Identification of a research protocol to study orthodontic tooth movement

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Abstract

Aim: The orthodontic movement is associated with a process of tissue remodeling together with the release of several chemical mediators in periodontal tissues. Each mediator is a potential marker of tooth movement and expresses biological processes as: tissue inflammation and bone remodeling. Different amounts of every mediator are present in several tissues and fluids of the oral cavity. Therefore, there are different methods that allow sampling with several degrees of invasiveness. Chemical mediators are also substances of different molecular nature, and multiple kind of analysis methods allow detection. The purpose of this study was to draft the best research protocol for an optimal study on orthodontic movement efficiency.
Methods: An analysis of the international literature have been made, to identify the gold standard of each aspect of the protocol: type of mediator, source and method of sampling and analysis method.
Results: From the analysis of the international literature was created an original research protocol for the study and the assessment of the orthodontic movement, by using the biomarkers of the tooth movement.
Conclusions: The protocol created is based on the choice of the gold standard of every aspect already analyzed in the literature and in existing protocols for the monitoring of orthodontic tooth movement through the markers of tooth movement. Clinical trials re required for the evaluation and validation of the protocol created.

Keywords: *Orthodontic tooth movement, biomarkers, protocol of study*

1.Introduction

The remodeling of periodontal tissue due to the application of an orthodontic force causes tooth movement.
Two different theories explain orthodontic tooth movement: the biochemical and the bioelectric theory. The biochemical theory states that tooth movement is related to the tension-compression phenomenon, after which, the blood supply of the periodontal ligament is altered; the result is a bone resorption in areas of compression and apposition in areas of tension [1,2].

The bioelectric theory states that, when the bone bends, piezoelectric currents, that alter bone metabolism, are generated [3].

Today, it is believed that currents of electrons are responsible for achieving the natural tropism of the bone. During orthodontic movement, the bioelectric phenomenon is of minor importance compared to the pressure-tension one. Important aspects to evaluate during therapy are: the efficiency of the orthodontic treatment, the extension and moving speed of the teeth. These features can be assessed with clinical and radiographic methods and allow the clinician to have a

check on the progress of treatment. But these methods do not allow to highlight the biological changes occurring during dental displacement. This study aims to identify the best protocol in order to monitoring all the aspects listed above with innovative, minimally invasive and easy to use methods, to integrate the clinical and radiographic data with the biological ones.

2. Material and Methods (Figure 1)

ANALYSIS OF THE LITERATURE

The present analysis was conducted according QUOROM statement suggestions [4]. The research was made on PubMed database in January 2014. The free text terms used were: markers, tooth movement, dental markers, analysis method, sampling method. The MESH terms used were: "biological markers", "analysis" and "isolation and purification".

Based on the analysis of the articles found, all the parameters of the protocol were selected: markers, sources and method of sampling, analysis method.

Markers

During the different stages of the orthodontic tooth movement, countless mediators are synthesized and released. The mediators expresses in a more or less specific way the ongoing biological process.

Significant markers of the inflammatory process are: lactate dehydrogenase (LDH) [5], aspartate aminotransferase (AST) [6], β-glucuronidase [7], cathepsin B (CTSB) [8], myeloperoxidase (MPO) [9].

Significant markers of bone remodeling are: C-terminal propeptide of type I procollagen (PICP), N-terminal propeptide of type I procollagen (PINP) [10], transforming growth factor β (TGF-β), bone morphogenetic proteins (BMPs) [2], insulin-like growth factor (IGF-1) [11], alkaline phosphatase (ALP)[12], interleukin-1β (IL-1β) [2], tumor necrosis factor-α (TNF-α) [13], prostaglandin E (PGE) [13], vascular endothelial growth factor (VEGF) [14], macrophage colony stimulating factor (GM-CSF) [15], the receptor activation of nuclear factor kappa B (RANK) [16], the ligand of the receptor activator of NF-kB (RANKL) [16], osteoprotegerin (OPG) [17], the tartrate-resistant acid phosphatase (TRAP) [18], nitric oxide (NO) [19], metalloproteinase (MMPs) [20].

During orthodontic treatment root resorption may occur [21). Two specific markers of root resorption are: root dentin phosphoprotein (DPP) and dentin sialoprotein (DSP) [22, 23].

Sources of sampling

Potential markers of tooth movement can be detected in different sources: gingival crevicular fluid (GCF), saliva, periodontal tissue and serum [24].

GCF is an inflammatory exudate found in the gingival sulcus whose amount, in conditions of health, it is usually little or no [25].

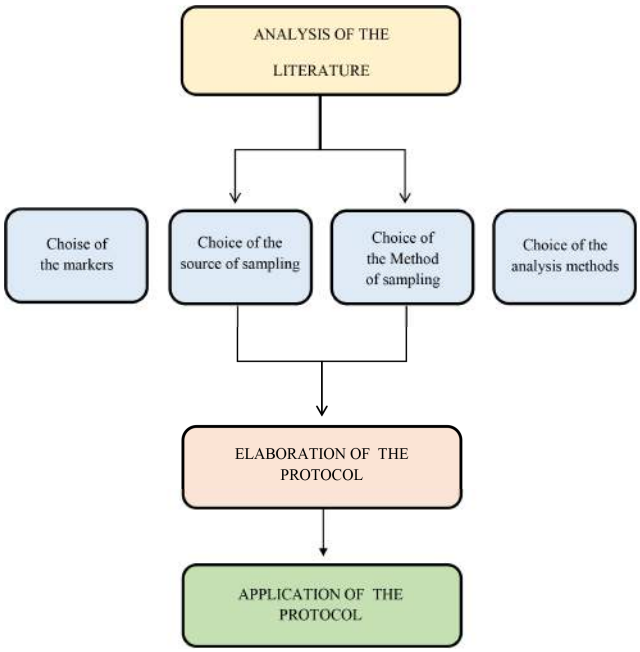

Figure 1. Processing scheme of the protocol

The quantity of GCF is influenced by several factors: inflammation, hormones, hard food chewing, use of toothbrush, hormonal contraceptives, smoking and periodontal therapy. GCF production is also characterized by a circadian periodicity.

The gingival crevicular fluid is of great interest for its unique ability to reflect, as constituted, the alterations occurring during orthodontic treatment.

Qualitative and quantitative changes of crevicular fluid accompanies the phases of tissue remodeling. In the GCF is possible to measure: cytokines, neurotransmitters, growth factors and metabolites of arachidonic acid.

Substances able to reflect the type and extent of tissue remodeling, taking place during orthodontic treatment, are also detectable in saliva [26]: a fluid composed of water (98%), electrolytes, glycoproteins, bicarbonates and phosphates, enzymes, immunoglobulins, bacterial and human cells. The microscopic and histological sampling of a part of the periodontal tissue surrounding the tooth, provides information about cellular and tissue alterations associated with the orthodontic movement.

The serum is used for many laboratory analyzes and containing inflammatory factors, secreted at the time of an inflammatory response, could be used as a source to detect the mediators of dental displacement.

Method of sampling

Methods of sampling include all the different procedures for collecting fluids and/or tissues, in relation

to the source of sampling chosen. Regarding the serum and the periodontal tissue proceed, respectively, through the blood sampling and the removal of a portion of tissue (biopsy technique).

The saliva can be collected by several methods: mechanical salivation, oral swab and cotton rope. Each method requires specific steps and techniques.

For the sampling of GCF different collection methods were tested: strips of absorbent paper, twisted wire, microcapillary tubes or micropipettes, micro-syringes and washings intracrevicular.

The main difficulty in the collection of crevicular fluid is related to the small size of the material. One of the most used techniques involves the use of strips of absorbent paper. It's a simple, fast and a little traumatic technique that provides the insertion of a strip of paper inside the sulcus (method intracrevicular) [27] or at the entrance of the same (method extracrevicular) [28]. The strip of paper must be kept in position for a standard period to allow the saturation of the filter paper.

Currently, to collect GCF, can be used strips of "Durapore": polivinilidiene fluoride (PVDF), available in both hydrophilic or hydrophobic. The hydrophilic membranes absorb proteins in much lower quantities than the hydrophobic ones (nitrocellulose, nylon or polytetrafluoroethylene (PTFE).

Analysis methods

To quantify the mediators of tooth movement and to monitor orthodontic treatment, there are several methods of analysis. The main technologies currently used to detect the proteins associated with orthodontic tooth movement are: mass spectrometry and immunoassays.

With mass spectrometry can be detected and quantified: LDH and MMP-8 and MMP-9 [29]. Inflammatory cytokines were not detected at all, or detected at very low levels, using mass spectrometry, probably because of their poor biological stability [29]; exception is represented by IL-1β [30, 31]. Ellias et al. [32], in their study, identified the levels of ALP, LDH and TRAP from saliva samples using mass spectrometry, to monitor the orthodontic treatment. They claim the necessity of a further validation of the method with a larger sample size and that movement [32, 33].

The immunoblotting checkerboard (CBIB) may potentially examine up to 45 different mediators on a single membrane [34], allowing the effectuation of site-specific correlations between these biomarkers.

The enzyme assays allow to quantify the levels of these markers of tooth movement: ALP [6], AST [12], cathepsin B [8], LDH [24], TRAP [24], myeloperoxidase [9], β-glucuronidase [7].

The cost of the necessary equipment can strongly limit the use of mass spectrometry in this sphere. They suggest a different method, such as ELISA (Enzyme-Linked ImmunoSorbent Assay), which is currently the gold standard for the identification and measurement of mediators of tooth A significant limitation of the ELISA assay is that cannot be detect multiple markers simultaneously in the same sample.

3. Results

IDENTIFICATION PROTOCOL

The literature showed that the most commonly studied markers to monitor the orthodontic movement were: IL-1β, PGE2, TNF-α and IL-6; less frequently were assayed levels of OPG, IL-8, GM-CSF, TGF-β, IL-2 and IGF.

After the literature analysis it was decided that IL-1β and OPG were the markers of choice to detect.

IL-1β is one of the most commonly markers studied and allows to use the protocol developed in future studies to monitor the orthodontic movement with new appliances, comparing the response of the periodontal tissues using conventional orthodontic appliances from the data present in the literature. Secondly, it was decided to analyze the levels of OPG, in order to make a comparison between the two markers of choice. The amount of the two mediators, in fact, vary with an opposite trend during tooth movement but both contribute to the regulation of the same process: the remodeling of the alveolar bone.

The gingival crevicular fluid (GCF) was chosen as source of sampling.

Today, the GCF is the most chosen source of sampling to monitor orthodontic tooth movement, because samples of GCF, unlike those of saliva, have the advantage of providing site-specific information [24].

The intracrevicular technique, with paper strips, was chosen as sampling method. This is a fast, simple, less invasive technique and guarantees the collection of site-specific samples.

The twine technique has been excluded because of its invasivity and because it allows only a quantitative but not qualitative analysis of the crevicular fluid. The crevicular washes method, that does not allow an accurate quantification of the GCF volume nor of the composition, because it cannot be determined the exact dilution factor, was excluded [35].

The micropipettes methods has not been chosen for the protocol because it requires a long time, up to 30 minutes, and because of the difficulty to completely remove the sample from the tube.

The selection of the method of sampling was one of the key aspect of choosing protocol because it has been found that the sampling technique influenced the composition of crevicular fluid [36].

For the quantification of IL-1β and OPG in the GCF, the sandwich ELISA test has been chosen as analysis

method. The ELISA test provides high sensitivity and specificity and is the gold standard for the detection of cytokines.

The levels of cytokines detected have been presented in terms of weight (pg=10-9g) rather than concentration (pg/μL), because of the problems relating to the accurate determination of the volume of the fluid [37].

The sampling sites are: the tooth test, subjected to orthodontic force, the control tooth, not subjected to orthodontic force, the tension and the compression site of both teeth.

Sampling sites must be isolated with cotton rolls; supragingival plaque, if present, should be removed with a cotton ball and the site should be washed and then dried for 5 seconds.

The residence time of the strip of absorbent paper in the sulcus must be of 30 seconds and the time between one sample and the subsequent of 1 minute.

The protocol provides for three different samples, in sequence, for each site in each time, to allow the analysis of three different mediators of tooth movement.

The times considered significant to evaluate the fluctuations in the levels of IL-1 β and OPG in GCF are: before the start of treatment (T0), at 1 hour (T1), 24 hours (T2), 1 week (T3), 1 year (T4) and 3 months (T5) from the application of orthodontic force.

The protocol provides for a total duration of 3 months.

Some authors believe that the study duration should be at least a month, to monitor the main phases of tooth movement [38], a prolonged duration of the protocol at 3 months was chosen in order to analyze the changes that occur even in the long term.

In planning the study were chosen two control points: the samples at T0, before the application of orthodontic force, and the tooth control (dental element not banded) on which was not applied any force. The results obtained, at each time, from tooth test should be analyzed and compared with the two control points established.

Samples at T0, T1, T2, T3, T4 and T5 were planned always at the same hour because in the literature it has been shown the existence of a circadian rhythm in the release of IL-1β, with lower levels in the morning and higher in the evening [39].

After the sampling it was decided to put the strips in a sterile plastic tube and store at -70 ° C until analysis.

The strips contaminated with blood or saliva must be eliminated.

To carry out the elution of the content of the strips was chosen centrifugation at 3000 g at 4 ° C for 15 minutes.

4. Discussion

The most studied, easy to sample and representative markers of orthodontic tooth movements were selected: IL-1β and OPG [38, 40-45]. Additionally the amount of the two mediators vary with an opposite trend during tooth movement, but both contribute to the regulation of the same process: the remodeling of the alveolar bone. This allow to make a comparison between the two markers of choice. Regarding to the sampling sorces and procedures, the gingival crevicular fluid (GCF) sampling is a non-invasive method and provides site-specific informations so it was chosen as source of sampling. The GCF is of great interest for its unique ability to reflect the alterations occurring during orthodontic treatment.

Sampling times were programmed according to the existing litterature [42-44, 46,47]. According to Toygar et al. [38] and Ren et al. [48] studies, performing the final sampling at 3 months, obtaining important scientific information about the change of the amount of the markers and a broader framework about their behavior, it was decided to terminate the studio just after 3 months from the first application of the force.

The Periopaper strips , as well as the ELISA test were chosen according to Ren et al . [45] review, which states that the two materials are among the most used for the execution of this type of studies.

In the experimental design of the study , was not considered any quantitative assessment of the volume of GCF sampled, nor of the volumetric changes of the GCF in function of the treatment time. In the literature, several studies, after collection of GCF with strips, used the Periotron, for the quantification of volume of fluid absorbed [38, 43,44,47,48]. Drummond et al. [49] showed that, although the inflammatory state, due to the application of orthodontic forces may causes volumetric changes of crevicular fluid, the GCF volume would not be a relevant factor to study tissue remodeling during orthodontic treatment. For this reason, this aspect has been neglected.

In the operating protocol has been given a lot of attention to the isolation and the removal of plaque from the sampling sites, to avoid possible contamination of the samples collected [50].

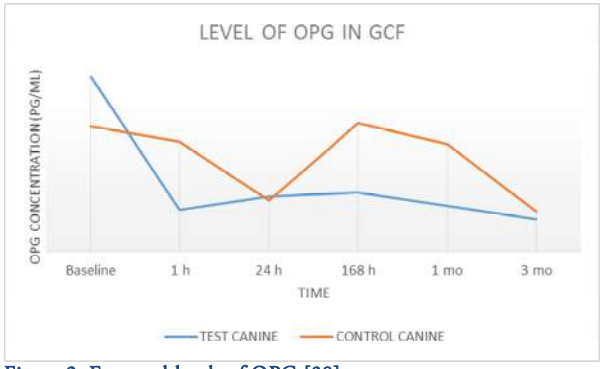

Figure 2. Expected levels of OPG [38].

Regarding the analysis methods ELISA (Enzyme-Linked ImmunoSorbent Assay) was choosen as the best. ELISA is currently the gold standard [33] for the

identification and measurement of mediators of tooth movement.

In the literature, has been detected a significant reduction in the levels of OPG in GCF samples of the tooth test during orthodontic treatment with conventional applinces. The reduction in the levels of OPG has a time-dependent trend; the concentration of OPG (pg/uL) decreases significantly during the first hour and remained at low levels during the 3 months of observation (38) (Figure 2).

Studies on canine retraction, by applying forces of different intensities, showed similar patterns of expression of IL-1β in time, with a peak in the initial stage of orthodontic movement, detected at 24 hours [42] or 72 hours by the application of force with a return to baseline levels after 7 days [47] (Figure 3).

The expected results may differ from those reported in the literature in relation to different orthodontic appliances used.

5. Conclusion

Knowing when and how the various mediators are released has advantages of relevance cynical: allows us to apply the orthodontic force in such manner and at the right time, eliminating or minimizing the damage to the tooth and supporting tissues, consequent to application of heavy and continuous forces; also allows us to reduce the time of treatment.

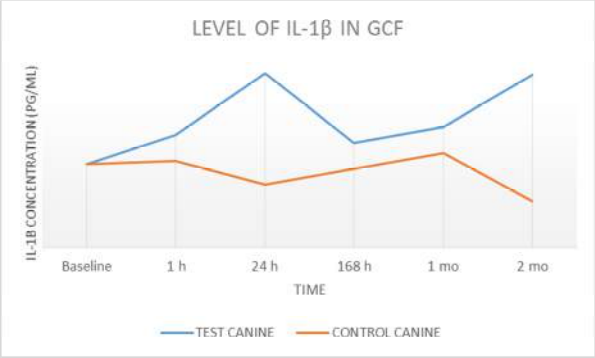

Figure 2. Expected levels of IL1-β [47].

This study offers a research protocol, among those found in the literature, which allows to effectively monitor the biological processes of orthodontic tooth movement. The main factors that influence the results, obtained from the analysis of GCF, do not only depend on the laboratory methods used, but also from the methods of collection of GCF. In fact, the sampling technique is a crucial aspect of the methodology in studies on the gingival fluid as it affects both the volume and the composition. The variables are many: the technique of sampling, the sampling sites, the time of immersion of the paper strip in the groove, the frequency of sampling and the storage temperature.

The standardization of sampling will improve the reliability of the results obtained from the analysis of crevicular fluid.

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