

The effect of piezosurgery with cvd tip in osteotomy in rat parietal bone: histologic and histomorphometric analysis.

O efeito da piezocirurgia com ponta cvd em osteotomia no osso parietal de ratos: estudo histológico e histomorfométrico.

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ABSTRACT

Objective: The aim of this study was to compare the response of bone tissue in osteotomy under piezosurgery using a CVD tip in rat parietal bone in contrast with the standard protocol using low-speed tungsten burs. **Material and Methods:** A bone defect model was created in parietal bone of 20 male Wistar rats using CVD tip and drill. Five animals were sacrificed after 3, 7, 14 and 28 days and bone containing the defects was submitted to the histologic and histomorphometric analysis. The results of histomorphometry were statistically analyzed using RM ANOVA with a significance level of $\alpha = 0.05$. The results were still subjected to Tukey's multiple comparison test. **Results:** The results showed statistically significant difference ($p < 0.05$) between the two types of treatment in the analyzed periods. Piezosurgery promoted slower and more precise cut with less bone loss and less bleeding during surgery, promoting conditions for a faster repair compared to the traditional method of osteotomy, fewer inflammatory cells, faster bone formation and cleaner surgical wound in the experimental group in all periods. **Conclusion:** The use of CVD tip in piezosurgery was proven to be valid for osteotomy, with more precise cuts, less tissue damage, less fewer pronounced inflammatory response and faster bone formation in the early healing periods when compared with low-speed tungsten burs.

KEYWORDS

Piezosurgery; Ultrasound; CVD; Bone repair.

RESUMO

Objetivo: O objetivo deste trabalho foi avaliar histológica e histomorfometricamente o efeito da piezocirurgia com ponta CVD em osteotomia realizada no osso parietal de ratos, comparativamente com a resposta do tecido ósseo frente ao protocolo padrão de osteotomia com brocas de tungstênio em baixa rotação. **Material e Métodos:** Defeitos foram feitos nos ossos parietais de 20 ratos Wistar machos, usando a ponta CVD e broca. Após 3, 7, 14 e 28 dias cinco animais foram sacrificados em cada período e o osso contendo os defeitos submetidos às análises histológica e histomorfométrica. Os resultados obtidos da histomorfometria foram submetidos à análise estatística RM ANOVA com nível de significância de $\alpha = 0,05$. Os resultados obtidos foram submetidos ainda ao teste de comparação múltipla de Tukey. **Resultados:** A piezocirurgia promoveu corte preciso e mais lento com menos perda óssea, menor sangramento durante a cirurgia, favorecendo condições para uma reparação mais rápida em comparação com o método tradicional de osteotomia. Os resultados mostraram que nos períodos analisados diferença estatisticamente significativa entre os dois tipos de tratamento ($p < 0,05$). Poucas células inflamatórias, formação óssea rápida, ferida cirúrgica limpa no grupo experimental em todos os períodos. **Conclusão:** A utilização da piezocirurgia com ponta CVD provou-se válida para a osteotomia, com cortes mais precisos, menos danos aos tecidos, resposta inflamatória menos pronunciada e formação óssea mais rápida nos primeiros períodos quando comparada com a broca de tungstênio em baixa velocidade.

PALAVRAS-CHAVE

Piezocirurgia; Ultrassom; CVD; reparação óssea.

INTRODUCTION

O sseointegration of endosseous implants is highly predictable when implants are installed completely inside bone tissue [1], since a minimal amount of bone is required in horizontal and vertical directions for the implant to be placed, following the best prosthetic positioning to fulfill aesthetic and functional goals within the stomatognathic system [2]. The surgical technique for installing endosseous implants requires the use of a protocol with special drills and copious irrigation to minimize trauma during osteotomy preparation of the alveoli that will receive them [3-5].

During osteotomy excessive heat generation shall be avoided, since subsequent necrosis often accompanies surgical procedures involving sharp instruments to remove the bone [6]. When a solid material such as the bone is being cut, energy is dissipated and heat is generated by tissue destruction and friction of the instrument. The heat generated increases the temperature about 50°C and initiates bone necrosis, while thermal damage to surrounding tissue becomes biologically irreparable at 70 °C. Eriksson et al. [7] demonstrated that temperature above 47 °C during one minute is associated with irreversible cell damage. In addition, the surgeon takes the risk, with the instrument of high rotation, to invade adjacent soft tissues such as the tongue, lips, gums, blood vessels and nerves, limiting operative access in the region and complicating the surgical procedure [8].

Ultrasonic bone surgery (Piezosurgery) is a new alternative technique for accurate bone surgery [9-11]. Ultrasound has been widely used in medicine and dentistry [2] as a diagnostic tool, for kidney stone fragmentation and removal of dental calculus. The principle is to introduce bone piezosurgery micro-vibrations metal tips with specific design at a vibration frequency of 29 to 32 kHz. Ceramic piezoelectric transducers can

vary dimensions when subjected to an intense electric field, generating vibrations. These deformations transmit micrometric mechanical forces causing the active tips to vibrate in a range of 60 to 210 μm selectively cutting hard tissue [12]. All ultrasound instruments have in common transforming electrical energy in a mechanical way, shortening and lengthening a transducer periodically. Soft tissues such as gums, blood vessels, nerves and sinus membrane are preserved of injury because they vibrate with the active tip of the instrument [2,13,14].

In this context it is understood that ultrasound is acceptable and indicated for a wide range of applications such as endodontic treatment, periodontal surgery, bone block removal, maxillary sinus lifting, dividing residual bone crest, lateralization of the inferior alveolar nerve, osseous resective surgery, fragmentation of bone grafting, tooth extraction, and fractured roots [14-18]. Moreover, less traumatic action may be indicated in patients in whom bone repair may be compromised, as the elderly and individuals with osteoporosis, diabetes or other systemic conditions.

Recently introduced in the dental market, CVD (chemical vapor deposition) tips and drills using ultrasound have been indicated in restorative dentistry [15,19-21] for cavity preparations in teeth. However, few clinical and experimental studies have been conducted with these tips with a ultrasonic unit in dental bone surgery.

The aim of this study was to evaluate the histologic and histomorphometric effects of piezosurgery with CVD tips in osteotomy in rat parietal bone comparing bone tissue response of the proposed protocol to the standard osteotomy protocol with low-speed tungsten drills.

METHODS

This study was conducted according to the Ethical Principles for Animal Experimentation adopted by the Brazilian College of Animal

Experimentation (COBEA) and submitted to the Ethics Committee in Research of ICT / SJC-UNESP, Protocol 032/2007-PA/CEP.

Twenty adult rats (*Rattus norvegicus* var. *Albinus*, Wistar), weighing approximately 350 g provided by the Institute of Science and Technology of São José dos Campos – UNESP were used. The animals were kept in cages, fed a solid diet before and during the experimental period, and received water *ad libitum*.

After trichotomy, antisepsis was performed at the area to be operated by using a solution of 1% iodine. Before surgical procedure the animals were weighed for the correct quantification of the anesthetic dose. Rats were anesthetized with a solution of Rompun® (Bayer Brazil) and Dopalen® (Agribrands of Brazil Ltda.). General anesthesia was performed by intramuscular injection of 13 mg/kg of a mixture of Rompun® (2% aqueous solution of ethyl 2-(2,6-xylylidine)-5,6-dihydro-4H-1.3-tiazina), which is a sedative, analgesic and muscle relaxant, and 33 mg/kg of general anesthetic Dopalen® (ketamine hydrochloride).

A semilunar incision in the median sagittal plane was performed using a #15 interchangeable blade, on a Bard-Parker scalpel, followed by a muscle divulsion plan to plan and mucoperiosteal incision to touch the bone. The separation was performed with periosteal elevators, a Molt periosteal elevator and curettes for exposing the parietal bones, so as to obtain a mucoperiosteal flap to expose and allow free access for the preparation of two linear bone defects approximately 10 mm long and 1.5 to 2 mm wide.

In the left parietal bone (control group) the preparation of the bone defect was performed with an aseptic electric motor (AEU-707 - MGF aseptic Inc., USA), with contra-angle reduction of 1/16, tungsten drills # 701, speed of 1500 rpm and copious irrigation with sterile 0.9% sodium chloride throughout bone manipulation. Bone instrumentation followed the surgical

protocol indicated for bone perforations in Implantology and related surgeries in order to make it less invasive and traumatic.

In the right parietal bone (test group), the preparation of the bone defect was performed with CVDentus 9.5107-8 and 9.5107-9 (CVDVale-Clorovale Diamond Industry and Commerce Ltda., São José dos Campos, São Paulo, Br) coupled to an ultrasound device Profi III Bios (Dabi Atlante, Ribeirão Preto, São Paulo, Br) with copious irrigation with sterile distilled water. Bone instrumentation with CVD tips and ultrasound followed the surgical protocol recommended by the manufacturer.

After completion of the defect, the area was irrigated with 0.9% sterile sodium chloride to remove bone splinters inside the defect using a syringe Luer. The flaps were closed with 4-0 silk intermittent sutures (Ethicon, Johnson & Johnson) and deeper planes were closed with absorbable Vicryl 5-0 suture (Ethicon, Johnson & Johnson). New asepsis was made with 1% povidone-iodine solution in the operated areas. After surgery a combination of antibiotics (penicillin) and anti-inflammatory (piroxicam), the Agrovit Plus (Ciba-Geygy Quimica SA, Sao Paulo, Brazil) was administered intramuscularly at a dose of 0.15 mL in order to prevent infection, decrease pain and postoperative edema.

Histologic and histomorphometric analysis

Five animals per group were killed with an overdose of anesthetic in the postoperative period, in a number of 5 at 3, 7, 14 and 28 days, to remove the bone fragment containing the treated region. After removal of soft tissues, bone fragments were obtained and fixed in a 10% formalin solution for at least 48 hours.

The specimens were decalcified in EDTA solution at 10% (etilendinetriolo tetraacetic acid, disodium salt dihydrate - Titriplex III® pa -, Merck KGaA, Darmstadt, Germany), which was agitated three times daily and replaced every three days for a period of approximately

90 days. These fragments were processed and embedded in paraffin. The decalcified samples were embedded in paraffin and 6- μm thick semi-serial sections were obtained and stained with hematoxylin-eosin for light microscopy analysis. For histologic analysis the inflammatory reaction and the general aspects of bone formation were evaluated in conventional light microscopy. Then histologic sections were analyzed for histomorphometric study.

To perform the histomorphometric analysis, the stereological method, which consists of determining three-dimensional quantitative parameters of anatomical structures from histological sections through geometry and statistics, was used. The stereological methods are based on a geometric-statistical principle, derived from the probability of the images of the structure profiles in histological section to coincide with an appropriate test system. Thus, the main feature of these methods is randomization of samples, eliminating the occurrence of an addition in the sample. This was accomplished through the application of randomization procedures at all stages of the experiment, such as: selection of animals, histological blocks of histological sections, cuts and microscopic fields.

Semi-serial sections were performed in 10 specimens for histological slides per animal per period. Among these sections, 5 were randomly separated, 3 histological fields, obtained longitudinally in the defect region, assessed by Axioskop 40 light microscope (Carl Zeiss, Germany) and Axiovision (Vision Imaging Systems, Carl Zeiss, Germany) program. For the measurement procedure, the objective 10x / 0.25 (A-PLAN, Carl Zeiss) and the ocular 10x (W-PI, Carl Zeiss) were used with a light microscope (Axioskop 40, Carl Zeiss, Germany). The images were captured by a digital camera (AxioCam MRC5, Carl Zeiss, Germany), representing an area of 439.28 x 329.12 μm^2 , in which the bone mass (area) at defect region was analyzed in the different observation periods. The results of histomorphometry were analyzed statistically using RM ANOVA with a significance level of

$\alpha = 0.05$. The results were also subjected to multiple comparisons Tukey test.

RESULTS

In the early periods (three days) in both groups, the histologic sections showed the presence of red blood cells, fibrin net, a small amount of polymorphonuclear inflammatory infiltrated cells and bone fragments close to the defect, especially in the face to the brain. In the experimental group, the edges of bone defects presented more regular margins and a basophilic line that was thinner than in the control group (Figures 1A and 1B).

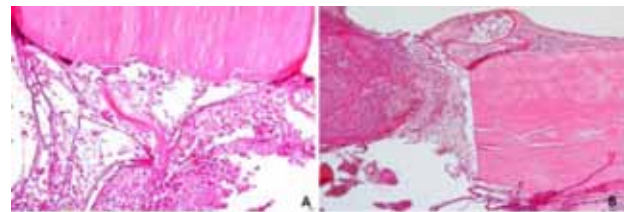


Figure 1 - Three days control group (A) with more irregular margins – HE x200. Experimental group (B) with more regular margins – HE x100.

After 7 days in both groups, the granulation tissue with fibroblasts and new blood vessels was present next to the freshly formed bone trabeculae. Fibrin, red blood cells and few inflammatory cells were still observed in the more central portion of the defect. Externally to cortical bone, new bone formation from the periosteum could be seen. The histological sections showed bone formation in the defect margins, characterized by immature trabeculae and thick osteoblasts in the experimental group (Figures 2A and 2B).

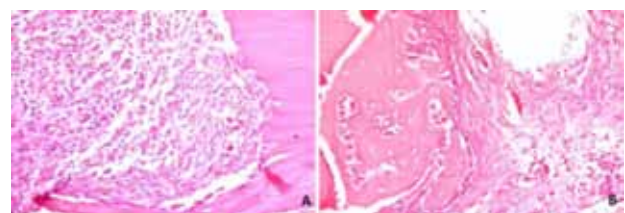


Figure 2 - Seven days control group (A) with granulation tissue next to the freshly formed bone trabeculae – HE x400. Experimental group (B) with immature trabeculae and thick osteoblasts in the defect margins – HE x200.

At 14 days, the control group showed osteoclasts and resorption lacunae, granulation tissue and small bone formation next to the edges. The defect was almost completely filled in some cases, with well-defined boundaries of bone defects, and it presented loose and more irregular tissue in the central portion of the defect. In the experimental group, increased bone formation with characteristics of a more mature tissue was observed. In some regions, discrete basophilic line in the edge of the defect and other imperceptible areas could be seen, merging with new bone containing thick osteoblasts. In the central portion of the defect there was loose tissue with irregularly arranged fibers (Figures 3A and 3B).

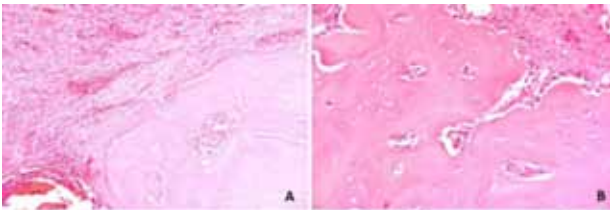


Figure 3 - Fourteen days control group (A) with lacunae resorption, osteoclasts, granulation tissue and small bone formation - HE x200. Experimental group (B) with more mature bone formation - HE x200.

At 28 days, the control group showed bone formation not filling out the defect, but showing maturity characteristics and visible limit. Externally to cortical, presence of bone formation, existence of osteoclast resorption lacunae and bone fragments in the center of the defect with inflammation. The experimental group showed the defect almost completely filled with new bone and practically imperceptible limit. Piezosurgery promoted better visibility by cavitation effect, slower and more precise cut with less bone loss, minimal tissue damage,

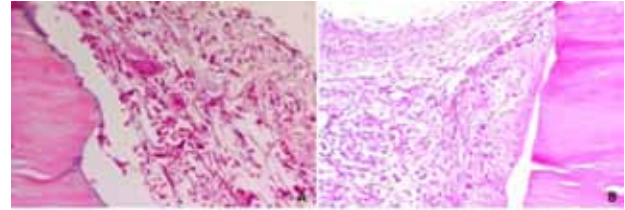


Figure 4 - Twenty-eight days control group (A) with bone formation not filling out the defect and bone fragments and inflammation in the center of the defect - HE x100. Experimental group (B) with defect almost completely filled with new bone and practically imperceptible limit - HE x200.

and less bleeding during surgery, promoting conditions for a faster repair compared to the traditional method of osteotomy (Figures 4A and 4B).

Histomorphometry

The results showed that in the analyzed period there was statistically significant difference between the two types of treatment ($p < 0.05$).

DISCUSSION

Piezosurgery, an ultrasonic bone surgery, has several advantages over conventional surgery with diamond drills and carbides in osteotomy and osteoplasty [22]. Among these advantages is the comfort of patients by removing noise instrumentation for root planning and high-speed drill [10, 23]. However, when compared to the action of low-speed burs, Moss [24], reporting the rate of cellular response in a perforated bone, determined that high-speed drills showed fewer unhealthy effects. Spatz [25] also observed better bone regeneration with higher than a low-speed.

Piezosurgery appears as an interesting alternative process due to its highly selective and precise nature with its target exclusively directed

Table 1 - Mean and standard deviation for bone formation measurements in the several groups (μm^2)

GROUPS	3C	3E	7C	7E	14C	14E	28C	28E
Mean	0.00 ^A	0.00 ^A	0.00 ^A	11.83 ^B	6.58 ^{AB}	26.29 ^C	33.91 ^C	69.02 ^D
DV	0.00	0.00	0.00	3.54	1.29	2.24	8.45	6.92

to cutting hard tissue [15,23]. The CVD tips on ultrasound were selected for this study because they exhibit characteristics of precision cutting, safety critical areas, cavitation effect, improves visibility and good irrigation. High-speed drills are harder to control, not only because of trepidation, but also for their high cutting power, requiring greater operator skills [26]. For this, trans-surgical greater stabilities were verified in this study showing better result as more regular margins and faster bone formation at the margins of the bone defects.

The effective refrigeration permitted by ultrasonic surgery reported in this study is extremely important because of the influence of heat on the regenerative potential of bone tissue, since higher temperatures than the critical level of 47 °C produce bone necrosis. Even with cooling, the temperature adjacent to the drill can often reach 60 °C or more, which is deleterious for bone tissue for denaturing proteins of hard tissue [11,27]. The extent of necrosis zone varies with the magnitude of the temperature reached and the duration of thermal injury. Several factors influence temperature increase: geometry of the drill, drill cutting, drilling speed, drilling depth, variations in the thickness of cortical bone, intermittent and gradual enlargement of the perforation, applied pressure and constant irrigation [9,25].

The histomorphometric results indicated significant differences between the control and the experimental groups and between analysis times for each group. The control group at 28 days showed an average bone tissue of 33.91%, while the experimental was 69.02%.

Corroborating the results of other studies [10,26], it could be verified that piezosurgery promoted better visibility by cavitation effect, slower and more precise cutting, with less bone loss, minimal tissue damage, and less bleeding during surgery [20,28,29]. Thus, it was identified that piezosurgery associated with CVD points favored a more rapid bone repair when compared to traditional osteotomy.

CONCLUSIONS

A critical evaluation of the results leads us to conclude that the use of the CVD tip was proven to be valid for osteotomy, with more precise cuts, less tissue damage, less pronounced inflammatory reaction and faster bone formation in the early periods when compared with low-speed tungsten drill.

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