

Short Communication

Evaluation of red light scattering in gingival tissue – *in vivo* study

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ABSTRACT

Antimicrobial photodynamic therapy (aPDT) has been used to treat periodontal disease, thus the aim of this study was to investigate red light ($\lambda = 660$ nm) attenuation in gingival tissue. This clinical trial included 30 patients with chronic periodontitis; three incisors from each patient were selected for the experimental procedures. A laser source with a radiant power output of 100 mW was used. Two digital photographs were taken of each selected incisor (in frontal and occlusal position). The images were analyzed in the *ImageJ* program. The results demonstrated that at a 3 mm distance from the laser probe, there is an attenuation of light intensity of 50%, along frontal and occlusal views. Light attenuation in gingival tissue should be considered when setting optimal parameters for antimicrobial photodynamic therapy or photobiomodulation.

1. Introduction

Lasers have many applications in Periodontology including root surface decontamination; soft tissue excision, incision and ablation; and calculus removal. The use of photobiomodulation (PBM) therapy has been proposed as a way to stimulate fibroblasts and accelerate the healing process, pain relief and minimize gingival inflammation [1,2]. Lasers can also be used with the objective of killing microbial cells, antimicrobial photodynamic therapy (aPDT) being an example of this. The applications of aPDT in dentistry are increasing substantially and this therapy has been efficacious in the treatment of bacterial, fungal, parasitic, and viral infections [3–7]. Antimicrobial PDT is a phototherapy that has been proposed as an adjuvant treatment for periodontal disease as an alternative to the use of antimicrobials [8–10]. Antimicrobial PDT is based on the association of a photoactive agent, known as a photosensitizer (PS), with a light source. The objective of this is to promote the generation of reactive species of oxygen (ROS) that are cytotoxic to the bacteria, leading to cell death [11–14]. Previous studies have investigated the effect of aPDT as coadjuvant treatment for periodontal disease; however, some researchers did not find any additional effects when using aPDT for periodontal treatment [15–17]. The main discrepancies among these studies are found in the parameters used, i.e., the differences in the irradiation time regime used, which varied from 10 to 90 s per site, and the photosensitizer

concentration, which ranged from 100 to 30,000 μM . These contradicting clinical findings indicate the need for further investigation of basic aPDT principles so that an effective treatment protocol can be developed.

When treating periodontitis with aPDT, there are many clinical variables involved which could interfere directly in the results of this therapy. These variables include the presence of bleeding and gingival fluid, different depths of probing and even different gingival biotypes that could interfere with the scattering of light in the gingival tissue. In addition, the literature shows that the optical properties of tissue with a pathological condition differ from that of healthy tissue. During the inflammatory process there are more light scattering centers (inflammatory cells), which can interfere with light decay in the inflamed gingival tissue [18].

Determination of the optical properties of gingival tissue is a challenge faced when considering how to optimize aPDT for the treatment of periodontal diseases. The distribution of light in the gingival tissue is determined by absorption and scattering phenomena. In combination with the photosensitizer concentration in the periodontal pockets, these parameters affect the efficacy of aPDT directly. Based on these issues and the lack of consistency within the literature, this clinical study was carried out to investigate the red light ($\lambda = 660$ nm) attenuation in gingival tissue.

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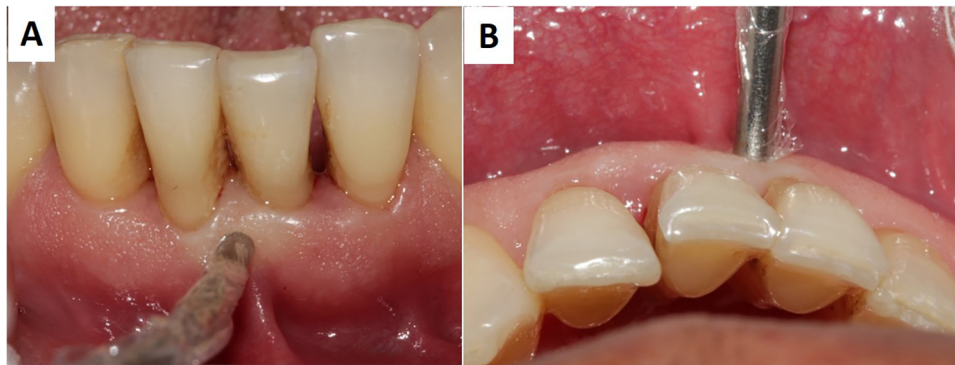


Fig. 1. Photographs taken in frontal (A) and occlusal positions (B) with a 100 mm macro lens to evaluate light scattering in the gingival tissue.

2. Materials and methods

This clinical trial was registered on ClinicalTrials.gov (NCT 03262077) and it involved 30 patients with chronic periodontitis. The patients were recruited from the Dental Clinic of Nove de Julho University (UNINOVE, Brazil). The project was approved by the Human Research Ethics Committee of the University (number 1.517.902). Patients who agreed to participate signed a statement of informed consent approved by the Research Ethics Committee from the same university.

Patients were included based on the following criteria: aged 18 years or older with chronic periodontitis [19], presence of at least 15 teeth and at least 3 incisors with probing depth greater than 4 mm [20,21]. The patients selected were receiving periodontal treatment at the university's dental clinic.

2.1. Photographs

Each patient had 3 incisors selected for the procedure. Two digital photographs were taken of each selected incisor - one in a frontal position (Fig. 1A) and one in an occlusal position (Fig. 1B). A Rebel T2i camera (Canon, Japan) with 100 mm macro lens was used and the following parameters were adopted for all photographs: 35 mm focal length, $f/22$ aperture, $1/100$ shutter speed and ISO 200. While the photographs were being taken, each selected incisor received irradiation, from a laser source with radiation emission at wavelength of 660 nm and radiant power output of 100 mW. The images were recorded for analysis in the *ImageJ* program.

3. Results

During the analysis of the frontal photographs, we observed light intensity zones around the laser probe indicating that light was scattering diffusively in the gingival tissue (Fig. 2). In the image the zones

of scattered light with a circular shape suggested that light intensity decreases from the center to the peripheral zone.

Using the images of scattered light captured by the camera, it was possible to extract the intensity variation using the *Image J* program. The results showed that at a 3 mm distance from the laser probe, there is an attenuation of light intensity of 50%, along frontal and occlusal views (Fig. 3). Due to the similar behavior of light attenuation, we observed that the light scattered in all directions in the same manner.

4. Conclusion

Photobiomodulation therapy has several applications in the area of Dentistry and it has been used to accelerate healing, bone repair, reduction of hypersensitivity, reduction of inflammation, pain relief and to provide other benefits as well [22,23]. The effects of PBM are caused by photochemical reactions within cell compounds, resulting in activation of cell proliferation, mitochondrial respiration, and collagen and ATP synthesis. In the process of periodontal repair, the effects of PBM encourage faster wound healing, when compared to conventional mechanical therapy. There are several studies which look at the effects of PBM on wound healing. Ustaoglu et al. investigated the effect of PBM after gingival surgery. The Ga-Al-As laser was used on wounds in an experimental group receiving doses of 8.6 J/cm^2 , with an irradiation time of 8 s and a constant output power of 3 W in continuous-wave mode (CW). The handpiece was positioned at a distance of 1 mm above the wound area, perpendicularly, in noncontact mode and the area of the probe was wider than that of the wound. The authors concluded that PBM had a positive effect on human palatal donor site healing after a gingival graft [24].

Another use for lasers is the reduction of the microbial load in periodontal pockets; this is done via antimicrobial photodynamic therapy (aPDT). However, the results of the studies, concerning this use, are inconsistent as to whether there exists an improved outcome when using aPDT to reduce subgingival bacteria [25]. Campanile et al.

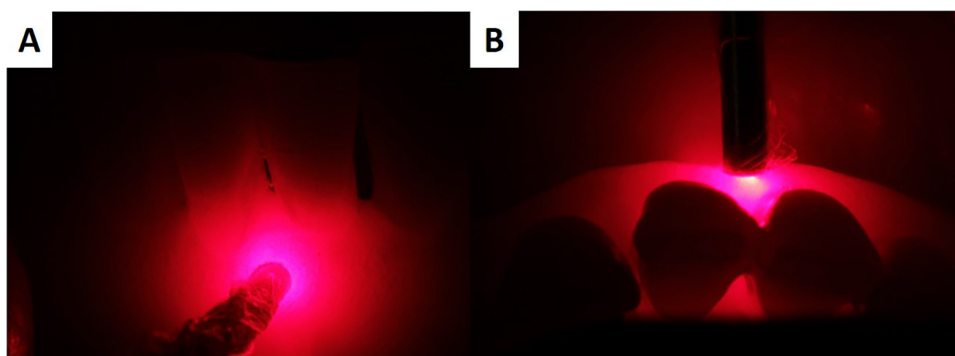


Fig. 2. Light scattering zones around the laser probe in the frontal view (A) and the occlusal view (B).

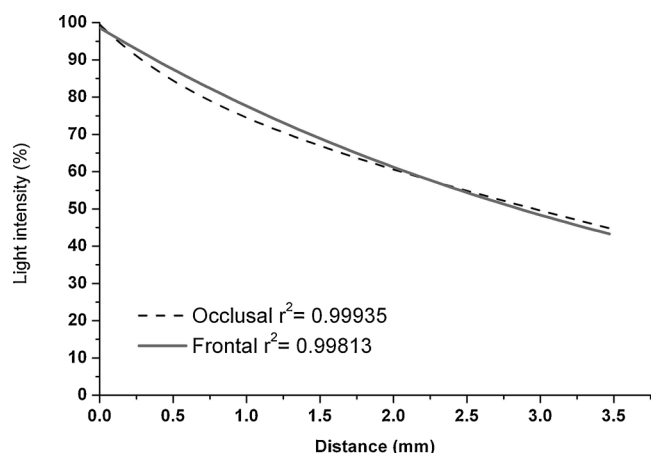


Fig. 3. Decay of light inside the gingival tissue.

studied the effect of aPDT on residual pockets. Methylene blue photosensitizer was applied to the pockets and the sites were irradiated for 60 s. The authors reported additional improvements with the use of aPDT [26].

In order to achieve good results with the use of a laser, it is essential to have a better understanding of the most optimal dosimetric parameters for items such as device power, irradiation time and energy delivered. Additionally, in the case of aPDT, the effectiveness of the treatment is influenced by the characteristics of the photosensitizer and light distribution in the irradiated tissue. Light attenuation in the tissue is caused by absorption, scattering, and, a small part, by reflectance. Chromophores, such as melanin, hemoglobin and proteins, may influence light scattering and absorption, and tissue surface may produce reflectance [27]. In our study, 5 patients presented gingival melanin pigmentation. However, the measurements were performed at sites without pigmentation. We also performed analysis without those patients; however, it did not change the results or the behavior of the data. Thus, further studies are necessary to investigate irradiation in gingival tissue with melanic pigmentation, as well as in healthy and inflamed tissues.

We observed 50% light attenuation at 3 mm from the laser probe. The significant decrease in light around the affected target may justify the wide range of results published by different clinical trials; depending on pocket probing depth, the light will not reach all sections and bacteria will remain viable in that region.

Accordingly, the light dosimetric parameters for clinical use of phototherapy on periodontal tissue should be addressed at the very beginning of study design, for clinical trials, or at the creation of a treatment plan when being used for daily practices. Light decay should be taken into account in order to optimize the light parameters used for aPDT or PBM when treating periodontal disease. As pocket probing depths vary widely, in deeper pockets more than one point of irradiation should be considered.

Declarations of interest

None.

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