Innovative Insights in Decontamination and Healing During Endodontic Treatment

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Chapter 1

General Introduction and Aim of this Thesis

Chapter 1

General Introduction

Endodontic treatment, also known as root canal treatment (RCT), aims to achieve wound healing by removing the source of infection and promoting favorable conditions for the transition of chronic inflammatory tissue into reparative tissue [1,2]. It happens since RCT promotes the reduction of intracanal bacterial load, and the root canal system is sealed to prevent the passage of pulpal antigens to the periapical tissues [2,3]. In this context, apical periodontitis emerges as an inflammatory response associated with pathogens (microorganisms) and their toxins that occupy the root canal system, presenting a high global prevalence [2,4].

The development of apical periodontitis necessitates contact between the vital pulp tissue and various oral microorganisms originating from sources such as dental caries, accidental trauma, inadequate restorations, pulp exposure, and other irritants [5-7]. In the absence of timely intervention, microbial colonization leads to necrosis of dental pulp tissue and the proliferation of infection through the root canal system, thereby impacting the periapical region of the teeth [8]. Activation of the host's immune response results in local acute and/or chronic inflammation, causing resorption and destruction of periapical tissues, ultimately leading to the formation of periapical lesions [9,10].

Apical periodontitis can develop even in previously root canal-treated teeth. Despite meticulous clinical procedures performed under aseptic conditions and the application of proper root canal filling techniques, periapical lesions may persist [11,12]. This persistence is often linked to the anatomical complexities of the root canal system, which includes regions that cannot be effectively debrided or obturated using current instruments, materials, and techniques. These inaccessible areas provide niches for persistent microbial infections [2,11].

Since infectious agents are implicated in the causation of apical periodontitis [2,4], it is important to comprehend the organization of microorganisms and how they are structured along the root canal system. Endodontic microbiota can include

fungi, archaea, and viruses, but bacteria are the most prevalent and dominant microorganisms [13]. They are usually observed as sessile multispecies communities, known as biofilms, attached to the dentinal root canal walls [9,13-15].

Biofilms structurally comprise sessile microbial communities adhered to surfaces. They consist of cells entangled within a self-produced matrix of extracellular polysaccharide substance (EPS), resulting in an altered phenotype regarding growth rate and gene expression [16]. The EPS matrix of biofilms serves a vital function in shielding bacteria from chemical and mechanical stresses [17], thereby playing a pivotal role in bacterial survival [18]. Considering the biofilm organization, bacterial interactions play an essential role in determining the overall virulence of the community, influencing the pathogenicity of apical periodontitis and its persistence [19].

The microbiome demonstrates variations according to infection types, which are linked to the condition of apical periodontitis [13]. In cases of primary apical periodontitis resulting from infection of the necrotic dental pulp, mixed bacterial communities primarily comprise obligate anaerobic species. These include several Gram-negative bacteria such as *Fusobacterium nucleatum*, *Porphyromonas endodontalis*, and *Tannerella forsythia*, along with several Gram-positive bacteria such as *Actinomyces*, *Streptococcus*, *Propionibacterium,* and *Lactobacillus* species. Within bacterial community, 20 to 30 species often compose the core microbiome [13,20-25].

In the case of a post-treatment apical periodontitis, a periapical lesion persists due to persistent or secondary intra-radicular infections. Within these scenarios, a combination of anaerobic and facultative bacteria may be detected, indicating a mixed infection [13,26]. Nonetheless, the microbiota tends to be less diverse compared to teeth affected by primary disease, particularly those with apparently adequate root canal fillings. Teeth with prior inadequate treatments exhibit bacterial richness akin to primary infections [26,27]. Between frequently

bacteria, *Streptococcus* and *Actinomyces* species are detected in abundance in post-treatment infections [26-28], and *Enterococcus faecalis* is among the most prevalent species found in the canals of teeth with post-treatment apical periodontitis [28].

It is worth noting that other conditions can present different microbial communities, such as those associated with different thirds of the root canal system. The types of microbial species present could be very different or even completely different in some teeth, considering the middle/coronal and apical thirds [13,29]. Despite the number of species present being very similar, the apical third of the root canal system is considered a critical zone because bacteria that cause apical periodontitis are mostly located in this region [2,13,29].

The chemomechanical preparation is a pivotal stage of RCT, being considered the main mechanism responsible for the elimination of biofilms [2], wherein chemical substances (irrigation solutions) are used in combination to the mechanical action of instruments (endodontic files) during the preparation of root canal walls [2,30-32]. These procedures aim to achieve efficient cleaning, canal shaping, debridement, and consequently reducing bacteria to levels that are compatible with periapical tissue healing [2,30,32].

Sodium hypochlorite (NaOCl) is the irrigation solution which has the highest disinfectant potential due to its organic dissolution and antimicrobial properties [31,32], considered as the gold standard for irrigation in Endodontics [33]. However, the mechanical action of endodontic files shaping and debriding the root canal walls and the chemical action of NaOCl in the organic content of dentine and pulp tissue remnants create a smear layer during this stage, which is a combination of organic and inorganic material [34]. This layer harbors bacteria and their by-products and obstructs access to dentinal tubules and could potentially shield microbiota within root dentine from antiseptic agents [35,36]. Moreover, the presence of the smear layer hampers the proper adaptation of root canal sealers to dentine walls [30,35,36].

The ethylenediaminetetraacetic acid (EDTA) is the most used chelating agent that eliminates the inorganic constituents of the smear layer [30,35]. Other chelating solutions are feasible to be utilized such as EDTA-T, a blend of EDTA and sodium lauryl ether sulfate. This combination enhances the effectiveness of EDTA by reducing surface tension and increasing antiseptic activity [37]. Additionally, combining a chelator with NaOCl is an alternative option, such as hydroxyethylidene bisphosphonate, known as etidronate (HEBP). Although HEBP is considered a weaker chelating agent compared to EDTA, it effectively reduces debris accumulation and removes inorganic components from the smear layer while preserving the properties of NaOCl and not interfering with its action [31,38].

However, the action of endodontic instruments and irrigation solutions during chemomechanical preparation is not capable of promoting a total antisepsis in the root canal system since untouched surfaces of canals may contain remaining microorganisms [2]. In pulp necrosis, not only bacteria, but also bacterial components can induce apical periodontitis [39], with higher levels of virulence factors [39]. Decontaminating all root canal space is a challenge mainly because smaller zones beyond the main root canal (Figure 1), such as lateral and apical ramifications [14] and the deep of dentinal tubules [3,40].

One strategy to complement disinfection of the root canal system is the use of intracanal medications between treatment sessions. Calcium hydroxide (CH) pastes are often the preferred approach to enhance disinfection or prevent the possibility of reinfection [40]. These pastes act through the contact of CH and the walls of the root canal system, providing alkalinization of the internal surfaces of the roots and neutralizing acidic products from the inflammatory process, facilitating tissue repair and antimicrobial activity [41,42].

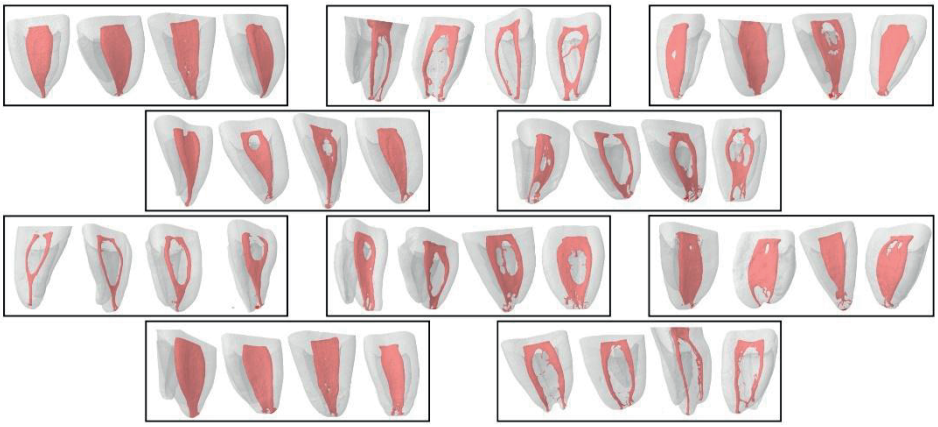

Figure 1. Images obtained by microcomputed tomography (micro-CT) and reconstructed in 3D for visualization of the anatomy of mesial root canals of mandibular molars, demonstrating the complexity of the root canal system.

Acknowledgement: Image kindly provided by Dr. Marcelo Haas Villas Bôas, adapted from: Villas Bôas, M. H. (2011). *Análise comparativa em microtomografia do preparo dos canais mesiais de molares inferiores por quatro sistemas rotatórios de níquel-titânio*. Master's Dissertation, Faculdade de Odontologia de Bauru, University of São Paulo, Bauru. doi:10.11606/D.25.2011.tde-09122011-095822. Retrieved 2024-07-16, from www.teses.usp.br

CH pastes are commercially available in different types of vehicles such as water soluble (aqueous and viscous), and non-water-soluble vehicles (oily) [43]. The vehicles associated with CH are known to be harmless to microorganisms; however, they can influence the properties of these medications [40-42]. Outcomes such as environmental alkalinization and antimicrobial action vary depending on the vehicle present in the CH paste formulation [40-43]. Then, to extrapolate the use of each CH paste for a clinical scope, it is necessary to know their properties while they are filling the main root canal. In other words, while there is contact between the CH paste and the root canal walls, it is important to consider a satisfactory interval between endodontic treatment sessions, depending on each case [40,42,43].

It is important to highlight that some cases presenting a superficial infection in the pulpal tissues are also possible, wherein the radicular pulp tissue is vital and inflamed and the root canal system is not entirely contaminated. It happens because microorganisms and their byproducts are restricted to the superficial pulp tissue, in contact with the microbial contamination source [44,45].

When pulp inflammation progresses to an irreversible stage, known as irreversible pulpitis, pulp extirpation and subsequent RCT are widely regarded as the first-line management by many clinicians to achieve immediate relief from painful symptoms [46]. The European Society of Endodontology (ESE) strongly recommends performing these procedures as part of a single-session RCT for vital pulp teeth [47]. However, the need for multiple sessions may arise in various situations, such as a lack of time to complete the RCT in a single visit, patient preferences [48], limitations in clinical planning, or limited operator experience. Additionally, RCT may require more than one session in cases where painful symptoms persist or when the root canal cannot be dried due to abundant bleeding [49], which is linked to hypervascularization and compromised pulpal hemostasis resulted from inflammation [50,51].

In such conditions, an emergency procedure for temporary pain relief may involve the removal of the affected portion of the pulp and the application of commercially available drugs containing a combination of antibiotics and corticosteroids. This approach provides simultaneous antibacterial and anti-inflammatory effects [52-54], helping to minimize the risk of infection during the interval between sessions and improving clinical outcomes by reducing inflammation and pain before completing the RCT [52,53,55]. This strategy differs from pulp capping procedures, which aim to preserve the vitality of the remaining pulpal tissue by maintaining an aseptic working field and applying a biomaterial directly onto the exposed pulp, followed by the immediate placement of a permanent restoration [47,56].

The utilization of this type of medication for such cases raises questions, particularly since the infectious condition is confined to a limited superficial area. The use of antibiotics may foster the selection of resistant bacteria, which can acquire the ability to exchange resistance genes, rendering them impervious to prescribed antibiotics [57,58]. This dilemma encourages the study and development of alternative strategies. For this reason, the concept of combining natural antimicrobial compounds with corticosteroid presents a promising and viable alternative.

Propolis (PRO), also known as bee glue, is a resinous substance collected by honeybees from various plants and used within their hives [59,60]. It possesses antibacterial properties akin to antibiotic medications [61], as well as significant anti-inflammatory and antioxidant properties due to the presence of flavonoids and other phenolic compounds [59,60]. Another noteworthy substance is copaiba oil-resin (COR), sourced from the Brazilian Amazon and extracted from trees of the genus *Copaifera*, also known as copaibeiras [62]. This resin exhibits anti-inflammatory and healing properties [63], as well as antimicrobial effects attributed to its constituents, including diterpenes and sesquiterpenes [63-65].

PRO has been proposed as a valuable substance for adjunctive disinfection of the root canal system due to its demonstrated anti-inflammatory and antibacterial activities [40,66]. Similarly, COR has demonstrated potential therapeutic properties for dental applications, including antimicrobial action against oral pathogens [63,65]. Not limited to intracanal medication possibilities, strategies could involve the use of PRO or COR to provide adjunctive disinfection of root canal systems within the scope of irrigation solutions [65-67].

These strategies aim to eliminate microorganisms since remaining biofilms after chemomechanical preparation may pose a risk of recurring apical periodontitis [68]. Moreover, root canal irrigation with NaOCl also presents disadvantages, such as toxicity to periapical tissues [69] and its proteolytic action on dentine tissues, leading to depletion of organic components and dentine moisture, which contributes

to tooth fragility [70]. However, it is important to note that the introduction of alternative solutions aims to provide complementary disinfection in RCT with nontoxic substances, targeting microorganisms and their byproducts not reached during chemomechanical preparation. Given the variety of endodontic microbiota, it is necessary to make as many biofilm comparisons as possible to provide consistent evidence about the antimicrobial performance of natural compounds and usual agents in Endodontics [22,40,42,71,72].

Due to the EPS matrix, an effective penetration of most antimicrobials is impeded [73,74]. This makes achieving satisfactory microbial elimination more challenging in the root canal system [71]. It is estimated that from 2050, antimicrobial-resistant bacterial infection may become the number one cause of death [75] with a massive socioeconomical impact [76]. The development of nanoparticles (NPs) that could diffuse and penetrate deep into biofilms appears to be an alternative since they could carrier antimicrobial agents deep into different biofilms [74,77,78].

The effectiveness of metallic nanoparticles such as Bismuth (Bi) based nanomaterials has been investigated [79,80]. In this context, bismuth sulfide (Bi_2S_3) NPs exhibit biocompatibility properties and have garnered significant attention in biomedical applications, being explored for drug delivery, photodynamic therapy, and to restore the sensitivity of bacteria with drug-resistant genes to prescribed antibiotics [80-82]. As a strategy to enhance the delivery of therapeutic agents, Polyethyleneimine (PEI) is often used in Bi NPs [82]. Investigating the penetration of PEI-Bi_2S_3 NPs into an endodontic biofilm may pave the way for further strategies to eliminate biofilms.

Chapter 1

Aim of this Thesis

The objective of this thesis is to explore disinfection protocols at various stages of endodontic treatment, including irrigation solution protocols, intracanal medications, and the investigation of alternative approaches such the application of natural antimicrobial compounds and nanoparticle penetration into biofilms.

References

[1]. Trowbridge HO. Immunological aspects of chronic inflammation and repair. J Endod. 1990;16(2):54-61.

[2]. Siqueira JF Jr, Pérez AR, Marceliano-Alves MF, Provenzano JC, Silva SG, Pires FR, Vieira GCS, Rôças IN, Alves FRF. What happens to unprepared root canal walls: a correlative analysis using micro-computed tomography and histology/scanning electron microscopy. Int Endod J. 2018;51(5):501-508.

[3]. Childs DR, Murthy AS. Overview of Wound Healing and Management. Surg Clin North Am. 2017;97(1):189-207.

[4]. Tibúrcio-Machado CS, Michelon C, Zanatta FB, Gomes MS, Marin JA, Bier CA. The global prevalence of apical periodontitis: a systematic review and meta-analysis. Int Endod J. 2021;54(5):712-735.

[5]. Kahler B, Taha NA, Lu J, Saoud TM. Vital pulp therapy for permanent teeth with diagnosis of irreversible pulpitis: biological basis and outcome. Aust Dent J. 2023;68 Suppl 1:S110-S122.

[6]. Levin LG, Law AS, Holland GR, Abbott PV, Roda RS. Identify and define all diagnostic terms for pulpal health and disease states. J Endod. 2009;35(12):1645-57.

[7]. Duncan HF. Present status and future directions-Vital pulp treatment and pulp preservation strategies. Int Endod J. 2022;55(Suppl 3):497-511.

[8]. Conrads G, About I. Pathophysiology of Dental Caries. Monogr Oral Sci. 2018;27:1-10.

[9]. Ricucci D, Siqueira JF Jr. Fate of the tissue in lateral canals and apical ramifications in response to pathologic conditions and treatment procedures. J Endod. 2010;36(1):1-15.

[10]. Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ, Cooper PR. Dental Pulp Defence and Repair Mechanisms in Dental Caries. Mediators Inflamm. 2015;2015:230251.

[11]. Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J. 2006;39(4):249-81.

[12]. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;99(2):231-52.

[13]. Siqueira JF Jr, Rôças IN. Present status and future directions: Microbiology of endodontic infections. Int Endod J. 2022;55(3):512-530.

[14]. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod. 2010;36(8):1277-88.

[15]. Pérez AR, Ricucci D, Vieira GCS, Provenzano JC, Alves FRF, Marceliano-Alves MF, Rôças IN, Siqueira JF Jr. Cleaning, Shaping, and Disinfecting Abilities of 2 Instrument Systems as Evaluated by a Correlative Micro-computed Tomographic and Histobacteriologic Approach. J Endod. 2020;46(6):846-857.

[16]. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1318-22.

[17]. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623-33.

[18]. Lei L, Shao M, Yang Y, Mao M, Yang Y, Hu T. Exopolysaccharide dispelled by calcium hydroxide with volatile vehicles related to bactericidal effect for root canal medication. J Appl Oral Sci. 2016;24(5):487-495.

[19]. Siqueira JF Jr, Rôças IN. Microbiology and treatment of acute apical abscesses. Clin Microbiol Rev. 2013;26(2):255-73.

[20]. Siqueira JF Jr. Endodontic infections: concepts, paradigms, and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94(3):281-93.

[21]. Gomes BP, Lilley JD, Drucker DB. Clinical significance of dental root canal microflora. J Dent. 1996;24(1-2):47-55.

[22]. Haapasalo M, Ranta H, Ranta K, Shah H. Black-pigmented Bacteroides spp. in human apical periodontitis. Infect Immun. 1986;53(1):149-53.

[23]. Keskin C, Demiryürek EÖ, Onuk EE. Pyrosequencing Analysis of Cryogenically Ground Samples from Primary and Secondary/Persistent Endodontic Infections. J Endod. 2017;43(8):1309-1316.

[24]. Sundqvist G. Associations between microbial species in dental root canal infections. Oral Microbiol Immunol. 1992;7(5):257-62.

[25]. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol. 1994;78(4):522-30.

[26]. Sakamoto M, Siqueira JF Jr, Rôças IN, Benno Y. Molecular analysis of the root canal microbiota associated with endodontic treatment failures. Oral Microbiol Immunol. 2008;23(4):275-81.

[27]. Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J. 2003;36(1):1-11.

[28]. Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain reaction. J Endod. 2008;34(5):537-40.

[29]. Alves FR, Siqueira JF Jr, Carmo FL, Santos AL, Peixoto RS, Rôças IN, Rosado AS. Bacterial community profiling of cryogenically ground samples from the apical

and coronal root segments of teeth with apical periodontitis. J Endod. 2009;35(4):486-92.

[30]. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod. 2005;31(11):817-20.

[31]. Arias-Moliz MT, Ordinola-Zapata R, Baca P, Ruiz-Linares M, García García E, Hungaro Duarte MA, Monteiro Bramante C, Ferrer-Luque CM. Antimicrobial activity of Chlorhexidine, Peracetic acid and Sodium hypochlorite/etidronate irrigant solutions against Enterococcus faecalis biofilms. Int Endod J. 2015;48(12):1188-93.

[32]. Martinho FC, Gomes BP. Quantification of endotoxins and cultivable bacteria in root canal infection before and after chemomechanical preparation with 2.5% sodium hypochlorite. J Endod. 2008;34(3):268-72.

[33]. Susila AV, Sai S, Sharma N, Balasubramaniam A, Veronica AK, Nivedhitha S. Can natural irrigants replace sodium hypochlorite? A systematic review. Clin Oral Investig. 2023;27(5):1831-1849.

[34]. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-98.

[35]. Kokkas AB, Boutsioukis ACh, Vassiliadis LP, Stavrianos CK. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. J Endod. 2004;30(2):100-2.

[36]. Violich DR, Chandler NP. The smear layer in endodontics - a review. Int Endod J. 2010;43(1):2-15.

[37]. Güzel C, Uzunoglu E, Dogan Buzoglu H. Effect of Low-surface Tension EDTA Solutions on the Bond Strength of Resin-based Sealer to Young and Old Root Canal Dentin. J Endod. 2018;44(3):485-488.

[38]. Tartari T, Guimarães BM, Amoras LS, Duarte MA, Silva e Souza PA, Bramante CM. Etidronate causes minimal changes in the ability of sodium hypochlorite to dissolve organic matter. Int Endod J. 2015;48(4):399-404.

[39]. Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, Souza-Filho FJ. Quantification of endotoxins in necrotic root canals from symptomatic and asymptomatic teeth. J Med Microbiol. 2005;54:777-783.

[40]. Pereira TC, da Silva Munhoz Vasconcelos LR, Graeff MSZ, Ribeiro MCM, Duarte MAH, de Andrade FB. Intratubular decontamination ability and physicochemical properties of calcium hydroxide pastes. Clin Oral Investig. 2019;23(3):1253-1262.

[41]. Sirén EK, Kerosuo E, Lavonius E, Meurman JH, Haapasalo M. Ca(OH)2 application modes: in vitro alkalinity and clinical effect on bacteria. Int Endod J. 2014;47(7):628-38.

[42]. Zancan RF, Vivan RR, Milanda Lopes MR, Weckwerth PH, de Andrade FB, Ponce JB, Duarte MA. Antimicrobial Activity and Physicochemical Properties of Calcium Hydroxide Pastes Used as Intracanal Medication. J Endod. 2016;42(12):1822-1828.

[43]. Ferreira FBA, Souza PAS, do Vale MS, de Moraes IG, Granjeiro JM. Evaluation of pH levels and calcium ion release in various calcium hydroxide endodontic dressings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97(3):388-92.

[44]. Wolters WJ, Duncan HF, Tomson PL, Karim IE, McKenna G, Dorri M, Stangvaltaite L, van der Sluis LWM. Minimally invasive endodontics: a new diagnostic system for assessing pulpitis and subsequent treatment needs. Int Endod J. 2017;50(9):825-829.

[45]. Ricucci D, Loghin S, Siqueira JF Jr. Correlation between clinical and histologic pulp diagnoses. J Endod. 2014;40(12):1932-9.

[46]. Rossi-Fedele G, Ng YL. Effectiveness of root canal treatment for vital pulps compared with necrotic pulps in the presence or absence of signs of periradicular pathosis: A systematic review and meta-analysis. Int Endod J. 2023;56(Suppl 3):370-394.

[47]. European Society of Endodontology (ESE) developed by: Duncan HF, Galler KM, Tomson PL, Simon S, El-Karim I, Kundzina R, Krastl G, Dammaschke T, Fransson H, Markvart M, Zehnder M, Bjørndal L. European Society of Endodontology position statement: Management of deep caries and the exposed pulp. Int Endod J. 2019;52(7):923-934.

[48]. Xavier AC, Martinho FC, Chung A, Oliveira LD, Jorge AO, Valera MC, Carvalho CA. One-visit versus two-visit root canal treatment: effectiveness in the removal of endotoxins and cultivable bacteria. J Endod. 2013;39(8):959-64.

[49]. Santos JM, Marques JA, Diogo P, Messias A, Sousa V, Sequeira D, Palma PJ. Influence of Preoperative Pulp Inflammation in the Outcome of Full Pulpotomy Using a Dog Model. J Endod. 2021;47(9):1417-1426.

[50]. Taha NA, Khazali MA. Partial Pulpotomy in Mature Permanent Teeth with Clinical Signs Indicative of Irreversible Pulpitis: A Randomized Clinical Trial. J Endod. 2017;43(9):1417-1421.

[51]. Levin LG, Law AS, Holland GR, Abbott PV, Roda RS. Identify and define all diagnostic terms for pulpal health and disease states. J Endod. 2009;35(12):1645-57.

[52]. Silva FB, Almeida JM, Sousa SM. Natural medicaments in endodontics -- a comparative study of the anti-inflammatory action. Braz Oral Res. 2004;18(2):174-9.

[53]. Cannon M, Cernigliaro J, Vieira A, Percinoto C, Jurado R. Effects of antibacterial agents on dental pulps of monkeys mechanically exposed and contaminated. J Clin Pediatr Dent. 2008;33(1):21-8.

[54]. Gallinari MO, Cintra LTÂ, Benetti F, Rahal V, Ervolino E, Briso ALF. Pulp response of rats submitted to bleaching and the use of different anti-inflammatory drugs. PLoS One. 2019;14(1):e0210338.

[55]. Chu FC, Leung WK, Tsang PC, Chow TW, Samaranayake LP. Identification of cultivable microorganisms from root canals with apical periodontitis following two-visit endodontic treatment with antibiotics/steroid or calcium hydroxide dressings. J Endod. 2006;32(1):17-23.

[56]. Bjørndal L, Simon S, Tomson PL, Duncan HF. Management of deep caries and the exposed pulp. Int Endod J. 2019;52(7):949-973.

[57]. Al-Ahmad A, Ameen H, Pelz K, Karygianni L, Wittmer A, Anderson AC, Spitzmüller B, Hellwig E. Antibiotic resistance and capacity for biofilm formation of different bacteria isolated from endodontic infections associated with root-filled teeth. J Endod. 2014;40(2):223-30.

[58]. Jungermann GB, Burns K, Nandakumar R, Tolba M, Venezia RA, Fouad AF. Antibiotic resistance in primary and persistent endodontic infections. J Endod. 2011;37(10):1337-44. Erratum in: J Endod. 2012;38(4):535.

[59]. Kayaoglu G, Ömürlü H, Akca G, Gürel M, Gençay Ö, Sorkun K, Salih B. Antibacterial activity of Propolis versus conventional endodontic disinfectants against Enterococcus faecalis in infected dentinal tubules. J Endod. 2011;37(3):376-81.

[60]. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA. Functional properties of honey, propolis, and royal jelly. J Food Sci. 2008;73(9):R117-24.

[61]. El-Tayeb MM, Abu-Seida AM, El Ashry SH, El-Hady SA. Evaluation of antibacterial activity of propolis on regenerative potential of necrotic immature permanent teeth in dogs. BMC Oral Health. 2019;19(1):174.

[62]. da Trindade R, da Silva JK, Setzer WN. *Copaifera* of the Neotropics: A Review of the Phytochemistry and Pharmacology. Int J Mol Sci. 2018;19(5):1511.

[63]. Menezes ACDS, Alves LDB, Goldemberg DC, de Melo AC, Antunes HS. Anti-inflammatory and wound healing effect of Copaiba oleoresin on the oral cavity: A systematic review. Heliyon. 2022;8(2):e08993.

[64]. Herrero-Jáuregui C, Casado MA, das Graças Bichara Zoghbi M, Célia Martins-da-Silva R. Chemical variability of Copaifera reticulata Ducke oleoresin. Chem Biodivers. 2011;8(4):674-85.

[65]. Bardají DK, da Silva JJ, Bianchi TC, de Souza Eugênio D, de Oliveira PF, Leandro LF, Rogez HL, Venezianni RC, Ambrosio SR, Tavares DC, Bastos JK, Martins CH. Copaifera reticulata oleoresin: Chemical characterization and antibacterial properties against oral pathogens. Anaerobe. 2016;40:18-27.

[66]. Cunha Neto MAD, Coêlho JA, Pinto KP, Cuellar MRC, Marcucci MC, Silva EJNL, Andrade FB, Sassone LM. Antibacterial Efficacy of Triple Antibiotic Medication With Macrogol (3Mix-MP), Traditional Triple Antibiotic Paste, Calcium Hydroxide, and Ethanol Extract of Propolis: An Intratubular Dentin Ex Vivo Confocal Laser Scanning Microscopic Study. J Endod. 2021;47(10):1609-1616.

[67]. Barros MC, Pedrinha VF, Oliveira FE, Marcucci MC, Gomes BPFA, 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. Biofouling. 2024;40(10):904–914.

[68]. Ohsumi T, Takenaka S, Wakamatsu R, Sakaue Y, Narisawa N, Senpuku H, Ohshima H, Terao Y, Okiji T. Residual structure of Streptococcus mutans biofilm following complete disinfection favors secondary bacterial adhesion and biofilm re-development. PLoS One. 2015;10(1):e0116647.

[69]. Bramante CM, Duque JA, Cavenago BC, Vivan RR, Bramante AS, de Andrade FB, Duarte MA. Use of a 660-nm Laser to Aid in the Healing of Necrotic Alveolar Mucosa Caused by Extruded Sodium Hypochlorite: A Case Report. J Endod. 2015;41(11):1899-902.

[70]. Gu LS, Huang XQ, Griffin B, Bergeron BR, Pashley DH, Niu LN, Tay FR. Primum non nocere - The effects of sodium hypochlorite on dentin as used in endodontics. Acta Biomater. 2017;61:144-156.

[71]. Pereira TC, Dijkstra RJB, Petridis X, Sharma PK, van de Meer WJ, van der Sluis LWM, de Andrade FB. Chemical and mechanical influence of root canal irrigation on biofilm removal from lateral morphological features of simulated root canals, dentine discs and dentinal tubules. Int Endod J. 2021;54(1):112-129.

[72]. Petridis X, Busanello FH, So MVR, Dijkstra RJB, Sharma PK, van der Sluis LWM. Factors affecting the chemical efficacy of 2% sodium hypochlorite against oral steady-state dual-species biofilms: Exposure time and volume application. Int Endod J. 2019;52(8):1182-1195.

[73]. Ciofu O, Tolker-Nielsen T, Jensen PØ, Wang H, Høiby N. Antimicrobial resistance, respiratory tract infections and role of biofilms in lung infections in cystic fibrosis patients. Adv Drug Deliv Rev. 2015;85:7-23.

[74]. Rozenbaum RT, Andrén OCJ, van der Mei HC, Woudstra W, Busscher HJ, Malkoch M, Sharma PK. Penetration and Accumulation of Dendrons with Different Peripheral Composition in *Pseudomonas aeruginosa* Biofilms. Nano Lett. 2019;19(7):4327-4333.

[75]. Humphreys G, Fleck F. United Nations Meeting on Antimicrobial Resistance. Bull. World Health Organ. 2016;94(9):638−639.

[76]. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629-655.

[77]. Huang R, Zhou Z, Lan X, Tang FK, Cheng T, Sun H, Cham-Fai Leung K, Li X, Jin L. Rapid synthesis of bismuth-organic frameworks as selective antimicrobial materials against microbial biofilms. Mater Today Bio. 2022;18:100507.

[78]. Li X, Yeh YC, Giri K, Mout R, Landis RF, Prakash YS, Rotello VM. Control of nanoparticle penetration into biofilms through surface design. Chem Commun (Camb). 2015;51(2):282-5.

[79]. Liu Y, Busscher HJ, Zhao B, Li Y, Zhang Z, van der Mei HC, Ren Y, Shi L. Surface-Adaptive, Antimicrobially Loaded, Micellar Nanocarriers with Enhanced Penetration and Killing Efficiency in Staphylococcal Biofilms. ACS Nano. 2016;10(4):4779-89.

[80]. Xu Y, Chen B, Xu L, Zhang G, Cao L, Liu N, Wang W, Qian H, Shao M. Urchin-like Fe_3O_4@Bi_2S_3 Nanospheres Enable the Destruction of Biofilm and Efficiently Antibacterial Activities. ACS Appl Mater Interfaces. 2024;16(3):3215-3231.

[81]. Wang R, Lai TP, Gao P, Zhang H, Ho PL, Woo PC, Ma G, Kao RY, Li H, Sun H. Bismuth antimicrobial drugs serve as broad-spectrum metallo-β-lactamase inhibitors. Nat Commun. 2018;9(1):439.

[82]. Zhang P, Wang L, Chen X, Li X, Yuan Q. Ultrasmall PEI-Decorated Bi2Se3 Nanodots as a Multifunctional Theranostic Nanoplatform for in vivo CT Imaging-Guided Cancer Photothermal Therapy. Front Pharmacol. 2021;12:795012.