Review Article

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Advanced Treatment Modalities For Endodontic Biofilms : A Narrative Review.

Abstract:
Endodontic disease is essentially a biofilm-mediated infection with broader bacterial diversity than previously anticipated. Removal of endodontic bacterial biofilms and preventing recontamination of root canal after treatment are the essential elements for successful outcomes of endodontic treatment. Nanoparticles and antibacterial photodynamic therapy corroborate to be emerging tools as they have proved to be effective in eradicating biofilms from the complex root canal system in various in-vitro studies. This article primarily aims to provide an exhaustive overview based on available literature on advanced treatment modalities to eradicate these biofilms.

Key-words: Biofilms, ozone, nanoparticles, lasers, morinda

Introduction:

The current notion emphasizes presence of biofilm to be the primary source of endodontic infection. Therefore for an endodontic treatment to be successful complete eradication of these biofilms is of utmost importance. Despite of meticulous chemo-mechanical preparation and obturation there is high prevalence of bacterial biofilms in uninstrumented portion of root canal system. This can be attributed to limitation with current disinfection strategies and challenges encountered within root canal system.[1] Table-1[2] illustrates major limitations with current approaches of treatment and Figure-1[1] demonstrates the various challenges encountered during root canal disinfection

Method	Major Limitations
Physical Debridement Hand/Rotary Endodontic Files	Limited contact with root canal walls; excessive dentin structure removal
Irrigant Solution	
Sodium Hypochlorite	Chemical irritancy, Chemical instability
Chlorhexidine	Limited Spectrum of activity
EDTA	No antimicrobial action ; inactivates sodium hypochlorite.

Table 1: Traditional methods used for the treatment of endodontic biofilms.[2]

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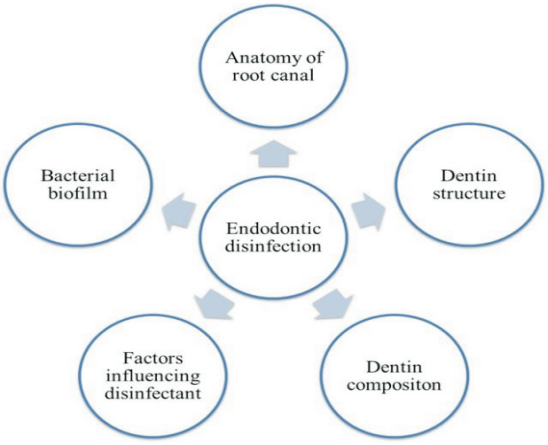

[1]Fig 1: Different challenges in the disinfection of endodontic biofilms. Courtesy: Anil Kishen

[1]RIDHIMA GUPTA, [2]ASHWINI B PRASAD, [3]DEEPAK RAISINGANI, [4]PRACHI MITAL
[1-4]Department of Conservative Dentistry and Endodontics Mahatma Gandhi Dental College and Hospital, Sitapura Industrial Area, Jaipur

Address for Correspondence: Dr. Ridhima Gupta
Department of Conservative Dentistry and Endodontics Mahatma Gandhi Dental College and Hospital, Sitapura Industrial Area, Jaipur
E-mail : ridhimagupta128@gmail.com

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Consequently to overcome these challenges, novel treatment modalities have been introduced with an objective to eliminate biofilm bacteria from uninstrumented portion and anatomical complexities without inducing untoward effects on dentin and periapical tissues.[1]

This article aims to describe in detail about the available contemporary therapeutic options for root canal biofilms.

Novel Therapeutic Strategies

1. Antibacterial Nanoparticles:

Nanoparticles are microscopic particles whose dimension is in the range of 1–100 nm. These particles due to there quantum size exhibit superior interaction with bacteria and dentin .Further high surface area, charge density associated with these particles and greater degree of interaction with cells, overall enhances thereantimicrobial/antibacterial activity[1]

Anti-bacterial nanoparticles exhibit broad spectrum of antimicrobial activity andin contrast to antibiotics do not induce microbial resistance[1]

Many nanomaterials, such as silver, copper oxide, zinc oxide nanoparticles, gold, titanium oxide, graphene, chitosan and bioactive glasses can be used to control biofilm formation .

Mechanism of Action:

There action is mediated via electrostatic interaction between nanoparticles (positively charged) and bacterial cells (negatively charged). This is followed by increase in membrane permeability and rapid loss of membrane function due to accumulation of a large number of nanoparticles on the bacterial cell membrane.[1]

An in vitro study was conducted by Hirashi et al to evaluate efficacy of silver diamine fluoride against 48 hrs old E.faecalisbioflim. Findings demonstrated that there was complete eradication of E. faecalis biofilm after interaction with silver diamine fluoride for 60 minutes; Silver diamine fluoride was also found to penetrate upto 40 mm into dentinal tubules.[4]

Another study[5] demonstrated that Ag-NPs antibacterial efficacy was more significant when used as medicament rather than irrigant; Ag-NP gel(0.02%) when used for 7 days as medicament showed superior activity in disrupting E.faecalis biofilm in comparision to syringe irrigation with Ag-NPs solution with higher concentration (0.1%) and calcium hydroxide groups. One possible explanation for this could be that when used as a medicament there might be prolonged interaction between positively charged Ag-NPs and negatively charged biofilm bacteria/structure resulting in this variation.[5]

Drawbacks of silver nanoparticles:

1. Potential discoloration of dentin
2. Toxicity towards mammalian cells [6]

Commercial Products :

1. Nanoseal SPrevestDenpro(Silver nanoparticle based antimicrobial sealer)

Shelf Life-3 years

Working time- <10 min

Setting Time-10-15 min

2. Orasil Endodontic irrigant (Silver nanoparticle based)
3. Nanocare Plus Silver Gold (Dental Nanotechnology, Katowice,Poland) (Silver and Gold nanoparticle based irrigant).

2. Herbal and enzyme alternatives

Use of natural extracts from plants to treat biofilm-mediated infection is a new area of research owing to there easy of availability, cost-effectiveness, low toxicity and lack of microbial resistance[1]

Spectrum of Antimicrobial efficacy-Broad spectrum, Bacteriostatic as well as bacteriocidal.[1,7,8]

A. Morindacitrifolia Juice (MCJ) –It is a herb comprised of antibacterial compounds L-asperuloside and alizarin. It exhibits broad range of activities. Besides being antibacterial, antiviral and antifungal it also possess analgesic, anti-inflammatory, and immune-enhancing effects .[1]

A study[9] compared the *in vitro* effectiveness of MCJ, sodium hypochlorite and chlorhexidine gluconate to remove the smear layer from the root canal. Findings revealed that effectiveness of MJC is similar to that of NaOCl when used in conjunction with EDTA as an intracanal irrigant.[1]

B. Turmeric (*Curcuma longa*) -Curcumin (diferuloylmethane), the main bio-active component of turmeric, have a wide spectrum of biological actions (anti-microbial, anti-inflammatory, and antioxidant activities). In accordance to recent evidence curcumin shows similar antimicrobial effect and endodontic disinfection as sodium hypochlorite and therefore could be used as an effective photosensitizer.[10,11,12]

C. Triphala-Fruits from three medicinal plants *Terminalia bellerica*, *Terminalia chebula*, and *Emblica officinalis that are dried and powdered* in equal proportions constitutes Triphala. Different compounds in such formulations may overall help to enhance the potency of the active compounds by there synergistic or additive effect. In a study following 6 minute interaction of Triphala with E.faecalis resulted in its complete eradication.[1,8]

D. Green tea-Tea plant *Camellia sinensis have* shown statistically significant antibacterial activity against *E. faecalis* biofilms formed on tooth substrates. The antibacterial action is mediated through polyphenols that are derived from its young shoots.[1,8]

E. Berberine– Plants such as Berberis Vulgaris (Barberry) ,Goldenseal and Coptis Chinesis are the main source for the quaternary ammonium salt of the isoquinolones group, Berberine. Berberine could be derived from various parts of plants such as root, leaf, and fruit juices[13] and is well known in Iran as a source of traditional drugs. It possesses a broad antimicrobial spectrum against bacteria, fungi, protozoans, virus, helminthes, and chlamydia. Its antibacterial activity is comparable to 5.25% sodium hypochlorite and 2% chlorhexidine when used in conjunction with 1% chlorhexidine.[12,13] . When used along with miconazole it has shown favorable antimicrobial activity against *Candida albicans* biofilms in a non-endodontic model.[14]

F. Trigonella foenum graecum seed extract (TFGSE)-Natural plant extracts such as Trigonella foenumare widely used in medical treatments. Mature Trigonella seed exhibits many active compounds such as fatty acids, amino acids, flavonoids, vitamins, saponins such as diosgenin and alkaloids. These compounds account for their wide range of medicinal properties. Based on its anti-inflammatory, antimicrobial and low cytotoxicity[15] it can be considered one of the suitable alternatives as an endodontic irrigant. Recently an in vitro study by Tosun etal demonstrated that TFGSE is more effective when compared with sodium hypochlorite in removing smear layer.[16]

3. Ozone:

Mechanism of Action:

Its action is based on oxidative cell damage of bacteria caused by singlet oxygen which is generated during dissociation of ozone, an unstable gaseous form of oxygen. It exhibits antimicrobial efficacy without inducing drug resistance[1]

Hems et al.[17] conducted an in vitro study to evaluate antibacterial efficacy of ozone. E.faecal is was grown both in planktonic and biofilm cultures on cellulose nitrate membrane filter. Interaction times ranging from 30sec-240 sec were applied in both 48 -hour old cultures. His findings demonstrated that ozone has significant antibacterial effect on planktonic E.facealis cells but little effect when cells are embedded in biofilm structure.[1]Further under the conditions tested in this study ozone antibacterial effectiveness was not comparable to that of sodium hypochlorite [17,18].

Overall inconsistent results has been yielded by Ozone gas (HealOzone, KaVo, Biberach, Germany)in its efficacy to eliminate endodontic pathogens. This could be attributed to difference in concentration used and duration of its application in different in -vitro studies. [1]

The conflicting evidence on its antimicrobial efficacy and reduced effects on sessile when compared to planktonic bacteria.[19]can be due to following reasons :

(i) Extracellular Polysaccharide portion of the biofilm may act as a physical/ chemical barrier thereby preventing deeper penetration of dissolved ozone in biofilm structure.[1]

(ii) Ultrastructure ofbiofilm is comprised of the water channels that surround microcolonies. Convective flow regulates the liquid movement through these channels. Any obstruction of these channels such as by the oxidation products of ozone may halt further diffusion of ozone into the inner layers of the biofilm structure[1] .

(iii) Enhanced resistance to antimicrobials possessed by morphologically altered microbial communities in the deeper aspects of the biofilm[1].

Commercial Product-HealOzone; KaVo, Biberach, Germany Concentration -4g/m[3] (non toxic to periapical and oral mucosal tissue).[1]

4. Antimicrobial Photodynamic Therapy:

APDT is a two-step technique which involves application of a photo sensitizer to tissue, followed by light illumination of the sensitized tissues. Light source used in this procedure could be either coherent (lasers) or non-coherent (lamps). APDT may be used in conjunction with the mechanical instrumentation and chemical antimicrobials.[1]

Mechanism of Action:

It is based on activation of photosensitizer (phenothiazinium chromophore group ,Methylene Blue and Toluidine Blue O (TBO)) by light of specific wavelength which induces photosensitizer to react with surrounding molecular oxygen to produce highly reactive singlet oxygen thereby causing phototoxic cell damage.[1]

Methylene Blue and TBO are phenothiazinium group of photosensitizers that are suitable for clinical application. Research over the last decade has shown that phenothiazinium chromophore are promising candidates for use as photosensitizers in APDT.[1]

Phenothiaziniums are chemical agents that find application in APDT as photosensitizers . Chemically they function as the chromophore which are cationic molecules composed ofa planar tricyclic aromatic ring system which forms its core structure. Besides phenothiaziniums, cationic porphyrins, phthalocyanines, and chlorinshave gained popularity as photosensitizers dueto there effectiveness against both Gram-positive and Gram-negative bacteria[1]

In accordance to George and Kishen[20,21] APDT can impair functional integrity of cell wall, membrane proteins and DNA of E. faecalis. The intensity of impairment to these targets is regulated by amount of photosensitizer solvent used during APDT.[22]

On comparing the effectiveness of APDT, standard root canal therapy and the combined treatment Garcez *et al* findings showed that there was 90% reduction in bacterial count with root canal therapy alone,95% with APDT and>98% when combined treatment was done. After 24 h bacterial regrowth observed was less for combined treatment when compared with either of single treatment.[22,23]

George and Kishen compared water based Methylene blue formulation with MIX- based Methylene blue formulation which is a mixture of glycerol: ethanol: water in a ratio of 30:20:50. They suggested that MIX based Methylene blue formulation resulted in significantly higher impairment of the bacterial cell wall and extensive damage to chromosomal DNA. This can be attributed to their effective penetration into dentinal tubules and enhanced singlet oxygen generation, which in turn improved bactericidal action.[1,24]

In order to improve antibacterial/antimicrobial efficacy and to reduce toxicity associated with photosensitizers, they are conjugated with various agents/chemical moieties. Fig 2 shows various strategies that have been used to combine nanoparticles with.[1]

Fig. 2. Schematic diagram showing different methods of combining nanoparticles and photosensitizers. Courtesy: Anil Kishen

Recently studies have also been conducted to evaluate effect of APDT on bacterial endotoxins. Endotoxins also known as lipopolysaccharide are large molecules encompassed in the cell wall of gram-negative bacteria. It is mainly composed of lipids, polysaccharides, and proteins.[25,26]

Shrestha *et al.*[29] evaluated the ability of APDT to inactivate endotoxins/LPS when used in conjunction with chitosan-conjugated rose bengal NPs (CSRBnps). Findings indicated that following application of photodynamically activated CSRBnps there was significant inactivation of endotoxins and subsequent decrease of all tested inflammatory markers from macrophages.[22]

Limitations of APDT in Endodontics:

The following limitations of APDT are tissue specific.

1. Restricted accessibility of the activating light energy in the root canal system
2. Limited diffusion of the optimum photosensitizer concentration into the infected tissue
3. Availability of oxygen in the infected tissue
4. Dentin discoloration induced by excess photosensitizer.[1]

5. Photon Induced Photoacoustic Streaming(PIPS)

Direct shock waves that are generated by an Er:YAG laser in the liquid irrigant forms the basis of PIPS. These lasers exhibitsubablative energies of 20 mJ at 15 Hz for an average power of 0.3W at 50 µs impulses[1]

Mechanism of Action:

The mechanism of action has been attributed to the efficient absorption of mid-infrared wavelength light by water that leads to formation of vapor bubbles of irrigant, which subsequently expand and implode with secondary cavitation effects. This process induces high-speed fluid motion into and out of the canal producing photomechanical effects[1]

According to an in -vitro study conducted by Alshahrani *et al.* [28] application of PIPS with 6% NaOCl is a more effective combination when compared to PIPS with water or irrigation with 6% NaOCl. Further Olivi *et al.*[29] showed that effect of irrigants commonly used in endodontics can be increased when used with PIPS.[22] With the PIPS technique, chelators and irrigants are strongly agitated in the root canals, increasing antibacterial and/or chelation potential of these solutions even distant from source, especially in apical 3rd of canal. One of these solutions is chitosan. Its superiority and antibacterial effects in terms of smear removal have been proven in previous studies.[30,31]

6. Gentlewave irrigation:

Generation of broad spectrum sound waves form the basis of disinfection of root canal by Gentlewave (GW) (Sonendo, Laguna Hills, CA, USA) system .[22]

When positioned inside the pulp chamber multisonic waves are initiated at the tip of GentleWaveTM handpiece, along with a stream of treatment solution. The stream of treatment fluidinteracts with the stationary fluid inside the chamberand create a force which causes hydrodynamic cavitation. A built-in vented suction in handpiece simultaneously remove the excess fluid.[22]

A study by Haapasalo *et al.* showed that the GW System provides eight to ten times faster tissue dissolution when compared with ultrasonic devices and needle irrigation.[32]

7. Lasers:

The prime use of lasers is to enhance the degree of microbial elimination subsequent to cleaning and shaping procedures. Infrared lasers such as CO_2, Nd:YAG, diode, and Erbium lasers are commonly used in dentistry for root canal disinfection.[1]

Mechanism of Action:

Its action is based on thermally induced alteration of the bacterial cell wall which leads to changes in the osmotic gradients, swelling, and cell death. Gram negative bacteria owing to their cell wall characterstics are more resistant to laser therapy than Gram positive bacteria.[1]

In a study by Noiri*et al.*[33] Er:YAG laser were found to be effective against biofilm of five bacterial species among the six bacterial species examined ,with the exception of those formed by L.casei. Findings demonstrated that following irradiation by Er:YAG laser there was a significant decrease in number of viable cells present in biofilm which is accompanied by certain morphological alterations of cells in terms of atrophic changes and reduction in biofilm cell density .Above findings indicate that Er: YAG lasers might be used as an adjunct for eradication of biofilms in endodontic treatments owing to there suppressive effect on biofilm growth.[34]

Earlier studies investigated possible concomitant / adverse effects of different lasers during root canal disinfection on ultrastructure of radicular dentin. According to these studies both near and mid-infrared lasers when used in dry root canal produce characteristic thermal effects on radicular dentin. Though radicular dentin have shown to present low absorption coefficients in near -infrared range, still Nd: YAG laser irradiation is sufficient to melt dentin surface[35]. Further studies also demonstrated that undesired effects of Erbium laser towards dentin can be prevented by presence of water in the root canal.[36]

Limitations:

1. Impossible to obtain uniform coverage of canal surface.
2. Possibility of thermal damage to periapical tissue.[1]

8. Irrigating Solution

A. MTAD

MTA Dwhen used as an irrigating solution serve dual role of removing the smear layer and simultaneously disinfecting

the root canal system.[37]

It is recommended as a final rinse after completion of conventional chemomechanical preparation. Citric acid and Doxycycline separately contributes to its chelating actionthereby facilitating removal of the smear layer and its low pH aid in removal of the inorganic components.[37]

Reported results for MTAD antibacterial efficacy are conflicting.[37] In accordance to Prabhakar and coworkers MTAD was effective in suppressing bacterial growth in a 3 week mature biofilm . On contrary, other studies revealed that MTAD is not effective against *E. faecalis*biofilms[38,39]

Composition-3% doxycycline hyclate, citric acid, 0.5% polysorbate-80 (Tween 80) detergent Commercial Product-BioPure MTAD, Dentsply Sirona Endodontics, York, PA, USA) Ph= ~2 Shelf Life- Single use Product. Store mixed solution under refrigentaion(0-3^0 C) and use within 48 hrs. Limitations-Tooth discoloration Expensive

B. Tetraclean:

Tetraclean is recommended as a final rinse after NaOClowing to superior efficacy in removing smear layer and polymicrobial biofilms when compared with MTAD.A possible explanation for greater antimicrobial action could be attributed to presence of CTR as detergent which is more potent than Tween 80 included in MTAD. However its antimicrobial activity is lower than NaOCland it cannot dissolve pulp tissue remnants.[37]

Composition:

Doxycycline-50mg/ml Citric acid -(10%) Detergents - Propylene glycol and 0.2% Cetylpyridinium Chloride and Cetrimide (CTR).

Commercial Product:

1. Tetraclean (Ogna Laboratorifarmaceutici, Muggio, Italy)
2. Tetraclean NA (OgnaLaboratorifarmaceutici, Muggio, Italy)-It is modified form of tetraclean that is basedon cetrimide and citric acid with superior antimicrobial activity.[37]

Drawbacks- Teeth discoloration Bacterial resistance:

Giardino et al conducted an in-vitro study to compare antimicrobial efficacy of MTAD, Tetraclean, cloreximid (CHX digluconate and cetrimide) and NaOCl . In accordance to this study MTAD and tetraclean were found to be highly effective against strictly anaerobic and facultative anaerobic bacteria,NaOCl against anaerobic bacteria, while chloremimid (CHX + cetrimide) showed the lowest antibacterial activity[1,40]

Drawbacks- Teeth discoloration:

Bacterial resistance:

C. Qmix

QMix 2in1 is a proprietary blend that comprises an effective smear layer-remover and a powerful antimicrobial agent. QMiX is a mixture primarily composed of CHX, EDTA and a detergent. It is used as a final rinse, with continuous irrigation done for 60-90 sec.[28] Its action against *Enterococcus fecalis* and mixed plaque bacteria in planktonic and biofilm states was found to be similar to NaOCl and superior to CHX.[3,41] Further it has been found to be as effective as 17%EDTA in removing smear layer.[42]

Commercial Product-Q Mix 2 in 1 by Dentsply Sirona. Ph-7.5-8 Shelf Life-2 years when stored at room temperature.

Conclusion:

Root canal system including isthmuses, accessory and lateral canals, ramifications harbors polymicrobial biofilms. These biofilms serve as main etiologic agent responsible for diverse periapical pathologies. The prime requisite of endodontic treatment is to eradicate these biofilms and to create a fluid impervious seal. Limitations associated with current therapeutic modalities have consequently lead to advent of novel treatment modalities. Though recent antimicrobial strategies have shown enhanced ability to disinfect root canal system by there tremendous inhibitory effects on most types of in -vitro microbial biofilms. However further studies are required in -vivo so as to determine their efficacy and any possible adverse effects on dentin and periradicular tissues.

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