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BIOFILM MANAGEMENT IN CONTEMPORARY AND PROSPECTIVE ENDODONTICS

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ABSTRACT
Endodontic biofilm existing in the form of aggregates and a co-aggregate attached by glycocalyx membrane is considered as primary etiology for endodontic infections. Endodontic microbiota is more complex and far more diverse than expected. The biofilm protects bacteria from host defenses and increase their resistance to intracanal disinfecting protocols. Understanding the virulence of these endodontic microbiota within biofilm is essential for the development of novel therapeutic procedures for intracanal disinfection. The primary goal of endodontic treatment is to eliminate the biofilm from root canal walls. Both the disruption of biofilms and the killing of their bacteria are necessary for effective treatment. Irrespective of the quality of the endodontic treatment, most treatment failures are due to persistence of infection. As stated by Siqueira et al., 'The very high frequency of biofilms in the root canals of treated teeth with post-treatment disease may be interpreted as indirect evidence that, depending on location and possible species composition, biofilms can be a challenge for proper root canal disinfection.' Thus, it is all the more imperative to focus on antibiofilm strategies in order to achieve successful endodontic outcomes. This review paper will aim to address the advanced protocols in Endodontic Biofilm management and their importance.

KEYWORDS: Endodontic Biofilm, Root canal disinfection, Intracanal irrigants, Antimicrobial activity, Antimicrobial photodynamic therapy, Laser disinfection, Antibacterial nanoparticles, Ultrasonic activation.

INTRODUCTION
Because biofilm is a way for bacteria to survive unfavourable environmental and nutritional conditions, it will be more likely to form in the root canal during both primary and post-treatment infections. Furthermore, the root canal systems' anatomical and geometrical complexity shields the adhering microbes from cleaning and shaping treatments.

The infected root canal harbors a polymicrobial population of aerobic, anaerobic, Gram-positive, and Gram-negative bacteria in a biofilm mode of growth. Gram-positive and Gram-negative bacteria have profound differences in their three-dimensional cell architecture. The membrane barrier of a bacterial cell limits the diffusion of antimicrobials into the cytosol. The membrane barriers of a Gram-positive bacterium consist of a relatively thicker but porous cell wall made up of inter-connected peptidoglycan layers surrounding a cytoplasmic membrane. The teichoic acid residues of the cell wall contribute to the negative charge, which serves as binding sites for cationic molecules. Conversely, the cell envelope of a Gram-negative bacterium is composed of an outer membrane, a thinner peptidoglycan layer, and a cytoplasmic membrane. Movement of molecules across a Gram-negative cell wall is strictly regulated at the outer membrane, which is rich in lipopolysaccharides.

Thus, the susceptibility of a bacterium to an antimicrobial will depend upon the type of cell wall it possesses. In addition to the inherent resistance to antimicrobials, bacteria are observed to demonstrate considerably high resistance to antimicrobials when they are in a biofilm mode of growth.[1]

Thereby, it is all the more essential to focus on biofilm management as part of our root canal disinfection protocol. The goal of this review paper is to discuss the significance of improved procedures in the management of endodontic biofilm.

Endodontic biofilms: microbiology and characteristics

Endodontic infection may be primary or secondary. In general, primary infection involves pulp inflammation and root canal infection following invasion by microbes or microbial by-products, eventually resulting in inflammation of the supporting tissues i.e., apical periodontitis. Secondary infection (or post-treatment infection) occurs either as reinfection (acquired or emergent), remnant (persistent) infection or recurrent infection (re-developed in teeth after apparent healing) in teeth that have been previously root canal treated.[2]

Primary endodontic infections are Polymicrobial. They are predominantly Bacteroides, Prophyromonas, Prevotella, Fusobacterium, Treponema, Peptostreptococcos, Eubacterium, and Camphylobacter species.[3]

It is believed that persistence of microorganisms within the root canal system after treatment is the major cause of treatment failure.[3] The ratios of the microbes in primary infections could be different after root canal treatment, as well as a shift in species propagation and quantity. The microbial flora found in secondary infections, typically, are able to survive harsh conditions such as a wide pH range and nutrient-limited conditions. There is a definite contrast in the microbial phenotypes in primary infections as compared to secondary infection, with the latter being predominated by gram-positive bacteria.[4-6] Studies have shown the prevalence of certain species in teeth with post-treatment infection, such as Enterococci, Streptococci, Lactobacilli, Actinomyces and fungi (such as Candida). In particular, a high proportion of Enterococcus fecalis in cases with persistent apical periodontitis was noted.[7,8]

Mixed infections are more common than single-organism isolates. Also, the wide variety of organisms found in root canals can be partially related to the principal interests and culture techniques of different investigators. Isolates from the exposed pulp are similar to the oral flora in which gram-positive cocci predominate, and approximately 25% of the isolates are anaerobes. Organisms associated with flare ups (which are emergency conditions characterized by pain and/or swelling) seem to share a similar composition as those from asymptomatic root canals.[9,10] Bacteria in the state of a biofilm are able to survive tough growth and environmental conditions, which, in part, is due to the protection offered by the extracellular matrix of the biofilms. This structure enables trapping of nutrients and allows metabolic cooperation among various resident bacteria of the same or different species. The organized internal compartmentalization in a biofilm allows bacteria of different growth requirements to survive in their own microenvironments.[3]

The polysaccharide matrix in biofilms retards diffusion of antibiotics and inactivating extracellular enzymes such as β-lactamase may become concentrated. Microbial cells communicate by quorum sensing to encourage the growth of species beneficial to biofilm structure. Subpopulations within a biofilm can alter gene expression to remain protected. Cells remain interiorly where they are protected from medicaments that act only on the microorganisms in the biofilms periphery. Bacterial cells grow more slowly with less metabolism in biofilms than when planktonic, and thereby elude antimicrobial agents. They halt growth with nutrient depletion or waste product accumulation, further protecting them from antibiotics. The altered pH and oxygen level within biofilms may further impair antibiotics.[11]

Challenges in root canal disinfection

The complexities of the root canal system, in addition to the structure and composition of the root dentin, are decisive limiting factors in endodontic disinfection. The root canal system is a highly complex anatomy. The accessory canals, lateral canals, apical ramifications, and transverse anastomoses all contribute to the complexities in root canal anatomy.[4] The inability of the endodontic irrigants/medicaments to penetrate the complexities of the root canal system will cause bacterial biofilms to persist in these niches after cleaning and shaping procedures. Nair et al. showed that following one-visit conventional endodontic treatment, the teeth revealed microbial biofilm in the inaccessible recesses and diverticula of instrumented main canals, the intercanal isthmus, and accessory canals.[12]

The structure of dentin is such that the tubular nature of dentinal tubules makes it a porous structure, and bacteria have been shown to possess the ability to invade dentinal tubules. The degree of bacterial penetration varies between different areas of a tooth and the number of patent dentinal tubules present. The inability of antimicrobials to penetrate the infected dentinal tubules results in the survival of the bacterial population within the dentin (reservoir of infection). Berutti et al. showed that irrigating the canal with sodium hypochlorite (after removing the smear layer) rendered the dentinal tubules bacteriafree only to a depth of 130 mm from the canal lumen, beyond which surviving bacteria were detected.[1]

It is important to realize that even the most powerful irrigant will be of no use if it cannot penetrate the apical portion (up to the working length) of the root canal, interact with the root canal wall, and exchange frequently within the root canal system. In an *in vivo* situation, a tooth root is enclosed in a bone socket and thus a root canal is believed to behave as a *closed-end channel*, which in turn causes gas entrapment at its closed end during irrigation (*vaporlock effect*). Recently there have been several computational fluid dynamics (CFD) analyses carried out to study the nature and pattern of irrigant flow within the root canal space. These studies have demonstrated that irrigants, when expressed into the apical portion of the root canal, experience a turbulent

flow near the exit (orifice) of the needle, followed by a reflux flow, and finally a laminar flow backwards toward the pulp chamber, allowing the irrigant to exit the root lumen. The irrigant flow was noted to be significant only to about 1–2 mm apically from the exit of the needle.[13] Furthermore, the shear stress exerted by the irrigant flow, which aids in the physical detachment of biofilms, was significantly less on the walls of the root canal compared to the center of the root canal lumen. In order to circumvent the above challenges, endodontic irrigation needs to be combined with strategies that apply pressure gradients to the irrigant, such as ultrasonic agitation, sonic agitation, or apical negative pressure.[14,15,1] The application of pressure gradients to irrigants can improve fluid dynamics within the root canal and subsequently result in significant biofilm elimination. These aspects of irrigation dynamics will not be covered here.[1]

The following sections of this article will briefly discuss contemporary treatment options and shed light on advanced therapeutic strategies to manage endodontic biofilms.

Contemporary Treatment Options in managing endodontic biofilms

1. Intracanal irrigants

Sodium hypochlorite (NaOCl) is regarded as the most potent disinfectant in endodontics due to its excellent ability to dissolve vital and necrotic tissues, in addition to its antimicrobial activity. In endodontic therapy, NaOCl is used in concentrations ranging from 0.5 to 6%, all of which demonstrate antibacterial activity. Studies show that the antimicrobial activity is not concentration dependent, but tissue dissolution and biofilm disruption are concentration dependent. The recommended irrigation regimen involves a sequential use of NaOCl and a decalcifying agent. Ozdemir et al., concluded that the combined application of 17% EDTA and 2.5% NaOCl reduces the amount of intracanal biofilm significantly. The effectiveness of sodium hypochlorite may be improved by warming the solution, use of agitation/activation methods, increasing the volume of the irrigant, and lowering the pH of the irrigant solution.[3]

Chlorhexidine (CHX) gluconate with a very broad antimicrobial spectrum is used as an oral antiseptic mouthwash for plaque control and as an irrigant for periodontal therapy and infected root canals. It has a lower grade of toxicity compared to sodium hypochlorite and sustained action i.e., substantivity. A concentration of 2% is recommended as a root canal irrigant. Arias-Moliz et al., showed that alternating the application of CHX and cetrimide resulted in a higher percentage reduction of Enterococcus fecalis compared to the combined use of these 2 agents. Cetrimide facilitates the destruction of EPS matrix allowing CHX to act more directly on Enterococcus fecalis thus resulting in a greater bactericidal potential.[3]

In two different studies, Baca et al., concluded that the combination of 2% CHX and 0.2% cetrimide as a final irrigating solution showed maximum residual and antimicrobial activity on Enterococcus fecalis biofilm.[16,17]

Another bisbiguanide, Alexidine (ALX) was introduced as a root canal irrigant quite recently.

Alexidine differs from CHX due to the presence of 2 hydrophobic ethylhexyl groups, which enables rapid antibacterial action. Compared to CHX, ALX has a greater affinity for lipoteicoic acids resulting in an increased permeability into the bacterial membrane.[3]

Ethylenediaminetetraacetic acid (EDTA) is a chelating agent recommended as an adjuvant in root canal therapy. Many authors have shown its efficacy for removing the inorganic portion of the smear layer. However, EDTA has little or no antimicrobial activity. Alternating the use of NaOCl and EDTA during root canal treatment appears to be a promising approach to remove the organic and inorganic debris, in addition to disrupting microbial biofilms.[3]

Another demineralizing agent, maleic acid has been shown to be effective against E. fecalis at a concentration of 0.88% for 30 seconds.[3]

MTAD (BioPure MTAD, Dentsply Sirona Endodontics, York, PA, USA) is a mixture of 3% doxycycline, 4.25% citric acid and 0.5% Tween 80. Prabhakar and coworkers showed complete inhibition of bacterial growth by MTAD in a 3 week old biofilm. In contrast, some studies have concluded that MTAD did not have good antibacterial activity against E. fecalis. QMiX is a mixture of CHX, EDTA and a detergent. It has been shown to be as effective as NaOCl and superior to CHX against Enterococcus fecalis and mixed plaque bacteria in planktonic and biofilm states. An interesting concept called continuous chelation involves mixing 5% sodium hypochlorite with 18% etidronic acid to serve as a single proteolytic-antibacterial-demineralising solution. Etidronic acid is a weak chelator and hence, when mixed with NaOCl, can be indicated as an irrigant during the entire instrumentation process. The continuous chelation protocol has been shown to bring about excellent antibiofilm activity against biofilms of E. fecalis.[3]

2. Intracanal medicaments

Calcium hydroxide (CH) is a widely used intracanal medicament that has broad antimicrobial activity, which is dependent on the release of aqueous hydroxyl ions to raise pH so that microbes cannot survive. Yet, intracanal CH was reported to be ineffective in preventing E. faecalis biofilm formation in root canals, while still being effective in eliminating their biofilm [38]. Brändle et al. evaluated the effects of growth condition (planktonic, mono- and multi-species biofilms) on the susceptibility of E. faecalis, Streptococcus sobrinus (S. sobrinus),

Candida albicans (C. albicans), Actinomyces naeslundii (A. naeslundii), and Fusobacterium nucleatum to alkaline stress. The findings showed that planktonic microorganisms were most susceptible; only E. faecalis and C. albicans survived in saturated solution for 10 minutes, and the latter also survived for 100 minutes. Dentin adhesion was the major factor in improving the resistance of E. faecalis and A. naeslundii to CH.[11]

Antibiotic combinations have been studied over the past few years as a regimen during regenerative endodontic strategies. The literature is inconsistent on the effectiveness of double and triple antibiotic pastes (DAP and TAP) respectively against mono- and multi-species biofilms. It has been shown that TAP is significantly better than calcium hydroxide and chlorhexidine in disrupting biofilms of E. fecalis. It has been suggested that 1 mg/mL DAP is needed to demonstrate any significant antibiofilm activity.[3]

Human beta defensins (HBDs) are cationic antimicrobial peptides that are critical host defense against microbes [49]. They bind to the negatively charged molecules on bacterial surface and disrupt bacterial membranes. Synthetic HBD-3 consisting of the C terminal 15 amino acids (HBD3-C15) was reported to be effective for disinfecting endodontic biofilm including C. albicans.[11]

Advanced Treatment Options in managing endodontic biofilms

1. Irrigant activating systems

Ultrasonic activation can cause dis-agglomeration of the bacterial biofilm, thus re-suspending the bacteria in planktonic form which are then, more susceptible to antimicrobial irrigants.

Also, any cavitation that may be produced, would cause temporary weakening of the cell membrane, thereby increasing the bacterial cell permeability to antimicrobial irrigants. There is wide variability in the endodontic literature with regards to effectiveness of sonic and ultrasonics in removal of smear layer as well as antibacterial activity. In part, this may be due to differences in the study design, with the parameters for using these activation methods being inconsistent. Notable differences concerned include the volume of irrigating solution used, concentration, activation cycle and replenishment cycle of the solution, which result in inconclusive results. From a logical standpoint, agitation of irrigating agents with sonic or ultrasonic should result in shear stresses that may cause detachment of the biofilms from the root canal walls.[18]

It will also enable better penetration of irrigating agents into the lateral channels of the root canal system allowing better disinfection. However, there appears to be no strong evidence to demonstrate the clinical effectiveness of this approach.[3]

2. Nanoparticles

Antibacterial nanoparticles have been found to have a broad spectrum of antimicrobial activity and a far lower propensity to induce microbial resistance than antibiotics. Studies have shown findings that highlight the fact that chemicals which alter the physico-chemical properties of dentin may influence the nature of bacterial adherence and the adhesion force to dentin. The quantum size effect of nanoparticles permits them to exhibit superior interaction with bacteria and dentin substrate. When cationic nanoparticles in an aqueous suspension are allowed to settle onto the dentin surface (negatively charged), the cationic nanoparticles adhere to the dentin surface via an electrostatic interaction. Although this interaction between nanoparticles and dentin is weak and easily disrupted, it can impede bacterial re-colonization and biofilm formation.[19]

Studies have also shown that the application of CS nanoparticles reduces the adherence of *E. faecalis* to root dentin. The treatment of root dentin with ZnO nanoparticles, ZnO–CS mixed nanoparticles, CS-layer-ZnO nanoparticles, or CS nanoparticles produces an 80–95% reduction in the adherence of *E. faecalis* to dentin. Root dentin treated with chlorhexidine and then with nanoparticulates shows the maximum reduction (97%) in bacterial adherence.[1]

Rose bengal-functionalized CS-np have been widely studied and appear to be effective against monospecies and multispecies biofilms, even in the presence of tissue inhibitors.[3]

Bioactive glass (BAG) has received considerable interest in root canal disinfection due to its antibacterial properties. BAG consists of SiO2, Na2O, CaO2, and P2O5 at different concentrations. Studies so far have shown that most tested nanoparticles possess high antibacterial properties when compared with their powder counterparts. The high reactivity resulting from the nanometric dimension and their ability to resist aging for longer durations are some of the advantages. Most cationic antibacterial particles show excellent interaction with biomaterials, bacteria, and biofilms. In root canal therapy, they may be applied as a slurry or in combination with sealers.[1]

3. Photodynamic Therapy

Antimicrobial photodynamic therapy (APDT) is a two-step procedure that involves the introduction of a photosensitizer (Step 1: photosensitization of the infected tissue) followed by light illumination (Step 2: irradiation of the photosensitized tissue) of the sensitized tissue, which would generate a toxic photochemistry on the target cell, leading to cell lysis. The wavelength of the light should be at the specific wavelength that corresponds to the absorption wavelength of the photosensitizer. A photosensitizer is a chemical agent that, when activated with light at a specific wavelength, reacts with the surrounding molecular oxygen to produce

highly reactive singlet oxygen. Toxicity may become an issue if a high concentration/volume of photosensitizer is applied to a tissue in order to obtain a more significant treatment response. The phenothiazinium group of photosensitizers such as Methylene blue and TBO are generally accepted photosensitizers for clinical application. Phenothiaziniums are usually cationic molecules with a core structure composed of a planar tricyclic aromatic ring system that functions as the chromophore. In addition to phenothiaziniums, cationic porphyrins, phthalocyanines, and chlorins have gained popularity as photosensitizers due to their ability to inactivate both Gram-positive and Gramnegative bacteria. Currently, photosensitizers such as Methylene blue, TBO, rose bengal, erythrosine, chlorin (e6), and hematoporphyrin have been investigated for their antimicrobial potential against oral pathogens.

Nanoparticles are ideal carriers of photosensitizer molecules for APDT. Recently, the effect of APDT on *E. faecalis* biofilm and human dental plaque bacteria was investigated *in vitro* using Methylene blue-loaded poly(lactic-co-glycolic) (PLGA) nanoparticles (positively and negatively charged) that activated with red light (wavelength 665 nm). The cationic Methylene blue-loaded nanoparticles exhibited greater bacterial phototoxicity in both planktonic and biofilm phases. The nanoparticles were found to concentrate mainly on the bacterial cell walls at all tested time points. It was concluded that cationic Methylene blue-loaded PLGA nanoparticles have the potential to be used as carriers of Methylene blue for antimicrobial APDT in endodontic treatment.[20,21]

In the Endodontic literature, Meire et al. and George & Kishen showed that *E. faecalis* could effectively be killed by APDT with photosensitizers such as Methylene blue and TBO along with red light. Soukos et al. conducted APDT experiments on a range of endodontic pathogens using Methylene blue as the photosensitizer and reported complete elimination of all of the bacteria except for *E. faecalis* (53%). In yet another study, significant antibacterial effects on suspensions of *S. intermedius*, *P. micros*, *P. intermedia*, and *F. nucleatum* were reported by Williams et al. following APDT with TBO and red light.[1]

Currently, APDT isn't viewed as a replacement for the current root canal disinfection techniques but rather as a potential supplement.

4. Laser assisted disinfection

Lasers that have a wavelength interacting with water molecules have been used to produce cavitation in liquids. When laser irradiation pulses, the cavitation effect produces a shockwave that can move the irrigating solution within the canal. One brand of Erbium:YAG (Er:YAG) laser propose its use in combination with a special tip to achieve the so-called Photon-induced photoacoustic streaming (PIPS) or irrigant in the canal. This device has been researched for removing debris and smear layer from the root canal system and the results seem positive Lasers that have a wavelength interacting with water molecules have been used to produce cavitation in liquids. When laser irradiation pulses, the cavitation effect produces a shockwave that can move the irrigating solution within the canal. One brand of Erbium:YAG (Er:YAG) laser propose its use in combination with a special tip to achieve the so-called Photon-induced photoacoustic streaming (PIPS) or irrigant in the canal. This device has been researched for removing debris and smear layer from the root canal system and the results seem positive.

There are only a few studies that evaluated laser activation of irrigants using a bioflm model; one of them examined the cleaning of biofilm-infected dentin on a bovine root canal comparing it with sonic or ultrasonic activation and needle irrigation. The authors showed favorable results for PIPS when compared to the other irrigant agitation methods. Neelakantan et al., demonstrated that both diode and Er:YAG lasers were more effective than ultrasonic activation or syringe irrigation method for removing E. fecalis biofilms. However, this study reported no significant difference between Er:YAG and diode laser when a new irrigating agent (sodium hypochlorite mixed with etidronic acid) was used.[3]

5. Ozone

Ozone (O3) is an energized, unstable gaseous form of oxygen that readily dissociates back into oxygen (O2), liberating a reactive form of oxygen, the singlet oxygen (O1). The singlet oxygen is capable of oxidizing cells. It has been suggested that ozone accomplishes its antimicrobial efficacy without developing drug resistance. Ozone gas (HealOzone; KaVo, Biberach, Germany) is currently used clinically for endodontic treatment. In order to achieve a concentration that is relatively non-toxic toward periapical and oral mucosal tissues, the ozone gas concentration currently used in Endodontics is 4 g/m3. This concentration has been shown to be slightly less cytotoxic than NaOCl (2.5%). Aqueous ozone (up to 20 mg/mL) showed essentially no toxicity to oral cells in vitro.

Hems et al. evaluated the potential of ozone as an antibacterial agent using E. faecalis as the test microbe, in both planktonic and biofilm cultures (48-hour-old biofilm grown on a cellulose nitrate membrane filter). Different interaction times ranging from 30 sec to 240 sec were applied to both cultures. It was concluded that ozone had an antibacterial effect on planktonic E. faecalis cells and those suspended in fluid, but little effect on cells embedded in a biofilm structure. The antibacterial efficacy of ozone was not comparable with that of sodium hypochlorite under the conditions tested in this study.[1] Huth et al. assessed the antimicrobial efficacy of aqueous (1.25–20 mg/mL) and gaseous ozone (1–53 g/m3) as an alternative antiseptic against

endodontic pathogens in suspension and in a biofilm model. E. faecalis, Candida albicans, Peptostreptococcus micros, and Pseudomonas aeruginosa were grown in planktonic culture or in mono-species biofilms in root canals for 3 weeks. It was concluded that highly concentrated gaseous and aqueous ozone was dose-, strain-, and time-dependently effective against the tested microorganisms in suspension and in the biofilm test model.[22]

6. Enzyme Derivatives

Some recent trends in anti-biofilm research are directed toward the application of natural extracts from plants to treat biofilm-mediated infection. Morinda citrifolia (MCJ) is an herb that has a broad range of antibacterial, antiviral, antifungal, analgesic, anti-inflammatory, and immune-enhancing effects.[1] MCJ contains the antibacterial compounds L-asperuloside and alizarin. An in vitro study investigated the antimicrobial activity of 2% chlorhexidine gel, propolis, MCJ, 2% povidone iodine (POV-I), and calcium hydroxide on E. faecalis-infected root dentin. It was observed that chlorhexidine gluconate produced better antimicrobial efficacy (100%), followed by 2% POV-I (87%), propolis (71%), MCJ (69%), and calcium hydroxide (55%).[23] Turmeric (*Curcuma longa*) is extensively used as a food preservative in Southeast Asia. It has been used in traditional medicine for the treatment of numerous diseases. A recent report suggested that curcumin in aqueous preparations exhibits phototoxic effects against Gram-positive and Gram negative bacteria. Triphala consists of the dried and powdered fruits from three medicinal plants: *Terminalia bellerica*, *Terminalia chebula*, and *Emblica officinalis*. Triphala achieved 100% killing of *E. faecalis* in 6 min.[24]

Green tea polyphenols is prepared from the young shoots of the tea plant *Camellia sinensis* and showed statistically significant antibacterial activity against *E. faecalis* biofilms formed on tooth substrates.[24]

7. Microbubble Emulsion

Halford et al., were the first to employ a microbubble emulsion to enhance the effect of sonic and ultrasonic agitation of sodium hypochlorite.[25] Essentially, the technique employs unstable gas-filled microbubbles that expand when exposed to ultrasonic waves. The dynamics thereby induced in the fluid would help in detaching surface adherent bacteria or biofilm destruction. In addition, it may also generate reactive oxygen species to exhibit an antibacterial effect. Microbubble emulsion in combination with ultrasonic agitation was shown to be superior than with sonic agitation.[25]

This strategy is clinically intriguing and merits further research.

8. Cold Atmospheric Plasma

CAP represents a promising non-antibiotic option for the eradication and control of biofilm infections. The CAP technique uses a highly reactive mix of ions and electrons, radical species, molecules in the ground or excited state and quanta of electromagnetic radiation (UV photons and visible light). Compared with conventional plasma technology, CAP is operated under atmospheric conditions, and is thus feasible for in vivo applications without damaging the surrounding tissues.

Recently, the CAP technology has been used in various dental applications including root canal treatment and dental implant surface modification. A CAP microjet device with argon/O2 as the working gas was used to disinfect root canals in single-rooted extracted human teeth. It was found the inactivation rate of planktonic E. faecalis gradually increased with the treatment time and reached 98.8% after an 8-min CAP treatment. Root canals treated with CAP for 40 min showed no detectable re-infection. It has been demonstrated that CAP was effective not only for young biofilms, but also for mature E. faecalis biofilms. After a 10-min of CAP treatment, the regular structure of a 100-μm biofilm was destroyed and replaced with ruptured bacteria.[26]

CONCLUSION

Complexity of the root canal anatomy and tenacious nature of the biofilms dictate that simple delivery of antimicrobial agents is not sufficient for disinfection of root canal systems. The focus is on developing methods that will satisfactorily deliver these antimicrobial agents into the complex anatomy, interfere with the adhesive mechanisms by inducing shear stress and disrupt the biofilms. Despite the increasing knowledge of the microbial status of root canal systems, much still remains unknown. The reported success rates of root canal treatment have not undergone significant improvement. From the clinical perspective, it is important to understand the aetiopathogenesis of periradicular periodontitis as a disease caused by microbial infection of the root canal system. Even though we know that root canal biofilms are complex, the literature unfortunately does not seem to offer due credence to understanding the dynamics between the components of a biofilm. Crosstalk between bacteria is a paradigm that has not be sufficiently studied thus far in the context of endodontic disease.[3]

Despite the recent achievements that are summarized in the present review, it should be emphasized that many challenges remain. Most currently available data is obtained from in vitro experiments and in preclinical models in the short term. Researchers must provide quantitative evidences that these reported strategies bring real benefits under in vivo conditions and confirm their antibacterial longevity. The safety profile and development of antimicrobial resistance for these antimicrobial compounds and nanomaterials is a matter of overriding significance and needs further assessment. Appropriately designed and well-structured multi-center clinical trials are critically needed to obtain reliable comparative data to identify the most effective

antibacterial solution and the most optimal parameters for utilizing these anti-biofilm strategies.[26]

Future research should give adequate consideration to the complexity of the microbial biofilm, analyse models to reassess the removal of biomass from root canals, and assess the impact of novel antimicrobial agents on complex biofilms.

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