Gingival crevicular fluid characterization during orthodontic treatment

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During Orthodontic tooth movement, teeth are moved through alveolar bone under applied forces. The applied mechanical loading force must be transferred to the alveolar bone via periodontal ligament (PDL) [1]. This process of mechanotransduction stimulates bone remodeling during which osteoblasts produce bone on the tension side and osteoclasts resorb bone on the compression side of the PDL [2-6]. Complex interactions between osteoblasts and osteoclasts involve numerous biologic molecules including cytokines and growth factors [7]. During the tooth movement, the expression of cytokines such as interleukin (IL)-1β, IL-6, IL-8, prostalglandin E2, RANKL and MMP1 in PDL will be up-regulated [8-10]. The sequence of events from the mechanotransduction commanding the tightly controlled accomplishment of osteogenesis attention sides and osteoclastogenesis at compressive sides is not completely understood [11, 12].

The gingival crevicular fluid (GCF) is a transudate of interstitial tissues which is produced by an osmotic gradient [13] and it is released into the crevicular crevices at a flow rate of about 3 ul/h [14]. Orthodontic treatment is triggered by an inflammatory process and it has been hypothesized that the quantification of specific biomarker within the GCF can be determined using Periotron [15]. However contrasting results have been reported in the literature, which studies showing both increased or unchanged GCF volumes incident to orthodontic treatment.

Given that the orthodontic treatment is triggered by a set of inflammatory cytokines which are released into the crevicular fluid during the mechanical loading, and its homeostasis is dependent on mechanical stimulation. An understanding of the biological response of crevicular fluid to mechanical loading could further advance the knowledge of orthodontic treatment.

In this study, we will investigate the biological response of gingival crevicular fluid before and after the initial wire placement of orthodontic treatment to determine the differentially expressed genes and proteins related to mechanotransduction.

Materials and Methods

Eligibility criteria for the collection of gingival crevicular fluid

One Hundred Healthy, non-smoking patients aged 11-35 years old who require orthodontic treatment with first premolar extraction at the orthodontic clinic at College of Dentistry, University of Illinois at Chicago (UIC) will be included. The informed consent will be presented to patients and parent(s) [legal guardian(s)] and signed before the procedure. The patient will be screened for the inclusion criteria at time of consent. The written assent will also be obtained from minor subjects. Before the treatment, periapical radiographs will be taken on both maxillary canines to verify the root length and morphology so the pre-fabricated power arms on the canine brackets will be closed to center of resistance of the canines. Fixed orthodontic appliances (0.018-in; MBT prescription) will be placed in the subject with an auxiliary vertical slot in the maxillary canine brackets (Dentsply International, York, NY) to accommodate the prefabricated power arms. The subjects will be referred for extraction of the maxillary first premolars. A temporary anchorage device will be placed between maxillary second premolar and maxillary first molar, 5 mm from alveolar crest for anchorage and reference purposes. The teeth in maxillary arch will be leveled and aligned before canine retraction. Once

0.016x0.016 stainless steel archwires (Dentsply International) are placed, the canine retraction will be achieved using calibrated 70-g [127] nickel-titanium closing-coil springs (Dentsply International) connected from a temporary anchorage device to the power arm on the canine brackets that allowed application of the force closer to the center of resistance of the tooth.

Collection of gingival crevicular fluid

Patients will be seated in the dental chair in an upright position. The selected site will be air-dried and isolated with cotton rolls. Without touching the marginal gingiva, supragingival plaque will be removed to avoid contamination of the paper strips. The absorbent paper strips will be placed gently in the sulcus and the absorbed paper strips will be pooled and placed in a sterile eppendorf tube containing 200 ul of phosphate buffer saline. The collected sample will be coded with unique ID numbers and kept at -80C until analyzed. The key for the IDs will be kept in a locked cabinet in Rm#237B , College of Dentistry. The patient will be informed not to take NSAIDs but paracetamol after the orthodontic treatment. The collection of GCF will be performed before bracket placement, before canine retraction, 2 weeks after canine retraction, 5 weeks after canine retraction and 7 weeks after canine retraction.

Compensation for the study

The subjects will be given the money compensation for travel expense for $100 after the completion of the sample collection.

Intraoral scanning

The patients' occlusion and jaws will be scanned using an intraoral digital scanner. The intraoral scanning will be performed at the highest resolution at each studied timepoint. The scanning data will be exported as stereolithography binary file format (.stl) and imported to a sophisticated processing software package (Geomagic® Control 14, Geomagic®, Research Triangle Park, NC, USA). The miniscrews and palatal rugae structure will be used as reference points

Wire placement and orthodontic treatment

The patients will be bonded with brackets and 0.016x0.016" stainless steel wire will be placed and ligated onto the brackets with rubber rings. The patient will be scheduled to come back for the orthodontic treatment according to the sample collection time and once a month after the study.

ELISA

The GCF samples will first be homogenized for 30 s and centrifuged for 5 min at 1500 g to elute. The elute will then used as sample for ELISA estimation from GCF samples. The GCF sample will be detected IL1, IL6, IL8, OPG and RANKL. The ration of OPG and RANKL will be calculated and determine the potential of PDL to activate osteoclasts.

microRNA analysis

Total RNA will be extracted from the stored GCF samples using miRNeasy serum/plasma kit (Qiagen, Valencia, CA). Quantity of the total RNA will be determined by NanoDrop spectrophotometer (Thermoscientific). The total RNA will be subjected to quantitative realtime RT-PCR using Taqman® microRNA RT-PCR assays specific to hsa-miR-21, -29a, b and c. hsa-let-7d, g and i will be used as an internal control [112, 129]. The samples from each subject will be analyzed using a 7900HT realtime PCR system (Applied Biosystems).

Statistical Analysis

All the analyses will be performed in triplicate for three independent experiments to confirm reproducibility of the results. Quantitative data will be presented as means ± SD from three independent experiments. Wilcoxon Sign-ranked analysis will be used to determine the mean difference before and after treatment at $P = 0.05$.

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