Journal of Molecular Structure 1176 (2019) 847–854

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Infrared microspectroscopy characterization of gingival crevicular fluid during orthodontic treatment

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ARTICLE INFO

Article history:
Received 6 April 2018
Received in revised form
10 September 2018
Accepted 11 September 2018
Available online 14 September 2018

Keywords:
FT-IR microspectroscopy
Gingival crevicular fluid
Orthodontic tooth movement
Biomolecular constituents

ABSTRACT

The gingival crevicular fluid (GCF) is a site-specific exudate deriving from the epithelium lining of the gingival sulcus. GCF analysis provides a simple and noninvasive diagnostic procedure to follow-up the periodontal ligament and bone remodeling in response to diseases or mechanical stimuli as the orthodontic tooth movement. The aim of this study was to use infrared (IR) microspectroscopy to assess GCF composition changes at different phases of fixed orthodontic treatment. GCF samples were collected using sterile paper cones from young patients before starting the orthodontic treatment (control) and after 2, 7 and 14 days of fixed orthodontic treatment. Infrared spectra were obtained on GCF extracted from cones. In these spectra, the contributions of main functional groups and the changes related to the different phases of orthodontic treatment were present. Different normalizations to significant peaks were performed to better evidence the changes occurring at different times of the orthodontic treatment. Useful information was also obtained by evaluating the ratio between the areas of selected bands related to protein, lipid and carbohydrate contents. The results here reported show that IR microspectroscopy analysis can give an important contribution for monitoring GCF changes during the early phases of the orthodontic tooth movement.

1. Introduction

Tooth movement by orthodontic force application is dependent on remodeling in periodontal ligament (PDL) and alveolar bone, correlated with several biological changes. This process involves the activation of many complex cellular and molecular mechanisms mediated by the release of chemical substance cascades [1]. The PDL is a membrane-like connective tissue interposed between the tooth root and the alveolar bone. It has an important role in supporting the tooth in the bone socket and in maintaining homeostasis of the surrounding tissues, such as alveolar bone and cementum [2,3]. Orthodontic tooth movement during treatment consists of initial phase, lag phase and postlag phase. In the first phase, an immediate and rapid movement is present. This phase occurs from 24 to 48 h after the first application of forces to the tooth. During the lag phase, lasting 20–30 days, very little tooth displacement occurs. The postlag phase is characterized by an increased rate of tooth movement [4]. Orthodontic force application reduces blood flow inducing local changes in the PDL, and an inflammatory process occurs as a physiological response to the tissue stress [5]. This process results in increase and modifications of the gingival crevicular fluid (GCF) into the periodontal space [6]. It has been shown that the GCF derives from the epithelium lining of the gingival pocket [7,8]. The range of the GCF constituents is very large, as it can contain both human and bacterial cells and many different molecules. For instance, among the most representative cellular components of the GCF, there are the leukocytes, and especially neutrophils, which have important roles in the antimicrobial defense of the periodontium. Investigations into the protein content of the GCF reported that in healthy gingival crevices the GCF has a similar protein concentration to interstitial fluid, which was notably lower than serum [9]. On the contrary, upon local inflammation or injury, the protein concentration raised in the GCF to a level similar to those of serum, subsequently GCF would become an inflammatory exudate [10,11]. The increased GCF flow contributes to host defense by flushing bacterial colonies and their

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https://doi.org/10.1016/j.molstruc.2018.09.013
0022-2860/

metabolites away from the sulcus, thus restricting their penetration into the tissue. GCF molecular contents represent a promising source of biomarkers in orthodontics in order to monitor cell death, tissue damage, inflammation, bone resorption, bone deposition and others, according to their specific biological functions [12,13]. For several of these biomarkers, associations between their levels and specific clinical conditions have been shown, for instance, in presence of periodontitis [14]. Recent studies on orthodontic tooth movement have used GCF because of its noninvasive nature and ease of repetitive sampling from the same site [14,15]. An almost exhaustive list of potential biomarkers involved in orthodontic tooth movement collected from GCF can be found in literature [4,13]. It must be noted that recent investigations seem to indicate that the use of multiple biomarkers is to be preferred to that of a single one [16]. In this framework, the role of infrared (IR) spectroscopy can be extremely useful in order to obtain an overall biochemical characterization of GCF and its changes during orthodontic treatment. In the last decades, IR spectroscopy has been demonstrated to be a very effective tool in many biomedical fields especially when applied to the analysis of biofluids as serum, plasma, blood, tears, urine, saliva and synovial fluid [17–21]. Biofluids can be considered as ideal media for routine clinical use (easily accessible, minimally invasive collection methods, and repeatedly available for monitoring disease progression or therapeutic response) and IR spectroscopy constitutes an efficient tool for analyzing biological specimens due to its sensitivity to subtle chemical and structural changes [18,20].

As far as concerns GCF analysis, IR spectroscopy has been already used to identify periodontitis. Xiang et al. showed that the contributions of amide I, amide II/tyrosine rings and symmetric and asymmetric PO_2^- stretching vibrations of phosphodiester groups in DNA showed in the infrared spectra obtained by the analysis of GCF, collected from subjects with and without periodontitis or gingivitis, can be used for identifying healthy and diseased periodontal tissues [22]. In addition, IR spectroscopy was also able to identify differences in infrared spectra of GCF from patients with chronic periodontitis affected or not by diabetes mellitus [23].

In the present paper, IR spectroscopy was adopted for investigating changes occurring in GCF during an orthodontic treatment with fixed appliances. As expected, IR spectra showed contributions of the main functional groups and significant changes were evidenced in the spectra of GCF samples pooled at different phases of tooth movement. The most relevant changes regarded protein, lipid, carbohydrates and acid nucleic contributions indicating that IR spectroscopy provides useful information on the complex processes occurring during the different phases of tooth movement in the alveolar bone and it can constitute a useful tool for monitoring the orthodontic treatment.

2. Materials and methods

2.1. Subjects

GCF samples were pooled from informed patients aged between 12 and 22 years recruited at the Orthodontic Program of the University of Campania “Luigi Vanvitelli” (Naples, Italy) before and during an orthodontic treatment with fixed appliances (Table 1). Informed consent was obtained from each minor patient's parents or adult patients after providing them detailed information about the clinical trial. The inclusion criteria were as follows: non-extraction fixed orthodontic treatment required, permanent dentition with similar incisor irregularity index in the mandibular dental arch ($\geq$2), a good general healthy periodontium with no radiographic evidence of bone loss, no gingival inflammation and probing depth of 3 mm in the whole dentition, a full-mouth plaque score (FMPS) and a full-mouth bleeding score (FMBS) $\leq$ 20%. Exclusion criteria included previous orthodontic treatment with fixed appliances, history of systemic diseases, congenital deformities, mandibular tooth agenesis, previous periodontal disease and the use of antibiotic or anti-inflammatory drugs in the month preceding the beginning of the study. Moreover, drug consumers and smokers were also removed from the study.

Table 1
Patients before and during orthodontic treatment with fixed appliances enrolled in the present experimental investigation.

Patient code	Age (years)	Sex
A	14	F
B	14	M
C	12	M
D	12	M
E	17	M
F	17	M
G	12	F
H	15	M
I	16	M
L	22	M
M	22	F
N	14	F
O	17	M
P	17	F
Q	13	M
R	12	M
S	13	M
T	12	M

The fixed orthodontic treatment of the patients included in the study consisted of metal brackets (MBT™; 3 M United; Monrovia; Cali, CO, USA) applied on the buccal surface of upper and/or lower permanent teeth and of a 0.014 NiTi archwire ligated with elastic ligatures. All the clinical procedures were performed by the same operator.

2.2. Collection of gingival crevicular fluid

The GCF samples were collected from each patient before bracket bonding (T0), and after 2 (T1), 7 (T2) and 14 (T3) days of treatment at the buccal side of the lateral incisors. The T0 samples represented the controls. Before GCF collection, the sample contamination by blood, saliva and plaque was minimized by using suction and self-retaining retractor, and the target teeth were carefully cleaned, gently dried with air stream for 5 s and isolated with cotton rolls. Standardized sterile absorbent paper cones were inserted 1 mm into the gingival crevice and left in situ for 30 s, without blood, saliva and plaque contamination. Two cones were consecutively used (1-min interval) in order to maximize GCF volume per site. Paper cones were transferred to sterile plastic vials and stored at −80 °C. Immediately before measurements, 10 μL of distilled water was added and the tubes were vortexed for 30 s and centrifuged (10 min, 800 g).

3. FT-IR microspectroscopy

3.1. Spectra acquisition

A Perkin Elmer Spectrum One FT-IR spectrometer equipped with a Perkin Elmer Multiscope system infrared microscope (Mercury Cadmium Telluride detector (MCT)) was used to record FT-IR spectra. Spectral acquisitions were performed in specular-reflection mode with a few microliter drop of sample put on a metallic IR-reflective surface and left to dry. The background spectrum was collected from the metallic IR-reflective surface in