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Nitric oxide in human gingival crevicular fluid after orthodontic force application

H. Ford [a], S. Suri [a], D. Nilforoushan [a], M. Manolson [b], S.G. Gong [a,b,*]

[a] Department of Orthodontics, Faculty of Dentistry, University of Toronto, Toronto, ON, Canada
[b] Dental Research Institute, Faculty of Dentistry, University of Toronto, Toronto, ON, Canada

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ABSTRACT

Nitric oxide (NO) is involved in bone remodelling and has been shown to play a role in regulating the rate of orthodontic tooth movement (OTM) in rat models. In humans, however, the role of NO in OTM remains less clear. In this study, NO concentration in gingival crevicular fluid (GCF) was measured in patients undergoing orthodontic treatment. Thirteen male participants (ages 11–18 years) planned for non-extraction fixed orthodontic therapy were recruited. Samples of GCF were collected from each maxillary central incisor and first and second molar immediately before (T0), 1 h after (T1), and 3–4 days after (T2) application of light orthodontic forces. The maxillary second molars were not included in the appliance and served as controls. Measureable NO levels were consistently obtained from all sampled sites. Total NO levels showed significantly higher NO levels ($p < 0.05$) at T1 at the buccal surfaces of the central incisors when compared to the first and second molars. The results indicate a possible role for NO in OTM at the pressure sites of incisors at early time points. Further studies are required to determine whether NO levels in the periodontal ligament tissues of human teeth during OTM are affected by a force gradient and the magnitude of the applied force.

1. Introduction

Reducing the duration of patient treatment is a primary goal for orthodontists.[1] The development of new methods to increase the rate of orthodontic tooth movement (OTM) have been sought by clinicians not only as a way to shorten treatment time, but also to reduce adverse effects such as pain, discomfort, dental caries, and periodontal disease. Additionally, shorter treatment times can minimize iatrogenic tissue damage, such as root resorption and the development of non-vital teeth.[2]

OTM occurs via sequential remodelling changes of the periodontal tissues to biomechanical forces.[3] During the application of an orthodontic force, the alveolar bone on the pressure side undergoes successive cycles of bone resorption and formation whereas the bone on the tension side predominantly undergoes continuous bone formation.[4,5] Cellular factors that influence the above cycles of cellular changes in the paradental tissues of teeth undergoing movement may alter the rate of OTM. Advancing the body of knowledge regarding some of these factors is thus of great interest in the field of orthodontics.

* *Corresponding author at*: Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, ON M5G 1G6, Canada. Tel.: +1 416 979 4917x4588.
E-mail address: sg.gong@dentistry.utoronto.ca (S.G. Gong).
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Nitric oxide (NO), a short-lived, highly-reactive free radical, has been shown to play a role during OTM. NO is generated by nitric oxide synthase (NOS) from oxygen and the amino acid L-arginine. Three isoforms of NOS have been identified: a neuronal form (nNOS), an endothelial form (eNOS), and an inducible form (iNOS).[6,7] NO mediates bone mechanical loading and regulates bone turnover and function.[8,9] It is released shortly after shear stress from osteoblasts and osteocytes.[10] NO has been shown to be involved in OTM in a number of animal models. When a general inhibitor of NOS activity and a NOS precursor were locally administered to the upper first molars or upper incisors of rat models undergoing controlled orthodontic forces, tooth movement was reduced and accelerated, respectively. Histopathologic analysis and/or measurement of tooth movement revealed that tooth movement and the number of osteoclasts, Howship's lacunae, and capillary vascularization was significantly increased in the NOS precursor groups and decreased in the NOS inhibitor groups, leading to the conclusion that NO plays a role in enhancing the rate of OTM in rat models.[1,11,12] In vivo, the expression of eNOS and iNOS in the periodontal ligament (PDL) decreased in an occlusal hypofunction model.[13] All NOS isoforms showed an increased expression on the tension side of teeth in a rat OTM model, with nNOS being more involved in early OTM events.[14] One human study demonstrated significantly greater levels of eNOS and iNOS expression in gingival tissue associated with teeth undergoing OTM than when compared to controls role.[15] As of yet, however, the literature on the role of NO in OTM is much more abundant in animal models as opposed to human subjects.

The usefulness of sampling gingival crevicular fluid (GCF) to assay the levels of a variety of biomarkers involved in OTM is well established.[5] Previous studies on implant stability and the effects of low-level laser therapy on OTM have shown that NO is detectible in GCF.[16,17] Since NO has been shown to enhance OTM in animals, improving our knowledge of NO in humans may aid in the study of approaches to potentially increase the rate of OTM. We, therefore, (i) explored whether NO levels can be consistently detected in GCF, and, (ii) investigated the changes in GCF concentrations of NO before and after the application of a continuous orthodontic force.

2. Materials and methods

2.1. *Participant recruitment and inclusion criteria*

Ethical approval was obtained from the University of Toronto Health Sciences Research Ethics Board (#26812). Informed consent was obtained verbally and in writing from all patients, or their parents as applicable.

Thirteen male participants were recruited from the Graduate Orthodontics Clinic at the University of Toronto and selected based on the following inclusion criteria:

1. Males (aged 11–18 years) treatment planned for non-extraction, fixed-edgewise orthodontic therapy. Females were excluded due to documented evidence of an enhancement of the female sex hormone, oestrogen, on NO production.[18]
2. Mild/moderate crowding with Carey's Analysis[19] exhibiting <6 mm of space discrepancy per arch.
3. Healthy systemic condition, non-smoker, no alcohol consumption and absence of use of anti-inflammatory drugs in the month preceding the beginning of the study.[15]
4. Healthy periodontal condition with probing depth values of <3 mm, loss of attachment <2 mm, and an absence of periodontal bone loss as revealed from bitewing radiographs.
5. Fair/good oral hygiene as determined by a Modified Gingival Index (MGI)[20] and Plaque Index (PI),[21] recorded for every site at each sampling time point. Scores >2 at any time would result in exclusion from the study.

2.2. *Sites sampled and timing of sample collection*

GCF samples were taken immediately prior to bonding with fixed edgewise appliances, 1 h after bonding and insertion of a light maxillary NiTi archwire (0.014″), and 3–4 days following bonding. Upon orthodontic appliance placement in a crowded arch, teeth tend to procline in order to align themselves.[22,23] In general, therefore, the buccal surfaces of teeth (especially anterior teeth) act as the "pressure" side whereas the lingual surfaces act as the "tension" side. To investigate potential difference in NO levels between the pressure and tension sides, GCF samples were collected on both the buccal and lingual of the maxillary central incisors, and first and second molars. The maxillary second molars were selected to serve as controls and therefore the archwire did not engage them. The second molar, either the left or the right, was bonded with a molar tube, while the other side remained non-bonded to serve as a control for plaque accumulation due to a bonded attachment. All twelve sites (six buccal and six lingual) were sampled at each time point for each patient (Fig. 1).

Participants were advised to take Acetaminophen (but not NSAIDS) as needed for pain relief during the course of the study due to the documented effects that anti-inflammatory

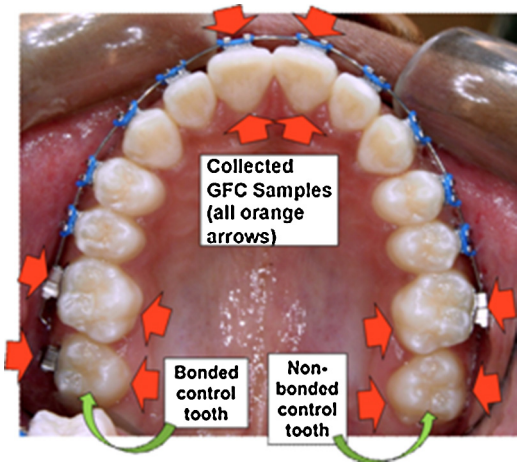

Fig. 1 – Sites of GCF collection. Arrows point to the buccal and lingual sampling sites of the central incisors, first molars, and second molars. The second molars served as bonded (with an attachment), and non-bonded (without an attachment) controls.