



Optical coherence tomography assessment of gingival epithelium inflammatory status in periodontal – Systemic affected patients[☆]

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ABSTRACT

Introduction: Optical coherence tomography (OCT) is an imaging tool used in various medical fields (ophthalmology, dermatology), which allows the observation of morphological particularities on the surface of tissues or internal constructive details of about 2–3 mms in depth. In periodontology, it has been used as an experimental tool for periodontal pocket analysis (depth, calculus deposits) but not for the assessment of periodontal inflammation in the gingival tissues, which has been the subject of our *in-vitro* study.

Material and method: Gingival samples were collected from three types of patients: patients with periodontal disease; patients with periodontal disease and a systemic comorbidity; periodontal and systemic healthy patients. The samples were scanned with an OCT light beam, resulting two-dimensional images of the gingival tissue (full thickness epithelium and partial connective tissue). The images were assessed using dedicated software, which allowed the quantification of pixels on a given segment in the epithelium. The average pixel densities were then calculated for each patient group and statistically analyzed.

Results: The resulted pixel densities were highest for the control group samples, while the lowest pixel densities were found in samples originating from periodontal patients with diabetes mellitus. For the other possible periodontal comorbidity, chronic hepatitis C, image assessment also exhibited lower pixel densities than those of the periodontal group, suggesting that this condition could also have an added effect on the tissular changes induced by periodontal disease.

Conclusion: OCT has proven that in an *in-vitro* environment it can be a useful tool for the assessment of periodontal inflammation in gingival samples of periodontal patients. In terms of inflammatory tissular changes observed by OCT analysis, chronic hepatitis C could be regarded as possible periodontal disease's comorbidity.

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1. Introduction

Optical coherence tomography (OCT) is a technique that provides optical sectional images. The technique is based on low-coherence interferometry in order to determine the echo time delay

and the magnitude of backscattered light reflected by a given studied surface (Huang et al., 1991). The light beam pointed at the surface is reflected in a different manner by its structural particularities, which creates interferences that are captured by a sensor. The penetration power of the method is about 2–3 mm deep inside the analyzed surface as long as it is penetrable by light. The output is delivered in the form of three dimensional images on a micrometric scale. Lateral and axial resolution may be even ten times higher than that of ultrasonography (Huang et al., 1991). OCT enables real-time observation of structural abnormalities of materials and tissues that

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are located either on their surface or just below it, so that they can be reached by the broadband light beam that the method uses. OCT is widely used in research purposes as well as industrial ones, such as material thickness and volume loss measurements (Merken et al., 2011) or even arts, where it is used for the restoration of important paintings (Xu et al., 1999).

After its development, OCT soon began to be used for biological purposes by analyzing samples of tissue. As the need for more non-invasive and early diagnosis tools rose, it started to be used in different fields of human medicine such as ophthalmology (Nolan et al., 2015), cardiology (Bezerra et al., 2009) or dermatology (Olsen et al., 2015). In comparison to other medical imagistic diagnosis tools, OCT has the advantage of being suitable for the visualization of soft tissues (in contrast to standard radiology) and also the fact that it does not produce any kind of harmful radiation for the patient (unlike computer tomography) as it uses light beams. Therefore, for the visualization of soft tissues, shallow or thin structures such as sarcomas or retina, it can be used for an early diagnosis (Srinivasan et al., 2017).

The most frequent medical use of OCT is in ophthalmology, where it is required to obtain images of the patient's retina and its characteristics (Garcia et al., 2012). This is of particular interest for conditions that affect the structure and function of the retina such as glaucoma, multiple sclerosis and macular degeneration. OCT test is also recommended for diabetic patients in order to assess the visual complications of the disease and to prevent them from happening (Kim et al., 2017b). Diabetes, the metabolic disorder in which elevated blood sugar levels are chronic due to pancreatic impairment or insulin resistance, can have multiple complications if left untreated. Mostly, these complications target peripheral blood circulation. For example, in diabetic patients, the retinopathy caused by inefficient blood flow in the retinal arteries leads to the development of macular edema and subsequent sight loss (Virgili et al., 2015). OCT is a valuable tool for assessing *in vivo* retinal alterations in these patients and for monitoring the onset of diabetes-related complications (Lee & Rosen, 2016).

In cardiology, OCT is used to assess the lumen of the coronal arteries and their permeability (Kubo et al., 2014). A catheter with fiber optic which produces high-resolution images of the interior of the blood vessels is placed inside the patient's body, up to the coronal arteries. In oncology, OCT can be used for the detection of soft tissue precancerous and cancerous lesions which would otherwise be difficult to observe, such as esophageal cancer or connective tissue sarcoma (Uno et al., 2015). The structural characteristics of the tumors can also be observed if they are thin enough to be thoroughly or partially penetrated by the light beam. In addition to this, OCT may also be used in gastroenterology, especially in cases of liver cancer such as cholangiocarcinoma (Zhu et al., 2015).

Periodontal disease is an inflammatory disease of the supporting tissues of the teeth. The disease has a bacterial etiology but it is also in close relationship with some patient-linked risk factors such as periodontal architecture, inflammatory response or systemic diseases and environment-linked ones like smoking, diet and oral hygiene. The disease debuts at gingival level, when bacteria cause inflammation of the soft tissues around the tooth (gingivitis) and then slowly progresses into the deeper structures that support the teeth such as the periodontal ligament. As the disease advances, periodontal pockets are formed around the tooth in which more bacterial plaque and calculus deposits can be found, further worsening the inflammation. The periodontal pocket width is an indicator of the disease's progression rate as it is a sign of attachment loss that eventually causes the teeth to become mobile (Newman et al., 2015). Periodontal disease has been linked in a bidirectional relationship to a number of systemic conditions such as diabetes mellitus, cardiovascular disease or rheumatoid arthritis (Holmstrup et al., 2017). Recent research has also revealed a possi-

ble link between periodontal disease and liver diseases, the subject requiring further assessment and analysis (Han et al., 2016).

The present *in vitro* study aims to point out the changes that occur on optical coherence tomography images of gingival tissue samples by assessing pixel density and making correlations with the gingival inflammatory status of patients with periodontal disease and chronic hepatitis C or type 2 diabetes. Secondly, the study intends to explore the possible influence that chronic hepatitis C has on inflammatory changes in periodontal patients with this disease. The use of this technique in an *in vivo* environment could provide the practitioner important information about the evolution of the periodontal disease and a more precise prognostic tool for its evolution.

2. Materials and methods

2.1. Patients

The patients participating in this study were selected among those who addressed the Periodontology Department and the Oral Surgery Clinic of the University of Medicine and Pharmacy of Craiova, Romania. The study received ethical approval by the Ethics Committee of the University of Medicine and Pharmacy of Craiova. The patients were offered complete information prior to examination and collection of the gingival samples and gave their informed consent for participation in the study.

The study comprised 18 patients (10 male, 8 female) who signed the informed consent, divided in four groups: first group: periodontal patients (P) $n=5$; second group: periodontal + diabetic patients (type 2, well-controlled) (P+D) $n=5$; third group: periodontal + hepatitis C patients (asymptomatic chronic hepatitis C) (P+HC) $n=5$; fourth group: periodontal and systemic healthy patients as control group (H) $n=3$. The patients suffering from chronic periodontal disease (aged between 35–48) were diagnosed following the proposed criteria of the 5th European Workshop in Periodontology (Tonetti & Claffey, 2005): 1. proximal attachment loss of ≥ 3 mm in ≥ 2 non-adjacent teeth; 2. proximal attachment loss of ≥ 5 mm in $\geq 30\%$ of existing teeth. All chronic periodontal patients exhibited moderate forms of periodontal disease, with maximum periodontal pocket depth of 5 mm. All the periodontal patients included in the first three study groups had at least one upper maxillary premolar with periodontal pockets, which allowed the standardized sampling of the gingival tissue. None of these clinical signs were found in the H group patients (aged between 32–38).

2.2. Gingival tissue sampling

Gingival samples were collected from a periodontal pocket during periodontal surgery performed as part of standard periodontal treatment strategy for the patients suffering from periodontal disease or during extraction of teeth for orthodontic reason with no periodontal alterations for the control group (teeth extracted for orthodontic treatment of dental anomalies). For sample standardization and scientific accuracy, all samples were collected from maxillary premolars, the vestibular (buccal) part of the gingiva. The samples were collected from the periodontal pocket of the periodontal patients and from the marginal gingiva for the control group. Subsequently, the samples were standardized to a square size of 3×3 mm. Afterwards they were placed in 10% formalin and refrigerated at $+8^\circ\text{C}$ for preservation until examination.

2.3. Optical coherence tomography measurements

The OCT setup was provided by Thorlabs (Thorlabs Inc., New Jersey, USA), model number OCS1300SS, with a power swept

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