Tissue response during Piezocision-assisted tooth movement: a histological study in rats


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SUMMARY
OBJECTIVES: Piezocision is a novel, minimally invasive technique combining micro-incisions and decortication made by a piezotome in order to enhance the rate of orthodontic tooth movement. The combined technique allows simultaneous hard and/or soft tissue grafting via selective tunnelling to correct gingival recessions or bone deficiencies. The present study was designed to evaluate the effects of Piezocision on bone with or without tooth movement on a rat model.

MATERIALS AND METHODS: Ninety-four Sprague-Dawley rats were divided into four groups: no treatment (n = 3), TM (tooth movement alone; n = 21), PS (Piezocision alone; n = 35), and PS + TM (Piezocision and tooth movement; n = 35). In each group, seven time points were studied: 1, 3, 7, 14, 28, 42, and 56 days. After sacrifice, the maxillae were removed, defleshed, stained with haematoxylin and eosin for morphometric analyses and tartrate-resistant acid phosphatase for osteoclastic activity.

RESULTS: Three days after the surgery, the bone content decreased significantly in the PS and PS + TM groups compared to baseline (P < 0.01) and the TM group (P < 0.05). This trend continued until Day 28 and was particularly evident in the PS + TM group. At Day 56, alveolar bone returned to its baseline levels in all groups. Osteoclastic activity followed similar change pattern found in the amount of bone, suggesting a strong role for the coupling of the resorptive and formative turnover of the bone. Osteoclastic activity increased as soon as Day 1 in the PS (29.0±3.0, P < 0.05) and PS + TM groups (39.0±6.0, P < 0.01) compared to baseline (22.0±4.0). The highest level of osteoclastic activity in TM group was observed at 3 days (64.3±8.0, P < 0.01) with a steady decrease thereafter. The Piezocision-induced osteoclastic activity showed a steady increase up to 7 days in both PS (39.0±7.0, P < 0.01) and PS + TM (51.8±7.0, P < 0.01) groups and decreased thereafter until Day 56.

CONCLUSIONS: Within the limitations of our study (number of animals, duration in time, and limited data on the anabolic activity), our preliminary results suggest that Piezocision-facilitated orthodontic tooth movement increases the rate of movement of the teeth undergoing orthodontic treatment through the coupled remodelling of the alveolar bone. This process is initiated by the osteoclastic activity following surgery and extended via the synergistic relationship between Piezocision and tooth movement.

Introduction

Turnover rate of alveolar bone determines the quantity and quality of the orthodontic tooth movement (Verna et al., 2000). Osteoclast-mediated catabolic activity is the limiting factor in the kinetics of this process where osteoclasts resorb the cortical lamina dura of the alveolus inducing the bone turnover where new bone apposition is stimulated by the osteoblasts (Roberts et al., 2004). In order to move teeth faster, the balance between resorption and apposition needs to be modified so that the waiting time for the alveolar cortex to resorb could be ‘by-passed’. There are several methods described and studied to accomplish this goal. In all such techniques, the basic tenet is not to cause irreversible damage to the periodontium while moving the teeth faster. Therefore, most of these approaches have yielded unpredictable outcomes. To this end, intentional injury of the cortical bone (corticotomy) to stimulate a robust and early osteoclastic resorption leading to a faster tooth movement has been one of the most predictable and successful techniques. The origin of this surgically accelerated orthodontic approach dates back to the end of the 19th century and has been extensively modified during the 20th century (Kole, 1959a,b,c). The latest refinement of this technique was proposed by Wilcko in 2001 with some modifications.
This approach involved full thickness labial and lingual alveolar flaps, with decortication barely reaching into the medullary bone adjacent to the roots of the teeth intended for movement and bone grafting (Wilcko et al., 2001). Tooth movement was initiated within 1 week after surgery and the fixed orthodontic appliances were activated every 2 weeks thereafter until the malocclusion was resolved (Wilcko et al., 2003). The authors suggested that the design of corticotomies and perforations was intended to maximize the trauma to the alveolus and to promote ample bleeding compared to creating blocks of bone, which was the basic principle in previous approaches aiming at corticotomy-facilitated orthodontics. Reportedly, no clinically significant periodontal problems were identified during the active treatment time; no disruption of the vitality of teeth was observed, no alveolar crest height changes occurred, and no significant apical root resorption was detected on the periapical radiographs. Based on these clinical findings, as well as radiographic monitoring, Wilcko et al. (2001, 2003) suggested that tooth movement in patients who underwent selective decortication might be due to a demineralization–remineralization process. This observation is part of a bigger event that is known in the orthopaedic literature as the regional acceleratory phenomenon (RAP) (Frost, 1983, 1989a,b) where at the site of osseous injury, a very dynamic healing process occurs, which is proportional to the extent of the surgical insult. A recent publication has shown that this clearly is the mechanism (Sebaoun et al., 2008). There is a localized surge in osteoclastic and osteoblastic activity, which results in a decrease in bone density with increased bone turnover. The RAP begins within a few days of the surgery, usually peaks in 1–2 months, and then slows down and disappears as remineralization sets in. Transient osteopenia after surgery was suggested to be responsible for the rapid tooth movement, as the teeth were now moving in a more ‘pliable’ environment. This pioneering work, combining alveolar decortication concomitant with bone grafting to expand the alveolar volume and allowing for rapid tooth movement into the newly expanded sites, stands out as seminal (Wilcko et al., 2007).

Although effective and highly predictable, corticotomies-assisted orthodontic treatment is highly invasive in nature as it requires extensive flap elevation and osseous surgery, which poses the potential to generate post-surgical discomfort as well as various post-operative complications. Therefore, the technique required further modifications to be widely embraced by the patient and the dental communities. Kim et al. (2009) introduced the corticision technique as a minimally invasive alternative to create surgical injury to the bone without flap reflection. In this technique, the authors used a reinforced scalpel and a mallet to go through the gingiva and cortical bone, without raising flaps. The surgical injury created was suggested to be enough to induce the RAP effect and move the teeth rapidly during orthodontic treatment (Mimura, 2013). The major drawback of this technique is the inability to graft soft or hard tissues during the procedure to correct inadequacies and reinforce the periodontium if needed. Vercellotti and Podesta (2007) proposed the use of piezosurgery in conjunction with the conventional flap elevations to create an environment conducive to rapid tooth movement. Dibart et al. (2009) developed a new minimally invasive procedure called ‘Piezocision’. It combines micro-incisions limited to the buccal gingiva that allow for the use of the piezoelectric knife to decorticate the alveolar bone to initiate RAP. Being minimally invasive, it also has the advantage of allowing for hard or soft tissue grafting via selective tunnelling to correct gingival recessions or bone deficiencies in patients. In order to understand the biological mechanism underlying this technique, we have designed the present proof-of-concept study and evaluated the effects of Piezocision with or without tooth movement on a rat model.

Materials and methods

Animal model and treatment procedures

This study has been approved by the Institutional Animal Care and Use Committee (protocol approval number AN-15061.2010.04). The animals were obtained and acclimatized in the Laboratory Animal Science Centre animal care facility for at least 2 days. A total of 94 young adult laboratory rats (Sprague–Dawley; 300–350 g, 9–10 weeks old) were used. The animals were divided into groups as baseline (no treatment; n = 3), TM (tooth movement alone; n = 21), PS (Piezocision alone; n = 35), and PS + TM (Piezocision and tooth movement combination; n = 35).

In the latter three groups, seven time points of 1, 3, 7, 14, 28, 42, and 56 days were studied. PS and PS + TM groups consisted of five animals per time point, while TM group consisted of three animals at each interval based on the established model’s efficiency in our previous work (Baloul et al., 2011). The animals were fed rat chow and water ad libitum, and weighed daily. The left side of each animal’s maxilla served as the experimental side, while the right side did not receive any treatment to ensure that the nourishment capacity of the animal was not impaired. All procedures were completed under general anaesthesia with intraperitoneally administered ketamine (8 mg/kg) and xylazine (5 mg/kg) combination.

Tooth movement was accomplished by a technique previously developed (Baloul et al., 2011). Prior to placing ligatures around the left first molar and the incisor teeth, shallow notches were made using a small round bur in order to enhance the stability of the ligation. Then, left maxillary first molar was mesially moved by ligating a 25 g Sentalloy stainless steel coil spring (GAC International LLC) to its buccal aspect (Figure 1A). The coil spring was activated for 10 mm and ligated around the left maxillary first molar and incisors. Piezocision was performed by
making a vertical palatal incision mesial and distal to the first molar using a microsurgical blade (IB6400, Hartzell instruments, Concord, California, USA). The BS1 insert of the Piezotome (Satelec, Acteon group, Merignac, France) was then inserted through that micro-opening to create the alveolar bone injury (Figure 1B). The cortical bone was penetrated to a depth of 0.5 mm mesially and distally (decorrations). The piezoelectric device settings were adjusted to deliver low-frequency ultrasonic waves (28–36 kHz). Under these conditions, the microvibrations that were created in the piezoelectric handpiece caused the inserts to vibrate linearly between 30 and 60 μm. In the PS + TM group, the activation of the wires was performed simultaneously with the Piezocision surgery. To monitor the changes in weight, baseline weight was taken at Day 0 for all animals before the procedures were performed and on a regular basis thereafter. After their recovery from general anaesthesia, all animals were permitted to move freely in their cages. All animals were housed in the same manner and provided rat chow and water ad libitum. The animals were sacrificed at 1, 3, 7, 14, 28, 42, and 56 days. For euthanasia, an overdose of carbon dioxide was used.

Histopathology

After the sacrifice, maxillae were removed, defleshed of soft tissue, and prepared for histology. Samples were fixed with 4 per cent paraformaldehyde (pH 7.2) for 48 hours, decalcified [in 10 per cent ethylenediaminetetraacetic acid (pH 7.2; Sigma Aldrich, St Louis, Missouri, USA) for 4–6 weeks], embedded in paraffin, and transversely sectioned (5 μm thick) through the first, second, and third molar roots using a microtome producing eight slide sections from apex to crown per hemiarch (125 μm between slides). Slides at three different vertical levels of the roots (apical, middle, and coronal) were chosen and stained with haematoxylin and eosin.

Intra-radicular bone. The area between the first molar roots including the roots (intra-radicular area) was morphometrically appraised. Histomorphometric analysis was performed according to the method previously described (Baloul et al., 2011). Briefly, the images of the first molars and the area between the first and second molars roots were captured at ×40 magnification (Zeiss Axiovert 200 microscope equipped with a Sony DFW-X700 digital video camera). Catabolic activity was measured by recording the number of TRAP-stained osteoclasts and preosteoclasts within the intra-radicular area. Osteoclasts and preosteoclasts were counted within the geometrical centre of the five roots of the first molar. Cell counts were performed by the same person (JS) on each slide and then averaged.

Catabolic activity in alveolar bone. In order to study the catabolic activity in the alveolar bone, slides at three different levels were stained with tartrate-resistant acid phosphatase (TRAP) following a previously described protocol (Baloul et al., 2011) and using a commercially available kit (Sigma Aldrich). Briefly, images of the first molars and the area between the first and second molars roots were captured at ×40 magnification (Zeiss Axiovert 200 microscope equipped with a Sony DFW-X700 digital video camera). Catabolic activity was measured by recording the number of TRAP-stained osteoclasts and preosteoclasts within the intra-radicular area. Osteoclasts and preosteoclasts were counted within the geometrical centre of the five roots of the first molar. Cell counts were performed by the same person (JS) on each slide and then averaged.

Figure 1 (A) Occlusal view of the rat’s maxilla, showing the experimental set up. (B) The Piezocision procedure mesial and distal of the first molar.

Figure 2 Horizontal cross-section of the rat’s maxilla showing the grid design used to capture data.
Demineralization extent after Piezocision. In order to evaluate the extent of alveolar bone loss that is induced by a piezoelectric cut, the degree of bone loss was measured in the mesiodistal direction for the Piezocision group alone. Briefly, the images of the first, second, and third molars were captured at ×25 magnification both for the experimental and control sides. The mesiodistal extent of bone loss was measured as the distance from the distal root of the first molar (site of Piezocision) to the most distal extent of visible bone recordable. Although the Piezocision cuts were done mesial and distal of the first molars, the distal site was selected as the Piezocision site that will lead to the most accurate measurement (anatomical limitations). This measurement was expressed in millimetres.

Statistical analyses

Inter-group comparisons were made using analysis of variance with Bonferroni’s correction for multiple groups. Intra-group comparisons were made using linear general model for repeated measurements. The significance level was set at 95 per cent probability.

Results

Histomorphometric assessment of changes in alveolar bone

Figure 3 shows the representative images from the test groups at different time points and quantitative assessment of changes in each group over time in the intra-radicular region. The quantification suggests that the PS led the changes in the PS + TM group where the bone content of the intra-radicular space decreased significantly at 3 days after the surgery compared to baseline (P < 0.01) and compared to the TM group (P < 0.05). While the TM group also showed a significant reduction compared to baseline by 3 days (P < 0.05), there was a pause in reduction of alveolar bone until 7 days during which the PS + TM group continued to show a steady alveolar bone resorption. The amount of alveolar bone in PS and PS + TM groups at 7 days was significantly less than the TM group (P < 0.01; Figure 4). PS + TM group continued to show a stable alveolar bone resorption until 28 days where TM group also resulted in a similar reduction. During this time, the impact of PS on alveolar bone content seemed to be stopped suggesting that TM was the driving force for alveolar bone resorption during the 14–28 days of treatment. There was no significant difference between TM and PS + TM groups at this time and thereafter, while the apposition of alveolar bone in PS + TM group showed a delay compared to TM and PS groups. Alveolar bone returns to its baseline levels in the intra-radicular space after 56 days in all groups.

Catabolic (osteoclastic) activity

The histologic observation that there was an early and rapid bone resorption led us to hypothesize that this phenomenon should be accompanied by increased osteoclastic activity. TRAP is an enzyme that is expressed in high amounts by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells (Burstone, 1959; Minkin, 1982). Figure 5 demonstrates representative images of TRAP + osteoclastic cells at 1 day. Figure 6 represents the counts of such cells in the test groups in the intra-radicular space. Piezocision led to a sharp and early increase in osteoclastic activity as soon as 1 day after the treatment compared to baseline (P < 0.05). This increase in osteoclastic activity was more significant in the PS + TM group (P < 0.01), suggesting that there was also a contributory effect of TM even if the TM alone does not show a change. The highest level of osteoclastic activity in TM group was observed at 3 days with a steady decrease thereafter, while the Piezocision-induced osteoclastic activity showed a steady increase up to 7 days. In the PS + TM group, all osteoclastic activity was diminished below the baseline levels by 42 days and stayed as such over 56 days.

Demineralization extent after Piezocision

In order to evaluate approximately the extent of alveolar bone loss induced by the use of the piezoelectric knife, we measured the distance of alveolar bone loss in the mesiodistal direction. Figure 7 shows the mesiodistal distance of demineralization in the Piezocision group. Alveolar bone loss following Piezocision alone increases dramatically after 1 day and continues at a steady state from Day 1 to Day 14 after which it decreases until the end of the study period. At Day 14, the alveolar bone loss extends approximately to a tooth and a half from the Piezocision site horizontally (Figure 8). This finding has some important clinical applications as it allows to ‘skip’ a tooth during Piezocision surgery when root proximity is a concern. For example, in a group of three adjacent teeth (i.e. teeth # 24, 25, and 26), if the root of the middle tooth (# 25) is too close to the others, and there is a fear of root damage during surgery, one can...
‘skip’ tooth # 25 as the effect of Piezocision done distal of # 24 and distal of # 27 will be enough to ‘bridge the gap’ and demineralize the bone around tooth # 25.

**Discussion**

Based on the clinical evidence that Piezocision could provide a viable alternative to corticotomy in surgically facilitated orthodontics (Dibart et al., 2009), the aim of this proof-of-concept study was to elucidate the biological mechanism underlying the clinical efficiency of this novel approach. In order to study the bone response in vivo, we have used a rat model that we have recently established and used to test the impact of corticotomy and corticotomy-facilitated orthodontic tooth movement (Sebaoun et al., 2008; Baloul et al., 2011). The results demonstrated that the Piezocision stimulates the alveolar bone turnover through increased osteoclastic activity as early as 1 day and leads to RAP, which forms the basis of rapid tooth movement compared to the conventional orthodontic treatment. Figure 3 shows how Piezocision + tooth movement leads to a faster and more profound demineralization of the bone surrounding the teeth when compared to tooth movement alone. Piezocision-assisted tooth movement also allows to ‘bypass’ the lag phase following the displacement phase that is characteristic of the tooth movement subjected to conventional orthodontics. It would appear as if tooth movement, when helped by Piezocision, would transition smoothly from the displacement phase to the acceleration and linear phase. Unlike conventional orthodontics and during the course of treatment in the adult patient, a sharp increase in tooth mobility is observed resulting from the transient osteopenia induced by the surgery. This is confirmed and explained by the amount of demineralization following Piezocision that we see histologically in our animal experiment. The forces that we apply to the teeth while treating the adult patient with this technique, by maintaining a constant mechanical stimulation of the alveolar bone, allow us to expand this transient osteopenic state and benefit from a longer ‘grace period’ in which teeth can be moved rapidly prior to the remineralization phase. It is the synergistic effect of Piezocision and tooth movement that allows this ‘extension of the biological effect in time and space’ as the RAP is transient but could be prolonged via the continuous mechanical stimulation of the teeth and bone; hence the need...
to see the patient and adjust the orthodontic appliance every 2 weeks during treatment. Understanding this conceptually and practically is of paramount importance for both orthodontist and surgeon. The surgically induced high tissue turnover is restricted to the surgical areas. Attention must be given to perform the bony incisions only around the teeth where tooth movement is planned. As such, the relative anchorage value of the teeth away from the surgical site remains high and anchorage value of teeth adjacent to the surgical site is low.

**Figure 5** Intra-radicular osteoclastic activity in the test groups at 1 day. (A) Piezocision alone. (B) Tooth movement alone. (C) Piezocision + tooth movement (tartrate-resistant acid phosphatase staining, ×400).

**Figure 6** The number of intra-radicular osteoclasts in the test groups (PS, TM, and PS + TM) at different time points.

**Figure 7** The mesiodistal extent of demineralization following Piezocision over time at one site.

**Figure 8** (A) Horizontal cross-section of the rat’s hemi maxilla at 14 days: the control side. (haematoxylin and eosin staining). (B) Horizontal cross-section of the rat’s hemi maxilla at 14 days after Piezocision alone. The vertical arrow points to the distal site of Piezocision (the mesial Piezocision site is not shown on the picture). Notice the amount and extent of demineralization taking place distal to the Piezocision cut (horizontal double ended arrow). It reaches to the rat’s third molar (perpendicular line; haematoxylin and eosin staining).
It is the orthodontist in the team that will dictate, after a thorough work up and treatment plan, where the surgeon needs to piezocise and/or graft in order to create a biological environment that will allow for a controlled rapid and safe movement of the dentition in part or in totality. Typically, in our adult population, Piezocision is done a week after the patient has been bracketed, and the wires activated every 2 weeks after that. Piezocison could be done for the whole dentition or only for some segments and could be repeated, if necessary, locally, over time (to reactivate the RAP).

The Piezocision approach for the acceleration of the orthodontic tooth movement is a novel concept and presents several advantages. While the clinical use has been reported in case reports (Dibart et al., 2009, 2010; Brugnami et al., 2013), there are no large-scale and randomized clinical trials. Therefore, it is difficult to speculate about the impact of this technique and come up with definitive suggestions. On the other hand, based on the collective evidence from the clinical experience thus far and the biological lessons learned from the current study, it can be argued that the technique is much less invasive compared to the surgical selective decortication. The healing at the clinical level is much more predictable and much less painful. Therefore, the recovery is better and more acceptable to the patient (Dibart et al., 2009, 2010; Brugnami et al., 2013). The tissue turnover seems to be restored in full and the rate of bone turnover is not impaired by the combination of the Piezocision with orthodontic tooth movement in rats. There is no evidence that the root resorption is increased, which could have been a risk in any surgical approach. The technique therefore seems to be safe for testing in large clinical trials in humans. The indications would include the class I malocclusions with moderate to severe crowding (non-extraction), alveolar or palatal expansion, correction of deep bite, selected class II malocclusions (end-on), rapid adult orthodontic treatment, Invisalign orthodontic treatment, rapid intrusion and extrusion of teeth, simultaneous correction of osseous and mucogingival defects, prevention of mucogingival defects that may occur during or after orthodontic treatment, in conjunction with impacted tooth exposure to assist in bone remodelling, and multidisciplinary treatments. The contraindications would include medically compromised patients, patients taking drugs modifying normal bone physiology (i.e. biphosphonates, corticosteroids, etc.), pre-existing root resorption, and ankylosed teeth.

Piezocision-facilitated tooth movement combines minimally invasive surgery and orthodontics as a powerful tool in the armamentarium of the 21st century dental team (Sebaoun et al., 2011). This technique is versatile as it allows for soft as well as hard tissue grafting in order to expand the volume and dimensions of the surrounding tissues of the jaws during tooth movement (Dibart et al., 2009). This helps prevent or correct pre-existing periodontal conditions such as bony fenestrations, dehiscences, gingival recessions, etc. that could be worsened by conventional orthodontic treatment. Another important characteristic of this technique is that only one side (e.g. buccal) of the alveolus is being operated upon. There is no need to intervene on the palate or the lingual aspect of the teeth as in previously described corticotomy-facilitated tooth movement techniques (Wilcko et al., 2001, 2003). In this study, we demonstrated that the combination of buccal interproximal micro-incisions and localized piezoelectric decortication results in significant amounts of demineralization around the teeth in the areas of tooth movement. The amount of demineralization created, through Piezocision, does not make an additional lingual or palatal approach necessary, hence minimizing trauma, discomfort, and enhancing patient’s acceptance. This is a very attractive alternative to the conventional and more aggressive technique using large full thickness periodontal flaps and the use of high speed handpiece along with surgical burs.

Conclusion

Piezocision is an innovative, minimally invasive technique that allows rapid orthodontic tooth movement without the down side of the extensive and traumatic classical surgical approach. Our results suggest that Piezocision-facilitated orthodontic tooth movement increases the rate of movement of the teeth undergoing orthodontic treatment (Figure 9) through the coupled remodelling of the alveolar bone. This process is initiated by the osteoclastic activity following surgery and extended via the synergistic relationship between Piezocision and tooth movement. Preliminary human studies using a split mouth design are being conducted and confirm what we are seeing in the animal model (teeth undergoing Piezocision-assisted orthodontics moving twice as fast as teeth without Piezocision-assisted orthodontics). These studies will be published at a further date.

Figure 9 The speed of tooth movement with and without Piezocision. The teeth in the Piezocision-assisted tooth movement group (PS + TM) move twice as fast as the ones in the tooth movement group (TM).
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