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Influence of Piezosurgery on Bone Healing around Titanium Implants: A Histological Study in Rats

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The aim of this study was to evaluate histomorphometrically the influence of two techniques of dental implant site preparation on bone healing around titanium implants. Fifteen male Wistar rats (±300 g) were used in the study. Each tibia was randomly assigned to receive the implant site preparation either with a conventional drilling technique (control – DRILL group) or with a piezoelectric device (PIEZO group). The animals were sacrificed after 30 days and then the following histomorphometric parameters were evaluated (percentage) separately for cortical and cancellous regions: proportion of mineralized tissue (PMT) adjacent to implant threads (500 µm adjacent); bone area within the threads (BA) and bone-implant contact (BIC). The results demonstrated that there were no statistically significant differences between both groups for cancellous BIC ($p>0.05$) and cortical PMT ($p>0.05$). On the other hand, a higher percentage of BA was observed in the PIEZO group in the cortical (71.50±6.91 and 78.28±4.38 for DRILL and PIEZO groups, respectively; $p<0.05$) and cancellous regions (9.62±4.06 and 19.94±14.18 for DRILL and PIEZO groups, respectively; $p<0.05$). The piezosurgery also showed higher PMT values in the cancellous zone (9.35±5.54 and 18.72±13.21 for DRILL and PIEZO groups, respectively; $p<0.05$). However, the DRILL group presented better results for BIC in cortical region (80.42±10.88 and 70.25±16.93 for DRILL and PIEZO groups, respectively; $p<0.05$). In conclusion, for the implant site preparation, the piezosurgery was beneficial to bone healing rates in the cancellous bone region, while the drill technique produced better results in the cortical bone.

Key Words: bone implant interaction, dental implants, piezosurgery, animal experiments.

Introduction

Ultrasound (US) has been widely used in periodontics with good results for decontamination of root surfaces, mainly because of its efficiency for calculus removal (1). The idea of using an ultrasonic device in surgery was well demonstrated by Horton et al. (2), showing good healing response compared to rotary bur. Recently, a new type of ultrasonic device proposed by Vercellotti (3) (developed by Mectron Medical Technology) known as piezosurgery broadened the possibilities of ultrasound use in clinical practice.

Piezosurgery has been employed in dentistry for clinical crown lengthening, dental extraction techniques, preparation of dental implant sites, maxillary sinus lifting, maxillofacial bone surgery, horizontal expansion of mandibular bone and bone block collection for autogenous grafting (4-7). Recently, piezosurgery has been used in other fields of medicine, such as orthopedics (hands and feet), spinal and cranial, due to its excellent cutting properties (8).

The increasing use of piezosurgery is based on its clinical advantages, such as precision (due to the micrometric amplitude of the tip oscillation) and selective cutting, avoiding soft tissues damage (obtained by the vibration frequency of the tip) (9), such as nerves, sinus membrane and dura mater. Furthermore should be highlighted the excellent visibility during procedures, since the saline solution used for continuous irrigation in engine and hydro pneumatic pressure causes a temporary stagnation of the bleeding for both hard and soft tissues (10-12). Despite these advantages, some aspects relative to bone repair after piezosurgery still need to be clarified, since this technology is quite recent.

The aim of the present study was to evaluate the impact of piezosurgery on bone healing around titanium implants when compared to conventional rotary drilling, as well as whether bone characteristics (i.e. cancellous or cortical bone) had influence on the results of each technique.

Material and Methods

Sample Design

The protocols were approved by the institutional Animal Care and Use Committee (CEUA) (Protocol #005/2013). Fifteen male Wistar rats (weighing approximately 300 g, mean age of three months) were used in the study. The animals were kept in plastic cages with access to food and water *ad libitum* . A day-night cycle of 12 per 12 h was used and the environment had controlled humidity and temperature.

Experimental Design

Each animal received two titanium implants (one in each tibia), randomized accordingly to the osteotomy technique used for implant site preparation, resulting in two groups: DRILL (control) group (n=15): sites were prepared with the conventional rotary drilling technique with a 16:1 reduction head coupled to an 800-rpm electric motor; PIEZO (teste) group (n=15): sites were prepared using a 50-W piezoelectric device, with 35 W power and 80 MO modulation.

Surgical Technique

All animals used in this study received two screw-shaped titanium implants (machined surface) 4.5 mm long and 2.2 mm diameter (INP Biomedical®, São Paulo, SP, Brazil), one in each tibia. The rats were weighed and according to their weight, anesthetized with an intramuscular injection of ketamine/xylazine (50 mg/kg and 10 mg/kg) (respectively Francotar® and Virbaxil®; Virbac do Brasil Indústria e Comércio Ltda, Roseira, SP, Brazil). Subsequently, the surgical sites were shaved and disinfected with iodine alcohol solution. A 1.5 cm incision was performed with a #15 scalpel, all tissues were elevated, providing access to the animal's medial surface of tibia. Similar bicortical implant sites were prepared on both sides under constant irrigation, with a difference in the preparation technique (drilling x piezosurgery). In the DRILL group, a contra-angle hand piece with a 16:1 reduction head coupled to an 800-rpm electric motor (BLM 500, VK Driller, São Paulo, SP, Brazil) was used. Initially a lancet bur was employed and then a 2 mm drill determined the final shape of the bi-cortical site. A similar implant site was prepared for the Piezo Group by a 50 W piezoelectric device (Piezosonic®, VK Driller) with 35 W power of and modulation of 80 MO, according to the manufacturer's recommendation. A lancet tip was used for the first osteotomy finished with a 2 mm-diameter diamond tip. The implants were placed manually until final stabilization in both cortical bone plates. The muscular sutures were performed with an absorbable suture (Vicryl®, Johnson & Johnson, São José dos Campos, SP, Brazil) and superficial tissues were sutured with a 5-0 monofilament nylon (Ethicon®, Johnson & Johnson). Iodine alcohol solution was then applied to the surgical area and a single-dose of intramuscular antibiotic was given to the animals (Pentabiótico®; Whitehall Ltda, São Paulo, SP, Brazil) (1 mL/kg). Thirty days after surgery, the animals were sacrificed with a ketamine/xylazine overdose (respectively 148 mg/kg and 30 mg/kg, intramuscular) and the experimental tissues were removed for histological preparation.

Histometric Analysis

After euthanasia, the tibia samples (implant ± surrounding bone) were fixed in 4% neutral formalin for 48 h, then dehydrated in ascending ethanol series (60-100%) and finally infiltrated with increasing solutions of methacrylate resin (Fluka Chemie AG, Buchs, Switzerland), starting with 50% up to 100%. Longitudinal sections were obtained using a precision saw (Exakt Saw 300 CP band system, Exakt Technologies, Ohlahoma City, OK, USA) equipped with a tungsten carbide knife. The sections were mounted onto slides, weathered with a polishing machine (Knuth-Rotor-3; Struers, Copenhagen, Denmark) until reaching 70-80 µm thickness and stained with an association of Stevenel's blue solution and alizarin S red (13), resulting in a central section for each sample, which was used for histometric analysis. A light microscope (BX60 optical microscope; Olympus, Tokyo, Japan) coupled to a digital camera (Olympus DP72 Camera, Olympus) was used to acquire the digital images. After that, a single blinded examiner used an image-analysis software (ImageJ®; Media Cybernetics, Silver Spring, MD, USA) to evaluate the following histometric parameters (percentage): bone-to-implant contact (BIC - linear measurements), bone area within the limits of the implant threads (BA - point counting technique) and the proportion of mineralized tissue in a 500 µm-wide zone adjacent to the implants (PMT - point counting technique). All parameters were separately evaluated for cortical and cancellous bone, as previously described (14) and shown in the schematic illustration (Fig. 1).

Statistical Analysis

Descriptive statistics (mean, standard error) was calculated for each histometric parameter and a general average was obtained for both groups. Intra-group analysis passed the normality test (Kolmogorov-Smirnov, p>0.05). Homogeneity of variances was tested using Levene's test (p>0.05). For the inter-group analysis the Student's t-test

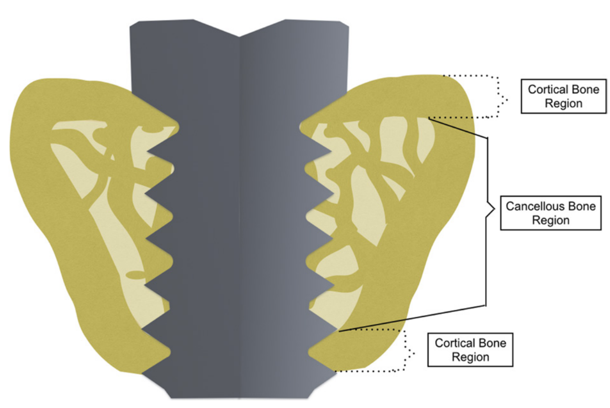

Figure 1. Schematic illustration representing the cortical and cancellous bone regions evaluated separately for all the parameters.

(p<0.05) was performed to identify statistically significant differences between the PIEZO and DRILL groups.

Results

Histological Description

All specimens in this study showed integration of the implant to the surrounding bone. Besides that, bone-to-implant contact and bone filling of the threads were observed in all samples. The histologic characteristic of the newly formed bone was similar to the original bone and this pattern was observed for all groups. Some areas of fibrosis were also observed around all implants, suggesting an osteoid region with newly formed bone. In addition, some areas exhibited mature harvesian matrix deposition constituting cortical and cancellous bone. Some adipose tissue may be observed around the implant in the cancellous bone region, due to the characteristics of the sample (rat's tibia).

Histometric Results

Data analysis showed that the PIEZO group presented higher mean values of BA in both zones and PMT in cancellous zone, compared with the DRILL group (p<0.05). However, the DRILL group was able to produce BIC values higher than the PIEZO group in the cortical region (p<0.05). Non-significant differences were observed for BIC results in cancellous bone and PMT in cortical bone. Figures 2 and 3 illustrate these results. The complete histometric results are in Table 1.

Discussion

The present study evaluated the effect of piezosurgery on bone formation around titanium implants compared to the conventional drilling technique. In addition, it

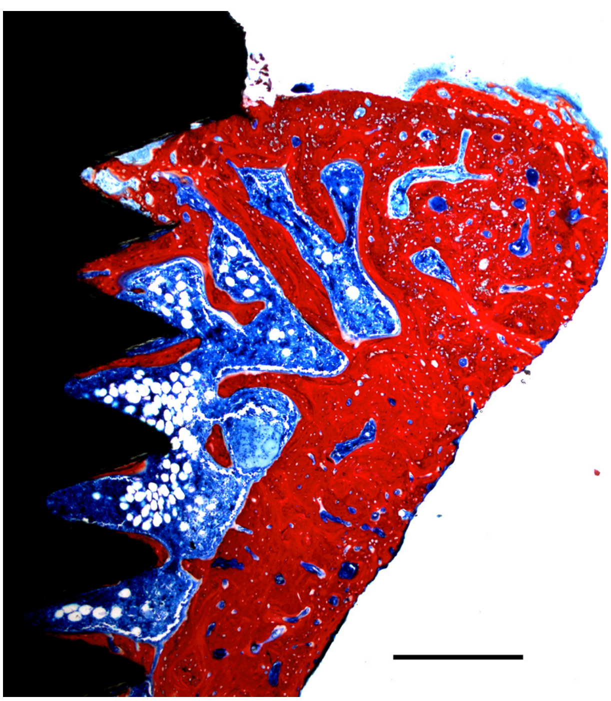

Figure 2. Photomicrograph illustrating the histological aspect of both cortical and cancellous regions in the PIEZO Group (Stevenel's blue and alizarin S red stain; bar - 500 µm).

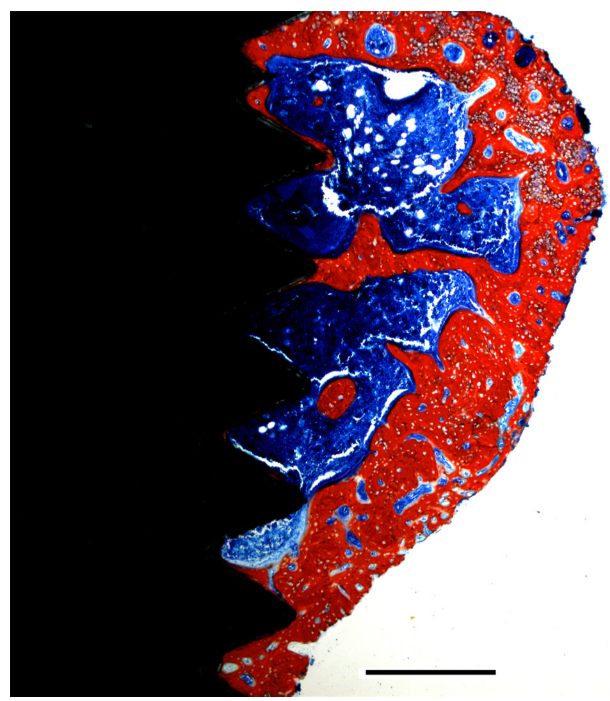

Figure 3. Photomicrograph illustrating the histological aspect of both cortical and cancellous regions in DRILL Group (Stevenel's blue and alizarin S red stain; bar - 500 µm).

Table 1. Histometric results of the present study (mean±standard deviation)

Group	Cortical			Cancellous		
	BIC	BA	PMT	BIC	BA	PMT
Drill	80.42*±10.88	71.50±6.91	84.33±10.42	35.32±18.47	9.62±4.06	9.35± 5.54
Piezo	70.25±16.93	78.28*±4.38	83.41±9.51	37.81±12.82	19.94*±14.18	18.72*± 13.21

BIC: bone-implant contact; BA: bone area within the threads; PMT: proportion of mineralized tissue adjacent to implant threads (500 µm adjacent). Statistically significant differences should be considered within each column (*p<0.05).

also evaluated the influence of bone region (cortical vs. cancellous zone) in this scenario. The results demonstrated that both techniques were able to prepare the beds for implant installation, allowing a favorable bone response, confirmed in the histological cuts. However, slightly different bone healing patterns were observed for each technique, depending on the bone region (cortical and cancellous). Piezosurgery presented better results than conventional drilling in both bone regions for BA and for PMT in cancellous bone. In cortical bone, the conventional technique (DRILL group) promoted higher mean values of BIC compared to piezosurgery. These findings may possibly help to determine future clinical indications for each technique. For example, it may be inferred that in regions with a greater amount of cortical bone component (i.e. bone type I and II), better results could be obtained with conventional drilling technique. On the other hand, it may be speculated that piezosurgery may be an adequate technique for implant bed preparation in regions with a predominant cancellous compartment, i.e. bone type III and IV, according to Jaffin classification (15). Another suggestion is that a combined approach could improve the results, using the drilling technique for initial perforation (in cortical region) and piezosurgery to complement the site preparation in the cancellous bone.

The increased values of BA and PMT favorable to the PIEZO group could be attributed, at least partially, to the decreased oscillation during implant bed preparation, when compared to conventional drilling. It can preserve the original bone tissue, resulting in a decreased clot thickness between implant surface and original bone. In addition, the positive results of PIEZO group in cancellous region may also be associated to the good cutting capacity of piezosurgery in this type of bone, allowing for a fast procedure with light tip pressure. In general, a higher tip pressure has to be applied to the cortical bone and consequently producing overheating of the bone (12,16). Some studies have shown that the heat produced following the implant site preparation may cause damage to bone tissues, such as necrosis (17), observed in the use of piezoelectric devices compared to conventional drilling techniques (12). Because of these observations, it is possible that some authors believed that the piezosurgery should be avoided in areas with predominance of the cortical bone component prevailed (3,9).

The contact area of piezoelectric device tip with the bone also influences the heat generation (12,18), which is extremely important to set the correct protocol for the use of this technology in the implant sites preparation. Stelzle et al. (12) found worse outcomes for piezosurgery compared with the conventional technique regarding surgical time and heat production. However, these authors used a single drill or tip (3 mm diameter for the tip), which may have a direct influence on the cutting ability of piezoelectric devices. Another important subject is the correct determination of the piezoelectric device power, frequency and modulation. In the present study, the parameters set used were 35 W power, 30 kHz frequency and 80 MO modulation. Hollstein et al. (19) found that these parameters can influence the roughness and temperature in bone tissue. Parmar et al. (16) found even higher vibration amplitudes and higher cutting power according to the raise of the load applied on the medullar bone component. According to the present results, the bone healing rates could improve by combining both techniques, using the drill for initial perforations (in cortical regions) and piezosurgery in the cancellous bone. Further studies are required to elucidate such questions. It is noteworthy that such inferences should be interpreted with caution, since the BIC parameter may have a different importance depending on the amount of mineralized tissue that fills the threads or is available in the regions adjacent to the implant. The opposite may also be true: the benefit of a high BIC can be negligible in the presence of a low BA or PTM.

The findings of the present study are in accordance with those of Di Alberti et al. (20), who found higher mean bone density values around implants inserted in piezoelectric-prepared beds, when compared to beds prepared with the traditional drilling procedure. Despite the interesting results, such data should to be interpreted with caution since they are based on a radiographic analysis.

A longer operative time using piezoelectric osteotomy has been reported in some studies (15,21). It may influence the indication of piezosurgery, as it directly affects patient's trans surgical and post-surgical comfort. In this study, the surgical time may have no significance because of the size of implant sites (4.5 mm long) and the number of employed drills/tips (only 2).

Despite these observations concerning heating and surgical time, Chiriac et al. (22) showed in an *in vitro* study that cortical bone chip samples collected by piezosurgery contained vital cells, with positive staining for alkaline phosphatase, therefore detecting osteoblast activity. Presence of intact and vital osteoblasts in regions near the osteotomy performed with the piezosurgery and the conventional technique has also been demonstrated (21). A comparative study (23) showed that the dynamics of bone healing after piezosurgery was similar to conventional drilling, with a slight but statistically significant bone formation at 30 days, favoring the defects created using piezoelectric ultrasound. Baker et al. (24) also demonstrated similar primary implant stability for both techniques, an important parameter for clinical practice.

Reside et al. (25) used an experimental rat model to evaluate bone repair after instrumentation with piezosurgery and conventional burs, at an earlier period (1 and 3 weeks after osteotomy), before completion of bone formation in those animals (5-6 weeks after osteotomies). Those authors demonstrated that there was greater bone preservation in the regions adjacent to the osteotomies when using two different piezoelectric devices (Piezotome® and Piezotome 2®), with higher percentage of bone fill and bone mineral density, compared to the conventional technique. These findings are consistent with the present results. The present study also found that there was greater bone formation around the implants placed in sites prepared with piezoelectric devices, mainly in the cancellous bone. Although interesting and promising data were found, it must be emphasized the importance of interpreting the present results with caution. The limitations of the present experimental model should be taken into account, as well as the anatomical differences between the experimental scenario in rats and the clinical scenario in humans.

Despite its extensive clinical use, the surgical method with piezoelectric ultrasound should be further elucidated regarding the molecular and cellular factors involved in bone repair. Moreover, few human studies addressed a comparison of this technology to the conventional technique, and there is no systematic clinical evidence on the subject. Within the limits of this methodology, it may be concluded that piezosurgery did not influence negatively the bone healing and even seemed to favor healing in some situations. However, more studies are required to determine its indications. Currently, due to the high equipment cost and longer surgery time, piezosurgery appears to be justified in borderline situations in which accuracy and absence of injuries to soft tissues have utmost importance to the clinical success or, at least, to decrease the risk of treatment failure.

Within the limits of the present study, it may be concluded that for implant site preparation, piezosurgery was beneficial to bone healing rates in the cancellous bone region, while the drill technique promoted better results in the cortical bone.

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Resumo

O objetivo deste estudo foi avaliar histomorfometricamente a influência de duas técnicas de preparo para implante dentário sobre a reparação óssea ao redor de implantes de titânio. Foram utilizados 15 ratos machos Wistar, com aproximadamente 300 g. Uma tíbia dos animais foi aleatoriamente selecionada para o preparo do leito para instalação de um implante de titânio com um motor rotatório convencional (Grupo ROTATÓRIO) e a outra com ultrassom cirúrgico piezoelétrico (Grupo PIEZO). Após 30 dias, os animais foram sacrificados e foram avaliados os seguintes parâmetros histomorfométricos (em porcentagem), separadamente, para a região cortical e medular: a proporção de tecido mineralizado (PTM) na região adjacente ao implante (500 µm adjacentes); área de tecido mineralizado (AO) dentro dos limites das roscas do implante e a extensão de tecido ósseo em contato direto (CD) com a superfície do implante. Os resultados deste estudo mostraram que não foram observadas diferenças para CD na região medular (p>0,05) e para PTM na região cortical (p>0,05). Por outro lado, um maior preenchimento das roscas foi observado quando utilizou-se ultrassom cirúrgico piezoelétrico tanto na região cortical (71,50±6,91 e 78,28±4,38 para os grupos ROTATÓRIO e PIEZO, respectivamente; p<0,05) quanto na região medular (9,62±4,06 e 19,94±14,18 para os grupos ROTATÓRIO e PIEZO, respectivamente; p<0,05). Resultados semelhantes foram observados para o parâmetro PTM na região medular (9,35±5,54 e 18,72±13,21 para os grupos ROTATÓRIO e PIEZO, respectivamente; p<0,05). No entanto, o grupo ROTATÓRIO foi superior ao grupo PIEZO em relação a CD na região cortical (80,42±10,88 e 70,25±16,93 para os grupos ROTATÓRIO e PIEZO, respectivamente; p<0,05). Pode-se concluir que, para o preparo do leito para implantes, a piezocirurgia favoreceu o reparo ósseo na região medular, enquanto a técnica convencional promoveu melhores resultados no osso cortical.

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