was observed.

The maximum temperature that bone could reach during drilling without affecting its survival is stated as 47°C for no more than 1 minute of drilling.[11] Scarano et al[42] published a study that evaluated the effect of reusing implant drills on alterations in temperature during osteotomies; they concluded that the increase in reuse of drills caused an increase in bone heating. This additional heat could cause an increase in osteoclastic activity and consequent bone resorption.[43]

In this study, no statistical correlation was found between the investigated variables, which was also observed in the study of Ercoli et al.[4] This fact could be attributed to the abundant irrigation employed throughout the procedure; this assumption was supported by the negative control procedure, which was performed without irrigation. It was verified that even the maximum thermal increase observed here (5.1°C) would be incapable of reaching the threshold value of 47°C. Therefore, even up to the 50th drilling, the bone would not reach a temperature that would make it unfeasible for later maturation to occur at the bone-implant interface.

Among the different factors that could influence bone heating, the shape of the drill has been mentioned in the literature but studied infrequently.[4,7,28] In the present study, the spear drill induced a threefold greater temperature increase during drilling, in comparison with the other drills. This suggests that dentists should take greater care during this initial step of implant bed preparation; precautions would include increasing the volume of irrigation fluid, ensuring that the irrigation reaches the drill–receptor bed interface, diminishing the force exerted on the drill, and drilling for the shortest time possible.

Researchers have reported various cell alterations in the literature after surgical trauma in bone tissue.[44,45] In this context, the importance of the osteocyte has been emphasized as a multifunctional cell in the dynamics of protein signaling after mechanical stimulus.[46,47] According to Bonewald,[13] these cells are capable of regulating bone resorption and neoformation while they are vital and even after they are dead.

According to Nomura and Takano-Yamamoto,[44] the bone matrix proteins present an essential function as signal transduction molecules that promote cell migration. Thus, an increase in the expression of these proteins occurs after tissue injury.[47] In this study, the influence of repeated drillings on immediate cell viability was analyzed through the expression of bone matrix proteins. This was possible because 2 to 3 minutes after tissue injury, there is synthesis and release of proteins to the cell cytoplasm, which may be detected immunohistochemically.[28,48]

The immunomarking of OPG in the superior cortex was observed in the osteoblastic cell line, as described by Suda et al.[21] This protein signals preparation for bone neoformation.[19] Therefore, in this study, OPG was correlated with the viability of the cells around the osteotomies in all the drillings performed.[49]

The balance of OPG and RANKL protein expression observed in all the groups confirmed the cell viability after osteotomies and signaled balanced bone dynamics, which is characteristic of a physiologic state of the bone tissue.[50] The immunomarking of osteocalcin in the superior cortex was very similar to that of the other proteins in the same area, as they are present in the same type of cell, as found in other studies.[28] Nevertheless, the increase in bone matrix protein expression suggests that there was greater tissue trauma in the latter group, as was also suggested by Lean et al.[51] In spite of the retained viability of the tissue, the greater surgical trauma could negatively influence repair of the defect and delay maturation of the bone-implant interface.

According to Ercoli et al,[4] the material from which a drill is fabricated, along with its mechanical properties, affects the efficiency and durability of its cutting power. In this study, the implant system from the company Conexão (Conexão Sistema de Próteses) was used, and the drills were made of M340 steel (Bholer), which is described as steel with high resistance to deformation, with qualities ideal for use in the dental industry. Various studies have evaluated deformation after reuse of drills, by means of SEM[4,7,28,42]; nevertheless, the absence of data for quantification of the deformed areas has made it impossible to compare studies.

The high level of correlation found between the variables *number of drillings* and *percentage of deformation* confirmed the hypothesis initially formulated, in agreement with other studies.[42] However, the low levels of deformation quantified at the active tip of drills, associated with the low rates of thermal oscillation found and good immediate cell viability described in the immunohistochemical findings, indicate that the evaluated system showed good behavior in the tested period.

According to various authors, the drill shape could influence the magnitude of bone heating during implant osteotomies.[4,7,28] In this study, it was observed that the drill shape also altered the rate of its deformation after reuse. This suggests that the spear-type drill must be replaced earlier than other drills in the system tested.

CONCLUSION

According to the methodology applied, it was possible to conclude that the evaluated drills did not cause significant bone heating in up to 50 episodes of reuse. Nevertheless, they caused greater tissue trauma in the 50th drilling operation. Thus, worn drills that are reused could cause excessive damage to the bone tissue, which could affect the osseointegration process.

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