Innovative Insights in Decontamination and Healing During Endodontic Treatment

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DOI:
10.33612/diss.1220804222

Summary

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The presence of microorganisms in biofilm form, along with their toxins within the root canal system, contributes to the development and persistence of apical periodontitis. Consequently, eliminating or reducing microbial loads to a safe level for periapical tissues presents a significant challenge, as bacteria can become lodged deep within dentine tubules or in areas of anatomical complexity. **Chapter 1** offers an overview of how endodontic infections occur and the pathways microorganisms take through the root canal system, where they thrive and function as biofilms. It also highlights the complexities of the canal's anatomy. Understanding how the characteristics of the root canal influence disinfection during endodontic treatment is essential. Furthermore, this chapter discusses the antimicrobial strategies employed for disinfecting and cleaning the root canal spaces, as root canal irrigation, intracanal medication, and brings some insights about adjunctive strategies like the application of natural antimicrobial compounds and nanoparticles (NPs) as potential candidates for enhancing disinfection in endodontic procedures.

In this regard, **Chapter 2** focuses on irrigation procedures using different chelating agents, followed by adjunctive mechanical agitation steps for root canals: ultrasonic activation and the use of the XP-Endo Finisher instrument. In this study, we evaluated the main canals, and the intratubular decontamination ability of sodium hypochlorite (NaOCl) followed by ethylenediaminetetraacetic acid (EDTA); a mixture of NaOCl with hydroxyethylidene bisphosphonate (HEBP); and NaOCl followed by EDTA-T (EDTA with sodium lauryl ether sulfate). All groups of teeth were subjected to ultrasonic activation or agitation by the XP-Endo Finisher instrument using saline solution. Colony-forming units (CFU)/mL counts were determined, and bacterial intratubular viability was analyzed via confocal laser scanning microscopy (CLSM) using Live/Dead staining. CFU/mL counting indicated equally effective decontamination across the experimental groups.

According to microscopy images, the use of irrigation solutions followed by agitation with the XP-Endo Finisher yielded better results.

In **Chapter 3**, we evaluated the physicochemical properties of calcium hydroxide (CH) pastes formulated with different vehicles, considering that CH is the most used intracanal medication between endodontic treatment sessions. The properties investigated included pH, volumetric alteration, antimicrobial action, and effects on biofilm matrix polysaccharides. *Enterococcus faecalis* was chosen for microbiological tests. Bacterial viability and extracellular matrix were quantified through direct contact evaluation (dentine discs) and at the intratubular level (dentine cylinders) using LIVE/DEAD BacLight and Calcofluor White dyes via CLSM. UltraCal XS and Metapex, which contain aqueous and oily vehicles respectively, exhibited lower values for the extracellular matrix. The pH of all CH pastes decreased over time and did not promote medium alkalization for up to 30 days. The CH pastes evaluated were able to reduce bacterial viability both through direct contact and at the intratubular level.

In **Chapter 4**, an investigation was conducted on the effects of natural antimicrobial compounds propolis (PRO) and copaiba oil-resin (COR), in combination with hydrocortisone (H), compared to Otosporin®. The study assessed cytotoxicity, genotoxicity, cytokine detection, and toxicity using the invertebrate *Galleria mellonella* model. Human periodontal ligament fibroblasts (PDLFs) were exposed to various drug concentrations and evaluated using the MTT assay. Drug combinations were tested at concentrations that did not compromise cell density. Genotoxicity was assessed through micronucleus counting, while cytokines IL-6 and TGF-β1 were detected in the cell supernatant using ELISA. PRO and COR showed a potential to promote PDLF proliferation in MTT tests, even when combined with H. Due to these proliferative effects, molecular docking simulations were performed based on the major compounds identified in PRO, COR, and H. Finally, increasing concentrations of PRO and COR were evaluated for acute toxicity in the *Galleria*

mellonella model. No changes in cell metabolism were observed in relation to cytokine levels of TGF-β1 and IL-6. The tested materials appeared to induce the release of AT_1R, potentially linked to PDLF proliferation through specific interactions. PRO and COR exhibited low toxicity in larvae, indicating safety at the tested levels.

In **Chapter 5**, we investigated disinfection strategies during root canal treatment. The antimicrobial efficacy of PRO and COR was compared to conventional agents in endodontics. Their effectiveness was tested against pathogens using macrodilution assays to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The biofilm-killing efficacy was assessed using two dual-species biofilms: Enterococcus faecalis (ATCC 29212) and *Streptococcus mutans* (ATCC 20523), as well as *Streptococcus oralis* (J22) and *Actinomyces naeslundii* (T14V-J1), which were grown on dentine discs. At the intratubular level (dentine cylinders), dentine tubule contamination was established with E. faecalis and S. mutans. The specimens were then exposed to the antimicrobials to simulate their use in various stages of root canal treatment, with bacterial viability quantified using Live/Dead staining via CLSM. Biofilm characteristics and the immediate removal of the *S. oralis* and *A. naeslundii* biofilm model were evaluated using optical coherence tomography (OCT) and CFU/cm^2 counting. PRO and COR demonstrated antimicrobial effects, indicating their potential as complementary approaches in root canal treatment to effectively reduce microbial load.

In **Chapter 6**, we studied bismuth sulfide nanoparticles (Bi_2S_3 NPs) nucleated by polyethyleneimine (PEI) for enhanced drug delivery in biomedical applications. The focus was on PEI-Bi_2S_3 NPs synthesized with varying PEI contents and their effects against planktonic bacteria and biofilms, particularly their penetration capabilities. Characterization techniques, including dynamic light scattering (DLS), transmission electron microscopy (TEM), and X-ray photoelectron spectroscopy

(XPS), revealed that the NPs were spherical in shape, with a significant reduction in size for those with higher PEI content. The antimicrobial activity was assessed under near-infrared (NIR) irradiation against *Streptococcus oralis* and *Actinomyces naeslundii*, showing moderate effects on planktonic cells but limited efficacy against biofilms. Penetration assays demonstrated that both 25 and 100 PEI-Bi_2S_3 NPs could infiltrate biofilms after 10 and 100 minutes of exposure. We concluded that higher PEI coating enhances the stability of PEI-Bi_2S_3 NPs, with penetration influenced by electrostatic interactions with the biofilm matrix, providing insights for optimizing antimicrobial NPs development.

Building on the results from the studies presented in the previous chapters, **Chapter 7** offers a comprehensive discussion of the findings related to antimicrobial strategies investigated. It explores alternatives for irrigation and intracanal medication, alongside the synthesis, characterization, and penetration testing of PEI-Bi_2S_3 NPs. Additionally, the chapter outlines future perspectives based on these studies, aimed at improving disinfection in root canal treatments, potentially facilitating the healing of apical periodontitis and maintaining system' homeostasis.

Summary

Samenvatting

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De aanwezigheid van micro-organismen in een biofilm draagt, samen met hun toxines, binnen het wortelkanaalsysteem bij aan de ontwikkeling en het in stand houden van apicale parodontitis. Het elimineren of reduceren van microbiële belasting tot een veilig niveau voor de periapicale weefsels vormt dan ook een aanzienlijke uitdaging, aangezien bacteriën zich diep in de dentine tubuli of anatomisch complexe gebieden kunnen nestelen. **Hoofdstuk 1** biedt een overzicht van het ontstaan van endodontische infecties en de route die micro-organismen volgen door het wortelkanaal, waar ze gedijen en functioneren in de vorm van biofilms. Het belicht ook de anatomische complexiteit van het wortelkanaal. Essentieel is ook het begrijpen van de beïnvloeding van de kenmerken van het wortelkanaal op de desinfectie tijdens endodontische behandelingen. Daarnaast bespreekt dit hoofdstuk de antimicrobiële strategieën die worden toegepast voor het desinfecteren en reinigen van de wortelkanaalruimtes, zoals wortelkanaalirrigatie en intrakanaal medicatie, en biedt het enkele inzichten over aanvullende strategieën, waaronder de toepassing van natuurlijke antimicrobiële verbindingen en nanodeeltjes nanoparticles (NP's) als potentiële kandidaten voor het verbeteren van de desinfectie in endodontische procedures.

In dit opzicht richt **Hoofdstuk 2** zich op irrigatieprocedures met verschillende chelerende middelen, gevolgd door aanvullende mechanische agitatiestappen voor het wortelkanaal: het gebruik van ultrageluid en XP-Endo Finisher-instrument. In deze studie hebben we, in zowel de hoofdkanalen als de intratubulaire kanalen, gekeken naar het decontaminatievermogen van natriumhypochloriet (NaClO) gevolgd door ethyleendiaminetetra-azijnzuur (EDTA); een mengsel van NaClO met hydroxyethylideen-difosfonaat (HEDP); en NaClO gevolgd door EDTA-T (EDTA met natriumlaurylethersulfaat). Alle groepen tanden werden onderworpen aan ultrageluidactivatie of agitatie met het XP-Endo Finisher-instrument, met behulp van een zoutoplossing. Het aantal kolonievormende eenheden Colony vorming units

(CFU)/mL werd bepaald en de levensvatbaarheid van de intratubulaire bacteriën werd geanalyseerd via confocale laserscanmicroscopie (CLSM) met een levend/dood kleuring. Het aantal kolonievormende eenheden toonde aan dat de decontaminatie in alle experimentele groepen even effectief was. Volgens microscopische beelden werden betere resultaten bereikt bij het gebruik van gebruik van irrigatie-oplossingen gevolgd door agitatie met de XP-Endo Finisher.

In **Hoofdstuk 3** hebben we de fysisch-chemische eigenschappen onderzocht van calciumhydroxide (CH)-pasta's geformuleerd met verschillende draagstoffen, aangezien CH het meest gebruikte intrakanaal medicijn is als overbrugging tussen endodontische behandelingssessies. De onderzochte eigenschappen omvatten pH, volumetrische veranderingen, antimicrobiële werking en het effect op de polysachariden van de biofilm matrix. De microbiologische testen zijn uitgevoerd met behulp van *Enterococcus faecalis*. De kwantificatie van bacteriële levensvatbaarheid en extracellulaire matrix werd uitgevoerd middels direct contact (dentine schijven) en op intratubulair niveau (dentine cilinders) met behulp van LIVE/DEAD BacLight en Calcofluor White-kleurstoffen via confocale laserscanmicroscopie (CLSM). UltraCal XS en Metapex, die een draagstof op respectievelijk water- of oliebasis bevatten, gaven lagere waarden voor de extracellulaire matrix. De pH van alle CH-pasta's nam over tijd af en stimuleerden geen alkalisatie van het medium gedurende maximaal 30 dagen. De onderzochte CH-pasta's waren in staat de levensvatbaarheid van bacteriën te verminderen, zowel via direct contact als op intratubulair niveau.

In **Hoofdstuk 4** werd een onderzoek uitgevoerd naar de effecten van de natuurlijke antimicrobiële verbindingen propolis (PRO) en copaiba olie (COR), in combinatie met hydrocortison (H), vergeleken met Otosporin®. De studie evalueerde cytotoxiciteit, genotoxiciteit, detectie van cytokines en toxiciteit met behulp van het ongewervelde *Galleria mellonella* model. Humane parodontale ligament fibroblasten (PDLF's) werden blootgesteld aan verschillende concentraties

geneesmiddelen en geëvalueerd met de MTT-test. Combinaties van geneesmiddelen werden getest bij concentraties die geen invloed hadden op de celdichtheid. Genotoxiciteit werd beoordeeld door het tellen van micronuclei en de cytokines IL-6 en TGF-β1 werden gedetecteerd in het celsupernatant middels ELISA. Zowel PRO en COR toonden potentie om de proliferatie van PDLF's in MTT-tests te bevorderen, zelfs wanneer gecombineerd met H. Wegens deze proliferatieve effecten werden simulaties van moleculaire docking uitgevoerd, gebaseerd op de belangrijkste verbindingen die in PRO, COR en H werden geïdentificeerd. Ten slotte werden oplopende concentraties van PRO en COR geëvalueerd voor acute toxiciteit in het *Galleria mellonella* model. Er werden geen veranderingen in de cellulaire stofwisseling waargenomen met betrekking tot de cytokineniveaus van TGF-β1 en IL-6. De geteste verbindingen leken de afgifte van AT_1R te induceren, wat mogelijk gelinkt is met PDLF-proliferatie middels specifieke interacties. PRO en COR vertoonden lage toxiciteit in larven, wat indiceert dat de geteste concentraties veilig zijn.

In **Hoofdstuk 5** onderzochten we desinfectie strategieën tijdens wortelkanaalbehandelingen. De antimicrobiële werkzaamheid van PRO en COR werd vergeleken met conventionele middelen in de endodontologie. De effectiviteit werd getest tegen pathogenen met behulp van een macrodilutie testen om de minimum remmende concentratie (MIC) en minimum bacteriedodende concentratie (MBC) te bepalen. De biofilm-dodende werkzaamheid werd beoordeeld met behulp van twee biofilms bestaande uit 2 soorten: *Enterococcus faecalis* (ATCC 29212) en *Streptococcus mutans* (ATCC 20523), evenals de combinatie van *Streptococcus oralis* (J22) en *Actinomyces naeslundii* (T14V-J1), die werden gekweekt op dentine schijven. Op intratubulair niveau (dentine cilinders) werd besmetting van de dentine tubuli opgezet met *E. faecalis* en *S. mutans*. De monsters werden vervolgens blootgesteld aan de antimicrobiële middelen om hun gebruik in verschillende stadia van de wortelkanaalbehandeling te simuleren, waarbij de bacteriële levensvatbaarheid werd gekwantificeerd met behulp van Live/Dead-kleuring via

CLSM. Kenmerken van de biofilm en de onmiddellijke eliminatie van de gecombineerde *S. oralis* en *A. naeslundii* biofilm werden geëvalueerd met behulp van optische coherentietomografie (OCT) en CFU/cm²-telling. PRO en COR toonden antimicrobiële effecten, wat een aanduiding is voor hun potentie als complementaire strategie in wortelkanaalbehandelingen om zo de microbiële belasting effectief te verminderen.

In **Hoofdstuk 6** bestudeerden we bismutsulfide nanodeeltjes (Bi_2S_3 NPs) gekernd door polyethyleenimine (PEI) voor verbeterde medicijnafgifte in biomedische toepassingen. De focus lag op PEI-Bi_2S_3 NPs, gesynthetiseerd met variërende PEI-inhoud, en de effecten van de NPs op planktonische bacteriën en biofilms, met name hun penetratiecapaciteiten. Technieken ter karakterisatie, zoals dynamische lichtverstrooiing (DLS), transmissie-elektronenmicroscopie (TEM) en röntgen-photoelektronenspectroscopie (XPS), onthulden dat de NPs sferisch van vorm waren, waarbij de grootte significant afnam naarmate de PEI-inhoud hoger werd. De antimicrobiële activiteit werd beoordeeld met nabij-infrarood (NIR) bestraling tegen *Streptococcus oralis* en *Actinomyces naeslundii*, wat een effect gaf op planktonische cellen maar een beperkte werkzaamheid tegen biofilms. Onderzoeken naar de penetratie toonden aan dat zowel 25 als 100 PEI-Bi_2S_3 NPs in staat waren om biofilms te infiltreren na respectievelijk 10 en 100 minuten blootstelling. We concludeerden dat een hogere PEI-coating de stabiliteit van PEI-Bi_2S_3 NPs verbetert, waarbij de penetratie wordt beïnvloed door elektrostatische interacties met de biofilm matrix, hetgeen inzichten biedt voor het optimaliseren van de ontwikkeling van antimicrobiële NPs.

Op basis van de resultaten van de in de voorgaande hoofdstukken gepresenteerde studies biedt **Hoofdstuk 7** een uitgebreide discussie van de bevindingen met betrekking tot de onderzochte antimicrobiële strategieën. Het onderzoekt alternatieven voor irrigatie en intrakanaal medicijngebruik, naast de synthese, karakterisering en penetratie testen van PEI-Bi_2S_3 NPs. Daarnaast schetst

het hoofdstuk toekomstige perspectieven op basis van deze studies, gericht op het verbeteren van de desinfectie tijdens wortelkanaalbehandelingen met als doel de genezing van apicale parodontitis te versnellen en de homeostase van het systeem te behouden.

Sumário

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A presença de microrganismos na forma de biofilme, junto com suas toxinas no sistema de canais radiculares, contribui para o desenvolvimento e a persistência da periodontite apical. Consequentemente, eliminar ou reduzir as cargas microbianas a um nível seguro para os tecidos periapicais apresenta um desafio significativo, pois as bactérias podem se alojar profundamente nos túbulos dentinários ou em áreas de complexidade anatômica. O **Capítulo 1** oferece uma visão geral de como ocorrem as infecções endodônticas e os caminhos percorridos pelos microrganismos no sistema de canais radiculares, onde eles prosperam e funcionam como biofilmes. Também destaca as complexidades da anatomia do canal. Compreender como as características do canal radicular influenciam a desinfecção durante o tratamento endodôntico é essencial. Além disso, este capítulo discute as estratégias antimicrobianas empregadas para desinfecção e limpeza dos espaços do canal radicular, como a irrigação dos canais, a medicação intracanal, e traz algumas percepções sobre estratégias complementares, como a aplicação de compostos antimicrobianos naturais e nanopartículas (NPs) como potenciais candidatas para aprimorar a desinfecção nos procedimentos endodônticos.

Nesse sentido, o **Capítulo 2** aborda os procedimentos de irrigação utilizando diferentes agentes quelantes, seguidos de etapas de agitação mecânica adjuntiva para os canais radiculares: ativação ultrassônica e o uso do instrumento XP-Endo Finisher. Neste estudo, avaliamos os canais principais e a capacidade de descontaminação intratubular do hipoclorito de sódio (NaOCl) seguido de ácido etilenodiamino tetra-acético (EDTA); uma mistura de NaOCl com hidroxi-etilideno difosfonato (HEBP); e NaOCl seguido de EDTA-T (EDTA com lauril éter sulfato de sódio). Todos os grupos de dentes foram submetidos à ativação ultrassônica ou agitação pelo instrumento XP-Endo Finisher utilizando solução salina. Foram determinadas as contagens de unidades formadoras de colônias (UFC)/mL, e a viabilidade bacteriana intratubular foi analisada por microscopia confocal de varredura a laser (MCVL)

utilizando a coloração Live/Dead. A contagem de UFC/mL indicou descontaminação igualmente eficaz entre os grupos experimentais. De acordo com as imagens de microscopia, o uso de soluções de irrigação seguidas de agitação com o XP-Endo Finisher apresentou melhores resultados.

No **Capítulo 3**, avaliamos as propriedades físico-químicas de pastas de hidróxido de cálcio (HC) formuladas com diferentes veículos, considerando que o HC é a medicação intracanal mais utilizada entre as sessões de tratamento endodôntico. As propriedades investigadas incluíram pH, alteração volumétrica, ação antimicrobiana e efeitos sobre os polissacarídeos da matriz do biofilme. *Enterococcus faecalis* foi escolhido para os testes microbiológicos. A viabilidade bacteriana e a matriz extracelular foram quantificadas por meio de avaliação de contato direto (discos de dentina) e no nível intratubular (cilindros de dentina) usando os corantes LIVE/DEAD BacLight e Calcofluor White via MCVL. UltraCal XS e Metapex, que contêm veículos aquoso e oleoso, respectivamente, apresentaram valores menores para a matriz extracelular. O pH de todas as pastas de HC diminuiu com o tempo e não promoveu alcalinização do meio por até 30 dias. As pastas de HC avaliadas foram capazes de reduzir a viabilidade bacteriana tanto por contato direto quanto no nível intratubular.

No **Capítulo 4**, foi conduzida uma investigação sobre os efeitos dos compostos antimicrobianos naturais própolis (PRO) e óleo-resina de copaíba (COR), em combinação com a hidrocortisona (H), em comparação ao Otosporin®. O estudo avaliou a citotoxicidade, genotoxicidade, detecção de citocinas e toxicidade utilizando o modelo invertebrado de *Galleria mellonella*. Fibroblastos do ligamento periodontal humano (FLPDs) foram expostos a várias concentrações de drogas e avaliados por meio do ensaio de MTT. As combinações de drogas foram testadas em concentrações que não comprometessem a densidade celular. A genotoxicidade foi avaliada por meio da contagem de micronúcleos, enquanto as citocinas IL-6 e TGF-β1 foram detectadas no sobrenadante celular por ELISA. PRO e COR mostraram

potencial para promover a proliferação de FLPDs nos testes de MTT, mesmo quando combinados com H. Devido a esses efeitos proliferativos, simulações de docking molecular foram realizadas com base nos principais compostos identificados em PRO, COR e H. Por fim, concentrações crescentes de PRO e COR foram avaliadas quanto à toxicidade aguda no modelo de *Galleria mellonella*. Não foram observadas alterações no metabolismo celular em relação aos níveis de citocinas TGF-β1 e IL-6. Os materiais testados parecem ter induzido a liberação de AT_1R, potencialmente ligada à proliferação de FLPDs por meio de interações específicas. PRO e COR exibiram baixa toxicidade em larvas, indicando segurança nos níveis testados.

No **Capítulo 5**, investigamos estratégias de desinfecção durante o tratamento de canal radicular. A eficácia antimicrobiana de PRO e COR foi comparada a agentes convencionais utilizados na endodontia. A eficácia desses compostos foi testada contra patógenos por meio de ensaios de macrodiluição para determinar a concentração inibitória mínima (CIM) e a concentração bactericida mínima (CBM). A eficácia contra biofilmes foi avaliada utilizando dois biofilmes de espécies duplas: *Enterococcus faecalis* (ATCC 29212) e *Streptococcus mutans* (ATCC 20523), assim como *Streptococcus oralis* (J22) e *Actinomyces naeslundii* (T14V-J1), que foram cultivados em discos de dentina. No nível intratubular (cilindros de dentina), a contaminação dos túbulos dentinários foi estabelecida com *E. faecalis* e *S. mutans*. Os espécimes foram então expostos aos antimicrobianos para simular seu uso em várias etapas do tratamento de canal, com a viabilidade bacteriana quantificada por meio de coloração Live/Dead via MCVL. As características dos biofilmes e a remoção imediata do modelo de biofilme de *S. oralis* e *A. naeslundii* foram avaliadas utilizando tomografia de coerência óptica (OCT) e contagem de UFC/cm^2. PRO e COR demonstraram efeitos antimicrobianos, indicando seu potencial como abordagens complementares no tratamento de canal para reduzir efetivamente a carga microbiana.

No **Capítulo 6**, estudamos nanopartículas de sulfeto de bismuto (NPs de Bi_2S_3) nucleadas com polietilenimina (PEI) para aprimorar a liberação de medicamentos em aplicações biomédicas. O foco foi nas NPs de PEI-Bi_2S_3 sintetizadas com diferentes quantidades de PEI e seus efeitos contra bactérias planctônicas e biofilmes, especialmente suas capacidades de penetração. As técnicas de caracterização, incluindo espalhamento de luz dinâmico (DLS), microscopia eletrônica de transmissão (TEM) e espectroscopia de fotoelétrons de raios-X (XPS), revelaram que as NPs eram esféricas, com uma redução significativa no tamanho para aquelas com maior teor de PEI. A atividade antimicrobiana foi avaliada sob irradiação de infravermelho próximo (NIR) contra *Streptococcus oralis* e *Actinomyces naeslundii*, mostrando efeitos moderados sobre células planctônicas, mas atividade limitada contra biofilmes. Ensaios de penetração demonstraram que tanto as NPs de 25 PEI-Bi_2S_3 quanto as de 100 PEI-Bi_2S_3 conseguiram infiltrar nos biofilmes após 10 e 100 minutos de exposição, embora a intensidade da fluorescência diminuísse nas camadas mais profundas, indicando penetração limitada. Concluímos que o maior conteúdo de PEI melhora a estabilidade das NPs de PEI-Bi_2S_3, com a penetração sendo influenciada por interações eletrostáticas com a matriz do biofilme, fornecendo conhecimento para otimizar o desenvolvimento de NPs carreadoras de agentes antimicrobianos.

Com base nos resultados dos estudos apresentados nos capítulos anteriores, o **Capítulo 7** oferece uma discussão abrangente sobre as estratégias antimicrobianas investigadas. Explora alternativas para irrigação e medicação intracanal, além da síntese, caracterização e testes de penetração de NPs de PEI-Bi_2S_3. Além disso, o capítulo apresenta perspectivas futuras baseadas nesses estudos, visando melhorar a desinfecção no tratamento de canais radiculares, potencialmente facilitando a cura da periodontite apical e mantendo a homeostase do sistema.

Acknowledgements

Acknowledgements

Before beginning to write this section, I spent some time reflecting, contemplating the blank space on this page, and recalling the long journey I have taken since starting my postgraduate studies in Belém, my hometown. I realized that this thesis reflects the journey along that road. Naturally, I remembered the people who helped me and made it all possible, feeling deeply grateful to have met incredible people in both Brazil and the Netherlands. This reflection presents a fitting opportunity to paraphrase a writer whom I greatly admire, J.R.R. Tolkien:

Many times, he used to say that there was only one Road; that it was like a great river: its springs were at every doorstep, and every path was its tributary. 'It's a dangerous business, Frodo, going out of your door,' he used to say. 'You step onto the Road, and if you don't keep your feet, there's no knowing where you might be swept off to.'

I first thank God for the opportunities I have been given in life and for providing a complex universe that compels us to keep studying to understand it.

To Our Lady of Nazareth, for being present in my family's history and for providing protection and assistance, especially in the moments when I needed it most.

To my supervisor in Brazil, **Prof. Dr. Flaviana Bombarda de Andrade**, for all the knowledge she has shared with me. There were countless moments of learning—through lectures, clinical activities, laboratory work, and research—when I realized I had a remarkable professional by my side, often providing opportunities and sharing invaluable life and professional experiences. Thank you for being that person on my journey. We are friends for life!

To my co-supervisor in Brazil, **Prof. Dr. Maria Cristina Marcucci**, for all the information and support provided for conducting experiments with natural antimicrobial compounds, which was pivotal to the development of my PhD research.

To my supervisor in the Netherlands, **Prof. Dr. Prashant Kumar Sharma**, for whom I have great admiration and with whom I feel privileged to have had the opportunity to work closely. Thank you for your punctuality, honesty, and constant willingness to listen whenever I needed it—and, of course, for supervising me throughout my journey in Groningen. I am also grateful for the opportunity to study at the University Medical Center Groningen, in the Department of Biomaterials and Biomedical Technology (BBT). This experience broadened my perspective as a researcher and enriched my view of the world and life.

To my co-supervisor in the Netherlands, **Prof. Dr. Mohammad-Ali Shahbazi**, for his guidance in my work, his assistance in the laboratory whenever I needed it, for all the knowledge he shared with me, and for guiding me throughout my learning process.

To **Prof. Dr. Carla Renata Sipert**, for all the assistance and guidance during several months of experiments at USP, in São Paulo, as well as for the enjoyable conversations, through which I came to appreciate the wonderful person and professional you are. Thank you so much!

To **Letícia Martins Santos**, for all her assistance during my experiments in São Paulo, for her friendship, and for the enjoyable moments we shared both in the laboratory and beyond. Thank you for everything.

To **Prof. Dr. Juliana Campos Junqueira**, for all the supervision during the in vivo experiments of my project at UNESP in São José dos Campos, where I had a wonderful experience working with the *Galleria mellonella* invertebrate model. Thank you so much.

To **Maíra Terra Garcia**, for sharing her knowledge and providing invaluable assistance during my experiments in São José dos Campos, and for the enjoyable moments we shared both in and out of the laboratory. Thank you for everything.

Acknowledgements

To my first supervisor in Endodontics, **Prof. Dr. Patrícia de Almeida Rodrigues**, who has guided me since my bachelor's degree and through my master's program at UFPA in Belém. Thank you for all the knowledge you shared, which was essential in shaping my choice of specialization, and for all the enjoyable moments we shared both within and beyond the university. I consider myself a lifelong learner of yours and am grateful to have gained not only a mentor but a friend for life. Thank you so much for everything!

To **Prof. Dr. Osmar Alves Lameira**, from EMBRAPA in Belém, for his support and guidance, as well as for the knowledge he shared with me regarding natural antimicrobial compounds, which was essential in designing several experiments for this thesis.

To **Márcia Sirlene Zardin Graeff**, for all her assistance with the Laser Scanning Confocal Microscope in Bauru, and for making my workdays enjoyable throughout my experiments. Thank you for being there during the times I needed it most.

To **Carolina Passarelli Gonçalves**, for her invaluable assistance and collaboration on the molecular docking aspect of my project, as well as for the knowledge she generously shared with me.

To **Ramón Andrés Pineda Álvarez** and **Kiyan Musaie**, for collaborating during different stages of my laboratory work and for the great times we shared at the BBT at UMCG. Thank you as well for all the knowledge you shared.

I would like to express my gratitude for the invaluable support provided by the technicians' team at BBT, especially **Marja Slomp**, for her assistance and involvement in my research during my time in Groningen. I truly appreciate the enjoyable moments we shared in the lab and her brilliant ideas when I struggled to find solutions to various problems.

I cannot overlook the invaluable support of another technician who aided me during my laboratory journey, **Willem Woudstra**. Thank you so much for all the help you provided. Your presence at BBT made my days much easier, knowing I could come to your office for assistance—or sometimes just to annoy you, which was always fun. Thank you for everything.

To all the technicians who supported my research at BBT: **Hans**, **Jelly**, **Ed**, **Willy**, **Gésinda**, and **Joop**. Thank you so much for all your assistance with my experiments.

To **Laís Ribovski**, for the conversations and helpful insights that assisted me. Thank you for being a part of this journey!

To **Prof. Dr. Henny van der Mei**, for providing me with some materials that facilitated my pilot experiments with biofilms at BBT.

To **Prof. Dr. Jelmer Sjollema**, for the opportunity to collaborate on microbiological experiments involving optical coherence tomography at BBT.

To **René Dijkstra**, for sharing many valuable insights and protocols that greatly assisted me.

To my Italian friend and one of my paranymphs, **Liliana Agresti**, for all the enjoyable moments we shared during my time in Groningen, the incredible experiences, and the fun times together. Thank you for always being there to listen when I needed it most. I miss our daily coffee breaks and having you as my neighbor like we were in Groningen. We need to take more pictures together in Rome soon. Non perdere mai il tuo splendore, mia amica! Grazie mile!

To my Colombian friend and paranymph, **Jhon Alexander Ramirez Idarraga**, whom I enjoyed disturbing whenever possible. Thank you for all the great moments at BBT, the conversations in our office, the fun tours around Groningen, and all the good times we shared. It was wonderful to share experiences and some funny stories from my life with you. ¡Muchas gracias, Cabezón!

Acknowledgements

To my Dutch friend, **Elles Boonstra**, who was always available to help me when I needed it most with her thoughtful approach and creative ideas for solving problems. It was truly fortunate to have you on my journey in the Netherlands and to share the same office at BBT. Thank you for all the great moments. Heel erg bedankt!

To **Jordy Alexander Larco Lasso**, for the friendship and all the enjoyable moments in Groningen and at BBT. I am already missing our walks in the city center. ¡Muchas gracias por tu amistad!

To the Brazilian group in Groningen, including my friends from BBT: **Veridianna Pattini**, **Clara Maas**, and **Beatriz Fumelli**, as well as other Brazilians: **Isaura Borges**, **Ana Beatriz Nasser**, and **Lucas de Freitas**. Thank you all for the wonderful moments and life experiences we shared. Your presence was truly important to me.

To **Mirela Cesar Barros**, for all your participation, collaboration, friendship, and the enjoyable times we had during our research projects and wonderful moments at the university and beyond. Muito obrigado por tudo!

To **Evelyn Giuliana Velásquez Espedilla**, thank you for your participation, collaboration, and friendship, especially during crucial moments of my PhD journey. I am also deeply grateful for all the good moments we shared. ¡Muchas gracias por tu amistad!

To all my friends from the postgraduate program at Bauru School of Dentistry: **Nailson Meneses**, **Maricel Cuellar**, **Pedro César Titato**, **Jéssica Coelho**, and many others with whom I shared time at the Endodontics Department. Thank you for all the enjoyable moments and the knowledge we shared.

To my friend **Vitor Muniz**, who welcomed me into his home in São Paulo during the months I was conducting experiments there. Thank you so much, my friend, for all the support, the laughter, the conversations, and the walks in that huge city that I love. Muito obrigado, meu amigo, tu mereces o mundo!

To all the members of the Assessment and Promotion Committee, thank you for taking the time to read and evaluate this thesis. I truly appreciate it.

I want to express my deepest gratitude to **Gabriel Aiello**, with whom I shared most of my PhD journey in Bauru and the Netherlands, even across the distance. His presence was essential and made my days brighter, providing the light, strength, and motivation I needed to keep going. I am so grateful to have him in my life; he has been the greatest surprise on my path. Thank you for everything—for standing by me, for all the support, and for the wonderful person you are!

I must also mention his family—**Marisa dos Santos**, **Acindino**, **Luciana**, and **Ignês Ferreira** (Vó Lola, *in memoriam*)—who became a second family to me and brought joy to my days while I was far from home. You are all unforgettable! Thank you for everything!

To all my friends from Belém—how I missed you all throughout this journey. Despite the distance, I never felt abandoned by you! I want to express my gratitude to: **Amanda Macedo**, **Carlos André**, **Carlos Guzzo Jr**., **Caroline Oliveira**, **Eduardo Auad**, **Francisco Jr**., **Gustavo Cardins**, **Letícia Lopes**, **Letícia Frade**, **Lucas Patrocínio**, **Maurício Solon**, **Marcelo Figueiredo**, **Rita de Cássia Ferreira**, **Sammla Castilho**, **Victor Fróes**, and **William Moraes**. Thank you all for staying close and supporting me along the way, even before my postgraduate studies.

To my grandparents (*in memoriam*), for the foundation you provided in shaping the person I am today. I feel that, in some way, each of you has been part of this journey. Thank you for everything!

To my parents and sister—**Maria Auxiliadora**, **Edison**, and **Camila**—thank you for all the support, understanding, and for being there during the most important moments and decisions of my life. I am grateful for the pride you have in me, even when my strength felt like it was faltering. I am part of you all, and you are my strength as well! Thank you for the sacrifices you have made to support me on my

professional path, and to my parents, I would like to especially thank you for teaching me to be a good person, to have faith, and for instilling the principles that guide me. You are my greatest example. I love you all!

To my uncles and aunts—**Paulo** and **Madalena**, **Ana** and **Peter**, and my aunt **Márcia**—thank you for all the assistance and support you provided whenever I needed it. You have always been a part of this journey. Thank you so much!

And to all my relatives who supported me, bringing happiness and positive energy to my life in so many ways and at so many points along this path—thank you all!

Once again, in the words of J.R.R. Tolkien, '*Not all those who wander are lost*.' With your presence, I am grateful to know that I was not alone.

Curriculum Vitae and Publications

Curriculum Vitae

Victor Feliz Pedrinha was born on the 18th of November 1992 in Belém, Pará, Brazil. At the age of 19, he began studying dentistry at the Faculty of Dentistry, Federal University of Pará (UFPA), located in his hometown. He engaged in various research projects during his bachelor's degree and served as a monitor for endodontic disciplines both in the laboratory and in clinical practices at the university. His undergraduate thesis was also focused on Endodontics.

In 2017 he embarked on his master's degree in dental clinic, concentration area in Endodontics, at the Postgraduation Program of Odontology, Faculty of Odontology, UFPA, conducting his experimental work at the Bauru School of Dentistry, University of São Paulo (FOB-USP). Relocating to Bauru, São Paulo, in 2019, he pursued specialization in Endodontics at the Bauru Foundation for Dental Studies (FUNBEO). A few months later, he commenced his Ph.D., participating in a double degree program between the University of São Paulo and the University of Groningen.

During the research phase in Brazil, he utilized various methodologies, conducting parts of his work at different universities such as the Faculty of Dentistry at the University of São Paulo (FO-USP), São Paulo, and the Institute of Science and Technology at São Paulo State University (UNESP), São José dos Campos. The research was sponsored by the Coordination of Higher Education and Post-Graduation (Capes) during the period conducted in Brazil and the Abel Tasman Talent Project (ATTP) from the Graduate School of Medical Sciences, University of Groningen, during his stays in the Netherlands.

Publications

1. **Pedrinha VF**, Barros MC, Portes JD, Slomp AM, Woudstra W, Lameira OA, Queiroga CL, Marcucci MC, Shahbazi MA, Sharma PK, Andrade FB. Antimicrobial Efficacy of Alternative Root Canal Disinfection Strategies: An Evaluation on Multiple Working Models (2025). *Biomedicine & Pharmacotherapy* 183, p. 117833.

2. Barros MC, **Pedrinha VF,** Oliveira FE, Marcucci MC, Gomes BPF, Oliveira LD, Andrade FB. Decrease from main root canal and intratubular Fusobacterium nucleatum and its endotoxin after ultrasonic activation of conventional and alternative irrigation solutions (2024). *Biofouling*, v. 2024, p. 1-11.

3. **Pedrinha VF**, Santos LM, Goncalves CP, Garcia MT, Lameira OA, Queiroga CL, Marcucci MC, Shahbazi MA, Sharma PK, Junqueira JC, Sipert CR, Andrade FB (2024) Effects of natural antimicrobial compounds propolis and copaiba on periodontal ligament fibroblasts, molecular docking, and in vivo study in Galleria mellonella. *Biomedicine & Pharmacotherapy* 171, 116139.

4. Cuellar MRC, Pereira TC, Vasconcelos LRSM, **Pedrinha VF**, Vivan RR, Duarte MAH, Andrade FB (2024) Reducing Apical Bacterial Extrusion: The Impact of Reciproc File Size and Irrigation Technique. *Iranian Endodontic Journal* 19, 176-182.

5. Cunha LMA, Shinomiya AS, Sá LL, Miranda ARLS, **Pedrinha VF**, Rodrigues PA (2024) Influence of different ultrasonic inserts and vehicles

associated with calcium hydroxide on intracanal medication remotion. *Dental Press Endodontics* 14, 75-96.

6. Melo WWP, **Pedrinha VF**, Silva LCOA, Gomes TC, Miranda ARLS, Rodrigues PA (2023) Comparison of different devices for removal of calcium hydroxide associated with two types of vehicles in simulated internal root reabsorption cavities. *Dental Press Endodontics* 13, 62-86.

7. Ramos MLG, **Pedrinha VF**, Barros MC, Bezerra RM, Andrade FB, Kuga MC, Vaz LG (2023) Antimicrobial effect of Pentaclethra Macroloba plant extract against Enterococcus Faecalis. *Brazilian Journal of Biology* 83, e272095.

8. Barros MC, **Pedrinha VF**, Graeff MSZ, Bramante CM, Duarte MAH, Andrade FB (2022) A new model of in vitro dentin intratubular contamination for Fusobacterium nucleatum: Validation by confocal laser scanning microscopy. *Heliyon* 9, e18042.

9. Silva LCOA, **Pedrinha VF**, Melo WWP, Gomes TC, Miranda ARLS, Rodrigues PA (2022) Tissue Dissolution Action of Different Irrigating Solutions Activated with Ultrasonics in Roots with Simulated Internal Resorption. *Dental Press Endodontics* 12, 74-81.

10. Barros MC, **Pedrinha VF**, Espedilla EGV, Cuellar MRC, Andrade FB (2022) Aerosols generated by high-speed handpiece and ultrasonic unit during endodontic coronal access alluding to the COVID-19 pandemic. *Scientific Reports* 12, 4783.

11. **Pedrinha VF**, Cuellar MRC, Barros MC, Titato PCG, Shahbazi MA, Sharma PK, Andrade FB (2022) The vehicles of calcium hydroxide pastes interfere with antimicrobial effect, biofilm polysaccharidic matrix and pastes? physicochemical properties. *Biomedicines* 10, 1-13.

12. Prescinoti R, Bramante CM, Bramante AS, Garrido LMA, **Pedrinha VF**, Andrade FB (2022) An unusual repair of perforating internal inflammatory root resorption; a case report of endodontic treatment. *Journal of Research in Dentistry* 10, 16-20.

13. **Pedrinha VF**, Cuellar MRC, Espedilla EGV, Duarte MAH, Andrade FB, Rodrigues PA (2021) Impact of irrigation protocols with some chelators and mechanical agitation on intratubular decontamination. *Brazilian Oral Research* 35, 1-12.

14. Silva A, Alencar CM, Jassé FA, **Pedrinha VF**, Zaniboni J, Dantas A, De Campos E, Kuga M (2021) Effect of post-space irrigation with acid solutions on bond strength and dentin penetrability using a self-adhesive cementation system. *Journal of Clinical and Experimental Dentistry* 13, e564-e571.

15. **Pedrinha VF**, Alencar CM, Jasse FFA, Zaniboni J, Dantas AAR, Andrade FB, Kuga MC (2021) Effect of the several epoxy resin-based sealer compositions on adhesion interface in radicular dentin after calcium hydroxide intracanal medication removal. *Journal of Clinical and Experimental Dentistry* 13, e913-e919.

16. Mendez DAC, Cuellar MRC, **Pedrinha VF**, Espedilla EGV, Andrade FB, Rodrigues PA, Cruvinel T (2021) Effects of curcumin-mediated

antimicrobial photodynamic therapy associated to different chelators against Enterococcus faecalis biofilms. *Photodiagnosis and Photodynamic Therapy* 35, 102464.

17. Titato PCG, Zancan RF, **Pedrinha VF**, Andrade FB, Vivan RR, Duarte MAH (2020) Influence of EDTA and its Association with Benzalkonium Chloride on Enterococcus faecalis Adhesion to Dentin. *International Journal of Odontostomatology* 14, 632-638.

18. Cuellar MRC, Espedilla EGV, **Pedrinha VF**, Vivan RR, Duarte MAH, Andrade FB (2020) Can Kinematics, file diameter and PUI influence the intracanal decontamination and apical bacterial extrusion? *Brazilian Oral Research* 35, 1-10.

19. Rodrigues PA, Nassar RSF, Silva TS, **Pedrinha VF**, Alexandrino LD (2019) Effects of Different NaOCl Concentrations Followed by 17% EDTA on Dentin Permeability. *The Journal of Contemporary Dental Practice* July, 838-841. 19.

20. Pontes FSC, Souza LL, **Pedrinha VF**, Pontes HAR (2018) Congenital ranula: A case report and literature review. *Journal of Clinical Pediatric Dentistry* 42, 1-4.
21. **Pedrinha VF**, Brandao JMS, Pessoa OF, Rodrigues PA (2018) Influence of File Motion on Shaping, Apical Debris Extrusion and Dentinal Defects: A Critical Review. *The Open Dentistry Journal* 12, 189-201.

22. Nogueira BML, Pereira TIC, **Pedrinha VF**, Rodrigues PA (2018) Effects of Different Irrigation Solutions and Protocols on the Mineral Content and

Ultrastructure of Root Canal Dentine. *Iranian Endodontic Journal* 13, 209-215.

23. Dominguez MCL, **Pedrinha VF**, Silva LCOA, Ribeiro MES, Loretto SC, Rodrigues PA (2018) Effects of Different Irrigation Solutions on Root Fracture Resistance: An in Vitro Study. *Iranian Endodontic Journal* 13, 367-372.

24. Alencar CM, **Pedrinha VF**, Araujo JLN, Esteves RA, Silveira ADS, Silva CM (2017) Effect of 10% Strontium Chloride and 5% Potassium Nitrate with Fluoride on Bleached Bovine Enamel. *The Open Dentistry Journal* 2017, 476-484.

25. Guimarães DM, Nascimento LS, **Pedrinha VF**, Pereira GG, Paradela CRF, Pontes FSC, Pontes HAR (2016) Avascular necrosis of the jaws as initial presentation of acute leukemia. *Quintessence International* 47, 791-796.