Article

Orthodontics Surgical Assistance (Piezosurgery®): Experimental Evidence According to Clinical Results

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Abstract: Orthodontic tooth movement (OTM) is based on intermitted or continuous forces applied to teeth, changing the mechanical loading of the system and arousing a cellular response that leads to bone adaptation. The traditional orthodontic movement causes a remodeling of the alveolar bone and changes in the periodontal structures that lead to tooth movement. The use of a piezoelectric instrument in orthodontic surgery has already shown great advantages. The purpose of this study is to rank the behavior of inflammatory mediators in accelerating orthodontic tooth movement. Ten patients with malocclusion underwent orthodontic surgical treatment, which included a first stage of surgically guided orthodontic movement (monocortical tooth dislocation and ligament distraction, MTDLD) to accelerate orthodontic movements. In all cases, corticotomy was performed by Piezosurgery. Bone and dental biopsy was executed to evaluate changes in the cytokines IL-1beta, TNF-alpha and IL-2 in different time intervals (1, 2, 7, 14 and 28 days). The molecular mediators are IL-1 beta, TNF-alpha and IL-2. Immediately after the surgical procedure there was a mild expression of the three molecular markers, while the assertion of IL-1 beta and TNF-alpha reached the maximum value after 24 h and 48 h, indicating a strong activation of the treated tissues. The Piezosurgery® surgical technique induces an evident stress in short times, within 24–48 h from the treatment, but it decreases significantly during the follow-up.

Keywords: Piezosurgery; orthodontics; cytokines

1. Introduction

OTM is a biological event that involves a series of signal transduction processes due to the remodeling of the periodontal ligament and alveolar bone. Chemical changes will in turn directly, or indirectly through other biologically active substances, stimulate a particular cell differentiation [1,2].

In the compression side of periodontal membrane, osteoclasts resorb bone and consent the tooth to move; in the tension side, osteoblasts induce formation bone.

Henneman et al. explained the main processes occurring after an orthodontic force application [3]. Four different moments are involved: the tension of the matrix and the flow of fluid, the cellular tension, the cellular activation and the differentiation, and finally the remodeling.

OTM begins with the expression of cytokines in the periodontal ligament determining strong modification in the periodontal ligament. Interleukin (IL)-1beta's concentration is the highest at the first level of orthodontic motion due to its effect on the resorption of the surrounding alveolar bone [4].

The level of IL-1 beta in human fibroblasts build up during all the orthodontic movements. In vitro, the periodontal ligament and gingival fibroblasts exposed to IL-1 beta let out prostaglandin, PGE2 and secrete metal proteinase of matrix (MMPs), which are among the major proteolytic enzymes involved in a process where collagens and other extracellular matrix (ECM) molecules become weaker. During inflammatory tissue responses, various subtypes of MMPs, MMP-2 and MMP-9 manifest. It stimulates the production of PG, other cytokines such as IL-2 and the activation and recruitment of other cells of the immune system [5–7]. IL-1beta plays a crucial role in the survival, blend and setting in motion of osteoclasts and assumes an important role since the amount of tooth movement is correlated with the correct amount of bone remodeling in the alveolar process [8,9]. IL-2 is a pro-inflammatory cytokine secreted by activated T cells and acts on both T and B lymphocytes and on cells of innate immunity such as mononuclear phagocytes and NK (natural killer) cells also. It is involved in the stimulation of osteoclast activity for bone remodeling and has an active role in the pathogenesis of periodontal condition [10].

Tumor necrosis factor-alpha (TNF-alpha) is a growth factor belonging to the cytokine group. It is a 17-kD protein, which is secreted by monocytes and macrophages at the site of inflammation. TNF-alpha precedes interleukin-1 beta (IL-1 beta) during the response to classic inflammatory inducers, such as bacterial endotoxin.

Orthodontic force induces expression of TNF-alpha in the compression side of the periodontal ligament, and has demonstrated to play an important role in OTM inducing osteoclast formation and regulating the distance of tooth movement. Moreover, TNF-alpha has an important role in increasing the expression level of RANK expressed on the surface of osteoclast precursor cells during OTM [11,12].

To date, there are numerous procedures in dentistry that require remodeling of the alveolar bone and orthodontics plays a crucial role among these [13–16].

In orthodontic treatment, with any type of appliance and material, including traditional brackets, ceramic brackets and EverStick, the number of adult patients has been increasing [17,18]. During the diagnostic phase it is essential to check the periodontal biotype, oral hygiene, dental structure (enamel and dentin) and the oral microbiology of the patient [19–23]. This kind of treatment must be performed looking at the cosmetic aspects and the minimum timing to reach the final goal. Indeed, the most frequent complaint is the long duration of the orthodontic therapy and hence the reason why most patients avoid the treatment [24].

For this reason, a series of surgical techniques have been described in the literature, so as to be able to effectively reduce orthodontic treatment time and significantly reduce the number of complications [25–31]. These include osteogenic distraction, corticotomies, cortical incisions, piezoelectric osteotomy, decortication, ligament dislocation and distraction technique (MTDLD). The MTDLD has optimized the orthodontic movement technique by shortening treatment time and preventing damage to periodontal tissue [32,33].

The application of a piezoelectric tool in orthodontic surgery has already shown great advantages, both from the psychological point of view of the patient, and by reducing the treatment time itself.

Piezosurgery® is the first ultrasonic instrument dedicated to bone cutting. The two principle concepts of bone microsurgery are:

1. Reduced surgical invasiveness: compared to traditional techniques, post-operative pain and swelling are reduced, also leading to favorable healing and reduced morbidity for the patient.
2. High predictability: this technique increases the therapeutic efficacy, the easy intra-operative control of the instrument and reduces bleeding. The precise and selective cut together with tissue healing optimizes surgical results even in cases of complex anatomy [34–36].

The aspiration of this study is to consider the performance of inflammatory mediators during tooth displacement through orthodontic microsurgery with Piezosurgery®.

2. Materials and Methods

2.1. Patients

At the Faculty of Medicine in Cluj Napoca (Romania) and in private practice, 10 Caucasian patients (aged range between 18 and 30 years old) were treated. The exclusion criteria were age below 18 years, acute or chronic local infections, bisphosphonate intake and therapy with corticosteroids, autoimmune disorders, oncological diseases, metabolic disorders and psychiatric disease that could interfere with treatment. The inclusion criteria were Class II or III malocclusion and no radiographic evidence of periodontal disease performed by full-mouth periapical radiograph examination. Before the treatment, all the patients received a standardized oral hygiene instruction by the same dental specialist and had a good oral hygiene profile.

The research was conducted in accordance with the Declaration of Helsinki.

Patients were told about both the advantages and risk involved in the therapy and gave their written informed consent for treatment and participation in the study.

2.2. Treatment

These patients underwent orthodontic surgical treatment, which included a first stage of surgically guided orthodontic movement (MTDLD) to speed up orthodontic movements. None of the patients on arrival declared to be suffering from relevant pathologies. Facial and intraoral orthodontic photography, cephalometric analysis were performed.

In all cases, corticotomy was performed by Piezosurgery® (Piezosurgery III—Mectron, Carasco, Italy), with the "cortical bone" setting and irrigation level 4. The surgery was performed by the same surgeon under local anesthesia with Mepivacaine 2% and 0.5 mL of Adrenaline 1:100,000.

A circumvestibular incision was made with a 15 blade scalpel on the gingiva exposing the periosteum with a periosteal elevator on the buccal bone surface of the maxilla or the mandible. Osteotomies were performed using the OT7 and OT7s insert. A Vycril 4-0 resorbable thread was used (Ethicon, Inc., Somerville, NJ, USA). Antibiotic therapy included administering oral amoxicillin associated with clavulanic acid (1 gr three times a day for five days), 8 mg dexamethasone one hour before and one hour after surgery to avoid post-operative edema, 20 mg omeprazole once a day for 12 days, while the pain was monitored with 100 mg Ketoprofen as needed.

The biomechanical orthodontic forces were applied the day before the surgery by the orthodontist. Self-ligating brackets with 0.22 of slots (SDS Ormco, Ultradent Product, 3 m and Unitech) and NiTi wires (0.32 inches, 0.17 at 0.25 and 0.32) were used. Orthodontic refinements were performed, whenever needed, using conventional techniques.

Follow-up examinations were performed at 1, 2, 7, 14 and 28 days, and subsequently every two weeks for the following two months.

2.3. Biomolecular Analysis

Bone and dental biopsy was performed to evaluate changes in the cytokines IL-1beta, TNF-alpha and IL-2 in various time intervals and it consisted of bone harvesting with the dental, trabecular and cortical component on the buccal side. The mucosa was infiltrated with 2% Mepivacaine associated with Adrenaline 1:100,000 and was collected with a micro

incision (2 mm) using a Piezosurgery® scalpel, with an EX1 insert at the level of the dental apex. The biopsy site was filled with a swine absorbable gelatin sponge (Spongostan, Ethicon). Polysorb 4-0 suture threads were used in order to fix the sponge. The sample was stored in a 2% Gluteraldehyde solution at 4°. The samples for the biomolecular evaluation were collected at 8, 24, 48 h and at 7, 14 and 28 days and analyzed by real-time RT—PCR technique.

The TRIzol reagent (Molecular Research Center Inc., Cincinnati, OH, USA) was applied to extract the total RNA from each sample, according to the methods used in the literature [37]. A microgram of total-RNA was subjected to be reported and transformed into cDNA using the Promega reverse transcription system (Promega Corp., Madison, WI, USA). Reverse transcriptase was summed up to the mixture that contained RT POX buffer, 2.5 mM of MgCl2, Oligo (dT) primer, dNTP Mix and Rnasin. The reagents were kept at 42 °C for 15 min and then heated at 95 °C for 5 min. Real-time RT-PCR was conducted with ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). PCR was used in a total volume of 25 μL, which contained 50 ng of cDNA samples, 1X TaqMan Universal PCR Master Mix, 300 nM of each primer and 250 nM of TaqMan probe. The phase conditions for IL-1 Beta, TNF and IL-2 were 30 s at 95 °C, 30 s at 60 °C, then 30 s at 72 °C. cDNA for IL-1 beta, TNF-alpha and IL-2 were then amplified for 40 cycles. In PCR, probes were used for hybridization of oligonucleotides, branded with 6-carboxyfluorescein to highlight fluorescence and with 6-carboxy-tetramethyl-rhodamine to terminate fluorescence. For each patient treated, the analysis of the expression values of the single mRNAs was carried out using the ANOVA test.

3. Results

The molecular mediators are IL-1 beta, TNF-alpha and IL-2. Expression values were analyzed at different time points (8, 24, 48 h and 7, 14, 28 days after surgery). The results of all mediators are statistically significant, so the change in values is due to time in which the variable was measured (and consequently to the treatment with respect to time 0). (Figures 1–3) The change is not due to factors related to the subjects or to the withdrawal or random analytical errors. All tests were performed with level = 0.05.

Subsequently, in order to verify whether and at which times the expression values of the mediators' mRNA are significantly changed, the Friedman test was used, and it was highlighted at what time the highest response was found.

It is notable that immediately after the surgical procedure, there was a mild expression of the three molecular markers, while after 24 h and 48 h, IL-1 beta and TNF-alpha reached the maximum value, indicating a strong activation of the treated tissues. The expression of IL-2 increased at longer times (48 h and 7 days post-surgery and load activation). In the later observation times (7, 14 and 28 days), the expression of the mRNA of the single markers seemed to diminish, indicating a significantly lower degree of inflammation and tissue stress. Above all the levels, IL-1 beta underwent a sharp decrease at 14 and 28 days.

The results for each analyzed marker are shown in Tables 1–3.

Table 1. IL-1 beta's values.

Observation Time Points	Medium Grade	Sum of the Degrees	Average
8 h	3.1	19	3.3
24 h	5	30.0	7.4
48 h	6	36.0	10.6
7 days	3.6	22	3.5
14 days	2.1	13	2.4
28 days	1	6	1.6

Table 2. TNF-alpha's values.

Observation Time Points	Medium Grade	Sum of the Degrees	Average
8 h	1.0	6.0	11.8
24 h	5.0	30.0	39.3
48 h	6.0	36.0	50.2
7 days	4.0	24.0	28.6
14 days	2.8	17.0	19.9
28 days	2.1	13.0	16.5

Table 3. IL-2′s values.

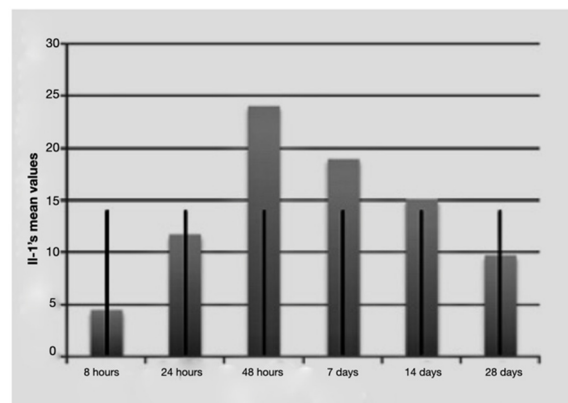| Observation Time Points	Medium Grade	Sum of the Degrees	Average
8 h	1.0	6.0	4.5
24 h	3.0	18.0	11.7
48 h	6.0	36.0	24.1
7 days	5.0	30.0	18.9
14 days	4.0	24.0	15.1
28 days	2.0	12.0	9.7

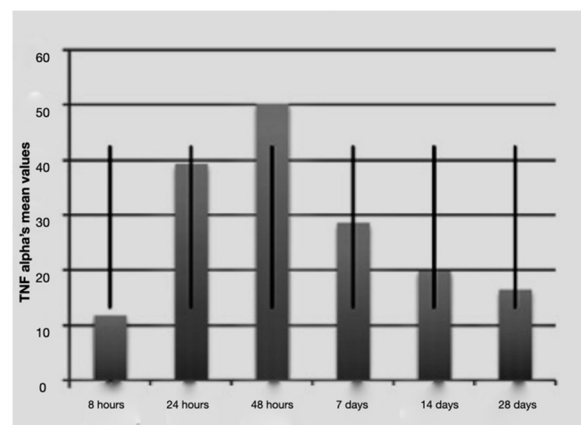**Figure 1.** Average IL-1 beta values over time with standard deviation.

Figure 2. Average TNF-alfa values over time with standard deviation.
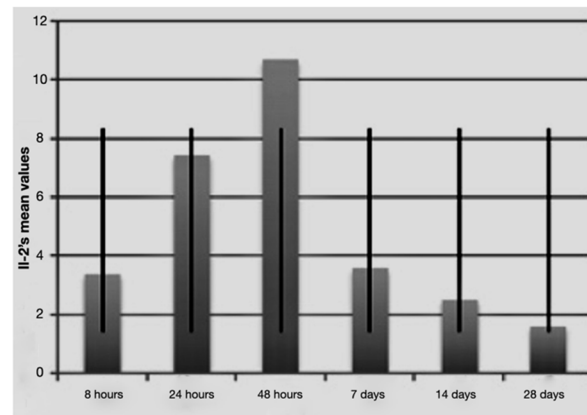
Figure 3. Average IL-2 values over time with standard deviation.

4. Discussion

As demonstrated in the literature, traditional orthodontics treatment acts as a trigger on the periodontal tissues [26,38,39]. Traditional orthodontic techniques have been described as causing damage to dental and periodontal tissues, such as recessions and root resorption [33,40–42]. Based on the protocols used in conventional orthodontics, profound changes in the periodontium were observed, such as thickening in the traction's zone and thinning in the compression's zone, with the appearance of areas of necrosis and bone remodeling.

The current gold standard is represented by Piezosurgery®, which aims to improve tooth alignment without adverse effects on periodontal structures through a standardized surgical practice protocol [43–48].

Gingival inflammation is related to the increase in inflammatory cytokines and/or MMPs in the periodontal ligament, and the turning on of inflammatory tissue destruction by gingival fibroblasts and periodontal ligament has been proposed.

Despite these functional and regional corrections, the role of gum tissue has been neglected with regard to orthodontic treatment [49,50]. To focus on biological events in gingival tissue, a prerequisite for this study was the exclusion of gingival tissue subjected to orthodontic improvement and the observation of primary subcellular mutations. In this study, a real-time RT-PCR was necessary since this allows a relatively reliable quantification of RNA, unlike conventional RT-PCR; without taking into account the number of PCR cycles, it quickly shows results in time real.

The IL-1 beta and MMP-9 mRNA levels were significantly high at all pressure sites for a relatively long period of time (2 weeks), and no substantial change was noted in the group of false activation, indicating that the changes were mainly due to pressure stimulation on the gum tissue.

Although many studies have pointed out growing pro-inflammatory cytokines in the periodontal ligament, the direct efficiency of the mechanical load on beta IL-1 from gingival fibroblasts is not yet clear.

Bode et al. demonstrated a voltage-induced increase in IL-6 secretion in tendon fibroblasts [51]. Itoh et al. found that the inflammatory response of tendon fibroblasts depended on the intensity of the traction, with an anti-inflammatory response at low levels and a proinflammatory response at high levels of strength [52]. Changes in IL-1 beta mRNA levels in this research mostly agree with those described in a previous report on IL-1beta secretion from gingival crevicular fluid. However, a relatively constant increase can be noted.

The decline in IL-1 beta on day 14 can be attributable to a decrease in the level of forces caused by the absence of reactivation, but the cytokines may be refractory to the application of a reactivation [53]. Iwasaki et al. reported that the concentration of IL-1 beta in gingival crevicular fluid rose and returned to baseline levels in 14–28 days [54]. However, a sustained increase of IL-1beta in the pressure side of the gingival tissue set against with

the traction site was a new discovery of our study, implying a possible role of gingival soft tissues in tissue remodeling [50,54].

In our research, the expression of MMP-9 was increased by performing orthodontic microsurgery followed by orthodontic loading. MMP-2 and MMP-9 were previously found, both in the pressure and PDL side, during orthodontic movements to assist collagen abasement.

Distler et al. reported, through in situ hybridization technique, that MMP-9 mRNA in the periodontal ligament was increased within 7 days of orthodontic loading and decreased after 14 days [55].

The primary increase in those key mRNA points in the gingiva suggest the following possibilities:

(i)   mRNAs could be directly implicated in gingival inflammation and/or destruction of the soft tissues surrounding the moving tooth;

(ii)   Inflammation mediators afforded in the gingiva could play a regulatory role in inducing bone remodeling in PDL.

However, a growth in inflammatory mediators under orthodontic loading in the gingiva put forward that the active biological effects of pressure on the gingival soft tissues are evident.

Kubota et al. showed the differences in cytokines and other metabolites between pressure and tension sides using gingival crevicular fluid and periodontal ligament [56,57]. A comparative examination on the pressure and tension side of the gingiva as well as on periodontal ligament cells will therefore be helpful for a broad understanding of the results expressed here. A study of cell cultures in vitro may also help to clarify the interplay between mediators.

Although the number of patients for this pilot clinical study is rather small (n = 10), in the subjects examining the analysis of the expression of mRNA levels for IL-1beta, TNF-alpha and IL-2 showed interesting differences during the observation period.

The Piezosurgery® surgical technique has been shown to induce an evident stress in short times, within 24–48 h from the treatment, but that decreases significantly at longer observation times [43,44,46].

This phenomenon indicates a re-adaptation of the treated tissue and a return within 14–28 days to the physiological conditions of the tissue.

5. Conclusions

In this study, the markedly lower level of molecular markers of inflammation and tissue stress one or two weeks from Piezosurgery® indicates that the surgical technique is minimally invasive and that after 28 days there is a restitutio ad integrum of the treated tissues, confirmed also by histological analyses [4].

Furthermore, it can be stated that Piezosurgery® should be considered as a helpful surgical technique in orthodontics due to the reduction of stress time to periodontal tissues.

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