

RANDOMIZIRANO KLINIČKO ISPITIVANJE UTJECAJA FOTODINAMSKE TERAPIJE I BIOKERAMIČKOG PUNILA KORIJENSKIH KANALA NA CIJELJENJE PERIAPIKALNE LEZIJE I POJAVNOST POSTOPERATIVNE BOLI NAKON ENDODONTSKOG LIJEČENJA

JAVNA OBRANA TEME DOKTORSKOG RADA

Mentor: izv.prof.dr.sc. Ivona Bago

izv.prof.dr.sc. Tomislav Lauc

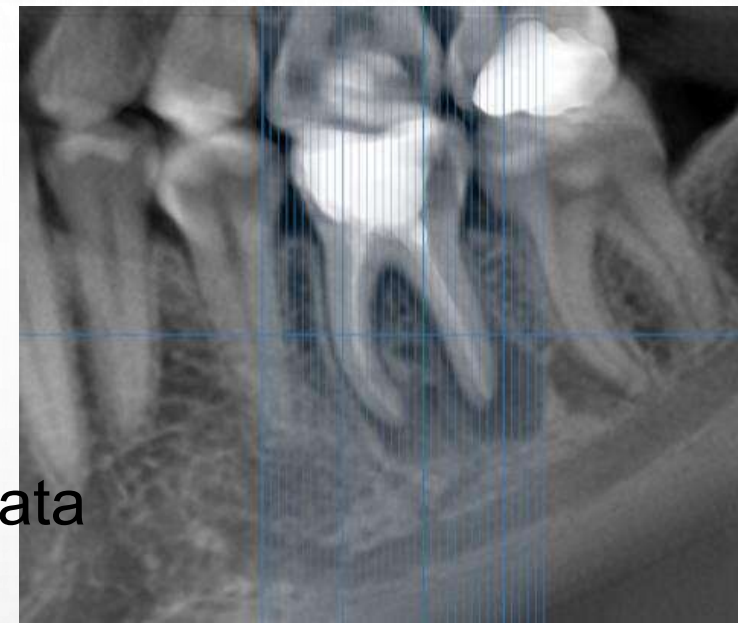
Zagreb, srpanj 2021.

UVOD

- Cijeljenje periapikalne lezije kao mjera uspješnosti primijenjene metode/materijala
- Utjecaj fotodinamske terapije (PDT) i biokeramičkog punila na cijeljenje periapikalne lezije
- Utjecaj PDT i biokeramičkog punila na pojavu i intenzitet postoperativne boli nakon jednoposjetne revizije
- Važnost dobro dizajniranih kliničkih istraživanja
- Smjernice za randomizirana klinička istraživanja u endodonciji*

KRITERIJI USPJEŠNOSTI ENDODONTSKOG LIJEČENJA ZUBA

- Radiološki i klinički kriteriji uspješnosti endodontskog zahvata
- Patohistološka evaluacija kao zlatni standard
- Rutinski se koriste prve dvije metode
- Dodatni moment 3D radiološkom dijagnostikom
- Klinički kriteriji



PREGLED DOSADAŠNJIH ISTRAŽIVANJA

- Utjecaj suplementnih metoda na eliminaciju bakterijske mikroflore
- In vitro istraživanja pokazala učinkovitost fotodinamske terapije



IN VITRO ISTRAŽIVANJA

AUTOR / GODINA	CILJ I MATERIJALI	METODE	ZAKLJUČAK
Garcez i sur, 2007.	Procijeniti uspješnost dezinfekcije korijenskog kanala kombinirajući konvencionalni endodontski tretman i PDT Proteus mirabilis Pseudomonas aeruginoza	PDT –polietilendiamin i klorin e6 kao PS, diodni laser (660nm)	Kombinacijom ispiranja + PDT smanjenje bakterija 98%.
Poggio i sur, 2011.	Ispitati učinkovitost PDT – a kao dodatne metode dezinfekcije. Enterococcus faecalis, Streptococcus mutans Streptococcus sanguis	FotoSan; CMS Dental, Copenhagen, Denmark Toluidinsko modriilo	PDT primjenjivan dulje vrijeme ili u kombinaciji s 5% NaOCl pokazuje značajno bolji antimikrobni učinak
Bago i sur, 2013.	Antimikrobni učinak PDT-a na Enterococcus faecalis s diodnim laserom, ispiranjem 2,5% NaOCl kroz 60sec, te zvučno aktiviranim NaOCl kroz 60 sec (Endoactivator)	Diodnim laser, ispiranje 2,5% NaOCl kroz 60sec, te zvučno aktivirani NaOCl kroz 60 sec (Endoactivator)	PDT i Endoactivator su bili uspješniji u eliminaciji infekcije u odnosu na pojedinačno primijenjene metode

Kishen A, Shrestha A. Photodynamic Therapy for Root Canal Disinfection
In: Bassrani B. Endodontic irrigation. 1st edition. Cham:
Springer; 2015. 237-253.

FOTODINAMSKA TERAPIJA (PDT)

engl. Photodynamic therapy, Photoactivated disinfection

- Svjetlosno inducirana inaktivacija stanica, mikroorganizama ili molekula
- Fotoaktivator kao netoksično bojilo
- Transfer apsorbirane energije na druge molekule
- Metilensko ili Toluidinsko modrilo
- Valna duljina apsorpcije 600 – 660 nm



PDT - RELEVANTNA KLINIČKA ISTRAŽIVANJA

AUTOR / GODINA	CILJ I MATERIJALI	METODE	ZAKLJUČAK
Bonsor i sur., 2006	Procijeniti uspješnost dezinfekcije korijenskog kanala kombinirajući konvencionalni endodontski tretman i PDT. Istraživanje je provedeno na 32 korijenska kanala 14 pacijenata.	Ispiranje s 20% limunskom kiselinom i 2,25% NaOCl, PDT – TBO i diodni laser (12.7 mg/L, 100 mW, 120 s).	Struganjem i ispiranjem korijenskih kanala uništene su bakterije u 86,7% uzoraka. Kombinacijom struganja i ispiranja + PDT uništene su bakterije u 96,7% uzoraka.
Bonsor i sur., 2006	Usporediti učinkovitost kombinacije 20% limunske kiseline i PDT-e nasuprot učinkovitosti kombinacije 20% limunske kiseline i 2,25% NaOCl-a na bakterije u korijenskom kanalu. 64 pacijenta su sudjelovala.	Protokol isti kao u prethodno prikazanoj studiji.	Kombinacijom 20% limunske kiseline i PDT uništene su bakterije u 91% uzoraka. 20% limunskom kiselinom i 2,25% NaOCl eradicirane su bakterije u 82% uzoraka.
Garcez i sur., 2008	Analizirati antimikrobni učinak PDT-e s endodontskim liječenjem. 20 pacijenata je uključeno. Prva faza: mehanička obrada i ispiranje korijenskih kanala + PDT. Druga faza: nakon prve faze kanal ispunjen kalcij hidroksidom i nakon jednog tjedna ponovljen PDT.	Ispiranje s 2,5% natrij hipokloritom, 3% hidrogen peroksidom i 17% EDTA-om. PDT – polietilenamin klorin e6 (2 min, 9,6 J, 240 s).	Nakon prve faze broj bakterija je smanjen za 98,5%. Nakon druge faze broj bakterija je smanjen za 99,9%. Druga faza se pokazala učinkovitijom od prve

Kishen A, Shrestha A. Photodynamic Therapy for Root Canal Disinfection. In: Bassrani B. Endodontic irrigation. 1st edition. Cham: Springer; 2015. 237-253.

PDT - RELEVANTNA KLINIČKA ISTRAŽIVANJA

<p>Garcez i sur, 2010.</p>	<p>Istražiti antimikrobni učinak PDT-e + konvencionalno endodontsko liječenje u pacijenata s nekrozom pulpe i infekcijom bakterijama rezistentnim na antibiotike. 30 zubi s periapikalnim lezijama od 21 pacijenta koji su već bili prethodno liječeni konvencionalnim endodontskim liječenjem i antibiotskom terapijom.</p>	<p>PDT – polietilamin klorin e6 kao PS i diodni laser (40 mW, 4 min, 9,6 J)</p>	<p>Samo endodontskom terapijom se znatno smanjio broj bakterijskih vrsta (ali samo su 3 zuba bila bez bakterija). Kombinacijom endodontskog liječenja i PDT-e uklonjene su sve vrste bakterija rezistentne na antibiotike i svi su zubi bili u potpunosti bez bakterija.</p>
<p>Silva i sur, 2012.</p>	<p>Istraživali su odgovor periapikalnog tkiva zuba pasa sa apikalnim parodontitisom nakon jednogodisnjeg endodontskog liječenja s i bez PDT-e.</p>	<p>PDT – fenotiazin klorid 10 mg/ml 3 min; diodni laser (660 nm, 60 mW, 1 min).</p>	<p>Grupa tretirana PDT-om pokazala je umjereno uvećano periapikalno područje, bez upalnih stanica, umjerenu angiogenezu i fibrinogenezu i najmanju periapikalnu leziju.</p>
<p>Bago Jurić i sur, 2014.</p>	<p>Ispitati dodatni učinak PDT-e u eradikaciji mikroorganizama nakon konvencionalnog postupka revizije kod pacijenata s perzistirajućim periapikalnim procesom 21 pacijent.</p>	<p>PDT - fenotijazin klorid kao PS + diodni laser (660 nm, 100 mW, 1 min zračenja), trodimenzionalni fiber - optički nastavak.</p>	<p>Iako je samo endodontsko liječenje (revizija) dovelo do značajnog smanjenja broja vrsta bakterija, kombinacija endodontskog liječenja i PDT-e bila je učinkovitija. Kod 11 zuba (od 21) nije otkriven porast bakterija.</p>

Kishen A, Shrestha A. Photodynamic Therapy for Root Canal Disinfection. In: Bassrani B. Endodontic irrigation. 1st edition. Cham: Springer; 2015. 237-253.

Photodynamic Therapy Associated with Conventional Endodontic Treatment in Patients with Antibiotic-resistant Microflora: A Preliminary Report

Aguinaldo S. Garcez, PhD,* Silvia C. Nuñez, PhD,[†] Michael R. Hamblin, PhD,^{‡,¶} Hideo Suzuki,* and Marbá S. Ribeiro, PhD[§]

Abstract

Introduction: This study reports the antimicrobial effect of photodynamic therapy (PDT) combined with endodontic treatment in patients with necrotic pulp infected with microflora resistant to a previous antibiotic therapy. **Methods:** Thirty anterior teeth from 21 patients with periapical lesions that had been treated with conventional endodontic treatment and antibiotic therapy were selected. Microbiological samples were taken (1) after accessing the root canal, (2) after endodontic therapy, and (3) after PDT. **Results:** All the patients had at least 1 microorganism resistant to antibiotics. PDT used polyethyleneimine chlorin(e6) as a photosensitizer and a diode laser as a light source (P = 40 mW, t = 4 minutes, E = 9.6 J). Endodontic therapy alone produced a significant reduction in numbers of microbial species but only 3 teeth were free of bacteria, whereas the combination of endodontic therapy with PDT eliminated all drug-resistant species and all teeth were bacteria-free. **Conclusions:** The use of PDT added to conventional endodontic treatment leads to a further major reduction of microbial load. PDT is an efficient treatment to kill multi-drug resistant microorganisms. (*J Endod* 2010;36:1463–1466)

Key Words

Antibiotic resistant bacteria, endodontic re-treatment, laser, photodynamic therapy

From the *Centro de Pesquisa e Pós-Graduação São Leopoldo Mandic, Campinas, SP, Brazil; [†]CETAD, São Paulo, SP, Brazil; [‡]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts; [§]Department of Dermatology, Harvard Medical School, Boston, Massachusetts; [¶]Harvard MIT Division of Health Science and Technology, Cambridge, Massachusetts; and [§]Center of Lasers and Applications, IPEN-CNEN/SP, São Paulo, SP, Brazil.

Address requests for reprints to Dr Aguinaldo Silva Garcez, São Leopoldo Mandic University, Campinas, SP, Brazil. E-mail address: garcez.segundo@terra.com.br.

0099-2399/10 - see front matter
Copyright © 2010 American Association of Endodontists.
doi:10.1016/j.joen.2010.06.001

Garcez AS, Nuñez SC, Hamblin MR, Suzuki H, Ribeiro MS. Photodynamic Therapy Associated With Conventional Endodontic Treatment In Patients With Antibiotic-resistant Microflora: A Preliminary Report. *J Endod*. 2010;36(9):1463-6

- Cilj: Ispitati utjecaj PDT – a u odnosu na konvencionalno ispiranje
- 30 zuba u 21 pacijenta
- Zaključak: PDT u svim uzorcima je dovela do eradikacije bakterijske flore

In the case of endodontic treatment failure, retreatment, surgical treatment, or extraction usually is carried out with the use of antibiotics and antiseptics as adjunctive therapies, but the long-term use of these agents can be rendered ineffective by resistance developing in the target organism (1). Currently, there is an emergence of bacteria with multiple resistances, and there is a need for alternative antimicrobial approaches (2–6).

The combination of conventional endodontic therapy and photodynamic therapy (PDT) has been shown as an effective approach in reducing bacterial load in *in vitro* and *in vivo* models (7–11).

This study investigated the combination of PDT with endodontic treatment in patients with necrotic pulp harboring microflora resistant to a previous antibiotic therapy.

Materials and Methods

Thirty teeth from 21 patients with periapical lesions who had been previously treated with endodontic treatment associated with antibiotic were selected. The patients were in good health and between the ages of 17 and 52 years. All the teeth presented signs and symptoms of periapical periodontitis and apical bone lesion detected by radiography, and some patients had pain by vertical percussion and/or local edema, all requiring root canal retreatment on teeth with closed apices. The same practitioner carried out this study in a private dental office in São Paulo, Brazil. The protocol was approved by the Institutional Review Board of the São Paulo University, and all procedures were conducted according to the principles of the Declaration of Helsinki.

Endodontic Treatment

Thirty root canals from anterior teeth were re-treated and received endodontic treatment followed by PDT. Microbiological samples were taken after accessing the root canal, after endodontic therapy, and after PDT. The first microbiological sample confirmed that all the patients had at least 1 microorganism resistant to antibiotic medication.

A periapical radiograph was taken for each case to determine the presence of apical lesion, the canal morphology, and its length.

The access to the pulp chamber was gained after installation of a rubber dam, and then the surrounding area received prophylactic asepsis and was irrigated with 5 mL of chlorhexidine solution at 2% to ensure that the crown of the tooth had minimal microbial load (8).

Once the canal was accessed, a Hedstrom file #15 (Maillefer Instruments SA, Ballaigues, Switzerland) was inserted inside the canal to remove the gutta-percha and root canal sealer obturation; then the root canal was irrigated with 1 mL of sterile saline solution. The canal was dried with 3 sterile paper points (Dentsply Latin America, Petropolis, Brazil) and left inside the root canal for 1 minute each. All 3 paper points were combined for microbiological analysis. This procedure was the first microbiological sampling representing the initial contamination. The paper points were deposited in a fresh sterile bottle with sterile nutrient broth.

Antimicrobial Photodynamic Therapy for the Treatment of Teeth with Apical Periodontitis: A Histopathological Evaluation

Lea Assed Bezerra Silva, DDS, MSc, PbD,* Artur B. Novaes, Jr, DDS, MSc, PbD,[†]

Rafael R. de Oliveira, DDS, MSc, PbD,[‡] Paulo Nelson-Filho, DDS, MSc, PbD,*

Milton Santamaria, Jr, DDS, MSc, PbD,* and Raquel Assed Bezerra Silva, DDS, MSc, PbD*

Abstract

Introduction: This study evaluated the *in vivo* response of apical and periapical tissues of dogs' teeth with apical periodontitis after one-session endodontic treatment with and without antimicrobial photodynamic therapy (aPDT). **Methods:** Sixty root canals with experimentally induced apical periodontitis were instrumented and assigned to 4 groups receiving aPDT and root canal filling (RCF) or not: group aPDT+/RCF+ (n = 20): aPDT (photosensitizer phenothiazine chloride at 10 mg/mL for 3 minutes and diode laser ($\lambda = 660$ nm, 60 mW/cm²) for 1 minute) and RCF in the same session; group aPDT+/RCF- (n = 10); group aPDT-/RCF+ (n = 20), and group aPDT-/RCF- (n = 10). Teeth were restored, and the animals were killed after 90 days. Sections from the maxillas and mandibles were stained with hematoxylin-eosin and Mallory trichrome and examined under light microscopy. Descriptive (ie, newly formed apical mineralized tissue, periapical inflammatory infiltrate, apical periodontal ligament thickness, and mineralized tissue resorption) and quantitative (ie, periapical lesion size and number of inflammatory cells) microscopic analysis was performed. Quantitative data were analyzed by the Kruskal-Wallis and Dunn tests ($\alpha = .05$). **Results:** In the aPDT-treated groups, the periapical region was moderately/severely enlarged with no inflammatory cells, moderate neoangiogenesis and fibrogenesis, and the smallest periapical lesions. **Conclusions:** Although apical closure by mineralized tissue deposition was not achieved, the absence of inflammatory cells, moderate neoangiogenesis, and fibrogenesis in the periapical region in the groups treated with aPDT indicate that this can be a promising adjunct therapy to cleaning and shaping procedures in teeth with apical periodontitis undergoing one-session endodontic treatment. (*J Endod* 2012;38:360–366)

Key Words

Antimicrobial photodynamic therapy, apical periodontitis, endodontic treatment

The effective control of bacterial infection in the root canal system is critical for the post-treatment success of endodontic therapy. It has been shown that the endodontic infection in teeth with pulp necrosis and apical periodontitis is of a polymicrobial nature with a high prevalence of anaerobic microorganisms, particularly gram-negative bacteria (1), which ultimately disseminate throughout the root canal system and reach the outer root surface (apical biofilm) (2). The extraradicular infection is inaccessible to the cleaning and shaping procedures, allowing persistence and multiplication of microorganisms and leading to low success rates when no antibiotic medication is used and one-appointment endodontic therapy is performed (3).

Photodynamic therapy (PDT) has emerged as a treatment strategy for eradicating target cells, involving the use of light of a specific wavelength to activate a nontoxic light-sensitive compound (known as photosensitizer) in the presence of oxygen (4, 5). The absorption of photons from the light source by the activated photosensitizer leads it to a triple state of excitation, resulting in energy or electron transfer to available molecular oxygen with consequent formation of highly reactive oxygen species (ROS), such as singlet oxygen and free radicals. This action produces a cascade of oxidative events that ultimately kill microorganisms by causing irreversible damage to essential intracellular molecules including proteins, membrane lipids, and nucleic acids (6). Photodynamic inactivation of microorganisms by local application of photosensitizer and light limits the action of ROS and avoids systemic harmful effects on "friendly" bacterial flora (7). In addition, unlike antibiotics, which have a single target in the microbial cell, ROS generated from the photodynamic reaction has a multifunctional nature and can damage multiple cellular structures, reducing the chances of the development of PDT-resistant bacterial strains (8).

As any treatment modality, antimicrobial PDT (aPDT) should ideally have the capacity to destroy the microorganisms responsible for the disease without causing damage to the host's surrounding healthy tissues. Low toxicity and rapidity of effect are desirable qualities of aPDT (9). It has been established that photosensitizers, which have a strong cationic charge, can rapidly bind and penetrate bacterial cells, and, thus, these compounds show a high degree of selectivity for microorganisms over host cells (10). However, even though studies (11, 12) have concluded that aPDT is less damaging to the host tissues, the concentration used for cytotoxicity assessment in these studies is usually lower than that of bacterial killing. Selectivity toward bacteria

Silva LA, Novaes AB Jr, De Oliveira RR, Nelson-Filho P, Santamaria M et al. Antimicrobial Photodynamic Therapy for the Treatment of Teeth with Apical Periodontitis: A Histopathological Evaluation. *J Endod*. 2012;38(3):360-6.

- Cilj: Upalni odgovor periapikalnog tkiva pasa nakon jednoposjetne endodoncije sa i bez PDT – a
- 30 zuba uključeno u istraživanje
- Zaključak: PDT skupina je pokazala najmanju periapikalnu leziju, umjerenu angiogenezu, fibrinogenezu, bez upalnih stanica

From the Departments of *Pediatric Dentistry, Preventive and Community Dentistry and †Oral and Maxillofacial Surgery and Traumatology and Periodontology, Dental School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

Address requests for reprints to Dr Lea Assed Bezerra Silva, Departamento de Clínica Infantil, Odontologia Preventiva e Social, Faculdade de Odontologia de Ribeirão Preto—Universidade de São Paulo, Av. do Café, s/n Monte Alegre, 14040-904, Ribeirão Preto-SP, Brazil. E-mail address: lea@fop.usp.br

0099-2399/5 - see front matter

Copyright © 2012 American Association of Endodontists.

doi:10.1016/j.joen.2011.12.023

The antimicrobial effectiveness of photodynamic therapy used as an addition to the conventional endodontic re-treatment: a clinical study

Ivona Bago Jurič¹, Vanda Plečko², Dragana Gabrić Pandurić³, Ivica Anić⁴

Affiliations + expand

PMID: 25461966 DOI: 10.1016/j.pdpdt.2014.10.004

Abstract

Background: The purpose of the study was to evaluate the efficacy of antimicrobial photodynamic therapy (aPDT) used as an adjunct to the endodontic re-treatment in the eradication of microorganisms from previously filled root canals.

Methods: The study sample consisted of 21 randomly selected patients with root filled and infected root canal system with chronic apical periodontitis on incisors or canines, who have had previously endodontic treatment. Microbiological samples from the root canals were collected after accessing the canal, following the endodontic re-treatment and after the aPDT procedure. During instrumentation, the root canals were irrigated with 2.5% sodium hypochlorite (NaOCl), and the final irrigation protocol included 17% ethylenediaminetetraacetic acid followed by NaOCl. Root canals were filled with a phenothiazinium chloride and irradiated with a diode laser ($\lambda=660$ nm, 100 mW) for 1 min. Microbiological samples from the root canals were cultivated on selective plates, and the identification was done by micromorphology, macromorphology and different API strips as well as bacterial counts (colony forming units).

Results: Fourteen bacteria species were isolated from the root canals initially, with a mean value of 4.57 species per canal. Although endodontic re-treatment alone produced a significant reduction in the number of bacteria species ($p<0.001$), the combination of endodontic treatment and aPDT was statistically more effective ($p<0.001$). No bacteria were cultivated from the main root canals of 11 teeth.

Conclusion: The results indicated that the aPDT used as an adjunct to the conventional endodontic therapy achieved a significant further reduction of intracanal microbial load.

Keywords: Endodontic re-treatment; Laser; Photodynamic therapy; Root canal infection.

Bago Jurič I , Plečko V, Gabrić Pandurić D, Anić I. The Antimicrobial Effectiveness of Photodynamic Therapy Used as an Addition to the Conventional Endodontic Re-treatment: A Clinical Study. Photodiagnosis Photodyn Ther. 2014;11(4):549-55.

- Cilj: ispitati adjuvantni učinak PDT – a u eradikaciji mikroorganizama
- Zaključak: Kombinacija s PDT – om je bila učinkovitija, kod 11 od 21 zuba došlo do potpune eradikacije bakterija

BIOKERAMIČKO PUNILO KORIJENSKIH KANALA

- Trikalcij fosfat, mineral trioksid (MTA) i trikalcij silikat
- Kalcij fosfat, MTA cementi, kalcij silikat
- Cirkonij oksid, trikalcij silikat, dikalcij silikat, koloidna silika, kalcij silikat, monobazični kalcij fosfat, kalcij hidroksid
- BioRoot RCS - trikalcij silikat, cirkonij dioksid, povidone



RESEARCH ARTICLE

Cytotoxic effects of four different root canal sealers on human osteoblasts

Susanne Jung¹, Sonja Sielker¹, Marcel R. Hanisch¹, Viktor Libricht^{1,2}, Edgar Schäfer², Till Damaschke^{2*}

1 Department of Cranio-Maxillofacial Surgery, Research Unit Vascular Biology of Oral Structures (VABOS), University Hospital Münster, Münster, Germany, 2 Department of Periodontology and Operative Dentistry, Westphalian Wilhelms-University, Münster, Germany, 3 Central Interdisciplinary Ambulance in the School of Dentistry, Münster, Germany

* tilda@uni-muenster.de

Abstract

The aim of this study was to evaluate the effect of an epoxy resin-based (AH-Plus), a zinc oxide eugenol containing (Pulp-Canal-Sealer) and two calcium silicate containing (MTA-Fillapex and BioRoot-RCS) sealers on primary human osteoblasts (hOB) in freshly mixed and set state. All sealers were mixed strictly according to the manufacturers' instructions and identically samples were produced. In a pretest cytotoxic sealer concentrations were determined. Thus, for the main cell culture study, dilutions of sealer extract 1:1, 1:2, and 1:10 were used. To simulate a clinical scenario, extracts from freshly mixed sealer were added to the cells on day one. Extracts from set sealers were used for subsequent culturing for 24h, 7d, 14d, and 21d. Cell viability was analyzed by living-cell-count, MTT-assay, and living/dead-staining, cytotoxicity by LDH-assay, and changes by Richardson-staining. All data were statistically evaluated by one way ANOVA and a posthoc analysis with Bonferroni-Holm testing ($p < 0.05$). AH-Plus was cytotoxic in a freshly mixed state, but not when the sealer was set. MTA-Fillapex and Pulp-Canal-Sealer were cytotoxic in a fresh as well as in a set state. BioRoot-RCS showed the lowest toxicity in both states; where as a regeneration of the cells could be observed over time ($p < 0.05$). Contact of freshly mixed AH-Plus to osteoblasts should be avoided. Pulp Canal Sealer and MTA-Fillapex showed no biocompatibility in contact with osteoblasts at all. BioRoot-RCS had a positive influence on the cell metabolism (bioactivity) and is biocompatible.

Introduction

The aim of a root canal filling is the three dimensional bacteria and fluid tight seal of the entire root canal system in order to prevent passage of microorganisms from coronal to apical or vice versa [1, 2]. Furthermore, root canal filling materials should be more or less insoluble to prevent dissolving by body fluids in the root canal. It is well known, that breakdown products from root canal sealers may have an adverse effect on the proliferative capability of periradicular cell populations [3]. It has to be kept in mind that, besides the apical foramen, numerous

Jung S, Sielker S, Hanisch MR, Libricht V, Schäfer E, Damaschke T. Cytotoxic Effects of Four Different Root Canal Sealers on Human Osteoblasts. Plos ONE.2018;13(3):e0194467

- Cilj: ispitati citotoksičnost 24h, 7 dana, 14 dana i 21 dan nakon miješanja
- Zaključak: BioRoot RCS (Septodont, Saint Maur Des Fosses, Cedex, France) pokazuje biokompatibilnost i nisku citotoksičnost



OPEN ACCESS

Citation: Jung S, Sielker S, Hanisch MR, Libricht V, Schäfer E, Damaschke T (2018) Cytotoxic effects of four different root canal sealers on human osteoblasts. PLoS ONE 13(3): e0194467. <https://doi.org/10.1371/journal.pone.0194467>

Editor: Chun-Pin Lin, National Taiwan University, school of dentistry, TAIWAN

Received: December 8, 2017

Accepted: March 2, 2018

Published: March 26, 2018

Copyright: © 2018 Jung et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Funding: We acknowledge support by Open Access Publication Fund of University of Münster for taking over the publication costs. No additional external funding was received for this study.

Competing interests: The authors have declared that no competing interests exist.

Biocompatibility and Mineralization Activity of Three Calcium Silicate-Based Root Canal Sealers Compared to Conventional Resin-Based Sealer in Human Dental Pulp Stem Cells

Deog-Gyu Seo ¹, Donghee Lee ², Yong-Min Kim ³, Dani Song ³ and Sin-Young Kim ^{3,*} 

¹ Department of Conservative Dentistry, Dentistry and Dental Research Institute, School of Dentistry, Seoul National University, Seoul 03080, Korea

² College of Medicine, The Catholic University of Korea, Seoul 06591, Korea

³ Department of Conservative Dentistry, Seoul St. Mary's Dental Hospital, College of Medicine, The Catholic University of Korea, Seoul 06591, Korea

* Correspondence: jeui99@catholic.ac.kr; Tel.: +82-2-2258-1787

Received: 1 July 2019; Accepted: 1 August 2019; Published: 5 August 2019



Abstract: The purpose of this study was to compare the cytotoxic effects and mineralization activity of three calcium silicate-based root canal sealers to those of a conventional resin-based sealer. Experiments were performed using human dental pulp stem cells grown in a monolayer culture. The root canal sealers tested in this study were EndoSequence BC Sealer (Brasseler), BioRoot RCS (Septodont), Endoseal MTA (Maruchi), and AH Plus (Dentsply DeTrey). Experimental disks 6 mm in diameter and 3 mm in height were made and stored in a 100% humidity chamber at 37 °C for 72 h to achieve setting. The cytotoxicity of various root canal sealers was evaluated using a methyl-thiazoldiphenyl-tetrazolium (MTT) assay. To evaluate cell migration ability, a scratch wound healing method was used, and images of the scratch area were taken using a phase-contrast microscope. Cell morphology was evaluated by a scanning electron microscope after direct exposure for 72 h to each sealer disk. In the cell viability assay, there were no significant differences between the EndoSequence BC, BioRoot RCS, Endoseal MTA, and control groups in any experimental period ($p > 0.05$). In the cell migration assay, there were no significant differences between the EndoSequence BC, Endoseal MTA, and control groups in any experimental period ($p > 0.05$). BioRoot RCS exhibited slower cell migration relative to EndoSequence BC and Endoseal MTA for up to 72 h ($p < 0.05$). Conversely, it showed a similar wound healing percentage at 96 h ($p > 0.05$). In an evaluation of cell morphology, cells in direct contact with EndoSequence BC, BioRoot RCS, and Endoseal MTA disks showed superior spreading compared to those in contact with the AH Plus disk. In an Alizarin red staining assay, EndoSequence BC, BioRoot RCS, and Endoseal MTA showed a significant increase in mineralized nodule formation compared to the AH Plus group ($p < 0.05$). In conclusion, all calcium silicate-based root canal sealers tested in this study showed good biological properties and mineralization activity compared to conventional resin-based sealer.

Keywords: cell viability; cell migration; scanning electron microscope; mineralization; calcium silicate-based sealer; conventional resin-based sealer

1. Introduction

The aim of root canal treatment is to provide three-dimensional obturation of the root canal system to prevent the entry of bacteria and fluid [1–3]. To provide hermetic sealing, core materials such as gutta-percha (GP) and root canal sealers are essential [4,5]. Sealers should be biocompatible

Seo D-g, Lee D, Kim Y-m, Song D, Kim S-y. **Biocompatibility and Mineralization Activity of Three Calcium Silicate-based Root Canal Sealers Compared to Conventional Resin-based Sealer in Human Dental Pulp Stem Cells.** *Materials* [Internet] 2019;12(15):2482. Available From: [10.3390/Ma12152482](https://doi.org/10.3390/Ma12152482)

- Cilj: Ispitivati tri kalcij – silikatna punila u odnosu na standard epoksi punilo
- Zaključak: Statistički značajna razlika u mineralizaciji svih kalcij – silikatnih punila u odnosu na AH+ (Dentsply Detrey)

CILJEVI DOKTORSKOG RADA

1. Ispitati utjecaj PDT-a, primijenjene na kraju kemomehaničke obrade kanala, na cijeljenje periapikalnog procesa kod pacijenata kod kojih je provedena revizija
2. Ispitati utjecaj punjenja korijenskog kanala biokeramičkim punilom na cijeljenje kronične periapikalne lezije
3. Ispitati utjecaj PDT-a, primijenjene na kraju kemomehaničke obrade kanala, na pojavnost i intenzitet postoperativne boli u odnosu na konvencionalni protokol dezinfekcije
4. Utjecaj punjenja korijenskog kanala biokeramičkim punilom na pojavnost postoperativne boli u usporedbi s punjenjem temeljenim na epoksi smoli

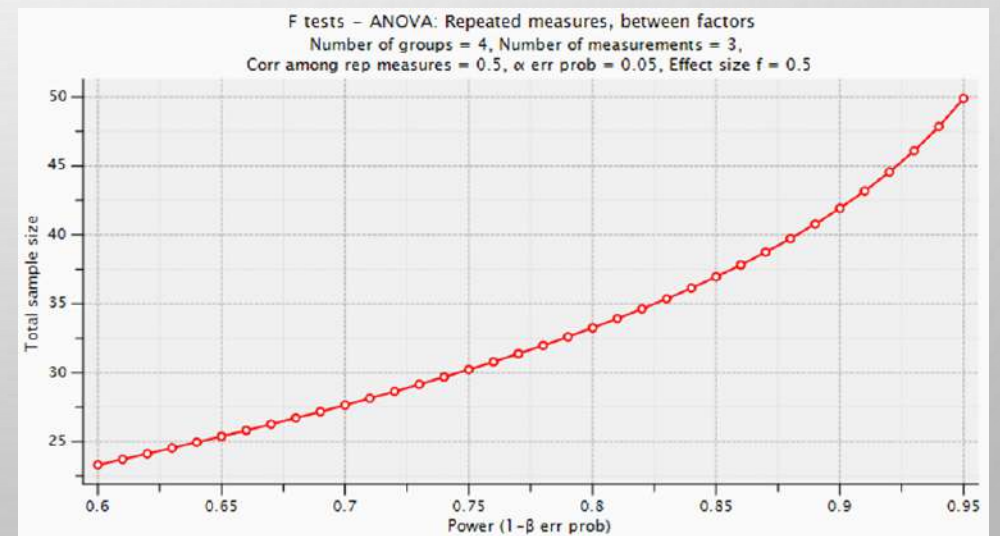
RADNE HIPOTEZE

1. Postoji statistički značajna razlika u cijeljenju periapikalne lezije kod punjenja kanala biokeramičkim punilom u odnosu na "zlatni standard" punjenja punilom baziranim na epoksi smoli
2. Postoji statistički značajna razlika u cijeljenju periapikalne lezije kod završnog protokola dezinfekcije s PDT-om u odnosu na pasivnu ultrazvučnu irigaciju
3. Postoji statistički značajna razlika u incidenciji postoperativne boli kod punjenja kanala biokeramičkim punilom u odnosu na punjenje bazirano na epoksi smoli
4. Postoji statistički značajna razlika u incidenciji postoperativne boli kod završnog protokola dezinfekcije s PDT-om u odnosu na pasivnu ultrazvučnu irigaciju

METODOLOGIJA I PLAN ISTRAŽIVANJA

- Kliničko istraživanje: Stomatološka poliklinika Zagreb lipanj 2019. – lipanj 2022.

Analizom snage testa : 4 ispitivane skupine, očekivani učinak veličine $f=0,5$, razina značajnosti $\alpha=0,05$ te snaga testa od 90%, u istraživanje je potrebno uključiti najmanje 44 uzoraka, odnosno 11 po skupini.



METODOLOGIJA I PLAN ISTRAŽIVANJA

SUDIONICI ISTRAŽIVANJA

- Redovni pacijenti upućeni radi revizije endodontskog liječenja na odjel EOPP Stomatološke poliklinike Zagreb
- Svi pacijenti potpisuju pisani informirani pristanak za sudjelovanje u istraživanju
- Odluka Etičkog povjerenstva Stomatološkog fakulteta o provođenju istraživanja
- Istraživanje se provodi u sklopu projekta HRZZ – a "Kliničko i eksperimentalno istraživanje laserski aktiviranog fotoakustičnog strujanja i fotoaktivirane dezinfekcije u endodontskom liječenju"
- Registracija protokola na clinicaltrials.gov id 021/002-19-208 NCT04072926

IZBOR PACIJENATA

- Klinički pregled, intraoralni rendgenogram
- Informirani pristanak
- Slučajni odabir (wheeldecide.com)
- Jednostruko slijepa studija

METODOLOGIJA I PLAN ISTRAŽIVANJA

KRITERIJI UKLJUČENJA PACIJENATA

- Simptomi periapikalne bolesti nakon prethodno provedenog endodontskog liječenja
- Zubi osjetljivi na perkusiju i palpaciju
- Sinus trakt i oticanje
- Asimptomatsko neadekvatno punjenje
- Periapikalne lezije veće od 5 mm

METODOLOGIJA I PLAN ISTRAŽIVANJA

KRITERIJI ISKLJUČENJA PACIJENATA

- Imunokompromitirani pacijenti
- Trudnoća
- Zubi s dubinom džepa većom od 3 mm
- Zubi sa znakovima i simptomima vertikalne frakture
- Zubi koje nije moguće restaurativno/protetski opskrbiti
- Antibiotiska terapija unutar posljednjih mjesec dana
- Akutni apikalni apsces

METODOLOGIJA I PLAN ISTRAŽIVANJA

RANDOMIZACIJA PACIJENATA

Dvije osnovne skupine ovisno o završnom protokolu dezinfekcije

Skupina 1: Fotodinamska terapija (PDT)

Podskupina A: Punjenje kanala BioRoot RCS punilom

Podskupina B: Punjenje kanala AH Plus punilom

Skupina 2: Pasivno ultrazvučno ispiranje (PUI)

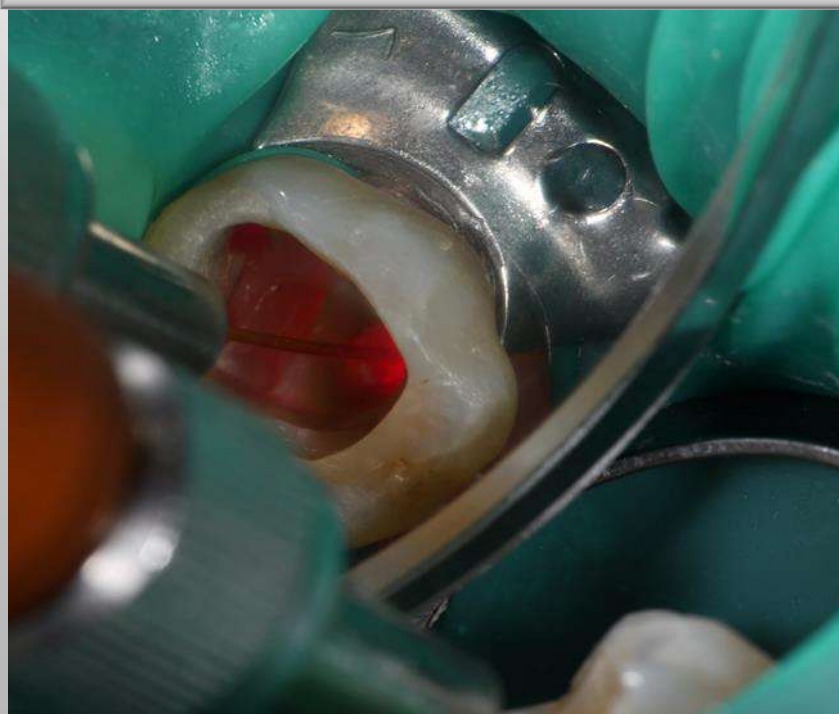
Podskupina A: Punjenje kanala BioRoot RCS punilom

Podskupina B: Punjenje kanala AH Plus punilom



KLINIČKI PROTOKOL

- Jednopusjetna revizija punjenja Endo – R (Micro-mega 5-12, Rue du Tunnel, 25006 Besancon Cedex, Francuska) strojnom obradom
- Završno punjenje single – cone TS2 (Micro-mega 5-12, Rue du Tunnel, 25006 Besancon Cedex, Francuska) gutaperkama i punilom
- Koronarni pečat FujilX (GC Corporation 3-2-14 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan)
- Radiološka kontrola



METODOLOGIJA I PLAN ISTRAŽIVANJA

KLINIČKI PROTOKOL- FOTODINAMSKA TERAPIJA

- Diodni laser (660nm, 100mW, 60 sec), fiberoptičko vlakno promjera 320 μ m
- PDT se provodi na kraju konvencionalnog ultrazvučno aktiviranog ispiranja
- Endo - PDT otopina toluidinskog modrila

METODOLOGIJA I PLAN ISTRAŽIVANJA

KLINIČKI PROTOKOL - PASIVNO ULTRAZVUČNO ISPIRANJE

- 2,5ml 3% NaOCl 30 sec pasivno
- 2ml 20% EDTA ultrazvučno aktivirano 60 sec
- 2,5ml 3% NaOCl ultrazvučno aktivirano 30 sec



METODOLOGIJA I PLAN ISTRAŽIVANJA

PROCJENA CIJELJENJA PERIAPIKALNOG PROCESA

- Radiološka volumetrijska procjena nakon godinu dana
- Egzaktni volumen u kubičnim milimetrima

Orstavik D. Time-course and risk analyses of the development and healing of chronic apical periodontitis in man. *Int Endod J.* 1996;29(3):150-5.

European Society of Endodontology. Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. *Int Endod J.* 2006;39(12):921-30.

METODOLOGIJA I PLAN ISTRAŽIVANJA

CBCT ANALIZA

Dvije CBCT snimke (FOV 5x5, 85 μ m veličina voxela, endomode):

1. Preoperativna CBCT snimka
2. Postoperativna, godinu dana nakon endodontskog liječenja
 - Klinički pregled, stanje restauracije, potreba za protetskim/kirurškim tretmanom
 - Segmentacija i mjerenje lezije u 3D mode – u OnDemand software - a
 - Redukcija veličine lezije kao mjera uspjeha zahvata

Opravdanost primjene CBCT - a

- Sve doze zračenja za pacijente bit će “as low as reasonably achievable” (ALARA)
- Učinkovita doza zračenja za mali FOV 13 - 44 μ Sv
- Zubi s lošom prognozom i velikom periapikalnom lezijom
- Primjena CBCT - a biti će prema uputama i pravilima Europskog endodontskog društva

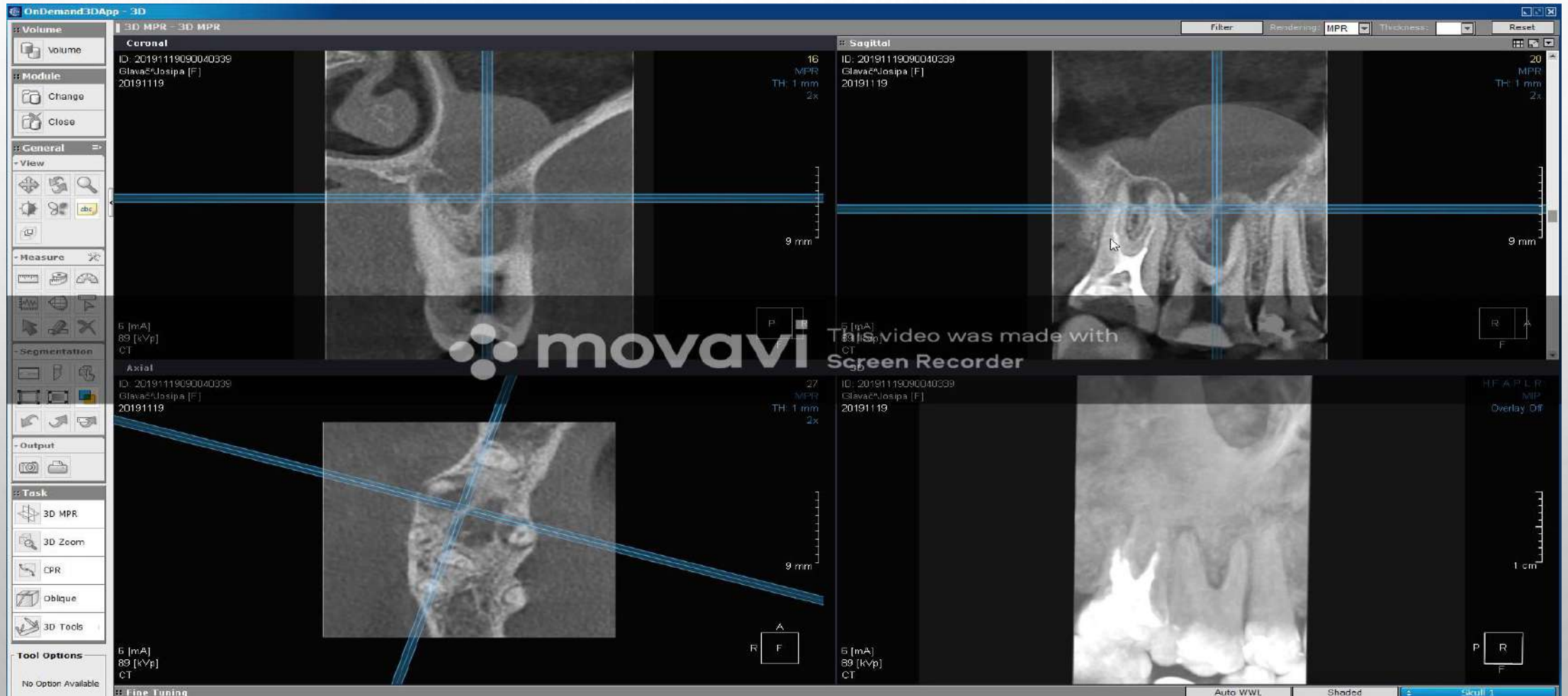
Kralik I, Faj D, Lauc T, Skarica M, Popic J, Brkic H. Dose area product in estimation of effective dose of the patients undergoing dental cone beam computed tomography examinations. J. Radiol Prot.2018; 38(4):1412-27.

Patel S, Brown J, Semper M, Abella F, Mannocci F. European Society of Endodontology position statement: Use of cone beam computed tomography in Endodontics: European Society of Endodontology (ESE) developed by. Int Endod J. 2019 Dec;52(12):1675-78.

Davies A, Mannocci F, Mitchell P, Andiappan M, Patel S. The detection of periapical pathoses in root filled teeth using single and parallaxperiapical radiographs versus cone beam computed tomography – a clinical study. Int Endod J. 2015;48(6):582-92.

METODOLOGIJA I PLAN ISTRAŽIVANJA

SEGMENTACIJA (3D MODE ONDEMAND SOFTWARE)



ANALIZA CIJELJENJA

- Da bi se postigla ponovljivost i točnost mjerenja:
- 30% CBCT – a će se ponovo mjeriti nakon mjesec dana
- 30% CBCT – a će mjeriti druga dva ispitivača uključujući radiologa

KATEGORIJE ISHODA ENDODONTSKOG LIJEČENJA

Rud J, Andreasen JO, Möller Jensen JE. Radiographic criteria for the assessment of healing after endodontic surgery. Int J of Oral Surg. 1972;1(4):195-214

- Nova periapikalna radiolucencija
- Povećana periapikalna radiolucencija
- Nepromijenjena periapikalna radiolucencija
- Smanjena periapikalna radiolucencija

IZVORNI ZNANSTVENI DOPRINOS ISTRAŽIVANJA

- Utjecaj primijenjenih metoda (PDT) i materijala (BioRootRCS) na cijeljenje periapikalne lezije
- Incidencija i intenzitet postoperativne boli ovisno o primijenjenim metodama/materijalima
- CBCT dijagnostika kao preciznija metoda evaluacije
- Učinkovitost, rizik, prognostički čimbenici, terapijske opcije
- Informirana odluka o terapiji na temelju dobivenih podataka