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Chapter 5

# Optimizing Antimicrobial Agents in Endodontics

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Additional information is available at the end of the chapter

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**Abstract**

In endodontic (root canal) treatment, a multispecies bacterial and fungal infection is present in a place that is inaccessible to the host immune system and which offers physical protection from applied topical agents. All current protocols for irrigation suffer various deficits in performance, which is why further research on alternative approaches to using antimicrobial substances is warranted. This chapter examines the technical and clinical factors which influence the performance of antimicrobial biocide-based therapies used in endodontics within dental practice, addressing issues around instability of biocides, the influence of pH, the role of physical agitation and the challenge of penetration into biofilms and into confined spaces. A range of methods to overcome the challenges in performance are described, including novel solvents and vehicles for biocides, stabilizing agents, physical agitation and the use of activation protocols including the use of intense light, ultrasound and laser-generated shockwaves to improve the effect of biocides. While specific examples are given from the dental setting of endodontics, the principles have broader application to medicine and to general industry.

**Keywords:** biofilms, endodontics, biocides, ultrasonics, lasers, disinfection

## 1. Introduction

Antimicrobial agents which act as biocides have an important place in modern health care as they overcome many of the limitations of antibiotic and antifungal medicines by attacking not one but many targets, making the development of resistance through spontaneous mutation difficult or impossible. There are a wide range of biocides in common use, and their major mechanisms of attack on bacteria and fungi vary according to the agent chosen (**Table 1**). Considering situations where infection is present and which are very challenging to treat with antibiotics, the situation of the infected root canal is the focus of this chapter.

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|---|
| **Oxidation of biomolecules**: Sodium hypochlorite (NaOCl), hydrogen peroxide ($H_2O_{2y}$, ozone ($O_3$), photoactivated disinfection (PAD). |
| **Cell membrane damage**: Chlorhexidine (CHX), phenolic agents, calcium hydroxide ($Ca(OH)_2$), functional antimicrobial peptides, nanoparticles (NPs) (including chitosan, gold (Au) and silver (Ag)). |
| **Protein coagulation**: Laser photothermal disinfection. |

**Table 1.** Primary mechanisms of action of broad-spectrum antimicrobial agents.

In this particular clinical situation, there is a polymicrobial infection with multiple species of bacteria as well as occasionally fungi also being present in a dense biofilm that penetrates into the tubules of the dentine of the tooth root. The organisms and their products including endotoxins cause severe inflammatory reactions in the adjacent bone and soft tissues. The location of the biofilm within the root canal system of the tooth makes it inaccessible to the host immune system, while the tubules give physical protection from agents which are applied topically. The goal of endodontic (root canal) treatment is to decontaminate the entire root canal system; however, in many cases, viable bacteria remain at the end of treatment, causing ongoing inflammation [1], with accompanying pain and other complications [2]. Retreatment using either non-surgical or surgical methods is focussed on removing persisting microbial contaminants [3].

## 2. The rationale for irrigation with disinfectant solutions

The use of physical instrumentation alone, such as files or ultrasonics, does not give adequate debridement of the root canal system because of its complex three-dimensional shape, but creates some space to permit better permeation of irrigation solutions [4, 5]. The mainstay of microbial therapy is the application of multiple chemical agents as partners to physical debridement. Most current protocols involve copious irrigation with 2.5–6% solutions of sodium hypochlorite (NaOCl), supplemented in some cases with further treatments using 2% chlorhexidine (CHX). The antimicrobial actions of such agents are related to their concentration, viscosity and ability to wet the walls of the root canal system [6]. Clinical studies have shown that supplementary steps such as placing a paste of calcium hydroxide ($Ca(OH)_2$) as an inter-appointment medications can further reduce the microbial bioburden [7].

The properties of an ideal irrigation solution are listed in **Table 2**. None of the current agents used alone fulfil all these requirements. This is why combinations of approaches and improved formulation are such an important goal and the focus of the current discussion.

Since all current irrigation protocols suffer various deficits in performance, further research on alternative approaches to using antimicrobial substances is warranted. The purpose of this chapter is to review the various ways that antimicrobial actions of these materials can be enhanced, using combinations of products, altered vehicles, chemical activation and physical agitation.

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| **Effectiveness**: Exerts a rapid antimicrobial action, effective on both Gram-positive and Gram-negative bacteria, effective on fungi, able to penetrate into biofilms, able to penetrate into dentine tubules, easy to dispense and use clinically, easily removed from the root canal, dissolves organic tissue, dissolves smear layer, removes debris from the canal, retains activity in the presence of organic matter. |
| **Safety**: Not allergenic, not mutagenic, not toxic to human cells, compatible with periapical tissues. |
| **Tooth considerations**: Non-staining, does not react adversely with dental materials, does not weaken tooth structure. |
| **Cost**: Low cost of manufacture, easily prepared, good long-term stability and long shelf life |

**Table 2.** Properties of an ideal disinfecting agent for endodontics.

## 3. Sodium hypochlorite

As a solution ranging in concentration from 0.5 to 6%, sodium hypochlorite (NaOCl) is currently the most popular irrigant used in endodontics [8]. The solutions used in dental practice for root canal treatment are a mixture of NaOCl with sodium chloride (to make the solution isotonic), sodium hydroxide (as a pH modifier) and a surfactant. They differ from domestic preparations used as disinfectants, e.g. for sanitizing baby bottles, by being more alkaline (with a pH from 10 to 12) and having the ability to dissolve vital and non-vital soft tissues [9, 10].

Altering the pH influences both the antibacterial effects and tissue-dissolving capacity of NaOCl preparations. In low pH solutions, hypochlorous acid (HOCl) predominates, which has disinfecting actions. This small neutral molecule enters bacterial cells where it oxidizes lipids and proteins, and also reacts with ferrous ions in a reaction similar to the Fenton reaction, to produce hydroxyl radicals [11]. At high pH, both hydroxyl and hypochlorite anions are present. The hypochlorite anion can destroy protein by the formation of chloramines, while the hydroxyl ion reacts with lipids in a saponification reaction to degrade them, thus increasing tissue-dissolving capabilities [12].

### 3.1. Advantages

NaOCl solutions have a broad antimicrobial spectrum and are effective on both bacteria and fungi such as *Candida albicans* [13, 14]. A particular challenge in endodontics is the presence of highly resistant organisms, particularly *Enterococcus faecalis*, that survive in extremes of acidic and alkaline pH and can withstand nutritional deprivation [15]. NaOCl can disrupt biofilms of *E. faecalis*, but this requires exposure for up to 5 min [16].

Solutions of NaOCl are able to dissolve necrotic soft tissue remnants [17], because it has strong but non-specific proteolytic activity [18, 19]. A further advantage is that when mixed with hydrogen peroxide ($H_2O_2$), effervescence is produced which can assist in physical debridement [20].

### 3.2. Limitations

Bacteria located on the surface are easily accessible and can readily be inactivated. Those lodged deep within the dentinal tubules are protected from contact and thus will persist in a

viable state. In the absence of aggressive agitation, NaOCl has a limited ability to eliminate *E. faecalis* when in the biofilm state and deep within dentine tubules [21].

The potency of NaOCl solutions, expressed as the available chlorine in parts per million, declines over time, because the solutions degrade readily, and even more so when exposed to heat and light. This can occur because the stock solutions are kept too long or have been dispensed into clear or translucent plastic syringes which allow in extraneous light [22].

NaOCl has an unpleasant chlorine odour, and it interacts adversely with certain antibiotic medicaments containing tetracycline, as well as with chlorhexidine, causing inactivation through oxidation. These reactions also produce highly staining end products which can cause tooth discolouration [23].

NaOCl corrodes metallic instruments, which may lead to their premature failure (breakage or separation). The proteolytic actions of NaOCl cause severe irritant reactions when only small volumes of NaOCl are extruded into soft tissues, causing dramatic swelling [24]. These reactions are more severe in formulations which include sodium hydroxide as an alkalinizing agent, because of caustic actions on soft tissues [25].

### 3.3. Enhancement

Adding calcium hydroxide ($Ca(OH)_2$) improves the effectiveness of NaOCl in terms of reducing bacterial counts as well as endotoxin levels [26]. Another chemical means of enhancing sodium hypochlorite is the addition of detergents to lower the surface tension and enhance penetration, for example, the quaternary ammonium compounds cetrimide and benzalkonium chloride, which themselves are antibacterial agents [27–29]. Adding in surfactants offers the option of using lower and thus less toxic concentrations of NaOCl for disinfection. The same strategy of adding detergents can be applied to other antimicrobial agents to enhance their effectiveness.

The actions of NaOCl can also be enhanced by physical activation, for example, using ultrasonics or pulsed middle infrared lasers (such as Er:YAG or Er,Cr:YSGG lasers) to agitate the solution. Lasers are better than ultrasonic agitation in this regard [30, 31]. Laser activation of NaOCl also enhances the removal of soft tissue and debris from regions that are difficult to clean [32]. Laser agitation employs low average power settings and short pulse durations; thus, there is no ablation of the root structure [33]. The general principle of using physical agitation to improve the effects of antimicrobial agents also applies to solutions of hydrogen peroxide and $Ca(OH)_2$.

## 4. Chlorhexidine

Chlorhexidine (CHX) comes in various forms with the gluconate and the acetate being the most widely used in dentistry as disinfectants. It is typically used as a 2% solution of the gluconate form [10, 14, 34, 35] as a final flush [10, 36, 37]. CHX has a narrow spectrum for Gram-negative bacteria but is effective against most Gram-positive bacteria and also fungi. CHX is the agent of choice when there are Gram-positive resistant enterococci present in the root canal, which may be the case in retreatment situations [38].

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### 4.1. Advantages

The actions of CHX in the root canal are strongly influenced by pH. At pH 5.5–6.0, chlorhexidine exists as a di-cation [39, 40]. This positive charge allows it to bind to negatively charged substances including the hydroxyapatite mineral of tooth structure, bacterial polysaccharides and particularly to Gram-positive bacteria [41] as well as onto the surface of biofilms [42]. Once adsorbed onto tooth structure, CHX can prevent subsequent microbial colonization on the surface [43].

CHX exerts antifungal actions against *C. albicans*, a property it shares with hydrogen peroxide and NaOCl [44]. It also has mild anti-collagenolytic activity, but no ability to dissolve necrotic tissues [42].

### 4.2. Limitations

While effective against key Gram-negative endodontic pathogens such as *Porphyromonas endodontalis*, *Porphyromonas gingivalis* and *Prevotella intermedia* [38], CHX suffers from problems of inherent resistance with pseudomonads and certain other Gram-negative bacteria. Resistance to CHX occurs in *Enterobacter* spp., *Pseudomonas* spp., *Proteus* spp. and *Providencia* spp. [45].

When used as an irrigant in the root canal in the absence of physical agitation, CHX has only a limited ability to eliminate *E. faecalis* because of poor penetration into the dentinal tubules [46]. Likewise, solutions of 2% CHX penetrate poorly into biofilms and are ineffective for dissolving biofilms [47].

When in contact with tissues, CHX is irritant and can delay healing [48, 49]. As with sodium hypochlorite, care must be taken to prevent accidental extrusion into soft tissues. Using a side-vented needle rather than a conventional needle can reduce the volume of fluid that is extruded during irrigation [50, 51].

Despite reported cases of allergy to CHX in medical settings, few have occurred in dental practice. IgE-mediated allergic responses to CHX manifest as redness, itching (urticaria) and swellings [52] and can progress to anaphylaxis [53]. The high propensity for allergy distinguishes CHX from all other disinfectants discussed in this chapter.

CHX suffers from moderate problems of stability as it can undergo cleavage with oxidation, both during storage and when in contact with oxidants such as hydrogen peroxide, ozone or NaOCl. The key end product of oxidative degradation of CHX is para-chloroaniline, an orange to brown coloured substance that is highly irritant and allergenic [23, 40]. As well as causing staining of tooth structure [40], para-chloroaniline may also interfere with the seal of the final root filling [54].

### 4.3. Enhancements

To enhance the actions of CHX, it can be combined with low concentrations of hydrogen peroxide, mixed freshly so as to limit the possible oxidation of the molecule [55]. Likewise, adding calcium hydroxide improves the antimicrobial actions of CHX [26].

Adding positively charged detergents such as cetrimide can potentiate the antibacterial actions of CHX [39, 56, 57]; however, because of its cationic nature, it cannot be mixed with anionic detergents [56], since these cause precipitation to occur.

## 5. Calcium hydroxide

Water-based pastes of calcium hydroxide ($Ca(OH)_2$) are the most commonly used inter-visit dressing material in endodontics [58]. When placed in the root canal, $Ca(OH)_2$ elevates the local pH, making this unfavourable for the growth of most bacteria [43, 59]. An in vivo study revealed that after 14 days of exposure to a $Ca(OH)_2$ paste, for 41 out of 44 endodontic pathogens, both the rate of detection in samples and mean bacterial counts declined [58]. $Ca(OH)_2$ both inactivates bacteria and reduces endotoxin levels [43], through release of hydroxyl ($OH^-$) ions over a prolonged period of time [43]. These destroy bacterial cell walls and cytoplasmic membranes by degrading fatty acids, thereby allowing the leakage of cellular components. They also inactivate bacterial enzymes. High pH levels within bacterial cells alter the charge of various organic molecules and so interfere with the transport of bacterial nutrients [60]. OH- ions also denature proteins and damage DNA [61]. Calcium ions may contribute to antibacterial actions by neutralizing negatively charged molecules [62].

### 5.1. Advantages

$Ca(OH)_2$ promotes the formation of dental hard tissues, a characteristic exploited in techniques such as apexification where continued formation of the root is intended. Enhanced mineralization is due to activation of alkaline phosphatase, resulting in the release of phosphate groups which then react with calcium ions [60]. While $Ca(OH)_2$ is biocompatible, the extremely irritant and caustic nature of sodium and potassium hydroxides makes these both unsuitable for use in the root canal of teeth. Neither sodium nor potassium hydroxides have mineralizing actions.

### 5.2. Limitations

A saturated solution or paste of $Ca(OH)_2$ in water has a pH of approximately 12.5–12.8. When placed in a tooth, buffering by dentine proteins and carbonate ions lowers the effective pH that can be achieved by water-based solutions and pastes by 1–2 pH units, making the effective pH achieved within the dentine around 10 [63]. This is a problem because *E. faecalis* can withstand a pH of 10 and thus is resistant to the effects of traditional water-based $Ca(OH)_2$ pastes [59, 64, 65]. Likewise, many water-based $Ca(OH)_2$ products exert limited antifungal activity [44].

### 5.3. Enhancements

While $Ca(OH)_2$ can be combined with CHX, the combination is not significantly more effective against *E. faecalis* [46]. In contrast, $Ca(OH)_2$ pastes that are enriched with ibuprofen or diclofenac become more effective [66].

$Ca(OH)_2$ has low solubility in water (0.17% by weight at room temperature), so only small amounts can be dissolved. While the dissolution characteristics can be improved marginally when nanoparticles are used, there remains an upper ceiling of pH 12.5–12.7 for $Ca(OH)_2$ in a water-based solvent, which explains why pH values of commercial products are always in this narrow range [43].

Work based on replacing water with non-aqueous solvents has shown that several biocompatible fluids are much better solvents for $Ca(OH)_2$ than water, including propylene glycol, polyethylene glycol (PEG) and glycerol. In each of these fluids, there is higher measured $OH^-$ release, as measured using special electrodes designed for non-aqueous solvents as well as by titration [67, 68]. A preferred solvent is a mixture of two forms of polyethylene glycol (PEG), one being the foundation (PEG 400) and the other being a thickener (PEG 3350), to generate the preferred creamy consistency required for application into the root canal. This PEG blend is a potent solvent for $Ca(OH)_2$ which has a high release of $OH^-$ ions into any water-based environment, since PEG 400 is miscible with water [69, 70]. This then translates into greater movement of $OH^-$ ions through the roots of human teeth than water-containing $Ca(OH)_2$ pastes [70]. This can be explained by the common ion effect, with $OH^-$ being the common ion in water.

## 6. Hydrogen peroxide

At a final concentration of 3–6%, $H_2O_2$ is a commonly used disinfectant. It generates oxygen radicals [71], of which the hydroxyl radical is the most important since it is the strongest oxidizer [72]. $H_2O_2$ generates effervescence which provides physical clearance of microbial deposits [73]. The elevated oxygen concentration it creates is unfavourable for the growth of strict anaerobes.

### 6.1. Limitations

Elevating the pH using an alkali metal hydroxide such as lithium, sodium or potassium hydroxide will accelerate the decomposition of $H_2O_2$ and provide an alkaline pH [72]. The limitation in this approach is that such hydroxides are inherently caustic.

### 6.2. Enhancements

The actions of $H_2O_2$ as a disinfectant can be enhanced by adding a suitable catalyst such as manganese or ferrous ions [74]. The latter can also be employed in the Fenton reaction, where the ferrous ion reacts with $H_2O_2$ to form hydroxyl and other radicals [74]. Blue, violet or ultraviolet light can provide photochemical activation for this reaction, enhancing further the generation of reactive oxygen species [75]. The Fenton reaction can also occur within bacterial cells, causing cell death [11]. Likewise, photolysis of $H_2O_2$ using 405 nm violet light combined with ultrasound activation can potentiate hydroxyl radical formation, with a synergistic antibacterial action between light and ultrasound activation [76].

The temperature of $H_2O_2$ and thus its rate of breakdown can be increased by using dyes that absorb the appropriate wavelength of intense light, for example, blue light into orange or yellow dyes. Titanium dioxide in forms similar to those used in sunscreens allows broad-spectrum light sources to be used to activate $H_2O_2$.

Mixing ozone ($O_3$) and $H_2O_2$ gives co-catalysis and enhanced effectiveness [77]. As with $H_2O_2$, physical agitation of solutions of $O_3$ in water using ultrasound improves their effectiveness [78]. When $H_2O_2$ is activated by ultrasonic agitation, the enhanced production of hydroxyl radicals by sonolysis causes increased bacterial killing [79]. This ultrasonic activation can be augmented with 405 nm violet light, as both activation pathways generate hydroxyl radicals in a synergistic manner [76].

The generation of oxygen gas bubbles from $H_2O_2$ and the associated disrupting effect of these on microbial deposits can be enhanced using middle infrared lasers which emit in the 2700–3000 nm wavelength range, such as the Er,Cr:YSGG and Er:YAG lasers. The energy from these lasers absorbs strongly into both water and $H_2O_2$, creating with each laser pulse bubbles of air, steam and oxygen. Shockwaves generated by bubble implosions create shear stresses on the walls of the root canal. Fluid movements at high speeds (in the order of 100 km/h) cause disruption of microbial deposits and smear layers [80, 81]. Because the streaming movements of such fluids causes most of the fluid movement being directed back towards the point of entry of the fibre into the tooth, the volume of fluids extruded by laser activation of various irrigation fluids is no more than when conventional irrigation is undertaken using syringes [50]. The frequent replacement of fluids also provides a cooling effect, so that heat does not accumulate [82, 83].

When $H_2O_2$ is added to pure water, the absorption curve of the mixture is left shifted from that of water alone, allowing lasers operating at wavelengths from 900 to 1100 nm to have much stronger absorption [84]. This approach allows handheld near-infrared lasers to be used for cavitation-based removal of bacterial deposits and smear layer from the root canal. There is accompanying photothermal disinfection of bacteria located deep in dentine tubules because such wavelengths have high transmission into dentine [85, 86]. When $H_2O_2$ is used in this clinical application, the concentration is below 6% by volume, as this is the threshold for soft tissue injury [73].

## 7. Phenolic agents

The use of phenolic agents such as camphorated monochlorophenol (CMCP) and other related hydrophobic antimicrobial agents such as essential oils has a long history in endodontics. Chlorination of phenols enhances their antibacterial action [61]. The antibacterial effect of CMCP is better at low pH. This agent has a high volatility, which may enhance its penetration into dentinal tubules [43].

### 7.1. Limitations

Many phenolic agents are hydrophobic and have low water solubility, so they are typically formulated in a hydrophobic solvent [87]. As a result, there are poor wetting of the root canal

walls, limited contact with biofilms in the canal and poor penetration into the water-rich environment of dentine tubules.

### 7.2. Enhancements

$Ca(OH)_2$ can be added to phenolic products to make them more effective antimicrobial agents, as this disrupts the biofilm matrix [88]. The contact between hydrophobic phenolic agents and the walls of the root canal can be enhanced by formulating these into a water base containing surfactants or by using water-miscible solvents such as low molecular weight forms of PEG. The proper choice of agents can provide not only disinfectant actions but also anti-inflammatory and analgesic effects. The latter are therapeutically desirable when patients present in pain from endodontic infections [69].

## 8. Nanoparticles

Nanoparticles (NPs) with diameters in the range of 1–100 nm have special properties, including increased chemical reactivity and a large surface area [89]. They can exist singly, in groups or in clusters. In endodontics, NPs suggested for use as adjuncts to conventional treatments include chitosan, poly(lactic-co-glycolic acid) (PLGA), silver (Ag), gold (Au) and $Ca(OH)_2$ [90]. Chitosan is derived from the chitin exoskeletons of various arthropods [91]. It is polycationic and attaches strongly to negatively charged bacterial cell walls causing their disruption, with subsequent leakage of cellular contents. Chitosan is also capable of disrupting the extracellular matrix of biofilms [92]. Low molecular weight forms of chitosan are more antimicrobial than those with high molecular weights [93]. Carboxymethyl chitosan has been included in endodontic sealers to provide antibacterial properties [94].

PLGA is a highly biocompatible copolymer of lactic and glycolic acid. Both PLGA and chitosan NPs can be loaded with photosensitizers for use in photoactivated disinfection (PAD). Functionalization of NPs enhances the action of photosensitizers such as rose bengal and methylene blue [95, 96].

NPs of metallic oxides, such as calcium oxide, magnesium oxide, zinc oxide and titanium oxide, can exert antibacterial actions by generating reactive oxygen species [92]. The high chemical reactivity and large surface area of NPs enhance these actions over their normal counterparts, as has been shown for calcium oxide [97]. NPs can also be made from $Ca(OH)_2$ [69, 98].

Of the various metals that form NPs, silver (Ag) is of particular interest because the antibacterial effects of silver are well known in endodontics. The same principles can be employed with ionic solutions of Ag compounds (such as silver fluoride) although issues of discolouration limit their clinical application. Using NPs of Ag in the 2–12 nm range can reduce problems of tooth discolouration. AgNPs may slowly release silver ions (Ag+) which then interact with biological systems, attracting electrons away from sulphur and nitrogen atoms in the sulfhydryl and amino groups of biological molecules such as proteins, or with the nitrogenous purines and pyrimidines of DNA and RNA [99].

Various methods are known for producing AgNPs, ranging from chemical reactions to biosynthesis through to electrochemical methods [100, 101]. Solutions containing AgNPs can be generated by high-voltage ultralow-current electrolysis of water using silver electrodes, followed by irradiation of the solution to violet light (400–430 nm wavelength). This alters the properties of the AgNPs and ensures only clusters remain rather than ions [102]. The solutions of such particles are optically clear, free of Ag ions and quite stable.

### 8.1. Limitations

While silver, gold, tin and zinc all have potential for use in ionic or NP forms, issues of staining and long-term stability need to be addressed. There are now systems for stabilizing stannous (tin) ions using sodium hexametaphosphate, as well as potassium iodide protocols for reducing staining from ionic silver. These have been employed in preventive and restorative dentistry but are yet to be optimized for endodontics [103, 104].

Toxicity issues with NPs require further investigation. AgNPs are not cytotoxic at 25 μg/mL or lower concentrations; however, this threshold is based on cell culture studies using a mouse fibroblast cell line (L929). Thus, for safe clinical use, animal and human studies are needed to assess the toxicological profile in greater detail [105].

### 8.2. Enhancements

Displacement of NPs into poorly accessible regions of the root canal, such as dentine tubules, can be achieved by using ultrasonic or pulsed laser agitation of the solution.

The application of NPs in solutions for irrigation is at an early stage of development. AgNPs have been used in combination with traditional $Ca(OH)_2$ to increase the killing of *E. faecalis* in biofilms [106]. There is also the possibility of combining metal NPs with traditional antibiotics to enhance their effectiveness. The interactions of metals with antibiotics need to be optimized, so that effectiveness is enhanced rather than impaired. Tetracyclines bind metal ions by chelation, and this alters their effectiveness. Tetracyclines with their long history of use in endodontics would be a logical first place to study such interactions. It is also possible that NPs can be functionalized with photosensitizers to increase their antimicrobial effectiveness [89]. There is already evidence that AgNPs can be combined with photosensitizers in a dual-treatment approach [107].

## 9. Functional peptides

Custom peptides could be designed which can inactivate particular bacterial pathogens such as *E. faecalis*. A typical primary root canal infection is a mixed flora of 20–30 species of microorganisms, so the first challenge in using antimicrobial peptides is to ensure that the coverage is sufficient. The second challenge is that some common Gram-negative endodontic pathogens such as *P. endodontalis* and *P. gingivalis* produce large quantities of proteases (such as the gingipains) [108], which could readily degrade such peptides and limit their useful life as therapeutic agents. A third challenge with peptides is their higher cost of manufacture and shelf life than other options.

## 10. Laser-based photothermal disinfection

Penetration of near-infrared light in the wavelength range from 800 to 1100 nm through dentine is high, allowing laser light to reach bacteria embedded deep within dentine tubules [109]. Absorption of laser energy into melanin, water, porphyrins and other molecules then denatures enzymes and so kills bacteria through photothermal actions. As the heat will then be transmitted into adjacent tooth structure and periodontal ligament, the laser energy must be delivered in pulsed mode to allow cooling. Typically, the laser is activated over several passes as the fibre is withdrawn, causing irradiation of the entire root canal system [110].

Laser energy used for photothermal disinfection can also exert biostimulatory effects and so enhance healing and reduce inflammation [111, 112]. A recent study using a 980 nm diode laser showed both bacterial inactivation and biostimulatory effects [113].

## 11. Photoactivated disinfection

Besides the term photoactivated disinfection (PAD), the literature in this area contains many terms that refer to the same process, such as photodynamic therapy (PDT), antimicrobial PDT (aPDT), light-activated disinfection (LAD), advanced non-invasive LAD (ANILAD) and lethal laser photosensitization (LLP).

Lasers or light-emitting diodes (LEDs) used as sources of intense light cause electronic activation of photosensitizers, which then produce ROS. Examples of light and dye combinations include visible red light with methylene blue or tolonium chloride [114], visible blue light with curcumin and green light with rhodamine B dye. The release of ROS is a photodynamic process, i.e. driven by light, with no accompanying thermal effects [115]. Even though the ROS produced are extremely short lived, photosensitizers are able to exert their actions on pathogens effect because they attach directly to microbial cell walls. The porphyrins found within many facultative and strict Gram-negative anaerobes can act as endogenous photosensitizers and absorb blue light, such that external dyes are not needed. Gram-positive bacteria and fungi do not contain large amounts of porphyrins and so are much less sensitive to the effects of blue light.

Maximal activation of photosensitizers requires many parameters to be optimized other than the specific absorption of the dye matching the emission wavelength of the light source. The dye concentration must be optimized, since too little means only a low concentration of ROS will be produced. When the dye solution is too concentrated, the optical density of the solution becomes too great and the penetration of light is limited to being superficial, and the effects become photothermal rather than photodynamic. Concentrated dyes also have the potential to stain tooth structure. Adequate amounts of oxygen need to be present locally to support the reaction. For this reason, some protocols include oxygen carriers or oxidizers such as $H_2O_2$ to enhance the action of the photosensitizers [116]. The pH of the solvent for the dye must be optimized for the concentration and type of ROS desired, e.g. a higher pH means more perhydroxyl radicals will form with greater antimicrobial actions. Inclusion of a surfactant is important for ensuring penetration of the dye into biofilms and into difficult-to-reach areas such as dentine tubules [117]. The optical fibre delivery systems used to deliver intense

light from lasers or LEDs may include special diffusers to optimize the angular distribution of light into the dye.

PAD should be used after conventional disinfection with NaOCl, but not as a complete replacement to traditional chemical disinfectants [118, 119]. It can also be used as a supplementary form of root canal disinfection in endodontic retreatment cases [120]. An animal study which used PAD as an adjunct to standard disinfection with NaOCl reported a larger reduction in periapical lesions and more periapical regeneration than standard treatment protocols [121]. While some in vivo studies have recommended PAD as a possible alternative to NaOCl [122, 123], there remains a need to standardize the protocol and optimize the light parameters [114, 124].

### 11.1. Enhancements

To achieve an even distribution of light across the dye solution, a range of optical fibre tips have been developed which have side-firing capabilities, giving even lateral dispersion of light because of surface patterning [125]. Pulsing the light source can enhance the production of ROS by the dye. This has been shown in studies of PAD using thick *E. faecalis* biofilms in the root canal [126]. Pulsing the light source lowers the requirements for cooling the diode laser or LED and also improves the stability of the wavelength emitted by reducing drift to longer wavelengths. Finally, ultrasonic agitation can increase the effectiveness of PAD [127]. Photosensitizer dyes can also be used in combination with NPs as discussed above, as well as with $H_2O_2$ [128, 129].

## 12. Fluorescence control of disinfection approaches

Both planktonic microorganisms and biofilms in the root canal system can be detected using fluorescence. The point where the canal is free of microbial contamination can be identified precisely, providing an endpoint to treatment [130, 131]. Fibres with special surface characteristics allowing light delivery for fluorescence excitation and light collection from fluorescence emissions have been designed, and their performance demonstrated in various situations [132–134].

### 12.1. Limitations

The major limitation to such systems being used is when the fluorescence properties of tooth structure have been enhanced because of incorporation of tetracycline. This causes increased fluorescence which must be corrected for [130, 131]. While few irrigants or medicaments used in modern endodontics give fluorescence emissions, certain potent oxidizers can quench (reduce) fluorescence emissions by bacteria. Using scavengers such as sodium ascorbate or sodium thiosulphate can remove such quenching actions and allow reliable fluorescence readings to be obtained.

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## 13. Conclusions

There are a range of options available for disinfection of the root canal. Recognizing that none of the existing agents used alone aligns with all the properties of an ideal agent (as listed in **Table 2**), the effort to enhance the capabilities of the existing agents and find new approaches must continue. Major directions for the future include improved formulations such as the use of non-aqueous solvents for $Ca(OH)_2$ for medicaments to remain in the root canal between successive appointments, as well as changes to clinical treatments delivered by the dentist during endodontic treatment. The latter include agitation of water-based disinfectant irrigants with lasers, and the combination of antimicrobial NPs with optical technologies, for synergy of effects as a final high-level disinfection step before the root canal is filled. The ideal protocol should address issues of clinical time, materials cost and complexity, as well as efficacy and safety. Using fluorescence to measure levels of pathogens can provide an endpoint to clinical interventions, to inform the practitioner when pathogens no longer remain. Including antimicrobial agents into dental materials used to fill or seal the root canal is a further avenue to explore, applying the principles discussed in this chapter.

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