Histopathological Evaluation of the Periodontal Ligament and Cementum of Extracted Teeth Subjected to Laser Ablation with Indocyanine Green

Device Investigated: Leonardo Diode Laser with the LEAP Laser Absorption Solution

Indication Studied: Pulpectomy. Specifically, a laser ablation step facilitated with the LEAP solution, added to the standard procedure for tooth pulpectomy, does not present a risk of damage to tissues and structures external of the treated root as a consequence of possible temperature increases.

Sponsor: CAO Group, Inc.

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Development Phase: Performance Validation

NCT ID: Not Yet Assigned

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DATA COLLECTION INSTRUMENT

Clinical Procedures and Sample Collection

Study design and population

The patients will be treated at the Unicesumar Dental Clinic. Ten healthy female and male patients, over 18 years old, and in need of tooth extraction, will be selected for the study. Patient selection will be carried out through anamnesis and clinical/imaginological examinations. The inclusion criterion are: lower incisors with indication for extraction.

The exclusion criteria are: other dental groups; teeth that have periodontal disease beyond the apical third of the root; and teeth that do not require tooth extraction.

At least ten teeth will be included in this study for the treatment group, and two other teeth will be used as a control group. The study is porposed under submission to the Human Research Ethics Committee (CAAE 70009423.2.0000.5539) and all patients who agreed to participate in the research will sign the Informed Consent Form.

Endodontic treatment procedure

Patients will be anesthetized with 3% Mepivacaine with 1:100,000 epinephrine (Mepiadre, DFL, RJ, Brazil), and the teeth will be isolated with a rubber dam (Madeitex, São José dos Campos, Brazil). The operative field will then be disinfected with a povidone-iodine solution. After coronary accesses, the root canals will be prepared to #35/0.04 reciprocating instrument (Blue S-one, Fanta Dental, Shanghai, China). All root canals will receive irrigation with 2.5% sodium hypochlorite, with a syringe (Descarpack, São Paulo, Brazil) and irrigation needle (Navitip, Ultradent, Utah, USA). At the end of preparation, each canal will receive the following final irrigation protocol: 10 ml of sodium hypochlorite, with 30-second activation (PUI) with an E1 ultrasonic insert (Helse, Santa Rosa do Viterbo, SP, Brazil), coupled to an ultrasound device (PM 200, EMS, Switzerland) at 10% power. Second activation with 10 ml of EDTA-T (Fórmula &

Ação, São Paulo, Brazil) for 30 seconds with the same parameters. Third activation with 10 ml of sodium hypochlorite for 30 seconds with the same parameters.

There will be no questionnaire.

Laser Ablation Protocol

After chemo-mechanical preparation, all root canals of all patients assigned to the trial intevention will receive laser ablation (LA) therapy with the indocyanine green solution (ICG). First, the root canals will be filled with 0.05% ICG solution (MP Biomedicals - Thermo Fisher Scientific, Pittsburgh, PA) which will remain in the root canal for 1 minute (pre-irradiation time). After this period, a #20 laser fiber will be inserted into the root canals to the working length. Then, 810 nm wavelength infrared diode laser (Leonardo, CAO Group, West Jordan-UT) will be activated with a power of 2.5 W, an emission interval of 300 milliseconds, and a pulse duration of 100 milliseconds. After activation commences, circular movements will be performed for 30 seconds together with the movement of removing the laser fiber. After the first activation, there will be a 30-second pause and a new 30-second activation cycle will be performed in the same way as the previous one. At the end of LA therapy with ICG, the root canals will be irrigated with 5 ml of saline solution and dried with absorbent paper cones (Dentsply Maillefer, Ballaigues, Switzerland).

Tooth extraction and histotechnical processing

After the previous procedure, an incision will be made in the gingival tissue around the tooth to be extracted, which will be gently moved away to expose the tooth neck. Then, with an atraumatic extraction kit, the dental element will be dislocated and, using forceps and/or an extractor, the element will be removed. Finally, a suture will be placed to bring the edges of the gingival tissue around the socket as close as possible.

From the dental element, the apical part of the element will be obtained together with the periodontal ligament, which will be immediately placed in individual bottles duly identified, containing 10% formalin solution, buffered with neutral pH, and stored for the first 20 hours and then washed in running water for another 12 hours to remove the fixing solution. After fixation, the pieces will be demineralized in a 10% EDTA solution28. Then, the pieces will be washed in running water, dehydrated in alcohol, cleared in xylene and embedded in paraffin. After inclusion, the pieces will be cut with semi-serial cuts, 3 μm thick, carried out using a microtome (Leica - RM 2045). For each specimen, 5 slides with 3 tissue sections will be prepared and stained with Hematoxylin-Eosin and Picrosirius red29.

Analysis of histological results

Analyzes will be performed at 400x magnification under light microscopy (DM 4000 B, Leica, Wetzlar, Germany) by a single, calibrated, and blinded operator. Histological analysis will be used to characterize the morphological structures of the periodontal ligament with the aim of identifying possible pathological changes. The results will be presented through descriptive and qualitative analysis. The analysis will consider: inflammatory infiltrate in terms of its intensity and extent; periodontal ligament organization; collagen fibers maturation; inflammatory and/or replacement resorption; and dental ankylosis. The two teeth used as controls will serve as baseline parameters to analyze possible morphological changes.

The inflammatory reaction will be scored according to the average number of inflammatory cells present in the middle third of the root of the teeth, being: Score 0, no or few cells (normal); Score 1, <25 cells (mild); Score 2, 25-125 cells (moderate); Score 3, >125 cells (severe).

For the periodontal ligament organization analysis, the percentage of organization will be considered, where: Score 0, total organization (100%), Score 1, organization greater than 75% and less than 100%; Score 2, greater than 50% and less than 75%; and Score 3, organization of less than 50%.

Inflammatory and/or replacement resorption and dental ankylosis will be considered according to their intensity and extent, where: Score 0, absent, without the presence of the mentioned events; Score 1, change in an area less than 25%; Score 2, change in an area greater than 25% and less than 50%; Score 3, change in an area greater than 50%.

Statistical Analysis Plan

All statistical analysis will be performed using SigmaPlot 12.0™ software (Chicago, IL, USA). The data obtained will be submitted to the Shapiro-Wilk normality test. Then, for comparisons between the Control and Treatment group, the Mann-Whitney U test will be used for non-parametric data. For parametric data, the Student t-test will be used. The level of significance will be set at 5%.