STUDY TITLE

The effects of a new irrigant solution on root canal bleeding during endodontic treatment: a randomized controlled study.

Protocol registration code (clinicaltrial.gov): NCT03336853.

Protocol Version : 1

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Administrative data

Promoter

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Coordinating Center

• SOD Odontostomatology

Main experimenter

Dr. Riccardo Pace

Declaration by the main investigator of compliance with the Helsinki Declaration, national legislation and protocol

I, Dr. Riccardo Pace, declare that I am aware of the study protocol and that I am the guarantor for this to be conducted as described and in accordance with:

• principles stated in the Helsinki Declaration

• Good Clinical Practice

• Community directives and national regulatory provisions

• Any changes to the procedures will be made exclusively to protect the safety, rights and welfare of the subjects involved in the study.

• I declare to coordinate the study ensuring that all those who will collaborate in its execution will be aware of the protocol and any amendments and will act in full awareness of its obligations.

Introduction

A large body of evidence shows that dental caries and periodontitis are plaque biofilm related diseases (1). Biofilm is a layer of polysaccharides, protein, and microbial cells forming a matrix that protects bacteria from antibiotics or the host immune response (2). Modern approaches to biofilm control seem to be mostly focused at the reduction of bacterial habitat in order to decrease the probability of pathogens survival in the biofilm.

A new device (HYBENX®, EPIEN Medical, Saint Paul, MN, USA) has been developed with the purpose of destroying dental biofilm. The material is a mixture of hydroxybenzenesulfonic acid (37%) and hydroxymethoxybenzene acids (23%), sulphuric acid (28%), and water (12%).

The product is currently marketed by the producer both for use in Periodontics (HybenX Oral Tissue Decontaminant) and for Endodontics (HybenX Root Canal Cleanser).
The two forms of the product, which have the same chemical composition, differ in consistency: more viscous gel for periodontal use and more liquid gel for endodontic use.

This device has been successfully used in periodontology. Recent studies (3, 4) have demonstrated the effectiveness of the oral tissue decontaminant material in the treatment of clinical cases showing acute periodontal abscess without the use of systemic or local antibiotics. Similar favorable effects were obtained in the treatment of peri-implant mucositis and peri-implantitis (5, 6). In addition, a randomized controlled trial (RCT) also demonstrated the beneficial effects of the material in the treatment of oral aphthae (7).

Based on this scientific information, a clinical and microbiological study has been planned using the decontaminant device in cases of teeth with necrotic pulp with the aim to destroy the dental biofilm of root canals. The ability of the decontaminant device to immediately stop bleeding after canal instrumentation was discovered accidentally during the procedures of this still unpublished trial.

Due to this surprising evidence, the Authors were interested in evaluating the coagulation property of the decontaminant material.

Aim of the study

The purpose of this study is to test the reduction of root canal bleeding in terms of significant percentage change for millimeters of blood in the canal at 2 different time points (before and after treatment).

Study Design

The study consists of a monocentric investigation and is configured as a randomized, controlled interventional study.

For each enlisted subject, an electronic data collection form, constructed "ad hoc", must be filled in to the inclusion in and at the end of it.

Study population

The study will include subjects, of both sexes, belonging to the O.U. Endodontics, clinic of Odontostomatology-AOUCareggi for a total of 60 patients in accordance with the inclusion and exclusion criteria below.

Inclusion criteria: patients aged between 20 and 60 years, able and willing to sign a consent form, single-rooted teeth with necrotic pulp confirmed by electric vitality test, associated with healthy periodontium, physiologic sulcus depth (<3 mm), and absence of bleeding on the probing of the involved teeth.

Exclusion criteria: patients with systemic diseases, using anticoagulants, antibiotics, or anti-inflammatory therapies in the last 30 days, patients with an allergy to sulphur in any form, and pregnancy. All subjects were informed of the nature and potential risks and benefits of their participation in the study. They also received information on the duration of the procedure, and the possible intraoperative and postoperative complications.

Endpoint

Primary endpoint

Reduction of bleeding in the root canal system

Study Procedures

During the baseline visit, after the signature of the subject's consent, the inclusion and exclusion criteria will be assessed. If the subject is responsive to all the inclusion and exclusion criteria, the following data will be collected and inserted into the electronic CRF prepared for the study: demography, remote and proximate pathological anamnesis, dental history.

Participation in the study may be interrupted by voluntary withdrawal of the subject for any reason

The reasons for leaving the subject from the study must be documented in CRF. Subjects who will not be judged to be enrolled on the inclusion / exclusion criteria or who will exit prematurely for any reason will no longer be eligible to enroll in the study.

The root canal therapy procedure will be standardized and limited to one experienced endodontist (R.P.). Treatments will be performed at the Endodontics Departments of the University Hospital of Florence Careggi, Italy, under local anesthesia using 1.8 mL mepivacaine hydrochloride with 1:100000 epinephrine (Optocaine; Molteni Dental SRL, Scandicci, Italy), and the teeth were isolated with rubber dams. After preparing the access cavity, the working length will be determined with an electronic apex locator (Propex II Dentsply Maillefer Instruments, Ballaigues, Switzerland) with a size 10 k-file and confirmed taking a periapical radiograph with the 10 k-file inserted in the root canal. Root canals will be shaped using ProTaper Universal NiTi files (Dentsply Maillefer Instruments, Ballaigues, Switzerland) in the following sequence: S1, S2, F1, F2, F3 until each instrument reached the working length. After each instrument, the root canals will be rinsed with NaClO (Niclor 5, Ogna, Italy) using a syringe with a side-vented 30 G needle. ProTaper NiTi instruments were driven with an endodontic motor (XSmart Endo Motor, Dentsply Maillefer Instruments, Ballaigues, Switzerland) with a 16:1 contrangle set up as suggested by the manufacturer.

After the root canal shaping will be performed, the root canal will be dried with four sterile paper points. A fifth 30 size-sterile paper point will be then introduced in the root canal, up to the working length, for 10 seconds to detect blood presence.

With a caliber, on the sterile paper point, the millimeters of blood present within the root canal will be

measured.

Only the teeth that at this point will show 1 or more millimeters of blood within the root canal will be included in the present study and randomize to the two experimental groups up to reach the estimated a priori sample size. In the patients excluded from the study, the ordinary endodontic treatment will be carried out.

Before further treatment, the teeth will be allocated to an experimental (HybenX) or a control group (sterile saline solution) according to an uneven block randomization designed by the statistician (A.N.). For each tooth, closed envelopes will be opened in a consecutive order, assigning the tooth to either the experimental or the control group.

HybenX is approved as a Class I CE medical device by the Italian Ministry of Health (no. 483768) on February 7, 2012.

Experimental Group

The decontaminant material will be introduced inside the root canal using the pre-dosed syringe, and activated for 20 seconds, with a sterile paper point, with an up and down movement up to the working length.
Finally, the canal will be rinsed with sterile water using a syringe with a side-vented 30 G needle 1 mm shorter than the working length.
The root canal will be then dried with four sterile paper points. Finally, a fifth 30 size-sterile paper point will be introduced in the root canal, up to the working length, for 10 seconds to detect the presence of blood; the millimeters of blood inside the root canal wll be measured again according the previous criteria.

Control Group

The root canal will be irrigated with sterile saline solution with a syringe and a side-vented 30G needle, activated for 20 seconds with a sterile paper point with an up and down movement up to the working length to ensure a flow of irrigant solution throughout the canal.
Finally, the canal will be rinsed with sterile water using a syringe with a side-vented 30 G needle 1 mm shorter than the working length. The root canal wll be then dried with four sterile paper points. Finally, a

fifth sterile paper point will be introduced in the root canal, up to the working length, for 10 seconds to detect the presence of blood; the millimeters of blood inside the root canal will be measured again according the previous criteria.

Statistic Plan

Sample Size Estimation

A total number of 60 patients (30 patients per arm) is evaluated to reject the null hypothesis of equality between the two experimental groups in terms of root canal bleeding on the basis of the following assumptions:

- Power of approximately 90% in rejecting the null hypothesis of equality
- Expected means at the baseline of 4 mm for both of the two groups
- Expected means gain after using the decontaminant material 2,5 mm (reduction of 40%) and 4 mm after using sterile saline solution (no reduction)
- Standard deviation of 1,7 mm in both the experimental group
- Overall significance level = 5% two-sided.

Statistical Analysis

Data will be analyzed using SAS Version 9.3 software (SAS Institute, Inc., Cary, NC).

A preliminary Levene's test will be performed to verify the homogeneity of variance for the percentage change in the two groups.

A t-test will be performed to verify the null hypothesis by using a type I error equal to 0.05.

Management of accidents and missed accidents

The reporting of any accidents or missed accidents will occur according to what is indicated by the legislation in force and through the appropriate form prepared by the Ministry of Health [Report of accident or non-incident by health workers to the Ministry of Health (Article 9 and 10, Legislative Decree n.46 of 1997, Article 11, Legislative Decree No. 507 of 1992)].

Ethical aspects and respect for confidentiality

The investigators ensure that the study will be conducted in full compliance with the provisions of international law [Dir. EU 2001/20 / EC] and its national implementation [DM 15 July 1997; D.Lvo 211/2003; D.L.vo 200/2007] regarding the clinical trial and the principles of the Helsinki Declaration in order to ensure maximum protection of the subjects involved. The principal investigator is committed to ensuring that the study is conducted in accordance with what is written in this protocol and the Good Clinical Practice (GCP). The promoter of the study is committed to the protection of sensitive personal data, clinical and otherwise, of the subjects involved in the study as established in the matter by national legislation [D.Lvo. 196/2003].

Informed consent

It will be the responsibility of the investigators, or of subjects appointed by them, to obtain the informed consent of the patients after adequate information about the aims, methods, expected benefits and foreseeable risks of the study. Investigators or appointees must also inform the participants that the non participation or the interruption of the same will not cause prejudice or damage towards them.

Ethics Committee and Competent Authorities

The promoter will provide the Reference Ethics Committee and the Competent Authorities (General Manager AOU Careggi) with the study protocol and any other related document provided to the patient (Informative Note and Informed Consent Form). The approval of the Ethics Committee and of the competent Authority must be obtained before the beginning of any procedure related to the study and must be documented by official communication to the investigator. If during the course of the study, changes to the

study protocol are necessary, the promoter will submit to the Ethical Committee of reference adequate request for amendment to the protocol, whose approval will follow the procedures established by the regulation of the Ethics Committee itself.

Data properties

The ownership of data, being an independent study pursuant to the D.M. December 17, 2004, belongs to the Promoter of the Study (D.M. 17 December 2004, Article 1, paragraph 2, letter c).

Final report and publication of results

According to the ICH-GCP, the Study Manager undertakes to produce a report on the study, publish all the data collected as described in the Protocol and to ensure that the data are reported responsibly and consistently. In particular, the publication of data deriving from this study will take place independently of the results obtained. The transmission or dissemination of data, through scientific publications and / or presentation in congresses, conferences and seminars, participation in multicentric studies, will take place exclusively following a purely statistical elaboration of the same, or in an absolutely anonymous form. Responsible for the entire research and therefore the data processing is Dr. Riccardo Pace, responsible for the study.

Independence of the study

The Study presents all the necessary requirements according to the D.M. 17 December 2004 (Art.1, Comma 1 and 2) for the definition of "clinical trial aimed at improving clinical practice as an integral part of health care and not for industrial purposes".

Contributions and conflict of interest

Study manager (s):

Dr Riccardo Pace who conceived and drafted this protocol.

Sources of contributions

This trial was conceived independently of any commercial organization and will be coordinated, managed and analyzed independently.

References

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4. Isola G, Matarese G, Williams RC, Siciliano VI, Alibrandi A, Cordasco G, Ramaglia R. The effects of a desiccant agent in the treatment of chronic periodontitis: a randomized, controlled clinical trial. Clin Oral Invest Jun 17.

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7. Porter SR, Al-Johani K, Fedele S, Moles DR. Randomised controlled trial of the efficacy of HybenX in the symptomatic treatment of recurrent aphthous stomatitis. Oral Dis 2009;15:155-61.