PROTOCOL

(Clinical Investigation Plan)

Comparative evaluation of the effectiveness of root canal preparation after irrigation using endodontic needle and inertial cavitation: A randomized controlled trial

Protocol version: 1.0

Protocol Date: 27.01.2022

SPONSOR SIGNATURE PAGE

The Sponsor have approved the Protocol (CIP) version 1.0 dated 27.01.2022 and confirm hereby to conduct the investigation according to the Protocol (CIP), the current version of the World Medical Association Declaration of Helsinki, ISO14155 norm, ICH-GCP as far as applicable, and the local legally applicable requirements.

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INVESTIGATOR STATEMENT

I have read and understood this Protocol (CIP) version 1, dated 27.01.2022 and agree that it contains all necessary details for carrying out the study described and in conformance with Good Clinical Practices (GCPs) and applicable regulatory requirements. I agree to conduct the investigation according to the Protocol, the current version of the World Medical Association Declaration of Helsinki, ISO 14155 norm, ICH-GCP and the local legally applicable requirements. I agree to maintain the confidentiality of all information received or developed in connection with the protocol.

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TABLE OF CONTENT

Comparative evaluation of the effectiveness of root canal preparation after irrigation using endodontic needle and inertial cavitation: A randomized controlled trial

Introduction

Postoperative pain after root canal treatment is a clinical challenge influenced by host-dependent (preoperative pain, occlusal trauma) **(Glennon et al. 2004, Yu 2004)** and operator-dependent factors (chemical, mechanical or microbiological factors) **(Siqueira & Barnett 2004)**. It is mainly attributed to the extrusion of infected debris into the periapical area. **(Siqueira JF 2003)**. Although the incidence of postoperative pain was reported to be around 40% of cases, it can reach 80% within the first 24 hours after the treatment **(Pak & White 2011)**. While host-dependent factors cannot be influenced, the operator-dependent factors such as instrumentation materials, shaping kinematics, irrigation material and method can be controlled to minimize or avoid postoperative pain **(Tanalp and Gungor 2014, Mostafa *et al*. 2020)**.

Chemo-mechanical debridement of the root canal system is important to achieve an optimal disinfection and shaping of the root canal and is a prerequisite for a successful treatment outcome **(Topcuoglu et al. 2018, Eyuboglu & Özcan 2019)**. Optimal shaping and disinfection require both mechanical and chemical debridement as large areas of the root canal system were shown to remain untouched even after the completion of root canal shaping **(Brito *et al*. 2009, Howard *et al*. 2011)**. The comparison of root canals using micro computed tomography before and after root canal treatment also presented non-prepared areas within the root canal system and shortcomings of mechanical shaping **(Peters *et al*. 2001)**. Therefore, root canal irrigation, the chemical part of the debridement, plays a key role in optimally disinfecting the root canal system, especially on these untouched areas **(Versiani *et al*. 2016, Estevez *et al*. 2017)**. However, there is no single, ideal irrigation solution that can penetrate the entirety of the root canal structure. This is not sufficiently improved by increasing the temperature, lowering pH, or increasing wettability. To this end, new irrigation solutions have been proposed such as combining sodium hypochlorite (NaOCl) with ethylenediaminetetraacetic acid (EDTA) as initial or final irrigation solution (**Zehnder 2006**).

Endodontic needles with differently shaped tips and designs are the most common way to deliver the irrigation solutions into the root canal system **(Boustsioukis et al. 2010)**. However, irrigation using endodontic needles does not remove all debris especially within the canal extensions at the apical third of the root canal system due to limited fluid penetration **(Tay *et al*. 2010, Chan *et al*. 2019).**

Different devices or methods for agitation or activation of the irrigation solution have been introduced to increase the efficacy of the irrigation solution within the root canal system and thus create optimal disinfection **(Gu *et al.* 2009, Dutner *et al*. 2012, van der Sluis *et al.* 2015)**.

During ultrasonically activated irrigation (UAI) the irrigation solution is activated with a noncutting oscillating tip within the root canal system **(Boustsioukis *et al.* 2013**). Acoustic microstreaming, a unidirectional circulation of fluid, is created by UAI. During the acoustic microstreaming, transient (inertial) cavitation was observed **(Macedo *et al*. 2014)**. Inertial cavitation can only be created under the right physical conditions within the root canal system **(Flynn (1975)a, Flynn (1975)b, Birkin et al. 2011).** There are several reasons for the disappearance of acoustic microstreaming within the root canal such as airlock formation in the apical third of the canal **(de Gregorio et al. 2009, Peeters et al.**

2013) or the oscillating tip getting stuck in the apical third thereby obstructing the oscillating movement **(Jensen *et al.* 1999)**.

In order to overcome such problems, a novel medical device "EndoClean" (Lumendo AG, Renens, Switzerland) has been developed that generates hydraulic cavitation to clean root canals deeper and more effectively than other systems described above. This method overcomes the problem of the stagnation plane formation (around 1-2 mm in front of a syringe **[Gulabivala et al.,2010),** permitting the irrigation solution to access deeper into the root canal system and thus providing a fast and efficient way of cleaning the root canal.

It is known that extrusion of irrigation solution may damage the periapical tissues and cause postoperative pain **(George & Walsh 2008).** Although there are data presenting similar amount of irrigation solution extrusion between UAI and manual irrigation **(Mitchell *et al*. 2011)**, no data regarding the short term (postoperative pain) effects of the novel device, EndoClean, is available. For this reason, a pilot clinical study for EndoClean is planned.

Objectives and hypothesis

The primary objective of this randomized controlled trial is to compare the postoperative pain intensity levels in patients with asymptomatic teeth diagnosed for non-surgical orthograde root canal treatment that are disinfected during the root canal treatment procedure by manual irrigation with sodium hypochlorite (NaOCl) solution or EndoClean. The secondary objective is to evaluate, if EndoClean could be used safely during root canal treatment as an alternative approach to manual irrigation. The null hypothesis is that there will not be a significant difference in postoperative pain intensity levels between patients who will undergo root canal treatment with manual irrigation with NaOCl or EndoClean.

STUDY PROTOCOL

The "Clinical Investigation" complies with the requirements of the World Medical Association Declaration of Helsinki (2013) and ISO 14155:2020 (Clinical investigation of medical devices for human subjects - Good Clinical Practice).

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3. Preparation of the Investigation:

3.1. Ethical Considerations:

The ethical considerations of the "Clinical Investigation" are based on the Declaration of Helsinki (2013). The approval by the "Ethics Committee" is the prerequisite for the start of the "Clinical Investigation".

3.2. Investigational device

3.2.1. General description of the investigational device

Endoclean generates hydraulic cavitation within the root canal. The cavitation provides a strong erosive effect that allows the removal of bacteria, smear layer, pulp tissue and dental debris based on mechanical action only. Endoclean intends to overcome the disadvantages of chemical irrigation system that either cannot fully reach all parts of the root canal system,is very cumbersome to use and is highly toxic to the surrounding tissue. It further intends to reduce the number and extend of mechanical filing steps, thus firstly reducing the overall treatment time andsecondly reducing the complication risk ofextensive mechanical filing.

Endoclean is applied following conventional access cavity preparation, pulp removal, glide path preparation and root canal length measurement. The Endoclean tip needs to reach within 3 mm from the apex. Accordingly, in very narrow canals minimal shaping is required. Endoclean is applied for 60 seconds in every root canal in an up-and-down movement.

3.2.2. Traceability and accountability of the investigational device

Traceability will be controlled during and after the investigation period by means of the lot number of the device. Only one device will be used in this study. The lot number of the device used in each finished clinical case will be included in the case report and recorded in a datasheet specifically-designed for that purpose.

The device will be delivered directly to the Site by Lumendo staff. The device will be stored and transported according to the regulations written in the instructions.

After completion of the study the used device will be returned to Lumendo by Lumendo staff or express shipping.

3.2.3. Intended purpose of the investigational device

Endoclean is intended to clean and remove bacteria, smear layer, pulp tissue, and dental debris from the root canal during root canal treatment.

3.2.4. Intended patient's population

Intended patients profile include individuals in need of root canal treatment (endodontic treatment), most commonly due to progressed tooth decay, faulty fillings, or tooth trauma which has resulted in an infection in the pulp and root of the tooth. The patients are otherwise in relatively good health, do not suffer from unrelated acute conditions or diseases and the chronic diseases, if present, are properly managed.

3.3. Study Design and Setting

The protocol of this prospective, 2-arm, randomized controlled clinical trial and consent forms for the patients will be approved by the ministry of health and ethics committee of the university. The study will be registered on www.clinicaltrials.gov. The study will be following the Consolidated Standards of Reporting Trials (CONSORT) guidelines **(Moher *et al*. 2010)**. All patients will be informed about the nature of the study, the treatment procedures, evaluation process, benefits, and the potential risks before they will be asked to sign a written consent form. The enrolment of patients will be executed from the outpatient clinic of Department of Endodontics, Faculty of Dentistry, Istanbul Medipol University, Turkey.

3.3.1. Patient Population:

3.3.1.1. Sample Size Calculation

In order to determine the number of samples, power analysis was performed using the G*Power (v3.1.9.2) program. The power of the study is expressed as 1-β (β = probability of type II error). Based on the postoperative 6th-hour pain measurements in the article by D. Liapis et al.*, the mean for

Group I was 13.9 SD:16.2; When the mean for Group II was taken as 4.9 SD: 7.3, the effect size (d) was found to be 0.716 in the calculation made to obtain 90% power at the α=0.05 level. Accordingly, it was calculated that there should be at least 42 people in each group and 84 people in total. Considering that there may be losses during the study process (20%), it was decided to take at least 50 people in each group.

3.3.1.2. Pilot Study

10 patients will be involved in a pilot study. There will be 5 patients in each group: manual final irrigation and EndoClean device. Pilot study will be conducted for two reasons. The first reason is to ensure the safety of the device for clinical use. Thesecond reason is to conduct an accurate power analysis and therefore, a sample group for the full clinical study. A full clinical investigation will be performed once the pilot study is done, and which will be powered according to the outcome of the pilot study.

3.3.1.3. Inclusion Criteria:

1. Physical status classification system described was used for patients' recruitment. Healthy patients, who were classified by the American Society of Anaesthesiologists (ASA) as ASA I and II
2. Patients with the age of 18 and over
3. Patients who were diagnosed as in need of non-surgical orthograde root canal treatment of an asymptomatic tooth
4. Tooth without signs of previously initiated Root Canal Therapy

3.3.1.4. Exclusion Criteria:

1. Pregnant and lactating females
2. Patients with chronic periodontal diseases
3. Patients with sensitivity or adverse reactions to any medication or materials that were used throughout the treatment procedure
4. Patients with acute periapical periodontitis
5. Patients with acute periodontal abscess
6. Patients who could not abide by the follow-up time
7. Patients who used analgesics 1-week prior or antibiotics 1-month prior to treatment
8. Uncooperative patients
9. Teeth that cannot be made functional nor restored or difficult to access teeth with no importance (wisdom teeth)
10. Teeth with poor prognosis, for example, due to deep root caries or big root resorption
11. Teeth with ≥4 mm periodontal pocket depth
12. Teeth with an inaccessible root end
13. Teeth with apical resorption or a radiologically not clearly defined apex
14. Fractured teeth
15. Teeth with immature or open apices
16. Teeth with root apices extending into the maxillary sinus

17. Teeth with external resorption communicating with the pulp

3.3.1.5. Withdrawal Criteria:

Subjects may be withdrawn from the study at the discretion of the Investigator or Sponsor due to a safety concern or if judged non-compliant with trial procedures.

A subject must be withdrawn from treatment if one of the following applies:

- Subject chooses to withdraw from the study at any time;
- Intolerable adverse effects;
- Other circumstances that would endanger the health of the subject if he/she were to continue his/her participation in the trial.

3.4. Diagnosis

The patient's dental and medical history, demographic data (age, gender) are recorded and a thorough clinical examination, as well as radiological examination, is performed. During clinical examination, the relevant tooth, and surrounding tissues, as well as adjacent lymph nodes, are examined. Percussion and palpation tests, and periodontal probing of the relevant tooth are performed. A cold test (Roeko Endo-Frost, Coltène/Whaledent Langenau, Germany) is used to assess sensitivity. Periapical radiographs (KODAK RVG 5100, Carestream Health, Rochester, NY, USA) are produced with a paralleling technique for evaluation of periradicular tissues (exposure time: 0.16 s, exposure dose: 1.22 mGy). All findings of patients will be recorded in patients' charts.

After being trained by a blinded investigator, all participants will be asked to grade their pain intensity level on a horizontal Visual Analogue Scale (VAS) before the treatment procedure. This scale is a horizontal 100 mm bar, marked in every 10mm with two ends. One end (0) indicated "no pain" and the other end (10) indicated "worst pain possible". Categorization of pain scores is as follows: 0-4 mm = No Pain, 5-44 mm = Mild Pain, 45-74 mm = Moderate Pain, 75-100 mm = severe pain. After grading the pain level, the distance between the marked point and the "no pain" endpoint will be measured with a ruler to calculate the pain intensity level. Clinical and radiological findings consistent with the asymptomatic tooth with nonvital pulp tissue with the preoperative pain intensity scores in the category of "no pain" will be included in the study. **(Eyuboglu & Kim 2020)**.

3.5. Randomization and Blinding

The patients will be randomly assigned to one of the two groups based on the irrigation protocol (Group 1-named "CONI" (conventional irrigation), and Group 2- named "INCA" (inertial cavitation)). To rule out the potential influence of the tooth type (molar, nonmolar) on postoperative pain, stratified randomization will be performed **(Nagendrababu & Gutmann 2017)**. Blocks with different sizes of 2, 4 and 6 and an allocation ratio of 1:1 will be prepared (https://www.randomizer.org).

All allocations containing the unique randomization number and group name (CONI or INCA) will be concealed in opaque sealed envelopes which will be marked with the abbreviations "M" (molar) and "NM" (non-molar).

Due to the nature of the study, both patient and the operator could not be blinded to the treatment protocol. However, the data collector will be blinded to the objectives and the design of the study.

4. Patient information and informed consent to participate:

All patients participating in the study have to be informed in oral form by the Investigator. Additionally, patients will receive an information form, on which they shall give their written consent to participate in the investigation. This form will be countersigned by the Investigator and the Patient.

5. Confidentiality:

Data will be collected with a browser-based electronic data capture system. No personal data such as name, address, date of birth will be collected with this system. The data will be accessible to the sponsor within this system. The identification codes, used by the "Investigator" to put the results of the study in a certain relation, have to be kept confidential by the Investigator after completion of the study.

6. Clinical Procedures

6.1. Endodontic procedures

All treatment procedures will be performed in a single visit by a single treatment provider. It will be assured that the operation room is quiet and isolated throughout the whole process to avoid any outside interference. Patients will be made comfortable and informed about the steps of the procedure a second time right before commencing the treatment protocol. Patients will be also reminded that they will get the necessary treatment even if they decline from the study and should not feel compelled to participate the study for a proper treatment. All root canal treatments will be performed using a dental loupe with a magnification of 4.50X (EyeMag Pro F; Carl Zeiss).

Each tooth will be anaesthetized with 2 mL local anaesthetic solution containing 40 mg/mL articaine hydrochloride and 12 mg/mL epinephrine hydrochloride (Ultracaine DS Forte; Aventis Pharma). After isolating the tooth with a rubber dam (Hygenic Dental Dam Kit; Coltene/Whaledent Gmbh & Co.kg, Langenau, Germany) the operation field will be disinfected using 5.25% NaOCl solution (Wizard; Rehber Chemistry) soaked cotton rolls.

After the access cavity preparation, a #10 C-File (MMC file, Coltene-Micro-Méga, Besançon, France) will be introduced into the root canal for scouting. Coronal flaring of root canals will be performed using a nickel-titanium flaring file (One Flare; Coltene-Micro-Méga) with an electric endodontic motor (Dual Move, Coltene-Micromega). Working length determination will be performed with a #10 C-file (MMC file, Coltene-Micro-Méga) and glidepath preparation will be completed with a One G (Coltene-Micro-Méga). The shaping of the root canals will be performed using One Curve (#25/0.06, Coltene-Micro-Méga). If further shaping of the canal will be needed 2 Shape F35 (#35/0.06) (Coltene-Micro-Méga) files will be used according to apical canal lumen. All rotary files will be used according

to the manufacturer's instructions. The filing protocols and files used will be recorded in the patient file.

In Group 1, 2 mL of NaOCl solution will be used after each file as an irrigation solution. After the shaping of the root canal is completed final irrigation with a 31G double side vented tip is performed in each canal using 2.5 mL of 5% EDTA (Wizard; Rehber Chemistry) for 1 minute, 2.5 mL of 5.25% NaOCl for 30 seconds and 5 mL of saline solution for 30 seconds respectively.

In Group 2, 2 mL of NaOCl solution will be used after each file as an irrigation solution. After the shaping of the root canal is completed, the NaOCl solution is removed from the root canal with 2.5 mL saline solution using a syringe with a 31G needle. EndoClean tip is introduced into the root canal, always maintaining a distance of at least 3mm from the apex. The EndoClean device is used for 60 seconds for each root canal.

In both groups, following root canal preparation, a matching gutta-percha cone (#25/0.06 or #35/0.06) (MM GP Points, Coltene-Micro-Méga) is introduced into the root canal coated with a calcium silicate-based root canal sealer (BioRoot RCS; Lancester PA, USA). Single-cone technique is used to fill the root canals.

Total-etch technique is used to restore the coronal part of each tooth. The cavities are etched with 35% H3PO4 for 15 seconds before a single layer application of an adhesive material (Adper Single Bond 2; 3M ESPE). After photopolymerization (Elipar S10, 3M ESPE) of the adhesive material for 20 seconds, a flowable bulk-fill composite material (Estelite, BulkFill Flow, Tokuyama Dental, Tokyo, Japan) is used to restore the coronal cavity incrementally with a thickness of 4 mm for every layer. Each layer is photopolymerized for 20 seconds.

6.2. Post-endodontic pain assessment

After the treatment, all participants will be provided with analgesics (75 mg diclofenac sodium) (Voltaren SR 75 mg, Novartis, Kurtköy, İstanbul) and will be advised to use them if needs be and record the time of use. A pain intensity evaluation diary including VAS diagrams with their respective follow-up evaluation times will be provided to each participant to mark their pain. All participants will be contacted on their follow-up time to remind them to mark their pain intensity scores. Pain intensity of patients will be evaluated at 6h, 24h, 48h, 72h and 1 week after the completion of root canal treatment as previously described. Any complication such as paraesthesia, acute exacerbations, flare-ups, swellings will be also recorded on patients' charts. Patients who fail to abide by the follow-up time will be traced via phone, e-mail or SMS to determine the possible reasons for dropout.

7. Concomitant Treatments

Medications given to patients are to be documented

8. Schedule

The duration of the investigation and the post-assessment has to be justified.

Study duration:

Step 1. Patients enrollement: 2 months

Step 2. Treatment duration: 1 visit to dentist

Step 3. Follow-up period: 1 week

Flow chart of the study:

Investigation Periods	**Consent (ICF) Screening**	**Treatment**	**Follow-up**				
Visit	1 On-site	2 On-site	3	4	5	6	7 On-site
Time (hour, day, week)	-1 week	0	6 hours	24 hours	48 hours	72 hours	1 week
Patient consent (ICF)	x						
Demographics	x						
Medical History	x						
In- /Exclusion Criteria	x	x					
Dental Examination	x						x
X-ray		x					
Randomization		x					
Medical device application		x					
Concomitant Therapy,	x	x	x	x	x	x	x
(Serious) Adverse Events, Adverse device effects	x	x	x	x	x	x	x
Pain intensity			x	x	x	x	x
Pain intensity diary dispensing		x					
Pain intensity diary collection							x

9. Reporting of Unexpected Serious Adverse Events, Serious Adverse Events and Adverse Events

Unexpected Serious Adverse Events and Serious Adverse Events must be reported to the sponsor within 24 hours after awareness of the event by the Investigator. Unexpected Serious Adverse Events must be reported to the Ethics Committee and Regulatory Authority within identified timeline as per the regulations.

10. Premature Termination of the Study

If excessive pain will be experienced by three concomitant patients the study will be terminated.

11. Statistical Analysis

Statistical analysis will be defined on later stage. It is a pilot study with exploratory statistics.

12. Financing and Insurance

12.1. Reimbursement of Patients' Expenses

The Sponsor shall provide the materials used for the patients.

12.2. Insurance for Patients

Insurance will be provided by the Sponsor. A copy of the certificate is filed in study site file and the sponsor file.

13. Publications policy

The study results are communicated to the sponsor after termination and evaluation of the study by E-Mail. To healthcare professionals, the public, and other relevant groups not participating directly in the study, the results are being presented via publication. It is not planned to use professional writers or to grant public access to the full protocol, participant-level dataset, and statistical code.

The decision to submit the report for publication as well as the ultimate authority over any of the activities lies with the Sponsor.

The trade secrets of the medical device are to be protected at all times.

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