Innovative Insights in Decontamination and Healing During Endodontic Treatment

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Chapter 7

General Discussion and Future Perspectives

General Discussion

The continuous development of new antimicrobial strategies is necessary in endodontics given that bacterial infections are the main cause of root canal treatment (RCT), leading to apical periodontitis if early intervention is not performed [1-4], and persistent infections occur due to challenges in biofilm eradication along the root canal system [5]. Additionally, clinical conditions present a range of situations wherein different antimicrobial approaches are required to achieve the healing of the tissue involved, whether it be the dental pulp or periapical tissues, as well as to maintain the homeostasis of the system [6-8]. Therefore, in this thesis, we have explored different strategies to combat bacterial infections and eliminate microorganisms in the root canal system, also considering biocompatible strategies to promote healing conditions. Furthermore, strategies for penetration into biofilms were investigated.

Disinfection procedures

Root canal irrigation with different chelating agents

The antimicrobial capacity of root canal irrigation provides a background for establishing clinical protocols and attributes to sodium hypochlorite (NaOCl) the status of the most used antimicrobial agent in endodontics [9-11]. NaOCl generates chemical reactions when in contact with the root dentine canal walls, organic components from the dental pulp tissue remnants [12,13], and the biofilm surface containing extracellular polymeric substances (EPS), which is responsible to enhance biofilm resistance and survival [14].

Due to the presence of the smear layer, generated by mechanical files during chemomechanical preparation and NaOCl action when in contact with root canal dentine walls and dental pulp tissue remnants, the use of a chelating agent is necessary [15-17]. Although chelating agents per se are not used aiming an antimicrobial activity in RCT [15,18], they are capable of interfere in the outcome of disinfection, acting in the inorganic content remotion of the smear layer which can potentially shield microbiota within root dentine from antiseptic agents and consequently avoiding an efficient root canal disinfection [16-19]. Bringing these setting to the clinical scope, an irrigation protocol alternating NaOCl and ethylenediaminetetraacetic acid (EDTA) consists in the most employed approach during root canal chemomechanical preparation [18,19]. Therefore, it is important to highlight that the use of chemical substances for cleaning and disinfection is essential in RCT.

In Chapter 2, we compared antibiofilm effects of chemomechanical preparation using NaOCl and different chelating agents in an intratubular biofilm model [20] of *Enterococcus faecalis* (*E. faecalis*). Despite a reduction in bacterial viability was demonstrated in all the treatments, the irrigation performance of NaOCl followed by EDTA-T (EDTA plus sodium lauryl ether sulfate) is an outcome to be highlighted. The sodium lauryl ether sulfate is an anionic surfactant widely used in the composition of cleaning products like detergents [21] and it presents antibacterial action through some mechanisms such as bacterial lysis, denaturation of proteins and enzymes, damage of cell membranes and changes in cell permeability [22]. Is possible to hypothesize that a blend of EDTA with sodium lauryl ether sulfate could also favor EDTA, reducing its surface tension and permeability into the dentinal tubules, reaching more bacteria, and consequently promoting cell disruptions.

However, these outcomes should be contemplated carefully, as adjunctive mechanical steps were performed to promote physical forces that have known efficacy in root canal disinfection, such as ultrasonic activation, which provides cavitation, acoustic flow, and the shockwave phenomenon, generating increased pressure and heating of the solution in the root canal [23,24]. Another adjunctive step evaluated was the use of the XP-Endo Finisher instrument, which expands and contracts when in contact with the root canal temperature, promoting agitation, allowing the solution to fill the root canal, and causing biofilm displacement [25,26].

Given the decontamination results, the authors recommend the use of NaOCl and an available chelating agent, followed by adjunctive mechanical steps to improve the disinfection capacity during RCT.

One critical drawback is that we tested the irrigation procedures using a mono-species biofilm. It is well known that endodontic biofilms consist of sessile multispecies communities attached to the dentinal root canal walls [6,27-29]. Despite an exact replicate of the clinical situation not being possible in *in vitro* studies, an intelligent selection must be made based on critical reasoning and validated research models. We used the microorganism *E. faecalis* due to its presence in the root canal, virulence factors, and survival characteristics, even under inhospitable conditions in the depths of dentinal tubules [30,31]. Thus, it was possible to state that a given irrigation procedure was effective against certain species associated with biofilm in dentinal tubules.

Efficacy of Calcium Hydroxide Paste

Calcium hydroxide (CH) paste is the most used intracanal medication applied between sessions of RCT [32]. It aims to provide a chemical-physical barrier to prevent contamination and promote antimicrobial activity against any microorganisms that may have survived the chemomechanical preparation [10,33,34]. Additionally, CH paste stimulates the periapical environment in tissue repair through a mineralization process involving alkaline phosphatase activation [35]. However, to achieve the efficacy of this medication, efficient chemomechanical preparation is essential. This preparation ensures proper shaping and debridement, enabling direct contact between the CH paste and the root canal walls [10,26,29]. Moreover, CH pastes are commercially available with various vehicles, which can influence their properties [35,36].

According to the literature, while the effectiveness of CH against bacteria in the planktonic phase is well established [32,37], its action on biofilms requires further investigation, especially using models that simulate endodontic conditions.

In Chapter 3, we evaluated the antibiofilm properties of four different CH pastes using two models: direct contact on dentine discs and the intratubular method in root dentine [20]. The latter aims to simulate antibiofilm activity at a distance evaluating the bacteria in superficial and deep dentinal tubules. Additionally, we investigated their action on EPS matrix as well as their physicochemical properties, such as pH and volumetric alterations.

E. faecalis is frequently found in secondary and persistent endodontic infections compared to primary infections, due to its resistance to intracanal disinfection procedures and its ability to withstand high pH conditions [29,38]. This suggests that CH may have limited effectivity against this bacterium [32,37]. For this reason, in Chapter 3, *E. faecalis* was chosen to investigate the disinfection efficacy of CH pastes.

All CH pastes tested showed a reduction in bacterial viability. Metapex, a commercial CH paste formulated in silicone oil (an oily vehicle) and containing iodoform, a radiopaque agent with antibacterial effects [39], exhibited the lowest bacterial viability and reduced EPS matrix content in both direct contact and intratubular tests. However, an acidic pH was observed for Metapex after 30 days, and the toxicity of iodoform raises concerns about its use [40,41].

For intracanal medication use, CH continuous ion release to maintain an alkaline environment is essential [34,36]. CH pastes with a more fluid consistency, such as those with water-soluble vehicles (aqueous or viscous), can achieve high pH and sustained calcium release in the periapical tissue [36,38,42]. In Chapter 3, CH pastes with these types of vehicles demonstrated high pH values after 7 days beyond similar volumetric alteration after 15 days. Notably, none of the CH pastes maintained the pH required for their reparative properties for up to 30 days. According to the literature, a pH of approximately 8.6 to 10.3 is necessary for the biological action of CH pastes [35].

CH + propylene glycol (CHP) and Metapaste (CH + propylene glycol + barium sulfate) are intracanal medications formulated in viscous vehicles, while UltraCal XS (CH + methylcellulose + barium sulfate) was chosen as a CH paste with an aqueous vehicle (as informed by the manufacturer) for comparison, with barium sulfate serving as a radiopaque agent. Under the tested conditions, CH pastes with water-soluble vehicles exhibited similar performance. It is reported that propylene glycol, as a viscous vehicle, induces a more favorable release of calcium and hydroxyl ions than aqueous vehicles, such as distilled water and saline solution, resulting in a higher pH [36,43,44]. However, the literature suggests that the methylcellulose present in UltraCal XS is an excellent option for drug delivery due to its biocompatible nature [45,46], likely contributing to the positive results observed in Chapter 3.

Chapter 3 presents the first investigation in the literature into EPS matrix results after the application of CH pastes. Analysis of the EPS matrix is important because it plays a significant protective role in biofilms, even when microorganisms enter a non-culturable state, continuing to express antigen components and virulence factors [14,47,48]. Additionally, residual biofilm can regrow after chemomechanical procedures, depending on EPS matrix presence and environmental conditions [48].

CH pastes with viscous and aqueous vehicles showed similar performance in reducing the EPS matrix. However, it is important to emphasize the need for mechanical procedures to disrupt the biofilm EPS matrix. Antimicrobial agents alone cannot effectively penetrate biofilms without the assistance of physical-mechanical action, especially when the biofilms are densely organized [14,47]

Thus, chemomechanical preparation is the primary method for eliminating biofilms during RCT [10,29,30,33]. Intracanal medication serves to target residual microorganisms that may remain after chemomechanical preparation, especially those partially dislodged from biofilms in hidden ramifications or located deep within the tubules, where they may not be associated with substantial EPS matrix [49].

Given these findings, the authors recommend the use of CH pastes with water-soluble vehicles. Notably, CH pastes formulated with viscous vehicles, such as propylene glycol, have been well-documented in the literature and have shown more favorable results [34,36,38,42-44,50,51]. This suggests they are effective in supporting the disinfection process when used as part of the overall RCT.

Present Status of Calcium Hydroxide Intracanal Medication

It is worth noting the ambiguity surrounding the use of intracanal medication in contemporary Endodontics. This discussion is closely tied to the debate on the number of appointments advocated for endodontic treatment, whether single-visit or multiple-visit approaches [52-56]. Studies have shown no significant difference in clinical outcomes, such as the resolution of apical periodontitis, between these approaches [52,57]. However, these studies did not account for several variables that may influence the outcomes like the effect of prognostic factors, and some included only cases with a favorable preoperative prognosis like asymptomatic teeth [57,58].

Despite the introduction of new technologies, such as advanced root canal preparation techniques and irrigation protocols, not all cases in dental practice result in a favorable prognosis [59]. Ordinola-Zapata *et al*. highlighted the importance of understanding the multifaceted nature of apical periodontitis to fully appreciate the advantages of a two-step treatment model and the use of intracanal medicaments. Several factors can influence the overall prognosis for the healing of apical periodontitis, including immune status, the size of the bone lesion, the diversity of the invading microbiome, persistent infection, tooth type, and the presence of cracks, among others [59].

The use of an intracanal medicament allows clinicians to evaluate the effects of fundamental endodontic procedures. If short-term outcomes, such as pain relief and the absence of percussion and/or palpation sensitivity, are satisfactory to both the operator and the patient, the case can then be completed and restored. These favorable clinical signs are indicative of effective root canal disinfection [59]. Thus, contemporary intracanal medicaments such as CH are useful not only for the elimination of microorganisms and inactivation of their by-products but also for the confirmation of initial signs of healing or symptom resolution before treatment completion [59-61].

When considering CH as an intracanal medication, it is essential to evaluate the specific clinical conditions of each case. In general, CH is recommended for cases involving extensive infections or when prolonged interappointment intervals are required, due to its well-documented properties in the literature [32–36,42–44].

The antimicrobial activity of CH is closely associated with the chemical reactions it generates within the root canal system due to its high alkalinity (pH ~12.5) [62]. The hydroxyl ions released by CH create free radicals that disrupt bacterial cell membranes and alter enzyme activity, leading to the inhibition of cellular metabolism and denaturation of structural proteins [62]. These ions can diffuse through the dentine mass, penetrating the dentinal tubules and increasing the pH of the surrounding environment [34,42-44,63].

As a physical barrier, CH limits the proliferation of residual microorganisms and prevents reinfection caused by coronal leakage [62]. It also can dissolve residual tissue within the root canal system [64]. Another critical mechanism of CH is its hydrolysis of the lipid A moiety of bacterial lipopolysaccharides (LPS), resulting in the release of free hydroxyl fatty acids [65,66].

The degradation of LPS is particularly significant because LPS, as an endotoxin and virulence factor, triggers the release of inflammatory bioactive mediators, including cytokines, and induces systemic reactions such as fever. It also activates the complement system and the metabolism of arachidonic acid, leading to inflammatory responses and bone resorption in the periapical region [66-68]. LPS is released during Gram-negative bacterial multiplication and death and has been detected throughout the root canal system in teeth with pulp necrosis [10,69]. Furthermore, its presence is strongly associated with clinical symptoms, including spontaneous pain, pain on palpation, and tenderness to percussion [10,69].

Moreover, clinical studies have shown that irrigation solutions commonly used in root canal preparation, such as NaOCl and chlorhexidine, are ineffective in eliminating endotoxins from root canal infections [10,69]. This reinforces the complementary role of CH as an interappointment dressing in infected root canal systems with apical periodontitis. The ability of CH to neutralize LPS underscores its importance in clinical endodontics, further enhancing its therapeutic benefits in RCT [59,66–68].

Natural Antimicrobial Compounds as Promising Alternatives

Anti-inflammatory and healing properties

The search for alternative substances in Endodontics remains continuous, as conventional agents and/or medications present limitations or even undesirable effects [70-76]. In this context, natural compounds such as propolis (PRO) and copaiba oil-resin (COR) have been evaluated to find promising approaches that could support or complement RCT.

In Chapter 4, the concept of combining these natural compounds with Hydrocortisone also arose from the clinical use of Otosporin® in vital pulp conditions. Otosporin® is an otologic drug that contains two different antibiotics, as well as H [77]. In such cases, medication is needed between treatment sessions to relieve inflammatory and painful symptoms and provide biological activities compatible with dental pulp tissue healing [7,78,79]. In vital pulp tissue conditions, some microorganisms are confined to the spaces closest to the source of infection, which is a superficial contamination condition [7,78]. This differs from necrotic conditions, where radical endodontic treatment is required, and several antimicrobial strategies are employed [2]. The use of antibiotics in such scenarios may foster the selection of resistant bacteria, which can acquire the capability to become impervious to the prescribed antibiotics [80-83].

Summarizing the effects aimed to be promoted by medication in a vital pulp condition between sessions of RCT: Firstly, dentists face a situation where antimicrobial activity is required to create a physicochemical barrier in the space corresponding to the removed dental pulp tissue [7,79]. This barrier must prevent contamination of microorganisms present in fluids such as saliva [33,34]. Secondly, when in contact with the radicular pulp tissue still occupying the root canal space, an anti-inflammatory effect is required [84]. Chapter 4 explored the anti-inflammatory and biological properties of the natural compounds PRO and COR, while their antimicrobial effects were examined in Chapter 5.

Chapter 4 also highlights the necessity of investigating standardized samples, as comparisons in the literature pose challenges due to variations in PRO and COR types evaluated and the methodologies used [85-87]. Instead of isolating individual bioactive compounds from PRO or COR, we chose to investigate the natural compounds containing their organic components in their naturally occurring chemical form, with a high content of bioactive compounds confirmed by chromatography. Previous studies suggested a synergistic effect of the different PRO compounds, being more active than were the individual fractions [88,89]. Moreover, we considered the roles of PRO and COR in their natural forms and the observed protective action of the pure compositions of them, possibly related to the PDLFs proliferation in the present study, could stem from their varied organic compounds.

In nature, PRO is primarily used for the construction and preservation of hives [90]. Its resin is characterized by naturally occurring chemical compounds, including various phenolic bioactive compounds, waxes, ashes, and volatile substances [90-92]. The content of these compounds can vary widely depending on the differences in plant ecosystems [91,92]. In addition, the green color of Brazilian PRO is a consequence of its botanical origin, from the young plant tissues of

Baccharis dracunculifolia DC, which contains a high concentration of chlorophyll [91]. The PRO type utilized, BRPX, is recognized for its high concentration of bioactive compounds such as derivates of caffeic acid and p-coumaric acids in addition to flavonoids [93,94]. PRO components present properties to reduce inflammation conditions, which was evaluated in different studies [95-97], being Artepillin C the main compound in Brazilian green propolis BRPX type [98].

Regarding COR, its anti-inflammatory properties have been notable over the centuries [99], prompting further investigation for various therapeutic applications. Many indigenous populations of the Amazon have historically used COR as traditional medicine. Early American settlers reported that indigenous populations applied this oil to the navels of newborns and the wounds of warriors after battles [100,101]. This indigenous use originated from the observation that animals, when wounded, rubbed themselves on the trunk of the copaiba tree to heal their wounds [100,102].

The biological properties of COR have been attributed to diterpenes and sesquiterpenes [103,104]. Among the most common sesquiterpenes are caryophyllene, α-copaene, β-bisabolene, and bergamotene, while the main diterpenes include kaurenoic and copalic acids [99,103,104]. Both Chapter 4 and Chapter 5 investigated COR samples, which were found to contain these bioactive compounds. The chemical composition, color, scent, and viscosity of oleoresin vary widely among species, and *Copaifera reticulata* oleoresin presents a thick yellowish-brown appearance [100]. In this study, the oleoresin was extracted from the tree species *Copaifera reticulata* Ducke, which is one of the most common species found in the Brazilian Amazon [100]. This type of oleoresin is extensively employed in folk medicine by people from diverse backgrounds and age groups [101,105].

Embracing conservative Endodontics, which involves all strategies aimed at preserving dental pulp vitality [106], Chapter 4 demonstrates that PRO and COR on human PDLFs yielded outcomes such as PDLFs proliferation, with or without conjunction with the corticosteroid hydrocortisone (H). Beyond that, no genotoxic activity was detected after exposing PDLFs to dilutions capable of maintaining cell density, and no changes were observed regarding cytokine levels of TGF-β1 and IL-6. These outcomes can be linked to studies exploring the anti-inflammatory and/or cytokine liberation from cells exposed to different compounds found in PRO and COR [107-109]. However, interestingly, the PDLFs proliferation is a result that needs to be highlighted.

In inflammatory conditions like in a microbial invasion, the Renin-angiotensin system (RAS) modulates cell behavior, forming angiotensin (Ang) II, which is linked to periodontal diseases through interactions with Ang II receptors (AT_1 and AT_2) [110,111]. Ang II also upregulates TGF-β1 in various cell types, contributing to pro-fibrotic mediation [112]. It was also reported that Ang II mediates signals in stretched cells to induce TGF-β1 expression [113]. Previous studies have shown that Ang II affects proliferation, cytokine production, and cell mineralization potential [111]. Considering that, Angiotensin II 1 Receptor (AT_1R) was analyzed as a biological target in molecular docking simulations.

Based on our results, we hypothesized that administering PRO and COR with H could induce Ang II synthesis, which might interact with AT_1R, potentially stimulating PDLFs proliferation. Significant binding, especially from PRO compounds like Artepillin C and Culifolin, was observed. These interactions, particularly hydrogen and hydrophobic bonds, play crucial roles in stabilizing energetically favored ligands [114]. Such interactions between PRO, COR, and H had not been previously documented in the literature.

Toxicity evaluation in an invertebrate model

In Chapter 4, *Galleria mellonella* (*G. mellonella*) larvae were used as an invertebrate model to assess the toxicity of PRO and COR. Beyond its simplicity and cost-effectiveness [115,116], this model shares a range of similarities with the human system [117]. The *G. mellonella* immune system exhibits both cellular and humoral immune responses. The cellular response is mediated by phagocytic cells (hemocytes) found within the hemolymph, akin to mammalian blood [117-118].

Consequently, the larvae can be infected by several human pathogens and can mount an immune response against them [115,117]. This response involves phagocytosis, encapsulation, and the release of antimicrobial peptides, like the responses of human innate immunity in infection pathways [117-119]. These characteristics make *G. mellonella* a valuable model for pharmacological studies. Therefore, we used this model to investigate the toxicity of PRO and COR after three escalating concentrations of injections. The outcomes suggest that PRO and COR can be metabolized in the larvae's system with no notable harmful effects, supporting their potential future clinical applications in Endodontics.

Antimicrobial properties

In Chapter 5, different planktonic bacterial species and models of dual-species biofilms were used to test the antimicrobial action of natural compounds, PRO and COR, as well as the combination of PRO + COR. We demonstrated that, at the concentrations tested, these natural compounds promote disinfection comparable to NaOCl and CH, what is in accordance with the literature [93,94,98,99,101,103,104,120].

Since microorganisms are found in various stages within an infected root canal system, including the planktonic phase, dense biofilms, and deep within dentinal tubules [121,122], the tested models provided valuable insights into the antimicrobial activities promoted by these natural compounds. After determining bactericidal concentrations through macrodilution assays against planktonic bacteria, the natural compounds were evaluated in different settings to compare their effectiveness as irrigation solutions and intracanal medications.

Unlike Chapters 2 and 3, the intratubular contamination model in Chapter 5 involved dual-species biofilms with tracer bacteria *E. faecalis* ATCC 29212 [123] and *Streptococcus mutans* (*S. mutans*) ATCC 20523, which are strongly associated with biofilm formation in the root canal system [124]. This dual-species biofilm was created on dentine discs [42,49], allowing for the investigation of antimicrobial agents' action on bacteria situated in the root dentine substrates closest to the main root canal walls and created inside root dentin specimens to observe decontamination deeper within the dentinal tubules [14,20,34,42,50,125].

We analyzed both a direct contact method (on dentine discs) and antimicrobial action at a distance (intratubular). It is worth noting that while bacterial penetration is more difficult in smaller dentinal tubules, this also hampers the penetration of antimicrobial agents.

The results obtained in Chapter 5 showed that the antimicrobial activity (fewer viable bacteria) of 10% PRO, 10% COR, and PRO + COR (1:1) were statistically like 2.5% NaOCl in an irrigation solution comparison and minimum bactericidal concentrations (CBM) of the natural compounds were statistically like CH in an intracanal medication comparison. These findings demonstrate the consistency of both biofilm models and the congruence of data between assays. Furthermore, it was inferred that the action mechanisms of PRO and COR, through their bioactive compounds promoting microbial membrane disruption [95-98,103,104], differ from the broad-spectrum oxidizing action of NaOCl [126] and the high pH of CH, which denatures proteins and disrupts cell membranes [127]. In this way, the natural compounds evaluated promote great disinfection when accurate concentrations are applied.

To investigate the immediate action of 10% PRO, 10% COR, and PRO + COR (1:1) compared to 2% NaOCl, a biofilm model using clinical isolates of *S. oralis* J22 and *A. naeslundii* T14V-J1 was created. These bacteria form coaggregation-mediated interactions, leading to robust biofilm formation from the earliest stages and are involved in persistent endodontic infections [14,128,129]. Biofilm reduction was assessed via optical coherence tomography (OCT), and antimicrobial activity was measured by colony forming units (CFU/cm^2). This type of analysis is important because it simulates a realistic contact surface area between the biofilm substrate and solutions Consequently, only diffusion-induced chemical effects could be investigated [46,124], with a minimal volume of solution (40 µL) applied to the biofilms for a short exposure time (30 seconds) statically, differing from the irrigation solution test conducted in direct contact settings and the intratubular test.

The application of 2% NaOCl resulted in the most significant biofilm reduction, presenting the lowest biofilm mean thickness (height) after 30 seconds, followed by 10% COR, PRO + COR (1:1), and lastly 10% PRO. This was consistent with CFU/cm^2 counts, where 2% NaOCl showed the most significant disinfection compared to the natural antimicrobial compounds, while 10% PRO exhibited the lowest disinfection. However, it is important to note that in root canal irrigation, higher volumes of solutions and longer exposure periods are used, as seen in direct contact and intratubular assays. Additionally, mechanical forces such as shear stress and irrigation flow rate significantly impact biofilm removal [14,130] but the study of mechanical stresses against the biofilms was not the goal of this research.

It is worth mentioning the importance of developing the optimal delivery systems for PRO and COR due to the hydrophobic characteristics of both [131,132]. In Chapter 5, different behaviors were showed when natural compounds were diluted in ethanol or propylene glycol via macrodilutions. Subsequently, propylene glycol was the vehicle selected to further analyzes on biofilms due to its well stablished application in Endodontics and non-antimicrobial activity [34,43,44,49,50]. Different vehicles such as oil-in-water emulsions with surfactants, and formulations like gels, pastes, controlled-release systems, and nanoencapsulation of these compounds should be considered to enhance their clinical application. Continued research is essential to develop products incorporating PRO and COR for endodontic practice, offering dentists effective alternatives to conventional antimicrobial agents.

Investigation of PEI-Bi_2S_3 Nanoparticles

In Chapter 6, bismuth sulfide nanoparticles (Bi_2S_3 NPs) synthesized with polyethyleneimine (PEI), a nucleating agent and cationic polymer that enhances drug delivery [133], were investigated by varying the PEI content during NP synthesis. The PEI provided stability to the Bi_2S_3 cores by imparting a positive surface charge, as observed in the zeta potential measurements. Electrostatic interactions occur between the negative charges in the EPS matrix of biofilms and the positively charged NPs [134-137], which also contribute to the accumulation of NPs within the biofilms. Studies have shown that positively charged NPs are less likely to be washed away [82,136,137].

Knowing that biofilms are notoriously difficult to treat and eliminate, not only during RCT but also in a wide range of biofilm-related human infections [1-5,82,137-139], the ability of both 25 and 100 PEI-Bi_2S_3 NPs to penetrate biofilms suggests that these NPs could be functionalized or loaded with therapeutic agents for delivering drugs deep within biofilms. This addresses one of the major challenges in treating biofilm-associated infections [82].

The authors consider this an interesting avenue for further research, as PRO and COR require an optimal delivery system to be integrated into the endodontic arsenal as adjunctive antimicrobial strategies. Moreover, the current PEI-Bi_2S_3 NPs setup did not reduce biofilm viability, despite its ability to penetrate biofilms, which underscores the need for further optimization. This includes conjugation of NPs with antimicrobial agents for more effective targeted drug delivery.

Future Perspectives

In this general discussion, we highlighted the primary goal of RCT: biofilm eradication to promote the healing of apical tissues. The research projects presented in this thesis confirm the challenges associated with biofilm removal. Furthermore, conventional techniques for root canal decontamination can have deleterious and undesirable effects, limiting their broad use and potentially leading to microbial resistance. This underscores the ongoing need for new and/or alternative agents.

Currently, there is an urgent and continuous demand for the development of natural products due to projections of increased human mortality from infections caused by resistant bacteria [83]. Another trend is the rising consumer preference for health-conscious and sustainable products, prompting the pharmaceutical industry to search for novel compounds, in addition to the economic and ecological benefits of utilizing natural sources [140,141].

From a sustainability perspective, this demand is expected to continue growing as global awareness of environmental sustainability, ethical consumption, and the need for natural bioactive compounds increases. In this thesis, we have explored natural antimicrobial compounds and provided insights into their potential new applications as nanoparticles delivery systems. Our aim is to pave the way for the safe use of these alternatives in the future, combining them with new technologies to enhance disinfection and promote the healing of apical periodontitis.

Returning to the goals intended to be addressed in this research, we conclude that the combination of mechanical and chemical stresses is essential for effectively disorganizing and eradicating biofilms, particularly in endodontic infections where microorganisms can remain protected deep within narrow spaces such as dentine tubules or anatomical complexities of the root canal system. Given the variety of clinical conditions in Endodontics and the challenge of microbial resistance in human infections, natural antimicrobial compounds can serve as complementary approaches to combat infections, demonstrating beneficial effects, including biocompatibility. However, to deeply penetrate biofilms, a drug carrier is required. PEI-Bi_2S_3 NPs demonstrated feasibility for this purpose using a dental biofilm model. To incorporate PRO, COR, or a combination of PRO + COR, further studies are necessary to develop a formulation that ensures the presence of at least the primary active agents of each compound.

References

[1]. Segura-Egea JJ, Cabanillas-Balsera D, Martín-González J, Cintra LTA. Impact of systemic health on treatment outcomes in endodontics. Int Endod J. 2023;56 Suppl 2:219-235.

[2]. Jakovljevic A, Nikolic N, Jacimovic J, Pavlovic O, Milicic B, Beljic-Ivanovic K, Miletic M, Andric M, Milasin J. Prevalence of Apical Periodontitis and Conventional Nonsurgical Root Canal Treatment in General Adult Population: An Updated Systematic Review and Meta-analysis of Cross-sectional Studies. J Endod. 2020;46(10):1371-1386.e8. Erratum in: J Endod. 2021;47(2):336.

[3]. 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.

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

[5]. 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.

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

[7]. 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.

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

[9]. Arias-Moliz MT, Ordinola-Zapata R, Baca P, Ruiz-Linares M, García EG, Duarte MAH, Bramante CM, 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.

[10]. 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.

[11]. 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.

[12]. Estrela C, Estrela CR, Barbin EL, Spanó JC, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13(2):113-7.

[13]. Tejada S, Baca P, Ferrer-Luque CM, Ruiz-Linares M, Valderrama MJ, Arias-Moliz MT. Influence of dentine debris and organic tissue on the properties of sodium hypochlorite solutions. Int Endod J. 2019;52(1):114-122.

[14]. 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.

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

[16]. 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.

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

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

[19]. Aslantas EE, Buzoglu HD, Altundasar E, Serper A. Effect of EDTA, sodium hypochlorite, and chlorhexidine gluconate with or without surface modifiers on dentin microhardness. J Endod. 2014;40(6):876-9.

[20]. Andrade FB, Arias MP, Maliza AG, Duarte MA, Graeff MS, Amoroso-Silva PA, Midena RZ, Moraes IG. A new improved protocol for in vitro intratubular dentinal bacterial contamination for antimicrobial endodontic tests: standardization and validation by confocal laser scanning microscopy. J Appl Oral Sci. 2015;23(6):591-598.

[21]. Paulo AMS, Aydin R, Dimitrov MR, Vreeling H, Cavaleiro AJ, García-Encina PA, Stams AJM, Plugge CM. Sodium lauryl ether sulfate (SLES) degradation by nitrate-reducing bacteria. Appl Microbiol Biotechnol. 2017;101(12):5163-5173.

[22]. Vehapi M, Özçimen D. Antimicrobial and bacteriostatic activity of surfactants against B. subtilis for microbial cleaner formulation. Arch Microbiol. 2021;203(6):3389-3397.

[23]. Pacheco-Yanes J, Provenzano JC, Marceliano-Alves MF, Gazzaneo I, Pérez AR, Gonçalves LS, Siqueira JF Jr. Distribution of sodium hypochlorite throughout the mesial root canal system of mandibular molars after adjunctive irrigant activation procedures: a micro-computed tomographic study. Clin Oral Investig. 2020;24(2):907-914.

[24]. van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J. 2007;40(6):415-26.

[25]. Alves FR, Andrade-Junior CV, Marceliano-Alves MF, Pérez AR, Rôças IN, Versiani MA, Sousa-Neto MD, Provenzano JC, Siqueira JF Jr. Adjunctive Steps for Disinfection of the Mandibular Molar Root Canal System: A Correlative

Bacteriologic, Micro-Computed Tomography, and Cryopulverization Approach. J Endod. 2016;42(11):1667-1672.

[26]. Bao P, Shen Y, Lin J, Haapasalo M. In Vitro Efficacy of XP-endo Finisher with 2 Different Protocols on Biofilm Removal from Apical Root Canals. J Endod. 2017;43(2):321-325.

[27]. 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.

[28]. 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.

[29]. 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.

[30]. Ferrer-Luque CM, Bejarano I, Ruiz-Linares M, Baca P. Reduction in Enteroccocus faecalis counts - a comparison between rotary and reciprocating systems. Int Endod J. 2014;47(4):380-6.

[31]. Liu H, Wei X, Ling J, Wang W, Huang X. Biofilm formation capability of Enterococcus faecalis cells in starvation phase and its susceptibility to sodium hypochlorite. J Endod. 2010;36(4):630-5.

[32]. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J. 2011;44(8):697-730.

[33]. Siqueira JF Jr, Magalhães KM, Rôças IN. Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/camphorated paramonochlorophenol paste as an intracanal dressing. J Endod. 2007;33(6):667-72.

[34]. 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.

[35]. Estrela C, Pécora JD, Souza-Neto MD, Estrela CR, Bammann LL. Effect of vehicle on antimicrobial properties of calcium hydroxide pastes. Braz Dent J. 1999;10(2):63-72.

[36]. Ferreira FBA, Silva e Souza PA, Vale MS, 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.

[37]. van der Waal SV, Connert T, Crielaard W, de Soet JJ. In mixed biofilms Enterococcus faecalis benefits from a calcium hydroxide challenge and culturing. Int Endod J. 2016;49(9):865-873.

[38]. Pacios MG, de la Casa ML, de Bulacio Ml, López ME. Influence of different vehicles on the pH of calcium hydroxide pastes. J Oral Sci. 2004;46(2):107-11.

[39]. Pallotta RC, Ribeiro MS, de Lima Machado ME. Determination of the minimum inhibitory concentration of four medicaments used as intracanal medication. Aust Endod J. 2007;33(3):107-11.

[40]. O'Connor AF, Freeland AP, Heal DJ, Rossouw DS. Iodoform toxicity following the use of B.I.P.P.: a potential hazard. J Laryngol Otol. 1977;91(10):903-7.

[41]. Dahake PT, Thosar N, Hande A, Joshi DA, Bhagat A. Hematological and Biochemical Responses of Newly Formulated Primary Root Canal Obturating Material: An In Vivo Study. Cureus. 2022;14(12):e32685.

[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. Erratum in: J Endod. 2017;43(5):826.

[43]. Simon ST, Bhat KS, Francis R. Effect of four vehicles on the pH of calcium hydroxide and the release of calcium ion. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;80(4):459-64.

[44]. Duarte MA, Midena RZ, Zeferino MA, Vivan RR, Weckwerth PH, Dos Santos F, Guerreiro-Tanomaru JM, Tanomaru-Filho M. Evaluation of pH and calcium ion release of calcium hydroxide pastes containing different substances. J Endod. 2009;35(9):1274-7.

[45]. Jacobs JC, Troxel A, Ehrlich Y, Spolnik K, Bringas JS, Gregory RL, Yassen GH. Antibacterial Effects of Antimicrobials Used in Regenerative Endodontics against Biofilm Bacteria Obtained from Mature and Immature Teeth with Necrotic Pulps. J Endod. 2017;43(4):575-579.

[46]. Altomare L, Cochis A, Carletta A, Rimondini L, Farè S. Thermo-responsive methylcellulose hydrogels as temporary substrate for cell sheet biofabrication. J Mater Sci Mater Med. 2016;27(5):95.

[47]. Busanello FH, Petridis X, So MVR, Dijkstra RJB, Sharma PK, van der Sluis LWM. Chemical biofilm removal capacity of endodontic irrigants as a function of biofilm structure: optical coherence tomography, confocal microscopy and viscoelasticity determination as integrated assessment tools. Int Endod J. 2019;52(4):461-474.

[48]. Tawakoli PN, Ragnarsson KT, Rechenberg DK, Mohn D, Zehnder M. Effect of endodontic irrigants on biofilm matrix polysaccharides. Int Endod J. 2017;50(2):153-160.

[49]. Pedrinha VF, Cuellar MRC, de Barros MC, Titato PCG, Shahbazi MA, Sharma PK, de Andrade FB. The Vehicles of Calcium Hydroxide Pastes Interfere with Antimicrobial Effect, Biofilm Polysaccharidic Matrix, and Pastes' Physicochemical Properties. Biomedicines. 2022;10(12):3123.

[50]. Pereira TC, Vasconcelos LR, Graeff MS, Duarte MA, Bramante CM, Andrade FB. Intratubular disinfection with tri-antibiotic and calcium hydroxide pastes. Acta Odontol Scand. 2017;75(2):87-93.

[51]. de Andrade FB, Vasconcelos LRSM, Pereira TC, Garcia RB, Bramante CM, Duarte MAH. Ultrasonic agitation reduces the time of calcium hydroxide antimicrobial effect and enhances its penetrability. J Mater Sci Mater Med. 2021;32(12):150.

[52]. Gesi A, Hakeberg M, Warfvinge J, Bergenholtz G. Incidence of periapical lesions and clinical symptoms after pulpectomy--a clinical and radiographic evaluation of 1- versus 2-session treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):379-88.

[53]. Moreira MS, Anuar ASN, Tedesco TK, Dos Santos M, Morimoto S. Endodontic Treatment in Single and Multiple Visits: An Overview of Systematic Reviews. J Endod. 2017;43(6):864-870.

[54]. 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.

[55]. Vera J, Siqueira JF Jr, Ricucci D, Loghin S, Fernández N, Flores B, Cruz AG. One- versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. J Endod. 2012;38(8):1040-52.

[56]. 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.

[57]. Paredes-Vieyra J, Enriquez FJ. Success rate of single- versus two-visit root canal treatment of teeth with apical periodontitis: a randomized controlled trial. J Endod. 2012;38(9):1164-9.

[58]. Penesis VA, Fitzgerald PI, Fayad MI, Wenckus CS, BeGole EA, Johnson BR. Outcome of one-visit and two-visit endodontic treatment of necrotic teeth with apical periodontitis: a randomized controlled trial with one-year evaluation. J Endod. 2008;34(3):251-7.

[59]. Ordinola-Zapata R, Noblett WC, Perez-Ron A, Ye Z, Vera J. Present status and future directions of intracanal medicaments. Int Endod J. 2022;55(Suppl 3):613-636.

[60]. Shuping GB, Ørstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod. 2000;26(12):751-5.

[61]. Chong BS, Pitt Ford TR. The role of intracanal medication in root canal treatment. Int Endod J. 1992;25(2):97-106.

[62]. Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J. 1999;32(5):361-9.

[63]. Tronstad L, Andreasen JO, Hasselgren G, Kristerson L, Riis I. pH changes in dental tissues after root canal filling with calcium hydroxide. J Endod. 1981;7(1):17-21.

[64]. Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. J Endod. 1988;14(3):125-7.

[65]. Safavi KE, Nichols FC. Alteration of biological properties of bacterial lipopolysaccharide by calcium hydroxide treatment. J Endod. 1994;20(3):127-9.

[66]. Silva L, Nelson-Filho P, Leonardo MR, Rossi MA, Pansani CA. Effect of calcium hydroxide on bacterial endotoxin in vivo. J Endod. 2002;28(2):94-8.

[67]. Barthel CR, Levin LG, Reisner HM, Trope M. TNF-alpha release in monocytes after exposure to calcium hydroxide treated Escherichia coli LPS. Int Endod J. 1997;30(3):155-9.

[68]. Horiba N, Maekawa Y, Yamauchi Y, Ito M, Matsumoto T, Nakamura H. Complement activation by lipopolysaccharides purified from gram-negative bacteria isolated from infected root canals. Oral Surg Oral Med Oral Pathol. 1992;74(5):648-51.

[69]. Gomes BP, Martinho FC, Vianna ME. Comparison of 2.5% sodium hypochlorite and 2% chlorhexidine gel on oral bacterial lipopolysaccharide reduction from primarily infected root canals. J Endod. 2009;35(10):1350-3.

[70]. Oyarzún A, Cordero AM, Whittle M. Immunohistochemical evaluation of the effects of sodium hypochlorite on dentin collagen and glycosaminoglycans. J Endod. 2002;28(3):152-6.

[71]. 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.

[72]. Xu H, Ye Z, Zhang A, Lin F, Fu J, Fok ASL. Effects of concentration of sodium hypochlorite as an endodontic irrigant on the mechanical and structural properties of root dentine: A laboratory study. Int Endod J. 2022;55(10):1091-1102.

[73]. 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.

[74]. Naseri M, Eftekhar L, Gholami F, Atai M, Dianat O. The Effect of Calcium Hydroxide and Nano-calcium Hydroxide on Microhardness and Superficial Chemical Structure of Root Canal Dentin: An Ex Vivo Study. J Endod. 2019;45(9):1148-1154.

[75]. Yassen GH, Vail MM, Chu TG, Platt JA. The effect of medicaments used in endodontic regeneration on root fracture and microhardness of radicular dentine. Int Endod J. 2013;46(7):688-95.

[76]. Swanljung O, Vehkalahti MM. Root Canal Irrigants and Medicaments in Endodontic Malpractice Cases: A Nationwide Longitudinal Observation. J Endod. 2018;44(4):559-564.

[77]. Ramos IF, Biz MT, Paulino N, Scremin A, Della Bona A, Barletta FB, Figueiredo JA. Histopathological analysis of corticosteroid-antibiotic preparation and propolis paste formulation as intracanal medication after pulpectomy: an in vivo study. J Appl Oral Sci. 2012;20(1):50-6.

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

[79]. Duncan HF, El-Karim I, Dummer PMH, Whitworth J, Nagendrababu V. Factors that influence the outcome of pulpotomy in permanent teeth. Int Endod J. 2023;56 Suppl 2:62-81.

[80]. 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.

[81]. 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.

[82]. 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.

[83]. Álvarez-Martínez FJ, Barrajón-Catalán E, Micol V. Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. Biomedicines. 2020;8(10):405.

[84]. Fava LR. Human pulpectomy: incidence of postoperative pain using two different intracanal dressings. Int Endod J. 1992;25(5):257-60.

[85]. Cardoso PCDS, Rocha CAMD, Leal MF, Bahia MO, Alcântara DDFÁ, Santos RAD, Gonçalves NDS, Ambrósio SR, Cavalcanti BC, Moreira-Nunes CA, Pessoa CDÓ, Burbano RMR. Effect of diterpenoid kaurenoic acid on genotoxicity and cell cycle progression in gastric cancer cell lines. Biomed Pharmacother. 2017;89:772-780.

[86]. Rodrigues CR, Plentz LC, Marcucci MC, Dihl RR, Lehmann M. In vivo evaluation of mutagenic and recombinagenic activities of Brazilian propolis. Food Chem Toxicol. 2016;96:117-21.

[87]. Pereira AD, de Andrade SF, de Oliveira Swerts MS, Maistro EL. First in vivo evaluation of the mutagenic effect of Brazilian green propolis by comet assay and micronucleus test. Food Chem Toxicol. 2008;46(7):2580-4.

[88]. Moncla BJ, Guevara PW, Wallace JA, Marcucci MC, Nor JE, Bretz WA. The inhibitory activity of typified propolis against Enterococcus species. Z Naturforsch C J Biosci. 2012;67(5-6):249-56.

[89]. Santos FA, Bastos EM, Uzeda M, Carvalho MA, Farias LM, Moreira ES, Braga FC. Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. J Ethnopharmacol. 2002;80(1):1-7.

[90]. Marcucci MC, Ferreres F, Custódio AR, Ferreira MM, Bankova VS, García-Viguera C, Bretz WA. Evaluation of phenolic compounds in Brazilian propolis from different geographic regions. Z Naturforsch C J Biosci. 2000;55(1-2):76-81.

[91]. Marcucci MC, Cunha IBS, Sanchez EMS, Gonçalves CP, Cedeño-Pinos C, Bañón S. Analysis of Brazilian Propolis by Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA). Characteristics of Crude Resin, Ethanolic Extracts, Wax and Isolated Compounds. Bee World. 2022;*99*(3):82–88.

[92]. Fikri A, Sulaeman A, Marliyati S, Fahrudin M. Antioxidant Activity and Total Phenolic Content of Stingless Bee Propolis from Indonesia. J Apic Sci. 2019;63(1): 139-147.

[93]. Veiga RS, De Mendonça S, Mendes PB, Paulino N, Mimica MJ, Lagareiro Netto AA, Lira IS, López BG, Negrão V, Marcucci MC. Artepillin C and phenolic compounds responsible for antimicrobial and antioxidant activity of green propolis and Baccharis dracunculifolia DC. J Appl Microbiol. 2017;122(4):911-920.

[94]. 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.

[95]. Ahangari Z, Naseri M, Jalili M, Mansouri Y, Mashhadiabbas F, Torkaman A. Effect of propolis on dentin regeneration and the potential role of dental pulp stem cell in Guinea pigs. Cell J. 2012;13(4):223-8.

[96]. 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):R117-24.7.

[97]. Wang K, Hu L, Jin X.-L, Ma Q-X, Marcucci MC, Netto AAL, Sawaya ACHF, Huang S, Ren W-K, Conlon MA, Topping DL, Hu F-L. Polyphenol-rich propolis extracts from China and Brazil exert anti-inflammatory effects by modulating

ubiquitination of TRAF6 during the activation of NF-κB. J Funct Foods. 2015;19:464–478.

[98]. Pedrinha VF, Santos LM, Gonçalves CP, Garcia MT, Lameira OA, Queiroga CL, Marcucci MC, Shahbazi MA, Sharma PK, Junqueira JC, Sipert CR, de Andrade FB. Effects of natural antimicrobial compounds propolis and copaiba on periodontal ligament fibroblasts, molecular docking, and in vivo study in Galleria mellonella. Biomed Pharmacother. 2024;171:116139.

[99]. Plowden, C. The ethnobotany of copaíba*(copaifera)* oleoresin in the amazoil. Econ Bot. 2004;**58**,729–733.

[100]. de Albuquerque KC, da Veiga AD, Silva JV, Brigido HP, Ferreira EP, Costa EV, Marinho AM, Percário S, Dolabela MF. Brazilian Amazon Traditional Medicine and the Treatment of Difficult to Heal Leishmaniasis Wounds with *Copaifera*. Evid Based Complement Alternat Med. 2017;2017:8350320.

[101]. 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.

[102]. Santos AO, Ueda-Nakamura T, Dias Filho BP, Veiga Junior VF, Pinto AC, Nakamura CV. Effect of Brazilian copaiba oils on Leishmania amazonensis. J Ethnopharmacol. 2008;120(2):204-8.

[103]. 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.

[104]. 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.

[105]. 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.

[106]. 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.

[107]. Kian D, Lancheros CAC, Assolini JP, Arakawa NS, Veiga-Júnior VF, Nakamura CV, Pinge-Filho P, Conchon-Costa I, Pavanelli WR, Yamada-Ogatta SF, Yamauchi LM. Trypanocidal activity of copaiba oil and kaurenoic acid does not depend on macrophage killing machinery. Biomed Pharmacother. 2018;103:1294-1301.

[108]. Askari VR, Rahimi VB, Zamani P, Fereydouni N, Rahmanian-Devin P, Sahebkar AH, Rakhshandeh H. Evaluation of the effects of Iranian propolis on the severity of post operational-induced peritoneal adhesion in rats. Biomed Pharmacother. 2018;99:346-353.

[109]. Chaihongsa N, Maneesai P, Sangartit W, Rattanakanokchai S, Potue P, Khamseekaew J, Bunbupha S, Pakdeechote P. Cardiorenal dysfunction and hypertrophy induced by renal artery occlusion are normalized by galangin treatment in rats. Biomed Pharmacother. 2022;152:113231.

[110]. Santos CF, Morandini AC, Dionísio TJ, Faria FA, Lima MC, Figueiredo CM, Colombini-Ishikiriama BL, Sipert CR, Maciel RP, Akashi AP, Souza GP, Garlet GP, Rodini CO, Amaral SL, Becari C, Salgado MC, Oliveira EB, Matus I, Didier DN, Greene AS. Functional Local Renin-Angiotensin System in Human and Rat Periodontal Tissue. PLoS One. 2015;10(8):e0134601.

[111]. Pizzatto LN, Meneses CCB, Diniz EA, Dionísio TJ, Santos CF, Sipert CR. Angiotensin II Regulates Proliferation and Function of Stem Cells of Apical Papilla. J Endod. 2020;46(6):810-817.

[112]. Duangrat R, Parichatikanond W, Mangmool S. Dual Blockade of TGF-β Receptor and Endothelin Receptor Synergistically Inhibits Angiotensin II-Induced Myofibroblast Differentiation: Role of $AT_1R/G_{\alpha q}$-Mediated TGF-β1 and ET-1 Signaling. Int J Mol Sci. 2023;24(8):6972.

[113]. Monnouchi S, Maeda H, Fujii S, Tomokiyo A, Kono K, Akamine A. The roles of angiotensin II in stretched periodontal ligament cells. J Dent Res. 2011;90(2):181-5.

[114]. Kati WM, Wolfenden R. Major enhancement of the affinity of an enzyme for a transition-state analog by a single hydroxyl group. Science. 1989;243(4898):1591-3.

[115]. Meccatti VM, Figueiredo-Godoi LMA, Pereira TC, de Lima PMN, Abu Hasna A, Senna LB, Marcucci MC, Junqueira JC, de Oliveira LD. The biocompatibility and antifungal effect of Rosmarinus officinalis against Candida albicans in Galleria mellonella model. Sci Rep. 2022;12(1):15611.

[116]. Lazarini JG, Sardi JCO, Franchin M, Nani BD, Freires IA, Infante J, Paschoal JAR, de Alencar SM, Rosalen PL. Bioprospection of Eugenia brasiliensis, a Brazilian native fruit, as a source of anti-inflammatory and antibiofilm compounds. Biomed Pharmacother. 2018;102:132-139.

[117]. Tsai CJ, Loh JM, Proft T. Galleria mellonella infection models for the study of bacterial diseases and for antimicrobial drug testing. Virulence. 2016;7(3):214-29.

[118]. Boman HG, Hultmark D. Cell-free immunity in insects. Annu Rev Microbiol. 1987;41:103-26.

[119]. Tojo S, Naganuma F, Arakawa1 K, Yokoo1 S. Involvement of both granular cells and plasmatocytes in phagocytic reactions in the greater wax moth, Galleria mellonella. J Insect Physiol. 2000;46(7):1129-1135.

[120]. Ferreira FB, Torres SA, Rosa OP, Ferreira CM, Garcia RB, Marcucci MC, Gomes BP. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104(5):709-716.

[121]. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34(11):1291-1301.e3.

[122]. 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.

[123]. Love RM. Enterococcus faecalis--a mechanism for its role in endodontic failure. Int Endod J. 2001;34(5):399-405.

[124]. Lima AR, Ganguly T, Walker AR, Acosta N, Francisco PA, Pileggi R, Lemos JA, Gomes BPFA, Abranches J. Phenotypic and Genotypic Characterization of Streptococcus mutans Strains Isolated from Endodontic Infections. J Endod. 2020;46(12):1876-1883.

[125]. Pedrinha VF, Cardenas Cuellar MR, Velásquez-Espedilla EG, Duarte MAH, Andrade FB, Rodrigues PA. Impact of irrigation protocols with some chelators and mechanical agitation on intratubular decontamination. Braz Oral Res. 2021;35:e127.

[126]. Petridis X, Busanello FH, So MVR, Dijkstra RJB, Sharma PK, van der Sluis LWM. Chemical efficacy of several NaOCl concentrations on biofilms of different architecture: new insights on NaOCl working mechanisms. Int Endod J. 2019;52(12):1773-1788.

[127]. 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.

[128]. He Y, Peterson BW, Jongsma MA, Ren Y, Sharma PK, Busscher HJ, van der Mei HC. Stress relaxation analysis facilitates a quantitative approach towards antimicrobial penetration into biofilms. PLoS One. 2013;8(5):e63750.

[129]. Palmer RJ Jr, Gordon SM, Cisar JO, Kolenbrander PE. Coaggregation-mediated interactions of streptococci and actinomyces detected in initial human dental plaque. J Bacteriol. 2003;185(11):3400-9.

[130]. 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.

[131]. Monteil J, Hadj-Sassi A, Dargelos É, Guzman-Barrera N, Poque E, Leal-Calderon F. Method to prepare aqueous propolis dispersions based on phase separation. Food Chem. 2022;389:133072.

[132]. Freitas C, Ribeiro R, Almeida E, Silva, Campello Y, da F. Chemistry, Biological Activities, and Uses of Copaiba Oil Resins. Springer eBooks. 2021 Dec 22;1–21.

[133]. Note C, Kosmella S, Koetz J. Poly(ethyleneimine) as reducing and stabilizing agent for the formation of gold nanoparticles in w/o microemulsions. Colloids Surf A: Physicochem Eng. 2006;290(1-3):150–6.

[134]. 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.

[135]. Liu Y, Shi L, Su L, van der Mei HC, Jutte PC, Ren Y, Busscher HJ. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem Soc Rev. 2019;48(2):428-446.

[136]. Carniello V, Peterson BW, van der Mei HC, Busscher HJ. Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth. Adv Colloid Interface Sci. 2018;261:1-14.

[137]. 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.

[138]. 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.

[139]. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012;75(3):311-35.

[140]. Silver LL. Natural products as a source of drug leads to overcome drug resistance. Future Microbiol. 2015;10(11):1711-8.

[141]. Katz L, Baltz RH. Natural product discovery: past, present, and future. J Ind Microbiol Biotechnol. 2016;43(2-3):155-76.