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Current Approaches In The Elimination Of Bacterial Biofilms From The Root Canal : A Review

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Abstract

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Introduction

Microorganisms are the primary etiological factors for pulpal and periradicular diseases. The oral micro organisms possess the ability to produce biofilms on different surfaces, including both hard and soft tissues. [1] They elicit specific mechanisms for initial attachment to a surface, development of a community structure and ecosystem, followed by detachment. [1] The bacterial cells can exist both in a sessile form within the biofilm as well as in a free-floating form known as the planktonic state.These forms exhibit significant differences primarily due to the protective nature of the biofilm matrix ,shielding them from environmental stresses,making them more resistant to host defence mechanism as well as to the use of antibiotics compared to their planktonic counterparts. [1] Hence , eradication of the bacterial biofilm remains a challenge for the clinicians , over the years.

Biofilm has been defined in a number of ways by various authors :

1. According to Grossman , Biofilm is defined as a community of microcolonies of microorganisms in an aqueous solution that is surrounded by a matrix made up of glycocalyx, which also attaches the bacterial cells to a solid substratum. [2]
2. In a study by Neelakantan et al , Biofilms have been defined as a highly organized structure consisting of bacterial cells enclosed in a self-produced extracellular polymeric matrix attached on a surface. [3]

COMPOSITION OF BIOFILMS - A fully developed biofilm can be described as a diverse arrangement of microbial cells on a solid surface. The fundamental building blocks of this structure are microcolonies or clusters of cells that adhere to the surface and are composed of a matrix consisting of : [1]

i. Proteins, polysaccharides, lipids, nucleic acids forming the organic content and minerals such as calcium, phosphorous, magnesium, fluoride comprising the inorganic content . Together they constitute approximately 85% of the volume of the biofilm. [1], [3]
ii. Remaining 15% consists of bacterial cells themselves. [1]

ENDODONTIC BIOFILMS - Initially, the root canal microflora primarily comprises of aerobes and facultative anaerobes. However, as the disease progresses, the ecological conditions within the root canal system undergo changes due to variations in oxygen levels when the root canals are exposed during treatment, with the use of various irrigating agents ; and shift in canal pH resulting from the introduction of different materials into the canal. [2],[7] The formation of biofilm on the surface of root canal dentin occurs through a number of distinct stages : Adhesion , colonization by various microorganisms , maturation of the biofilm followed by dissolution.

The structural and morphological complexities of the root canal systems, such as deltas and isthmuses make it difficult for cleaning and shaping procedures to effectively remove the microrganisms. [1],[9]. Endodontic biofilms are classified as follows:

1. Intracanal microbial biofilms –
   a. Intracanal biofilms which were initially identified by Nair in 1987 using transmission electron microscopy , refer to the microbial biofilms that develop on the dentin of an infected root canal.
   b. The majority of organisms within these biofilms were observed as loosely arranged filaments, spirochetes, cocci, and rods. [1],[2]
2. Extraradicular microbial biofilms –
   c. Extraradicular biofilms formed on the root surface adjacent to the root apex of endodontically infected teeth are root surface biofilms.
   d. P.gingivalis, F.nucleatum, and Tannerella forsythensis were found to be associated with the biofilm by using polymerase chain reaction (PCR)-based 16s rRNA gene assay. [2] ,[7]
3. Periapical microbial biofilms –
   e. Periapical biofilms in the periapical region of endodontically infected teeth are isolated biofilms which can be seen even in the absence of root canal infections.[2]
4. Foreign body centered biofilms –
   f. A foreign body-centered biofilm emerges when bacterias adhere to a surface of artificial biomaterial and results in structured biofilm formation. This phenomenon is commonly referred to as biomaterial-centered infection. [1],[2]
   g. Takemura et al. discovered that facultative anaerobic gram-positive bacteria colonize and create an extracellular polymeric matrix around gutta-percha, with serum playing a crucial role in the formation of biofilms. [7]

Endodontic disease is a biofilm mediated infection, hence in order to achieve a successful treatment the primary aim is the elimination of the biofilm from the root canal system.[1] Incomplete or inadequate removal during root canal treatment can lead to clinical failure. [1],[2]

Methods Of Removal Of Endodontic Biofilms –

Irrigation of the root canal is one of the effective methods for removal of biofilm which also includes the dissolution of vital or necrotic pulp tissues, neutralization of endotoxins, and removal of the smear layer. [3]

The challenge in endodontic treatments is for the irrigants to reach the minute areas and facilitate the removal of the inflamed or necrotic tissues within the biofilm. [3]

The methods of removal of endodontic biofilm are : [1], [3], [7]

i. Traditional methods
ii. Contemporary approaches.

Traditional methods used for the treatment of endodontic biofilms –

Biofilms within the root canal system of teeth exists in a protected environment with low oxygen tension , low pH and little if any flow of fluid. The absence of shear forces distinguishes these biofilms from oral biofilms such as dental plaque on the surfaces of teeth. The structure of these biofilms limit diffusion of active ingredients from irrigating solutions and medicaments. Hence , achieving reliable and complete decontamination and disinfection of the root canal remains a major challenge. [11]

The various traditional methods which have been used over the years for removal of biofilms from the root canal , with their limitations are as follows

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Method	Major Limitations
Physical debridement	
Hand endodontic files	Non-contact with walls of the root canal; instrument breakage
Rotary endodontic files	Limited contact with walls; excessive root structure removal
Ultrasonic endodontic files	Limited activation of irrigant fluid; apical fluid extrusion
Irrigant solutions	
Sodium hypochlorite	Chemical irritancy; chemical instability; instrument corrosion
Hydrogen peroxide	Chemical irritancy; interactions with other irrigant solutions
Chlorhexidine	Limited spectrum of activity; chemical degradation
EDTA	No antimicrobial actions; inactivates sodium hypochlorite
Medicament pastes	
Calcium hydroxide	Limited alkaline pH for aqueous preparations
Phenolic compounds	Limited spectrum of activity; chemical irritancy
Tetracyclines	Staining of roots from first generation tetracyclines
Clindamycin	Inherent resistance of *E. faecalis*; adverse reactions

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Because of these challenges, a range of novel approaches have been developed for detecting and treating biofilms within the root canals of teeth.

Contemporary approaches for the treatment of endodontic biofilms

METHODS	EXAMPLES	MECHANISM OF ACTION
1. Instrumentation of root canal using newer file designs	i. XP-endo Shaper ii. Hyflex EDM iii. One Curve	Files are made from alloys with different manufacturing processes which permits them to expand from original size giving the instruments a semi-circular shape that helps to perform eccentric rotary motion against the canal walls.
2. Improved irrigating solutions A) Addition of detergants	Cetrimide, Benzalkonium chloride,etc	Reduces the surface tension & improves penetration of solution into dentin.
B) Physical activation of the solution	i. Ultrasonic activation ii. Laser activation – a) Er:YAG	Acoustic streaming of irrigants Orchestrated cavitation

	b) Er,Cr : YSGG c) Photon-induced photoacoustic streaming(PIPS) iii. Sonic irrigation using Polyamide tip	
C) Continuous chelation	Bisphosphonates like Clodronate	Produces stable mixture to remove the biofilm
D) Use of nanoparticles	Chitosan , Zinc oxide , Silver nanoparticles, etc.	Small size, good reactivity producing broad spectrum antimicrobial activity
E) Enzymatic irrigation	Trypsin, Proteinase K , etc	Interference with bacterial cell wall
3. Improved antimicrobial medicaments	Triple antibiotic paste , CHX gel	High alkaline pH inactivating microorganisms in the canal
4. Antimicrobial Photodynamic therapy		Light activated disinfection of the root canal.
5. Plasma	a) AC argon/oxygen cold plasma	Low temperature disrupting the biofilms in the canal

I) Instrumentation of the root canal –

Biofilms in the root canal are difficult to reach with round endodontic files hence, files with novel shapes that can move in eccentric or oval paths have been developed. These designs are more effective in canals with oval cross-sections than the circular files. [11]

-XP-endo Shaper, Hyflex EDM, One Curve files have shown significant results in eliminating root canal biofilms in a study by Hamed et al.[12]

Bartoluzzi et al conducted a study (2015) on the efficacy of 3D conforming nickel titanium rotary instruments in eliminating bacteria from oval-shaped root canals . Results have shown that the 3D conforming nickel titanium instruments which were tested, removed significantly more bacterial biofilms from the surface of canal walls than a contemporary concentrically rotating nickel titanium instrument system. [13]

Nevertheless, any given rotary instrumentation system needs to be combined with a suitable antimicrobial irrigating solution, and a suitable delivery or agitation technique for that solution, to achieve effective chemo-mechanical debridement of the root canal.[11]

II) Improved irrigating solutions –

Traditionally, extensive irrigation with 2.5–6% sodium hypochlorite (NaOCl) solutions has been typically employed over the years to remove the endodontic biofilms. They have shown to exhibit antimicrobial effects against both bacteria and fungi, however , certain highly resistant organisms, particularly E. faecalis, necessitate longer exposure time of up to 5 minutes for inactivation when it is in the biofilm state .Bacteria deeply lodged within dentinal tubules remain shielded from direct contact with NaOCl, and the high pH of the irrigating solution is buffered by the adjacent dentin.[11]

Additionally, physical activation of the solution can be achieved using ultrasonic instruments or pulsed middle infrared lasers (such as Er:YAG or Er,Cr:YSGG lasers) which has shown to provide effective results.

Ultrasonic agitation with piezoelectric instruments require a moving tip, and creates random cavitation events, while laser agitation uses a stationary tip and creates orchestrated cavitation events. This explains the superiority of lasers over the ultrasonic instruments for agitation.

Lasers that have a wavelength interacting with water molecules have been used to produce cavitation in liquids. This cavitation effect produces a shockwave that can move the irrigating solution within the canal. [21]

Photon induced photoacoustic streaming (PIPS) –

Photon-induced photoacoustic streaming (PIPS) uses Er:YAG laser energy (2940 nm) at subablative power levels (0.3 W, 20 mJ at 15 Hz) and a single short pulse duration (50 μs).[24]

The goal of PIPS is to improve biofilm removal by creating photoacoustic shockwaves that would travel through the root canal system , filled with an irrigant [25] . When applying the PIPS technique, the laser tip is usually positioned in the access cavity (pulp chamber or canal entrance).

-A study by Seal GJ et al have shown that low power laser directed at the access cavity combined with a photosensitizing agent was bactericidal on S.intermedius biofilms in root canals, but less effective than NaOCl (3%) irrigation . [21]

-Recently , Golob et al. (2017) suggested a modified PIPS protocol, which offered promising results in disinfection of root canals .Unlike the conventional PIPS protocol, the authors introduced PIPS with EDTA, before NaOCl irrigation, which removed the mineralized part of the smear layer, thus opening dentinal tubules and enabling deeper penetration of NaOCl. [30]

Sonic activated irrigation using Polyamide tip –

-Sonic-activated irrigation (SAI) is based on acoustic streaming agitation using a frequency of 1–6 kHz, which is lower than that used in ultrasonic activated irrigation [31]. Recently, SAI using polyamide tips (EDDY for root canal agitation) has been introduced. . The polyamide tip is characterized by a low elastic modulus that can follow the curved canal without preparing the canal wall. Hence, oscillation of polyamide tips with a frequency in the kHz range may achieve effective biofilm removal, through not only agitation of the irrigant but also direct contact with a wider area of the root canal wall for the propagation of vibratory force. [32]

Continuous chelation-

The continuous chelation approach is a newly introduced protocol where both a chelating agent as well as sodium hypochlorite are mixed together and applied into the canal.The agents do not react with NaOCl and may be able to reduce time and complexity, as well as improve the quality of debridement of the canal by its more potent actions on biofilms and smear layer . [11],[13]

Use of nanoparticles –

In recent years, the use of nanoparticles to disinfect root canals has gained popularity due to their broad spectrum antibacterial activity. Nanoparticles with reactive molecules have the potential to combat microorganism resistance, since they have the advantages of very small sizes, a large surface area to mass ratio and very good reactivity. [7] Chitosan (CS-np), zinc oxide (ZnO-np) and silver (Ag-np) nanoparticles possess a broad spectrum of antimicrobial activity by altering bacterial cell wall permeability ,resulting in cell death.

Enzymatic irrigation –

It was introduced by Niazi and coworkers, who evaluated the effectiveness of 1% trypsin and 1% proteinase K, with or without ultrasonic activation, on a multi-species biofilm. Trypsin with ultrasonic activation was able to effectively kill both aerobic and anaerobic bacteria and has the capability of disrupting the biofilm. [39] Agents that interfere with the cell wall, such as D-amino acids, specifically D-leucine has been demonstrated to bring about efficient dispersal of biofilms and it has been suggested that the biofilm dispersal by sub-toxic concentrations of this agent reduces the success of resistant organisms.[39],[40]

III) Improved antimicrobial medicaments –

Microorganisms should be kept at a pH level of over 11 to be inactivated, but this pH is not achieved in the root canal when water-based calcium hydroxide pastes are used, because of pH buffering by dentinal proteins .

Other than calcium hydroxide, there is a growing interest in non-antibiotic antimicrobial agents which can penetrate biofilms. Plant-derived phenolics, and nanoparticles such as chitosan which can inactivate both fungi and bacteria have been incorporated in the medicaments to improve antibacterial efficacy. [11]

An in vitro study by Mozayeni MA et al (2014) showed that the triple antibiotic paste and CHX gel showed better antibacterial activity than calcium hydroxide (CH) and so can be used as an alternative medicaments in endodontic treatment . [41]

AUTHOR	METHOD EMPLOYED	AGENTS USED	MECHANISM OF ACTION	RESULTS
1.Wang et al (2012) [14]	Addition of detergent to the conventionally used irrigating solution of 2% Sodium hypochlorite.	0.1% Cetrimide	Improved flow of the irrigants into the anatomical complexities of the canal, due to reduced surface tension.	Increased antibacterial efficacy against *E. faecalis* in the dentinal tubules.
2. Aleksandr Baron et al (2016) [15]	Incorporation of detergent to 6% Sodium hypochlorite solution.	0.008% Benzalkonium chloride	Reduction in the surface tension of the irrigant with enhanced wettability of dentin surface.	Significant reduction in the number of bacterias in the endodontic biofilm after irrigation compared to NaOCl alone.
3. Frederik Bukiet et al (2012) [16]	Solutions of detergent were mixed in 2.4% sodium hypochlorite	0-1% benzalkonium chloride	Reduction in the contact angle and surface energy of the dentin without affecting the chloride content.	Superior eradication of biofilm bacteria from the root canal .

4.Laurence J Walsh (2017) [17]	Ultrasonic agitation of NaOCl and EDTA based on piezoelectric unit	Ultrasonic tip	Ultrasonic energy creates cavitation at the tip of the instrument. The explosions and implosions produce shear stress, which can disrupt biofilms and damage microorganisms.	The performance of alkaline solutions of NaOCl and EDTA improved significantly when agitated using ultrasonic energy.
5. Anastasios Retsas et al (2022) [18]	Ultrasonic activation of NaOCl with piezoelectric instrument	Ultrasonic tip	Ultrasonic agitation requires a moving tip, and creates random cavitation events referred to as acoustic streaming.	Three cycles of intermittent ultrasonic activation of NaOCl irrigation resulted in more effective biofilm removal from the lateral canal than without activation.
6.de Groot et al (2013) [19]	Laser activated irrigation using 2% sodium hypochlorite.	Diode laser	Streaming, caused by the collapse of the laser-induced bubble is the main mechanism behind removal of bacterial biofilms.	More effective removal of biofilm from the apical part of the root canal than passive ultrasonic irrigation or hand irrigation when activated for 20 seconds.
7. Neelakantan et al (2014) [20]	Laser induced activation of irrigants	Diode laser , Er:YAG laser.	Cavitation produced improves penetration of the irrigants	Superior to ultrasonics in biofilm removal and disinfection of the canal.
8. Meire et al (2012)[22]	Laser activated irrigation using sodium hypochlorite	Er : Yag		Combination of Er:Yag irradiation and NaOCl irrigation can provide better biofilm removal than Er:Yag laser alone
9. Cheng et al. (2012) [23]	Laser activated irrigation using sodium hypochlorite	Er : Yag		Removal of E. faecalis from deep dentin layers was achieved by applying Er:Yag laser and NaOCl as irrigant together as it supports penetration of NaOCl.

10. Neelakantan et al [20]	Laser activated irrigation using sodium hypochlorite	Er : Yag		More effective than ultrasonic activation or syringe irrigation for removing biofilms.
11. Cheng et al (2012) [23]	Laser activated irrigation using sodium hypochlorite	Er : Yag and Er,Cr:YSGG		Superior removal of biofilms from the canal when compared to Er,Cr:YSGG laser irradiation alone.
12.De Meyer et al. (2017) [24], [25]	Inserted the PIPS tip into the root canal, 6 mm short of the working length.	PIPS tip		Equal effect of PIPS was observed, regardless of the position of the laser tip.
13. Balic et al. (2016) and Hage et al. (2019) [26],[27]	Compared biofilm removal efficacy of sonic irrigation and PIPS.	PIPS tip and sonic irrigation with NaOCl		Both PIPS and sonic irrigation of NaOCL remove biofilm evenly from the root canal .
3.Kasic et al (2017) [28]	Compared the effect of PIPS to Er,Cr:YSGG laser in dual-species biofilm removal.	PIPS tip and laser		Er,Cr:YSGG laser agitation of non-antimicrobial agent performed better at E. faecalis and C. albicans biofilm removal in comparison to PIPS.
14. Swimberghe et al (2019) [29]	Compared the anti-biofilm activity of PIPS with Sonic devices.	PIPS tip and sonic irrigation		PIPS was more efficient than sonic devices in removing hydrogel from the isthmus when using only water as irrigant.
15.Yahata Y et al (2023) [32]	Sonic activated irrigation	Polyamide tip	The primary mechanism of root canal cleaning with polyamide tips is agitation of the irrigant caused by acoustic streaming around the tip. In contrast to conventional Ultrasonic and Sonic tips, which mainly oscillate in one	A polyamide tip oscillating with a frequency in the kHz range is an effective strategy for biofilm removal, which was achieved not only by agitating the irrigant but also by mechanical action on the root canal wall.

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			direction the EDDY tip has an oval oscillation trajectory suggesting that tip oscillation is distributed over the entire circumference.	
16.Wu et al (2013) [34]	Application of nanoparticles as medicament in the canal.	Silver nanoparticles of 10-100nm	Powerful antibacterial activity against gram-positive and gram-negative bacteria.	When used as a medicament potentially eliminated residual bacterial biofilms during root canal disinfection.
17. Veerapandian M et al. (2011) [35]		Mesoporous bioactive calcium silicate nanoparticles and bioactive glass powder loaded with silver nanoparticles	Potent antimicrobial action against biofilm bacteria.	Significant reduction in adhesion of E. fecalis biofilms .
18.Srestha et al (2016) [36]		Chitosan nanoparticles	Effective against monospecies and multispecies biofilms, even in the presence of tissue inhibitors	Improved antibiofilm efficacy from the root canal.
19. Zhang et al. (2016)[37]		Chlorhexidine with silver nanoparticles	Inbition of bacterial cells in the biofilm	Significant inhibitory effect on the biofilm of E. faecalis.
20. Louwakul et al.(2016)[38]		Chlorhexidine nanoparticles and Calcium oxide nanoparticles	Promoting bacterial cell death	CHX nanoparticles were more efficient than calcium oxide nanoparticles in bacterial elimination in dentinal tubules
21. Neelakantan et al (2017) [20]		Chitosan nanoparticles added to Calcium hydroxide	Bacterial inhibition	Enhanced bacterial killing in a multi species biofilm over a period of 7 to 14 days was seen compared to use of Calcium hydroxide alone.

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22. P. P. Wright et al (2019) [33]	Clodronate mixed equally with 10% NaoCl and applied for continuous chelation in the canal.	Bisphosphonate like Alkaline Clodronate		Stable mixture showing significant removal of endodontic biofilm.
23.Laurence J.Walsh(2020) [11]	Addition of Clodronate with Sodium hypochlorite irrigant.	Non-nitrogen containing Clodronate		Excellent biofilm removal capabilities seen .

IV) Antimicrobial photodynamic therapy (APDT) [Light activated disinfection of the root canal] -

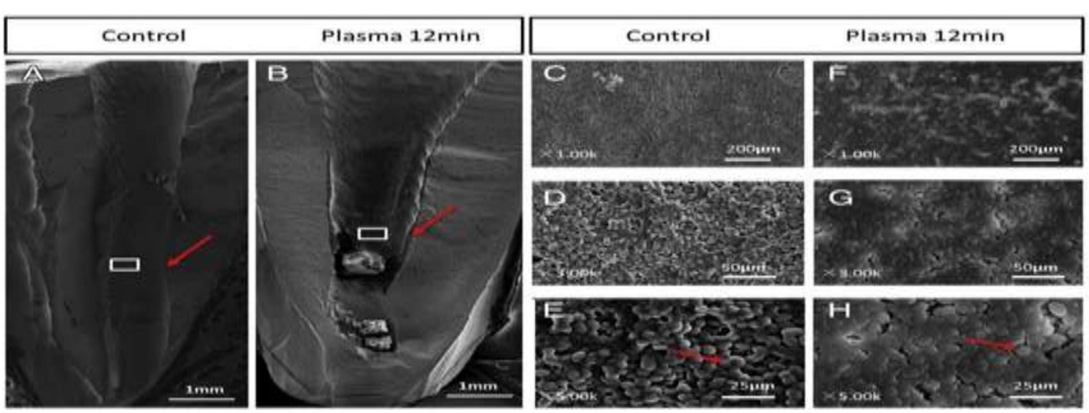
APDT is a two-step procedure that involves applying a photosensitizer, followed by illuminating the sensitized tissues with light. This generates a toxic reaction on target cells, leading to the killing of microorganisms according to studies by Dai T et al(2009), deOliveira et al(2014) ,Kishen A et al(2015).

Nowadays, APDT is considered as an adjunct to traditional protocols for disinfecting canals. To enhance the antimicrobial efficacy of APDT in endodontics, recent research has developed new formulations of photosensitizers that effectively penetrate dentinal tubules, anatomical complexities, and possess antibiofilm properties. [15]

APDT can be combined with the usual mechanical instrumentation and chemical antimicrobials.

V) Role of Plasma –

Atmospheric pressure cold plasma is an effective therapy in endodontics for its strong sterilization effect on fully matured biofilm within a few minutes. It has been accepted to be mechanically safe for its low temperature and does not alter the microhardness and roughness of root canal dentin significantly. [49]



Biofilm Modifiers – A very recent approach is the use of biofilm modifiers in the root canal. These agents modify the biofilm without hampering or killing all the useful microorganisms in the biofilm. The various biofilm modifiers acting against different bacterial strains include Furanone C-30 , Quercetin , Antimicrobial peptide Cec4 , etc. They act by downregulating the quorum sensing genes and affect the motility of the microorganisms. [51]

Conclusion –

Endodontic biofilms are well protected within the root canals of teeth and are difficult to access. Their complete elimination is essential for long-term clinical success and remains a major goal for endodontic research. Recent advances in root canal disinfection using new technologies and approaches may improve the ability to disinfect the root canal system. Further more research and long term studies are required for better understanding of the interactions between microbes in biofilms and how each organism influences the other. This, in conjunction with the various recent therapeutic strategies may help to remove the biofilm and improve the success rates of endodontic treatment. Such strategies must focus on a step-wise approach from monospecies to multispecies biofilms eradication from the canal so as to develop a sufficient knowledge on their mechanisms at a cellular level . [1],[7]

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