The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics

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Abstract
Bacteria have been implicated in the pathogenesis and progression of pulp and periapical diseases. The primary aim of endodontic treatment is to remove as many bacteria as possible from the root canal system and then to create an environment in which any remaining organisms cannot survive. This can only be achieved through the use of a combination of aseptic treatment techniques, chemomechanical preparation of the root canal, antimicrobial irrigating solutions and intracanal medicaments. The choice of which intracanal medicament to use is dependent on having an accurate diagnosis of the condition being treated, as well as a thorough knowledge of the type of organisms likely to be involved and their mechanisms of growth and survival. Since the disease is likely to have been caused by the presence of bacteria within the root canal, the use of an antimicrobial agent is essential. Many medicaments have been used in an attempt to achieve the above aims but no single preparation has been found to be completely predictable or effective. Commonly used medicaments include calcium hydroxide, antibiotics, non-phenolic biocides, phenolic biocides and iodine compounds. Each has advantages and disadvantages, and further research is required to determine which is best suited for root canal infections.

Key words: Endodontics, bacteria, antimicrobial, medicaments.

Abbreviations and acronyms: Ca(OH)2 = calcium hydroxide; CFU = colony forming units; CHX = chlorhexidine; CMP = camphorated monochlorophenol; CP = camphorated phenol; IPI = iodine potassium iodide; LPS = lipopolysaccharide; NaOCl = sodium hypochlorite; PEG = polyethylene glycol; PMCP = paranitrochlorophenol; QAC = quaternary ammonium compounds.

INTRODUCTION
Bacteria play a major role in the development and progression of pulp and periapical diseases, as shown by many authors.14 Infections within the root canal system of a tooth cause periapical inflammatory responses which usually manifest as periapical radiolucencies and occasionally as radiopacities on radiographs.14 Bacteria also play a major role in the development of apical periodontitis associated with root-filled teeth, although studies have shown that the microflora differs in these teeth from that present when there has been pulp necrosis with infection.14

Bacteria can exist within the root canal itself, or within other related regions such as the dentinal tubules, accessory canals, canal ramifications, apical deltas, fins, and transverse anastomosis.1 Apart from the canal itself, all of these other areas are inaccessible to mechanical instrumentation procedures and to the irrigating solutions used during endodontic treatment. In order to predictably eliminate as many bacteria as possible from the entire root canal system, a combination of mechanical instrumentation and irrigating solutions is used to remove or dissolve organic and inorganic debris, to destroy bacteria, to remove the smear layer and to maintain dentine permeability. However, several studies have shown that mechanical instrumentation with antibacterial irrigation will only render 50–70 per cent of infected canals free of microorganisms, depending on which irrigants are used.15 Since there is no entirely predictable way, in one treatment session, to ensure complete elimination of root canal bacteria, an effective antimicrobial agent in the root canal is required for a predetermined time period to predictably eradicate or destroy any remaining bacteria.14 Therefore, antimicrobial agents used as inter-appointment medicaments must be able to penetrate through the dental tissues in the presence of microbes to reach a sufficiently high concentration in order to eliminate the disease-causing bacteria in a predictable manner.14

Microbial invasion of dentine
Microbial invasion of the root canal system is time related and bacterial species dependent. Hence, endodontic treatment of a tooth should minimize the number of micro-organisms lodged in the dentinal tubes.15 Micro-organisms in dentinal tubules may constitute a reservoir from which root canal and surrounding tissue infection and re-infection may occur. Bacteria located inside dentinal tubules are protected from host defence cells, systemic antibiotics and chemomechanical preparation. Therefore, endodontic medicaments must be able to penetrate into dentinal tubules and kill bacteria within them.15
When bacteria invade the dentinal tubules, not all
infections will have been invaded to the same extent. Both in vitro and in vivo observations show that
cellular penetration into dentinal tubules occurs as a
random process, with bacterial colonies seen as
sporadic, dense accumulations of cells (rather than as a
continuous film), extending out from the main canal
towards the periphery. The reported frequency of
dentinal tubule invasion in necrotic, infected teeth
varies between 50 and 90 per cent.11

When a root canal infection develops, the predentine
is readily infected but the calcified dentine is less readily
infected. Bacterial species that penetrate the dentine are
dominated by Gram-positive rods (68 per cent) and
 cocci (27 per cent). The predominant genera are
Lactobacillus (30 per cent), Streptococcus (13 per
cent), and Propionibacterium (9 per cent).11 The
presence of Gram-negative bacteria in root canal
dentine has been confirmed indirectly by the detection
of high concentrations of lipopolysaccharide (LPS) in
the inner layers up to 3000 μm in depth.11 One study11
reported that E. faecalis did not invade the dentine
until after two weeks of incubation when cementum was intact. By three weeks, the organisms
had penetrated more than halfway through the dentine
in the cervical third of the root, up to halfway through
dentine in the middle third, and only a third of the
distance through the dentine in the apical third of
the root. E. faecalis has been shown to penetrate between
50–300 μm in human dentine.12 13 Dentine penetration
by other species has also been reported.12 13

Medicaments

Medicaments are used as an aid to improve the
predictability and prognosis of endodontic treatment.
They are used in endodontic therapy in order to:
• eliminate or destroy any remaining viable bacteria
in the root canal system that have not been
destroyed by the chemomechanical preparation
processes (i.e., instrumentation and irrigation),
• reduce periapical inflammation and hence
reduce pain,
• help eliminate apical exudate if it is present,
• prevent or arrest inflammatory root resorption if it
is present, and
• prevent re-infection of the root canal system by
acting as both a chemical and a physical barrier if
the temporary or interim restoration breaks down.

Inter-appointment intracanal medication has been
unequivocally shown to contribute to favourable
outcomes when treating endodontic infections.1 2 3 The
need for intracanal medication is greater in those cases
where bacteria are resistant to routine treatment, and
where the therapy cannot be successfully completed
due to the presence of pain or continuing exudate.3

Some endodontic conditions are ideally treated over
several appointments which may be extended over a
long period of time. This allows various medicaments
to be used depending on the status of the pulp, the
periapical tissues, the hard dental tissues (such as
cementum) and the condition of the apical foramen
(i.e., "open", or fully developed and unaffected by
resorption). The minimum inter-appointment time
interval should be no less than 14 days, since
inflammation takes at least 10–14 days to subside or
heal, but longer periods are generally more desirable
as most medicaments take 3–4 weeks to reach their
maximum concentration within the peripheral
dentine. In addition, if signs or symptoms are not
subsiding, then a longer period of medication time or
an alternative medicament may be necessary.

Many hand and rotary instrumentation techniques
tend to produce round preparations (especially in oval
canals) leaving some areas uninstrumented and hence
possibly containing infected debris. It has been estimated
that as much as 50 per cent of the canal wall may
remain uninstrumented during preparation. The
remaining necrotic tissue remnants may provide a
source of nutrition for any surviving bacteria.4 5 The
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source of nutrition for any surviving bacteria.4 5 In
addition, bacteria are likely to remain in dentinal
tubules after instrumentation. If this occurs, calcium
hydroxide and other disinfectants that require direct
physical contact with pathogens may be ineffective.6

The longstanding popular notion of entombment and
perishing of intraradicular microbes following
treatment lacks scientific validity.6 7 The presence of microorganisms inside a root canal may not necessarily lead
to the failure of treatment, but their absence will
certainly favour healing.8

Five groups of antimicrobial substances have been
used as root canal medicaments: (a) calcium hydroxide;
(b) antibiotics; (c) non-phenolic biocides; (d) phenolic
biocides; and (e) iodine compounds.

(a) Calcium hydroxide

Calcium hydroxide (Ca(OH)₂) has been used
exensively in dentistry since the 1920s. Today, it is still
the mosly commonly used endodontic medicament
throughout the world.6 10 Calcium hydroxide has low
solubility in water, an inherently high pH (approximately 12.5–12.8), and is insoluble in alcohol.
Its low water solubility is a useful characteristic because
a long period is necessary before it becomes soluble in
tissue fluids when in direct contact with vital tissues.4 5
Calcium hydroxide paste kills bacteria by direct contact
through pH effects, and hence it should occupy the
apical region in a sufficient quantity to permit its
biological effect to be exerted in close proximity to the
appropriate tissues.6 The antimicrobial activity of
calcium hydroxide is due to the release and diffusion of
hydroxyl ions (OH⁻) leading to a highly alkaline
environment which is not conducive to the survival of
micro-organisms. The rate of diffusion of hydroxyl ions
is slow due to the inherent buffering capacity of the
dentine.6 11 12 Availability of calcium ions at the site of
action appears to be useful for exerting therapeutic
effects which are mediated through ion channels. The
role of calcium ions in cell stimulation, migration, proliferation and mineralization is well established. Calcium hydroxide also inactivates LPS and in so doing can assist periapical tissue repair.33

The lethal effects of calcium hydroxide are due to several mechanisms,12,34 namely:

(a) a chemical action through:

- damage to the microbial cytoplasmic membrane by the direct action of hydroxyl ions,
- suppression of enzyme activity and disruption of cellular metabolism,
- inhibition of DNA replication by splitting DNA,

and

(b) physically by:

- acting as a physical barrier that fills the space within the canal and prevents the ingress of bacteria into the root canal system, and
- killing the remaining micro-organisms by withholding substrates for growth and limiting space for multiplication.

The biological properties of calcium hydroxide include:

- biocompatibility (due to its low solubility in water and limited diffusion),
- the ability to encourage periapical hard tissue healing around teeth with infected canals, and
- inhibition of root resorption and stimulation of periapical healing after trauma.

These properties are due to its antimicrobial activity, its ability to inactivate LPS, its ability to promote hard tissue formation, and its long-lasting action.55,56

The limited effectiveness of the short-term use of calcium hydroxide in disinfecting dentinal tubules is due to several factors,12 namely:

- inhibition by dentinal protein buffering, particularly in terms of the ability of hydroxyl ions to reach the apical third and have an antibacterial effect,
- the low solubility and diffusibility of calcium hydroxide may make it difficult to gain a rapid increase in pH to reach the level necessary to eliminate or kill bacteria within the dentinal tubules and anatomical variations,
- the varying alkaline potential of different formulations,
- dense biofilms of bacteria located within the dentinal tubules can protect those located deeper inside the tubules,
- necrotic tissue in ramifications, isthmuses and irregularities may protect bacteria from the action of calcium hydroxide,
- the ability of E. faecalis to colonize within dentinal tubules and thus evade the hydroxyl ions, and
- calcium hydroxide promotes the adhesion of bacteria to collagen (the main organic component of dentine) which increases the extent of tubule invasion and thereby resistance to further disinfection.57

Other disadvantages of calcium hydroxide are the difficulties associated with removing it from the root canal walls and its effect on decreasing the setting times of zinc oxide-based root canal cements. Some cements have brittle consistencies when set and are granular in structure after contact with calcium hydroxide.14 Since calcium hydroxide kills bacteria through the effects of the hydroxyl ions, its efficacy depends largely on the availability of these ions in solution which in turn is dependent on the vehicle in which the calcium hydroxide is carried.9

Nerwich et al.9 demonstrated that when calcium hydroxide dressings were placed into the root canals of extracted teeth, the hydroxyl ions diffused more quickly through dentine in the cervical third of the root than in the apical third because there are less tubules and they have a smaller diameter in the apical third. They also showed that the hydroxyl ions diffused in a matter of hours into the inner root dentine (i.e., adjacent to the root canal). However, a time period of 1–7 days was required for the hydroxyl ions to reach the outer root dentine (i.e., near the cementum), and 3–4 weeks to reach peak pH levels and to stabilize at these levels.15 It took nearly seven days for the pH to rise to 9.1, a level at which many bacteria do not grow.9 A study by Esberard et al.16 showed similar results. The pH in cavities on the root surface rapidly increased from control values (pH 7.8) to greater than pH 9.0 within three days, followed by a small decline to pH 9.0 over the next 18 days before finally rising and remaining near pH 10.0 for 120 days without paste replacement. Of note, aqueous calcium hydroxide and calcium hydroxide mixed with camphorated monochlorophenol released hydroxyl ions more rapidly than a commercial form of calcium hydroxide (Pulpdent paste, Pulpdent Corporation of America, Watertown, Massachusetts, USA), particularly in the apical region where the pH in canals filled with Pulpdent paste remained almost a full pH unit below the pH levels attained with the other two medicament preparations.15

Gomes et al.11 showed that the concentration of calcium ions peaked and stabilized at 2–3 weeks after packing root canals with calcium hydroxide. When calcium hydroxide comes into contact with carbon dioxide or carbonate ions (e.g., from bacterial metabolism), calcium carbonate is formed. This material has a very low solubility, creates only a mildly alkaline pH of 8.0, and as a result has neither biological nor antibacterial properties.14 Kwon et al.12 showed that 10 per cent of the calcium hydroxide was converted to calcium carbonate in the apical region within two days.
and the remainder was unchanged after six weeks. Little calcium carbonate was detected in samples from the middle portion of the root canal even after six weeks.

The use of calcium hydroxide has been suggested as a factor contributing to the continual presence of E. faecalis after endodontic treatment, because of its relative inefficacy as an antimicrobial agent against this organism. A saturated calcium hydroxide solution has been shown to be unable to kill E. faecalis in the presence of dentine, hydroxyapatite and bovine serum albumin. The alkaline environment created by the calcium hydroxide in the dentine may also interfere with the resorptive activity of demineralization which require an acid environment to achieve mineral dissolution. Hence, calcium hydroxide cannot be considered to be a universal intracanal medicament for all cases of infected root canal systems with apical periodontitis.

Haapasalo et al. showed that dentine powder had an inhibitory effect on all endodontic medicaments tested and that the effect was concentration dependent as well as dependent upon the length of time the medicament was pre-incubated with dentine powder. The effect of saturated calcium hydroxide solution on E. faecalis was totally abolished by the presence of dentine powder, a powerful indication of the buffering of the alkalinity of calcium hydroxide by dentine which can occur. Portenier et al. also studied the effects on medicaments of dentine powder, hydroxyapatite (a major component of dentine), and bovine serum albumin (representing inflammatory exudate) and found, as did Haapasalo et al., that saturated calcium hydroxide had lost all of its antibacterial activity against E. faecalis after 24 hours in the presence of dentine, hydroxyapatite and bovine serum albumin.

Many studies indicate that E. faecalis may be encountered in root-filled teeth with periradicular lesions where there are recoverable micro-organisms in the root canal, with the prevalence of this species ranging from 12 to 70 per cent. Hence, the use of calcium hydroxide as the disinfectant of choice in endodontic re-treatment of infected root-filled teeth with apical periodontitis has been questioned. In a study by Sundqvist et al., calcium hydroxide eliminated E. faecalis when they were present in low numbers (as in infected teeth without previous root fillings) but not in teeth with previous root fillings where E. faecalis was found in higher numbers. De Souza et al. used calcium hydroxide and reported a reduction in most of the species initially detected, but a modest increase in the number of A. actinomycetemcomitans, E. corrodens and E. nodatum organisms. Waltimo et al. showed in an in vitro study that Candida species are more resistant to saturated calcium hydroxide than E. faecalis. All Candida species were highly resistant to saturated aqueous calcium hydroxide. The majority of yeast strains survived incubation for between one and six hours, while E. faecalis was killed within 10–20 minutes. Thus, in view of its limited action on E. faecalis and Candida spp., calcium hydroxide cannot be considered as a panacea for all cases of infected root canals.

(b) Antibiotics

Commercial preparations in this group contain either one or a combination of antibiotics, and sometimes incorporate other compounds such as corticosteroids. Antibiotics can be used as an adjunct to endodontic treatment in a number of ways: locally (i.e., intracanal), systemically, and prophylactically. The focus for this review will be the local use of antibiotics in the form of intracanal medicaments. As discussed above, bacteria may be present within areas of the root canal system that are inaccessible to irrigants and to the mechanical cleaning processes within the canal. Hence, an antibiotic contained within an intracanal medicament must be able to diffuse into these areas to reduce the number of viable bacteria. If such a reduction is achieved, an improved periapical healing response would be expected.

The first reported local use of an antibiotic in endodontic treatment was in 1951 when Grossman used a polyantibiotic paste known as PBSC. PBSC contained penicillin to target Gram-positive organisms, bacitracin for penicillin-resistant strains, streptomycin for Gram-negative organisms, and caprylate sodium to target yeasts - these compounds were all suspended in a silicone vehicle. Although clinical evaluation suggested that the paste conferred a therapeutic effect, the composition was ineffective against anaerobic species which are now appreciated as being the dominant organisms responsible for endodontic diseases. In 1975, the USA Food and Drug Administration banned PBSC for endodontic use primarily because of the risks of sensitization and allergic reactions attributed to penicillin.

The two most common antibiotic-containing commercial paste preparations currently available are Ledermix™ paste (Lederle Pharmaceuticals, Wolfsbrachenhausen, Germany) and Septomixine Forte™ paste (Septodont, Saint-Maur, France). Both of these preparations also contain corticosteroids as anti-inflammatory agents.

Septomixine Forte contains two antibiotics – neomycin and polymixin B sulphate. Neither of these can be considered as suitable for use against the commonly reported endodontic bacteria because of their inappropriate spectra of activity. Neomycin is bactericidal against Gram-negative bacilli but it is ineffective against Bacteroides and related species, as well as against fungi. Polymixin B sulphate is ineffective against Gram-positive bacteria, as shown by Tang et al. who demonstrated that a routine one-week application of Septomixine Forte was not effective in inhibiting residual intracanal bacterial growth between appointments. In addition, although the anti-inflammatory (corticosteroid) agent, dexamethasone
(at a concentration of 0.05%), is clinically effective, triamcinolone is considered to have less systemic side effects.\textsuperscript{41}

In 1948, the first synthetic tetracycline, chlortetracycline, was developed and marketed by Lederle Pharmaceuticals.\textsuperscript{42} Subsequently, this company developed the drug demethylchlortetracycline HCl (also known as demeclocycline HCl) which became the antibiotic component of Ledermix paste. Ledermix paste was developed by Schroeder and Triadan in 1960, and was released for sale in Europe by Lederle Pharmaceuticals in 1962.\textsuperscript{43} The primary interest of Schroeder and Triadan in the development of Ledermix paste was based on the use of corticosteroids to control pain and inflammation while the antimicrobial properties at the time were catered for by a formalin-based paste called Asphalin (introduced in 1921). The sole reason for adding the antibiotic component to Ledermix paste was to compensate for what was perceived to be a possible corticoid-induced reduction in the host immune response. Schroeder and Triadan initially incorporated chloramphenicol in their first trials but when Lederle Pharmaceuticals became the manufacturer, the antibiotic was changed to demeclocycline HCl. Today, Ledermix paste remains a combination of the same tetracycline antibiotic, demeclocycline HCl (at a concentration of 3.2%), and a corticosteroid, triamcinolone acetonide (concentration 1%), in a polyethylene glycol base.

The two therapeutic components of Ledermix paste (i.e., triamcinolone and demeclocycline) are capable of diffusing through dentinal tubules and cementum to reach the periodontal and periapical tissues.\textsuperscript{44} Abbott \textit{et al.}\textsuperscript{45} showed that the dentinal tubules were the major supply route of the active components to the periapical tissues, while the apical foramen was not as significant as a supply route. The concentration of demeclocycline within Ledermix paste itself (i.e., as it would be when placed within the root canal) is high enough to be effective against susceptible species of bacteria. However, within the peripheral parts of the dentine and in the periodontal tissues, the concentration achieved through diffusion is insufficient to inactivate bacteria, especially over time.\textsuperscript{46} Immediately adjacent to the root canal wall, inhibitory levels of demeclocycline are achieved for all reported bacteria within the first day of application but this level drops to about one-tenth of the initial level after one week in both the mid-root and apical third levels. Further away from the root canal towards the cementum, the concentration of demeclocycline after one day is not high enough to inhibit growth of 12 of the 13 strains of commonly reported endodontic bacteria.\textsuperscript{47}

Tetracyclines are bacteriostatic rather than bacterical, and it is well known that yeasts are resistant to tetracyclines.\textsuperscript{48} Tetracyclines exhibit a level of substantivity due to their ability to form complexes with bivalent and trivalent cations. It is for this same reason that they are deposited in teeth and bones during calcification.\textsuperscript{49} Abbott \textit{et al.}\textsuperscript{50} demonstrated that tetracyclines form a strong reversible bond with hard tissues and that they exhibit slow release over an extended period of time.

The combination of antibiotics with a corticosteroid paste, as in Ledermix paste, has been used to arrest external inflammatory root resorption, and this effect has been documented histologically in an \textit{in vivo} study.\textsuperscript{51} Since it does not have damaging effects upon the periodontal tissues, Ledermix is an effective medication for the treatment of inflammatory root resorption in traumatized teeth. The immediate or early use of Ca(OH)\textsubscript{2} following replantation has been shown to exacerbate replacement resorption due to its high pH and toxicity, and should therefore be discontinued.\textsuperscript{52,53} Ledermix is now the preferred medicament to use immediately after replantation as it reduces both inflammatory and replacement resorption. While not all authorities would agree with this view, the beneficial effects in terms of reducing resorption have been demonstrated in dogs by Bryson \textit{et al.}\textsuperscript{54} In teeth with 60 minutes of dry extra-alveolar time, immediate placement of Ledermix paste resulted in 59 per cent of the root surface showing favourable healing compared with only 14 per cent when calcium hydroxide was used immediately following replantation. Ledermix paste also resulted in greater preservation of root mass, hence maintaining function of the replanted teeth for longer.\textsuperscript{55}

The combination of Ledermix paste with calcium hydroxide was advocated by Schroeder, initially for the treatment of necrotic teeth with incomplete root formation.\textsuperscript{56} A 50:50 mixture of Ledermix paste and calcium hydroxide has also been advocated as an intracanal dressing in cases of infected root canals, pulp necrosis and infection with incomplete root formation as an initial dressing prior to using calcium hydroxide alone for apexitification, perforations, inflammatory root resorption, inflammatory periapical bone resorption and for the treatment of large periapical radiolucent lesions. It has been shown that the 50:50 mixture results in slower release and diffusion of the active components of Ledermix paste which makes the medicament last longer in the canal.\textsuperscript{7} This in turn helps to maintain the sterility of the canal for longer and also maintains a higher concentration of all components within the canal.

The 50:50 mixture of Ledermix paste and calcium hydroxide pastes does not alter the pH to any noticeable extent\textsuperscript{57} and therefore it is expected that the mixture will act in a similar manner to when calcium hydroxide is used alone. Taylor \textit{et al.}\textsuperscript{58} also showed that for two indicator micro-organisms, \textit{Lactobacillus casei} and \textit{Streptococcus mutans} (which are cariogenic), the 50:50 mixture was marginally more effective than either paste used alone. However, Seow\textsuperscript{59} showed that for \textit{Streptococcus sanguis} and \textit{Staphylococcus aureus}, the addition of only 2.5 per cent by volume of Calcyd (a calcium hydroxide in saline paste) (Otto and Co., Frankfurt, Germany) to Ledermix converted the zone
of complete inhibition originally seen in Ledermix to one of only partial inhibition. This latter study suggested that some medicaments should not be used in combination, and that when two medicaments with strong antimicrobial activity are combined there may be additive or synergistic effects.

Due to the complexity of root canal infections, it is unlikely that any one single antibiotic could result in effective and predictable disinfection of all canals. More likely, a combination would be needed to address the diverse flora encountered. A combination of antibiotics would also decrease the likelihood of the development of resistant bacterial strains.15 Hoshino et al.15 determined that a combination of ciprofloxacin, metronidazole and minocycline, each at a concentration of 25μg per ml (0.0025 per cent) of paste, was able to disinfect infected root dentine in vitro. Sato et al.16 found that this combination at 50μg of each antibiotic per ml (0.005 per cent) was sufficient to disinfect infected root dentine in situ. However, it is questionable whether this concentration would be adequate in vivo, particularly in immature teeth which present many challenges for their disinfection, including the potential for periapically-derived fluid to have a washing-out effect on the antibiotic paste via the open apical foramen.

As already discussed, Portenier et al.17 demonstrated that dentine itself can have an inhibitory effect on the bactericidal activity of intracanal medicaments. Therefore, Windley et al.4 used metronidazole, ciprofloxacin and minocycline in a thick paste at a concentration of 20μg of each drug per mL (i.e., 2 per cent) to counteract these potential effects. Of the 30 samples from which bacteria were cultured before treatment, 90 per cent remained positive following irrigation with 10ml of sodium hypochlorite, but this dropped to 30 per cent following the application of the triple antibiotic paste for two weeks.4

In a study by Chu et al.,14 Ledermix paste, Septomixine Forte, and Calasept (a calcium hydroxide in saline paste) (Nordiska Dental, Angleheden, Sweden) were spiraled into root canals and left for seven days. Bacteriological samples were taken before and after the two-visit endodontic treatment. The mean number of colony forming units (CFU) was reduced to 0.39 per cent after seven days, and the percentages of canals that remained with positive growth were 48 per cent, 31 per cent and 31 per cent respectively for each medicament. There were no significant differences in the number of canals with positive growth or mean CFU after instrumentation, irrigation and medication with Ledermix, Septomixine Forte or Calasept. It was postulated that the results may be due to the synergistic balance between strict anaerobes, facultative anaerobes and other aerobes in endodontic infections being disrupted by antibiotics not specifically targeting the predominant flora.

The incidence of postoperative pain following endodontic treatment varies from 16 to 48.5 per cent, and this symptom can last for several hours up to several days.16 The effectiveness of corticosteroid preparations in decreasing periapical inflammation secondary to chemomechanical instrumentation of the root canals was demonstrated by Smith et al.,17 who also showed histologically that fibroblastic and osteoblastic activity was not eradicated by the use of 2.5% hydrocortisone. Ledermix paste has also been shown to be useful in reducing the incidence of pain following chemomechanical preparation of root canals.18 Dentine acts as a slow release mechanism for triamcinolone to the periodontal tissues which is the probable basis of its long-acting therapeutic effect.19 Ehrmann et al.20 have shown that Ledermix paste provides greater postoperative pain relief compared to teeth medicated with calcium hydroxide, and thus has been confirmed by several other studies.20-22 As an example, Negra23 reported that more than 85 per cent of cases had complete relief of pain after a one hour interval and more than 92 per cent were free of pain within 24 hours of treatment.

(c) Non-phenolic biocides

Biocides comprise a large group of diverse chemical agents that are capable of inactivating a variety of micro-organisms. Biocides have a long history of safety and therefore are used in a large variety of applications (e.g., as antiseptics and disinfectants in public hospitals, oral rinses, water purification and as preservatives).4 Some of the commonly used biocides include alcohols (e.g., ethanol), aldehydes (e.g., formaldehyde, glutaraldehyde), biguanides (e.g., chlorhexidine), quaternary ammonium compounds (QACs), zinc, and phenolic compounds including essential oils and phenylethers (e.g., triclosan). Some biocides (e.g., chlorhexidine salts and QACs) are used predominately as antiseptics, disinfectants and preservatives whereas others (e.g., glutaraldehyde) are used predominately for disinfection of endoscopes and water cooling towers in large air conditioning systems.4

While antibiotics affect a specific target site in micro-organisms resulting in bacteriostatic and bactericidal effects at therapeutic concentrations, biocides have a broader spectrum of activity as they work on multiple target sites. Hence, bacterial resistance to biocides is unlikely to develop.4 In general, biocides bind to target molecules within the cell wall, which becomes disrupted and this allows the agent to then penetrate into the cell and interact with the cytoplasmic constituents. The modes of action of biocides include membrane damage and leakage, protein denaturation, binding of rhod groups, initiation of autolysis, and congealing of cytoplasmic contents at higher concentrations.4 Biocide susceptibility is a function of the permeability of the biocide through the cell wall; gram-positive bacteria are more permeable and susceptible to biocides, whereas mycobacteria and gram-negative bacteria, which have a more complex cell wall, are less permeable and susceptible.4
When used at their recommended concentrations, biocides have multiple targets within the microbial cell which confer bactericidal activity and prevent the emergence of resistant bacteria.6,15 While mutational resistance to antibiotics is well known, target site mutations are rare with biocides and it is unlikely that mutation to high level resistance occurs with biocides.15 Laboratory studies have shown evidence of intrinsic or acquired mechanisms of non-susceptibility to biocides, but clinical failure has not yet emerged.6 Despite this, sub-optimal biocide concentrations may result in the emergence of non-susceptible organisms which could also be resistant to biocides.6

Epidemiological surveys show no increase in antibiotic resistance in clinical isolates of oral bacteria from subjects using anti-plaque biocide formulations. Similarly, analysis of the skin microflora from oral health care workers routinely using biocides for hand disinfection in dental clinics has indicated no alterations in the composition of the microbial flora and no resistance to biocides.23

Biocide activity is affected by several factors – notably concentration, period of contact, pH, temperature, and the presence of organic matter.44 Concentration is a factor of prime importance – QACs, chlorhexidine (CHX) and glutaraldehyde retain much of their activity when diluted but phenolics and alcohols rapidly lose activity readily on dilution.44 Glutaraldehyde and cationic biocides (CHX, QACs) are most active at alkaline pH, whereas hypochlorites and phenolics are most potent at acid pH. Interaction with organic matter (such as blood, serum, pus) and non-ionic surfactants can adversely affect the efficacy of some biocides.44 The activity of biocides can be enhanced by the use of chemical agents (e.g., EDTA, polylisine) which increase the permeability of bacterial cell membranes.84

**Chlorhexidine**

Chlorhexidine has a reasonably wide range of activity against aerobic and anaerobic organisms as well as Candida species. It is more effective at alkaline than at acid pH, and its action is inhibited by the presence of soaps and organic matter.86,29 CHX is sporostatic (prevents the outgrowth but not the germination of bacterial spores) but not sporicidal towards bacterial spores.48 CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism.49 Its efficacy is based on the interaction between the positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall. This increases the permeability of the cell wall which allows the chlorhexidine molecule to penetrate into the bacteria with intracellular toxic effects. CHX at low concentrations will result in a bacteriostatic effect but at higher concentrations, it is bactericidal due to precipitation and/or coagulation of the cytoplasm which is probably caused by protein cross-linking.13 The beneficial effect of CHX is due to its antibacterial, substantive properties and its ability to inhibit adherence of certain bacteria.46 Attachment of bacteria to oral surfaces represents a critical step in the pathogenic process, CHX has been shown to inhibit adherence key pathogens (e.g., that of P. gingivalis to host cells).47

CHX has much greater activity against Gram-positive than Gram-negative organisms. The least susceptible of the Gram-negative micro-organisms include strains of Proteus, followed by Pseudomonas, Enterobacter, Actinobacter and Klebsiella.49 There is no evidence that CHX resistance in Gram-negative bacteria is plasmid-borne or transferable.6 This may explain why long-term application of CHX in dogs leads to a domination in plaque samples of Gram-negative rods. The efficacy of CHX against Gram-positive bacteria may thus cause an over-estimation of the clinical usefulness of this agent.66

CHX in gel formulations has a low toxicity to the periapical tissues. The viscosity of the gel keeps the active agent in contact with the root canal walls and dentinal tubules.14 According to Barthel et al.,30 when used to medicate root canals, CHX gel does not interfere with the sealing properties of root filling materials. However, unlike sodium hypochlorite, CHX does not dissolve organic tissue or inactivate bacterial LPS16,31 and therefore its use during the mechanical instrumentation and cleaning of canals is questionable.

CHX has a unique feature in that dentine medicated with it acquires antimicrobial substantivity.28,69,71 The positively-charged molecules of CHX can adsorb onto dentine and prevent microbial colonization on the dentine surface for some time beyond the actual medication period32 – this may inhibit re-infection of the canal subsequent to treatment69 during that time period. CHX is retained in root canal dentine in levels sufficient to exert antimicrobial effects for at least 12 weeks.80 In order to achieve this substantivity, prolonged interaction is required to allow saturation of the dentine with the CHX. Thus, it should be applied as an intracanal medicament between appointments for at least seven days rather than being used only as an irrigant.69 Antimicrobial substantivity depends on the number of CHX molecules available to interact with the dentine. Therefore, medicating the canal with a more concentrated CHX preparation should result in increased resistance to microbial colonization.82 In large bovine root canals, 0.2% CHX imparted substantivity whereas in smaller human root canals a higher concentration of CHX was required to achieve a comparable effect.82

When CHX is used as an irrigating solution, it has a relatively short effective exposure time in the root canal (a few minutes at most) and this does not allow the medication to apply its full antibacterial action. As a result, a large number of bacteria may persist within the dentinal tubules and remain viable.27 The limited
The antibacterial effect of CHX irrigation is a result of the lack of time of application. The dentine must reach saturation point before there is any persistence of the antibacterial activity. During the first hour of exposure, the dentine is absorbing the medication and therefore it is not yet active. In a study by Lin et al., a slow release device containing 5% CHX was placed in root canals for seven days to allow sufficient time for penetration into the dentinal tubules and for CHX to reach its maximum antibacterial effect. This resulted in no bacteria being detected in bovine dentine up to 500μm into the dentinal tubules.

When used as an intracanal medicament, CHX was more effective than calcium hydroxide in eliminating E. faecalis from inside dentinal tubules. In a study by Alamiroudi et al., all of the chlorhexidine formulations used, including a CHX/Ca(OH)2 50:50 mix, were efficient in eliminating E. faecalis from the dentinal tubules with a 1% CHX gel working slightly better than the other preparations. These findings were corroborated by Gomes et al. in bovine dentine and Schaper et al. in human dentine where 2% CHX gel had greater activity against E. faecalis, followed by CHX + Ca(OH)2, and then Ca(OH)2 used alone.

In a study using agar diffusion, Haenni et al. did not demonstrate any additive antibacterial effect by mixing Ca(OH)2 powder with CHX (0.5 per cent). In fact, they showed that the CHX had a reduced antibacterial action. However, Ca(OH)2 did not lose its antibacterial properties in such a mixture. This may be due to the deprotonation of CHX at a pH greater than 10 which reduces its solubility and alters its interaction with bacterial surfaces as a result of the altered charge of the molecule. In an in vitro study using human teeth, Erkan et al. showed 2% CHX gel was the most effective agent against E. faecalis inside dentinal tubules, followed by a Ca(OH)2/2% CHX mix, whilst Ca(OH)2 alone was totally ineffective, even after 30 days. The 2% CHX gel was also significantly more effective than the Ca(OH)2/2% CHX mix against C. albicans at seven days, although there was no significant difference at 15 and 30 days. Ca(OH)2 alone was completely ineffective against C. albicans. In another in vitro study using primary teeth, a 1% CHX gluconate gel, both with and without Ca(OH)2, was more effective against E. faecalis than Ca(OH)2 alone within a 48 hour period.

Schaper et al. reported that 2% CHX gluconate was significantly more effective against E. faecalis than a Ca(OH)2 paste (Calxyl) (Oto and Co., Frankfurt, Germany) used alone, or a mixture of the two. This was also confirmed by Lin et al. although in a study by Evans et al. using bovine dentine, 2% CHX with Ca(OH)2, was shown to be more effective than Ca(OH)2 in water. In an animal study, Lindskog et al. reported that teeth dressed with CHX for four weeks had reduced inflammatory reactions in the periodontium (both apically and marginally) and less root resorption. Wahlimo et al. reported that 0.5% CHX acetate was more effective at killing C. albicans than saturated Ca(OH)2, while Ca(OH)2, combined with CHX was more effective than Ca(OH)2 used alone. The high pH of Ca(OH)2 was unaffected when combined with CHX in this study.

CHX has been used extensively in commercially available oral rinses and subgingival irrigants at a concentration of 2% without any apparent adverse effects being reported. In vivo and in vitro studies conducted by Wennberg showed CHX was no more toxic or irritant to tissues than other endodontic agents. Filho et al. found that 2% CHX gluconate produced the same inflammatory response in mice as phosphate buffered saline, while Tanomaru et al. found that 2.0% CHX digluconate solution did not induce a significant inflammatory response nor damage tissue, again indicating its biocompatibility. Hence, 0.1% to 2% chlorhexidine solutions are classified as toxicologically safe. Even at higher concentrations, CHX has very low toxicity.

(d) Phenolic agents

These medicaments have been applied either on a cotton wool pellet placed in the pulp chamber or on a paper point placed in the root canal, with the rationale being that the antimicrobial effect is delivered through vaporization of the medicament. Endodontic medicaments such as camphorated monochlorophenol (CMCP) depend on the diffusion of their vapours to spread the material throughout the root canal system and bring it into contact with micro-organisms remaining in the canal following chemomechanical instrumentation and irrigation. The antimicrobial action in the apical portion of the root and within the dentinal tubules is therefore dependent on the volatility of the medicament. Hence it must convert to the vapour phase and penetrate the entire root canal system to come into direct contact with the micro-organisms. Depending on the volatility of the agent used, the amount which can be loaded onto a cotton pellet or paper point is small and some of the medicament will be lost through evaporation into the atmosphere before the access cavity is closed. If this loss were great, the duration of effectiveness of the remaining material within the canal will be limited. In studies by Menezes et al. and Messer and Chen, there was 90 per cent loss of the CMCP from cotton pellets inserted into pulp chambers within 1–2 days of insertion. Vapours may be effective but the delivery to the apical part of the root canal is unpredictable. Hence an endodontic antiseptic that is not in direct contact with the root canal walls in the very apical end of the pulp space must be considered as being unreliable at best.

The antibacterial action of phenolic materials may not persist for prolonged periods of time. Hence some bacteria may survive and have the opportunity to multiply and persist in the root canal system. CMCP can diffuse beyond the apical foramen and since it is not firmly bound to periapical proteins, it is rapidly
removed by the blood circulation with 80 per cent of it being excreted by the kidney. Bystrom et al. reported that calcium hydroxide had a higher antibacterial effect in the root canal than camphorated phenol (CP) or CMCP. When infected root canals were treated with CP or CMCP, bacteria persisted in 33 per cent of the canals. When compared with mechanical cleansing and irrigation, the antibacterial effect of CP and CMCP was not impressive. When paramonochlorophenol (PMCP) was combined with Calen (a Ca(OH)2 paste) (S.S. White, Rio de Janeiro, Brazil), the amount of PMCP released was of sufficiently low concentration not to harm the periapical tissues but sufficient to exert bactericidal effects. A loss of approximately 50 per cent of the PMCP occurred within 48 hours but there was no further significant loss after 14 days. In contrast, the combination of Calen and CMCP paste was reported as the most effective intracanal medicament compared to a variety of medicaments including 2% CHX solution, Calen paste and 2.5% sodium hypochlorite (NaOCl) for the elimination of E. faecalis and C. albicans using sampling with sterile paper points after 15 days in human teeth.

In a study by Ayhan et al., Cresophe (a mix of 30% paramonochloro-phenol, 5% thymol, 0.1% dexamethasone) (Septodont, Saint-Maur, France) had one of the highest levels of antimicrobial activity using agar diffusion when compared with five other irrigants. However, the authors cautioned against using Cresophe due to its known cytotoxic and possible carcinogenic, mutagenic and teratogenic properties.

CMCP is the most toxic and irritating phenolic antiseptic agent followed by Cresatin, formocresol, and camphorated phenol (CP), but all four have similar antimicrobial properties. The problematic biocompatibility of some phenol compounds such as CMCP was corroborated by Collet et al. The toxic effects of CP ended when the dilution exceeded 1:70, while CMCP required 1:2000 times dilution to eliminate toxicity to cultured cells. CMCP has low solubility in water and a slow diffusion rate in agar – hence, when assessed in vitro, it appears to have only a limited antimicrobial effect. In contrast, serial dilution experiments indicate that CMCP is a highly effective antiseptic agent.

(e) Iodine compounds

Iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal. Aqueous iodine solutions are unstable, with molecular iodine (I2) being mostly responsible for the antimicrobial activity. Iodophors (iodine carriers) are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active free iodine. In 1976, Tornec advocated the use of povidone-iodine solution as an endodontic irrigant. This was based on its rapid antiseptic action against a broad range of micro-organisms, low toxicity, hypoaalergenicity, and greatly decreased tendency to stain dentine than other iodine containing antiseptics.

The antimicrobial action of iodine is rapid, even at low concentrations, but the exact mode of action is not fully known. It is thought that iodine attacks key groups such as proteins, nucleotides, and fatty acids, resulting in cell death. In endodontics, a 2% preparation of iodine potassium iodide (IPI) has been used, which has been shown to be less toxic or tissue irritating than Formocresol, CMCP, and Cresatin. In 2% IPI-treated root canals in human teeth, a period of 1–2 hours was required to prevent growth of E. faecalis in dentinal tubules, whilst the calcium hydroxide specimens still had positive cultures after 24 hours.

Molander et al. failed to demonstrate an enhanced antibacterial effect by combining 5% IPI for a period of 3–7 days with a subsequent two-month period of calcium hydroxide dressing in human teeth where the smear layer was not removed. When IPI was used, 44 per cent of the 50 cases examined showed positive growth, whilst the addition of calcium hydroxide to IPI resulted in only 20 per cent positive cultures. Peculiene et al. studied the effect of calcium hydroxide medication for 10–14 days on 20 root-filled teeth with apical periodontitis. Bacteria were isolated in 16 of the 20 teeth prior to instrumentation, and in five teeth after instrumentation and irrigation (with smear layer removed). The third sample taken after five minutes of irrigation with 2% IPI in 4% potassium iodide (2% IPI 4%) showed growth from only one canal.

When the antifungal effects of different medicaments and their combinations were compared, 2% IPI 4% killed all C. albicans cells within 30 seconds and a 10-fold dilution showed complete killing within five minutes. A 2% IPI 4%-saturated calcium hydroxide combination was significantly less effective than 2% IPI 4%.

As with other medicaments, the presence of dentine and its components are responsible for different inhibitory patterns on the activity of the iodine solutions. Haapasalo et al. demonstrated that dentine powder effectively abolished the effect of 0.2% IPI 0.4%, whilst dentine powder had a very limited capacity to inactivate 2% IPI 4%, where it took only five minutes to kill E. faecalis. Portenier et al. showed that hydroxyapatite caused little or no inhibition, whilst the collagen matrix in dentine effectively inhibited 0.1% IPI 0.2%.

Abdullah et al. showed that a 10% povidone iodine solution resulted in 100 per cent bacterial reduction after 30 minutes in an E. faecalis planktonic suspension, and 30 minutes in an E. faecalis biofilm model, whilst calcium hydroxide was unable to achieve 100 per cent bacterial reduction even after 60 minutes. In a model using bovine teeth infected with E. faecalis, Baker et al. showed that every iodine containing agent tested (2% IPI, 2% IPI plus Tween 20, 10% povidone iodine, 10% povidone iodine plus detergent) performed significantly better than 10% calcium hydroxide. The most effective irrigant was 2% IPI, with...
nearly total elimination of *E. faecalis* within 15 minutes, whilst 10% calcium hydroxide failed to eliminate *E. faecalis* from any of the samples. There was also no residual effect of the iodine-containing medicaments.

Siren et al.\(^6\) used *E. faecalis* infected bovine root blocks to test the effectiveness of one-day and seven-days incubation of medicaments. The 2% IKI medicament showed results with no growth up to 700 mp and 950 mp at one and seven days. However after seven days, 2% IKI saturated with calcium hydroxide had the same effect as 0.5% chlorhexidine acetate and 2% IKI. Cwilka et al.\(^{10}\) used human single-rooted teeth infected with *E. faecalis* and showed that Ca(OH)$_2$/silicone oil was the most effective combination followed by 2% IKI/Ca(OH)$_2$ and then Ca(OH)$_2$.

**Medicament vehicles**

The medicament vehicle plays a very important role in the overall disinfection process because it determines the degree of ion dissociation causing the paste to be solubilized and resorbed at various rates by the periapical tissues and from within the root canal. The lower the viscosity, the higher will be the ion dissociation. The high molecular weight of commonly-used vehicles minimizes the dispersion of calcium hydroxide into the tissue and maintains the paste in the desired area for longer periods of time.\(^4\)

There are three main types of paste vehicles:\(^4, 5\)

(a) Water-soluble substances such as: water, saline, dental anaesthetics, Ringers solution, methylcellulose, carboxymethylcellulose, anionic detergent solutions (including sodium lauryl sulphate and sodium lauryl diethyleneglycol). Some examples of water-soluble proprietary medicaments include Calxyl, Pulpdent, Calasept, Hypocor (Ellman Co., New York, USA), and DT temporary dressing (Dental Therapeutics AB, Nacka, Sweden).

(b) Viscous vehicles such as: glycerine, polyethylene glycol (PEG) and propylene glycol. Some examples of viscous proprietary medicaments are Calen and Ledemix paste.

(c) Oil-based vehicles such as: olive oil, silicone oil, camphor (the essential oil of camphorated perchlorophenol), metacresylacetate, eugenol and some fatty acids (including oleic, linoleic and isostearic acids). Some examples of oil-based proprietary medicaments include Endoapex (Lab. Inodon Ltda. Porto Alegre, RS, Brazil) and Vitapex (Neo Dental Chemical Products Co. Ltd, Tokyo, Japan).

Calcium hydroxide should be combined with a liquid carrier because the delivery of dry calcium hydroxide powder alone is difficult or impossible in narrow, curved canals, and water is required for hydroxyl ion release. Sterile water or saline are the most commonly used carriers. Aqueous solutions promote rapid ion liberation and should be used in clinical situations. Although dental local anaesthetics have an acidic pH range (between 4–5), they provide an adequate mixing agent since calcium hydroxide is a very strong base which is only minimally affected by acid. The effects of glycerine and propylene glycol mixing vehicles on the pH of calcium hydroxide preparations was investigated using conductivity testing by Safavi et al.\(^{13}\) A range of 10–30 per cent for a glycerine/water mixture and 10–40 per cent for a propylene glycol/water mixture resulted in the greatest amount of conductivity. They reported that the higher concentrations of these vehicles may decrease the effectiveness of calcium hydroxide as a root canal dressing.

Viscous vehicles are also water soluble substances that release calcium and hydroxyl ions more slowly and for extended periods.\(^4\) A viscous vehicle may remain within root canals for 2–4 months and hence the number of appointments required to change the dressing will be reduced.\(^4\) Only vehicles have restricted applications—they are not recommended since they are difficult to remove and leave an oily film on the canal walls which will affect the adherence of the root canal cement or other material used to fill the canal.

As well as the type of vehicle used, the thickness of the paste can influence the antimicrobial activity, especially for calcium hydroxide. This was seen in a study by Behn et al.\(^2\) in which thick mixtures of calcium hydroxide showed a significant reduction of antibacterial activity against *E. faecalis* in dentine tubules compared to a thin mix and the commercial product, Pulpdent paste.

PEG is one of the most commonly used vehicles used in root canal medicaments and it possesses an ideal array of properties including very low toxicity, excellent solubility in aqueous solutions and extremely low immunogenicity and antigenicity.\(^11\) Concentrated PEG 400 solutions have their own significant antibacterial activity against various pathogenic bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*\(^3\) which is in addition to any other substances added to the PEG base as a medicament. In a study by Cameos et al.,\(^{12}\) the pH in an aqueous medium was tested outside the roots of human teeth when various vehicles (aqueous or viscous) were used with calcium hydroxide. They reported that vehicles with glycerine and PEG 400 showed a trend to acidification during the first eight days after filling (pH 6.85 to 6.4 – PEG 400) but then the pH returned to similar levels to other groups after 42 days (pH 7.1 – PEG 400).

**Biofilms**

Micro-organisms do not normally exist as isolated single cells suspended in an aqueous environment (i.e., the planktonic model); rather, most in vivo populations of bacteria grow as adherent biofilms.\(^3\) Biofilms are composed of micro-colonies of bacterial
cells that are non-randomly distributed in a matrix of polysaccharides, proteins, salts and cell material in an aqueous solution. Since biofilms are the preferred method of growth on a surface for most species of bacteria, it is likely that bacteria are present in biofilms on the dentinal wall or on the external surface of the root tip. Infection of the root canal system ultimately leads to liquefaction necrosis of the pulp where the bacteria form biofilms on the root canal walls with the tissue remnants in the canal. Planktonic bacteria that are either leaving or joining the biofilm surround it and this constant detachment of cells serves as a steady source for chronic infection.

The presence of biofilm will affect the efficacy of antimicrobial agents since biofilms are much more resistant to such agents as a result of their diffusion barriers and altered bacterial cell metabolism and replication rates. Micro-organisms remaining in the root canal may survive and their effects will depend on the micro-organisms possessing pathogenicity, reaching sufficient numbers and gaining access to the periapical tissues to induce or maintain periapical inflammation. The survival of bacteria is recognized as the main cause of persistent infections and the induction of inflammatory responses in the periapical tissues following root canal treatment.

Oral biofilms are complex and may comprise 30 or more bacterial species. Biofilm formation in the root canal varies depending on the cause of the pulp breakdown. Root canals may not always be fluid-filled and the canals of teeth with necrotic pulps often appear dry on entering them, at least in the coronal portion. Hence the question remains as to whether bacterial condensations in a biofilm structure can develop or be retained in sites of the root canal system other than the apex where host-derived proteins and bacteriologically-produced adhesive substances may provide the proper prerequisites.

Nair et al. found that even after instrumentation, irrigation and obturation in a one-visit treatment, microbes existed as biofilms in untouched locations in the main canal, isthmuses and accessory canals in 14 of the 16 root-treated teeth examined. Bacterial biofilms are reported to be the most common cause of persistent inflammation and apical periodontitis is considered to be the result of an intraradicular biofilm-induced chronic disease.

Mechanisms of resistance of bacterial biofilms to antimicrobial agents include:

- the polysaccharide matrix retards diffusion of the antibiotic. Extracellular enzymes such as β-lactamase may become trapped and concentrated in the matrix thereby inactivating β-lactam antibiotics;
- communication with one another (known as "quorum sensing") which can influence the structure of the biofilm by encouraging growth of species beneficial to the biofilm. Transfer of genetic information (antibiotic resistance and virulence traits) to other bacteria within the biofilm may provide an effective defence against host protective mechanisms and antimicrobial agents producing a highly resistant phenotypic state,
- chemical changes to the environment in the biofilm where the lack of oxygen inhibits some antibiotics and accumulated acidic waste leads to a difference in pH which has an antagonizing effect on the antibiotic,
- depletion of nutrients or accumulation of waste products can result in bacteria entering a non-growing state which protects bacteria from the antibiotics, as well as the dose and frequency of exposure to the antimicrobial agent,
- protecting themselves by being located within the interior part of a biofilm – hence medications will only act on the micro-organisms in the peripheral portion of the biofilm. Bacterial cells residing within a biofilm grow more slowly than planktonic cells and as a result antimicrobial agents act more slowly,
- subpopulations of bacteria in a biofilm form a phenotypic state (altered gene expression) where they are highly protected – this form of cell differentiation is similar to spore formation, and biofilm bacteria existing in a low metabolic state, a slower growth rate and production of exopolysaccharides.

Biofilms and microbial aggregates are a common mechanism for the survival of bacteria in nature. Coaggregation is highly specific and is considered a virulence factor which is believed to play a role in the development of dental plaque and other biofilms. In a study by Khemaleelakkul et al., coaggregation was very prominent between Streptococci-Prevotella, Streptococci-Staphylococci, and Streptococci-Corynebacteria. Prevotella and Streptococci were the genera that most often coaggregated with multiple bacterial species. Fusobacteria isolated from endodontic abscesses demonstrated the ability to coaggregate with members of every genus tested and it also had the ability to auto-aggregate. Prevotella spp. is considered to play a key role in the pathogenesis of endodontic infections due to its ability to coaggregate with other species, especially Streptococci (regarded as early colonizers). It seems likely that multi-generic coaggregation may occur in infected root canals and periapical tissues in the same manner as dental plaque biofilms. The aggregation of bacteria in biofilms is likely to result in these bacteria being more resistant to antibiotics and other antimicrobials as well as being protected from the host defenses.

Wilson has pointed out that the above refers to mono-species biofilms. At present, there are no studies to show what happens in multi-species biofilms with low oxygen content, low oxidation-reduction potential and high concentrations of metabolite end-products which may lead to increased growth rates for anerobic
bacteria. It would be useful to know their antimicrobial susceptibilities in relation to their planktonic counterparts. The use of the membrane filter-based model using single species biofilms offers a simple and more accurate assessment for determining the susceptibility of oral bacterial biofilms to antimicrobial agents.\textsuperscript{40}

It is estimated that bacteria grown in a biofilm have a 1000–1500 times greater resistance to antibiotics than planktonically-grown bacteria.\textsuperscript{41,42} For example, the biofilm inhibitory concentrations for chlorhexidine and amine fluoride are 300 and 75 times greater, respectively, when Streptococcus sobrinus is grown in biofilm compared with the minimum bactericidal concentration for planktonic cells.\textsuperscript{42} With regard to host defences, bacteria in biofilms are less easily phagocytosed and less susceptible to complement than their planktonic counterparts.\textsuperscript{44} Bacterial viability may vary throughout dental plaque biofilm with the most viable bacteria being located in the central part of the plaque. Lining the voids and channels are layers of viable bacteria packed in layers of dead material.\textsuperscript{19}

During the irrigation of root canals, the outer layer of the biofilm will be directly affected by the high concentration of the irrigating solution but the extracellular matrix of the biofilm may prevent the solution penetrating into the deeper layers at full strength.\textsuperscript{19} Endodontic instrumentation helps to disrupt and expose the full thickness of the biofilm to the irrigant solution. However, since not all of the root canal wall are contacted by instruments, some micro-organisms may still remain in the canal and within the tubules.\textsuperscript{19}

Thrower et al.\textsuperscript{12} showed that chlorhexidine was by far the superior agent in the membrane-based biofilm model and the planktonic model of Actinomyces viscosus, followed by cetylpyridinium chloride, with triclosan being the least active. In a study by Lima et al.,\textsuperscript{135} medications containing 2% chlorhexidine gluconate were able to completely eliminate most of the biofilms while medications containing clindamycin, with or without metronidazole, did not significantly affect the biofilms. Biofilms of oral bacteria have also been found to be more resistant to amoxicillin, doxycycline and metronidazole.\textsuperscript{11}

In polymicrobial (bacteria and fungal) biofilms, bacterial species have been found to be more resistant to biocide treatment than when in single species biofilms.\textsuperscript{11} Hence Clegg et al.\textsuperscript{135} used polymicrobial biofilms to assess the antimicrobial effectiveness of various irrigants including 2% CHX. The root apices were incubated for seven days to allow biofilm formation and immersed in test solutions for 15 minutes. No cultivable organisms were found after dentine sections were placed in 2% CHX for 15 minutes. However, when the samples were viewed under SEM, the biofilm was virtually intact. This result may be due to the potential fixation of cells by CHX. If CHX is to be used as an irrigant, additional agents (e.g., sodium hypochlorite) may be needed to physically disrupt the biofilm.

E. faecalis has been shown to form biofilms in the root canals of human teeth, with or without intracanal medicaments, after only two days with depths of 2 μm for 86-day biofilms and 28–30 μm for 160-day biofilms. Root canals inoculated with E. faecalis for 86 days resulted in the bacteria becoming embedded in branching filamentous material which represented an extracellular polysaccharide produced by bacteria. Biofilms maintained for 160 days had a highly organized structure consisting of mushroom-shaped clumps of bacteria with vacant areas which were thought to contain water channels for the delivery of nutrients and to remove waste from the biofilm bacteria.\textsuperscript{137}

Alterations in the micro-environment of root canals (as occurs during endodontic treatment) could stimulate calcification of E. faecalis biofilms, as shown in a study by George et al.\textsuperscript{138} They also reported that biofilms which formed under nutrient rich conditions had increased calcium levels within the biofilm whereas nutrient poor conditions did not produce this effect. This implies a possible role of the mineralized matrix as a shelter for viable bacteria which helps to protect them against antimicrobial agents.\textsuperscript{138}

Biofilms produced in the laboratory are often less adherent and less virulent than those found in nature and this should be taken into account when trying to achieve in vivo-in vitro correlations.\textsuperscript{139} More studies are needed to explore the conditions that may affect the efficacy of endodontic antimicrobial agents in vivo so that their clinical effects can be better predicted. Indeed, conclusions reached on studies of monocultures on standard laboratory surfaces must be interpreted with great caution as such models do not reflect the clinical scenario and may give misleading data especially in terms of the effects of antimicrobials on bacteria within biofilms.\textsuperscript{140}

Antibiotic resistance

Anaerobes and Gram-negative rods which are found frequently in untreated infected root canals are often associated with acute exacerbations of chronic apical periodontitis. Since the Gram-negative cell wall is more fragile, these bacteria are typically more sensitive to biocides and easier to eliminate than Gram-positive and/or facultative bacteria.\textsuperscript{149,150} Bacteria have been able to develop or acquire resistance to every class of antibiotics in use and, as a result of this, every antibiotic has a limited period of use before resistance emerges.\textsuperscript{151}

Clindamycin offers no additional antimicrobial advantage over conventional root canal medicaments such as calcium hydroxide and therefore it has not been recommended for routine use in endodontic therapy.\textsuperscript{152} Dahlén et al.\textsuperscript{153} reported that 26 isolates of E. faecalis and three isolates of E. faecium were found in 17 of 29 teeth that had calcium hydroxide dressings and 4 of 29 teeth that had iodine tincture as root canal dressings. All strains were resistant to metronidazole and most strains were resistant to clindamycin.

In another study by Sedgley and Clewell,\textsuperscript{154} all but one of the E. faecalis isolates from oral rinse samples
were resistant to clindamycin and aminoglycosides. The LSA gene is responsible for intrinsic resistance to
tincosamidcs (such as clindamycin) and strepto-
gramins.141 Tetracycline resistance is present in at least
60 to 80 per cent of enterococcal isolates, even though
tese antibiotics are not routinely used to treat
tenterococcal infections.141,142 Tetracycline resistance has
ealso been associated with oral isolates of Prevotella
intermedia and Prevotella nigrescens. Tsai et al.143
found 21 per cent of the P. intermedia and 34 per cent
of the P. nigrescens isolates either showed intermediate
susceptibility or were resistant to tetracycline, with
resistance carried by the tet Q gene.

Bacterial resistance to tetracycline usually results in
cross resistance to other tetracyclines and this was
observed by Bystrom et al.144 where the strains resistant
to tetracycline were also resistant to doxycycline.
Gulabivala145 reported that Enterococcus,
Staphylococcus and Lactobacillus species contributed
to the majority of strains that were resistant and they
also had the highest degree of multiple antibiotic
resistance. This was also noted by Noda et al.146
Enterococcus strains showed low susceptibility for
azithromycin and erythromycin while clindamycin and
the cephalosporins were not clinically effective for
Enterococcus spp.147

Sublethal concentrations of antibiotics (tetracycline
and chloramphenicol) can act as inducers of multi-drug
resistance.4c It is possible that during root canal therapy,
only sublethal doses may be in contact with the infecting
organisms, particularly in the narrow and more
inaccessible parts of the canals.4d

The virulence of E. faecalis

Enterococcus species are now second only to E. coli
as the most common nosocomial pathogen isolated
overall. They are the leading cause of surgical site
infections and the third leading cause of urinary tract
infections.179 Enterococcus faecalis (formerly designated
Streptococcus faecalis) accounts for 85–90 per cent
of infections caused by enterococci and Enterococcus
faecium accounts for 5–10 per cent.192,193,194 Many
studies indicate that E. faecalis may be encountered in
root-filled Teeth with periapical lesions where there are
recoverable microorganisms in the root canal with
the prevalence ranging from 12 to 70 per cent.19

E. faecalis is a normal intestinal organism and may
inhabit the oral cavity and gingival sulcus. In its intestinal
environment, it is considered a commensal organism
that contributes to carbohydrate, amino acid and
vitamin metabolism. However, a subset of this species
appears to be pathogenic because it has acquired a
number of genes which confer infectivity and virulence,
plus resistance to multiple antibiotics.192

The low prevalence of E. faecalis reported in primary
endodontic infections (i.e. where there has been no
previous endodontic treatment) may be a result of other
species inhibiting its growth. Thus, the possibility exists
that during chemomechanical preparation, the most

prevalent anaerobic bacterial species (which are Gram-
negative) are more likely to be eliminated than the
facultative Gram-positive E. faecalis, which is resistant
to many intracanal antimicrobial procedures and
medications.

Despite its ability to adapt extremely well to the
surrounding environment, E. faecalis does not appear to
ever major pathogenic actions within the root canal
system when it is present as a mono-infection.121 It has
been shown that the addition of E. faecalis to a four-
strain combination increased the survival and patho-
genicity of the other bacteria in the root canal system.141
Increased pathogenicity of E. faecalis has also been
found in animal models.142 Currently, it is unclear
whether E. faecalis is the major pathogen associated
with ongoing apical periodontitis after endodontic
treatment or merely a survivor that takes advantage of
its ability to endure adverse conditions within filled
root canals.172 Studies investigating this have several
limitations173 which include:

- a periapical lesion may be present in some cases
  but will remain radiographically undetectable until
  a certain amount of bone has been resorbed,
- for a disease to develop, a certain amount of
  bacterial load is required; hence it is possible that
  the number of E. faecalis organisms may be lower
  in root-filled teeth without apical periodontitis
  lesions,
- a more virulent clone of E. faecalis is responsible
  for teeth with periapical disease, and
- host resistance differs from subject to subject and
  may result in different patterns of microbiological
  infection.

Enterococci have both intrinsic and acquired
resistance to many antibiotics.193,194 Intrinsic resistance
is a usual species characteristic in all or most of the
strains where the gene for intrinsic resistance resides on
the chromosome, whereas acquired resistance is
resistance due to either a mutation in the existing DNA
or acquisition of new DNA. Enterococci are inherently
more resistant to antimicrobial drugs than other
clinically-important Gram-positive bacteria.19c They
inhabit the gastrointestinal tract and environments
contaminated by human waste and therefore they may be
exposed to antibiotics that pass through the
gastrointestinal tract. The intrinsic resistance of
enterococci to many antimicrobials might have resulted
from their need to survive and persist in highly
competitive and potentially detrimental ecosystems.19d

Enterococci are intrinsically resistant to many
antimicrobial agents, including cephalosporins,
clindamycin, penicillinase-resistant penicillins and
vancomycin.194,195,196 E. faecalis has intrinsic resistance
to clindamycin, cephalosporins and aminoglycosides.1941
In addition to these intrinsic resistances, enterococci
have acquired genetic determinants for resistance to
many classes of antimicrobials, including tetracycline,
doxycycline, erythromycin, chloramphenicol,
ciprofloxacin and vancomycin.194,195,196
Table 1. Antibiotic resistance in *Enterococci species*, based on several studies

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Mechanisms of resistance</th>
<th>Type of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactams (e.g., penicillins, carbapenems, cephalosporins)</td>
<td>(a) Overproduction of low-affinity penicillin binding proteins and/or decreased affinity for binding β-Lactams (NB: Intrinsic resistance to almost all cephalosporins) (b) β-Lactamase production</td>
<td>Intrinsic</td>
</tr>
<tr>
<td>Tetacyclines</td>
<td>(a) Ribosomal protection systems (Tet L, Tet N, Tet O gene) (b) Efflux pump</td>
<td>Acquired</td>
</tr>
<tr>
<td>Linosamides (e.g., clindamycin)</td>
<td>(a) Low level (b) High level</td>
<td>Intrinsic</td>
</tr>
<tr>
<td>Macrolides (e.g., erythromycin, azithromycin, clarithromycin)</td>
<td>(a) MLSβ phenotype-methylation in 23S ribosomal RNA (b) Efflux pump</td>
<td>Acquired</td>
</tr>
<tr>
<td>Streptogramin B (e.g., quinupristin)</td>
<td>MLSβ phenotype (NB: Virtually all <em>E. faecalis</em> isolates are resistant)</td>
<td>Acquired Intrinsic</td>
</tr>
<tr>
<td>Streptogramin A (e.g., dalbopristin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides (e.g., gentamicin, streptomycin, kanamycin)</td>
<td>(a) Aminoglycoside modifying enzyme (b) Low level (limiting transport of drug across cell membrane)</td>
<td>Acquired Intrinsic</td>
</tr>
<tr>
<td>Glycopeptides (e.g., vancomycin, teicoplanin)</td>
<td>(a) Van A phenotype (b) Van B phenotype (susceptible to teicoplanin)</td>
<td>Acquired Acquired</td>
</tr>
<tr>
<td>Fluoroquinolones (e.g., moxifloxacin)</td>
<td>(a) DNA gyrase (topoisomerase II,IV) (b) Efflux pump</td>
<td>Acquired Acquired</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>(a) Chloramphenicol acetyltransferase enzyme (b) Efflux pump (NB: 50% of enterococci are resistant to chloramphenicol)</td>
<td>Acquired</td>
</tr>
<tr>
<td>Trimethoprim and Sulfa derivatives</td>
<td>Chromosomal mutations in gene encode dihydrofolate reductase</td>
<td>Acquired</td>
</tr>
</tbody>
</table>

*E. faecalis* has many remarkable and distinct features which make it an exceptional survivor. It can:
- live and persist in the poor nutrient environment of endodontically-treated teeth,
- survive in the presence of several medications, sodium hypochlorite, clindamycin and the most popular medication, Ca(OH)₂,
- form biofilms in medicated canals,
- invade and metabolize fluids within the dentinal tubules and adhere to collagen in the presence of human serum,
- convert into a viable but non-cultivable state (VBNC),
- endure prolonged periods of starvation and utilize tissue fluid (human serum) that flow from the periodontal ligament and bathe alveolar bone to recover,
- establish mono-infections in medicated root canals,
- acquire gene-encoding antibiotic resistance combined with natural resistance to various antimicrobials agents, and
- survive in extreme environments with low pH, high salinity, and high temperatures.

Sedgley et al. have shown that all isolates of *E. faecalis* had ACE and the gel E gene, 61 percent had ESP gene, 93 percent expressed gelatinase activity in primary endodontic infections and 25 percent in retreatment cases. Due to the proximity of bacteria in biofilms, *E. faecalis* can participate in plasmid-mediated horizontal transfer of virulence determinants. It also has the ability to acquire plasmid-encoded resistance genes from other bacteria which results in intra-species propagation of resistance. Resistance is a major issue with this bacterial species (Table 1). When the opportunity arises, *E. faecalis* could be released from dentinal tubules into the root canal space and act as a source of bacteria for re-infection of the canal.

*E. faecalis* can gain entry into the root canal system during endodontic treatment, between appointments or even after the endodontic treatment has been completed. Hence, current methods used to eliminate *E. faecalis* include:
- using aseptic techniques consisting of a pretreatment CHX rinse, placement of rubber dam, disinfection of the tooth and the rubber dam with CHX or NaOCl and disinfection of the gutta-percha with NaOCl,
- minimum instrumentation of the apical third of the canal to a size 30 file with sufficient taper of the canal towards the coronal orifice in order to allow further penetration of the irrigating solutions,
- irrigation with 1% NaOCl, 17% EDTAC and 2% CHX solution,
- intracanal medicaments consisting of 2% CHX gel or 2% CHX gel plus calcium hydroxide, and
- the use of a root canal cement with a well placed and adapted coronal restoration.

**CONCLUSIONS**

Bacteria have been implicated in the pathogenesis and progression of pulp and periapical diseases. Hence,
the primary aim of endodontic treatment is to remove as many bacteria as possible from the root canal system and then to create an environment in which any remaining organisms cannot survive. Many medications have been used in an attempt to achieve these aims but no single preparation has been found to be completely predictable in its efficacy and hence much further research is required.

The choice of which intracanal medicament to use during endodontic treatment is dependent on having an accurate diagnosis of the condition being treated. If the primary aim is to control inflammation, then a corticosteroid-antibiotic mixture is indicated. However, since the inflammation is likely to have been caused by the presence of bacteria within the root canal and the currently available antibiotic-containing preparations may not be the most ideal antimicrobial agents, a further intracanal medicament with increased anti-microbial action may be indicated. In such cases, calcium hydroxide is the most commonly used agent. Chlorhexidine could also be considered but more research on its effectiveness for this purpose is required.

The current literature indicates that:
- Calcium hydroxide may not be the most ideal medicament for all cases with infected root canal systems with or without apical periodontitis and in previously root-filled teeth.
- Antibiotics may not be ideal as the active component for intracanal medicaments.
- Corticosteroid/antibiotic pastes are best suited in situations where pain control is required.
- The use of a combination of calcium hydroxide and Ledermix pastes needs further investigation.
- Current medicaments used in association with instrumentation and irrigation are unlikely to predictably achieve a bacteria-free root canal system.
- Chlorhexidine may have promise as an intracanal medicament in teeth with infected canals – both when there has not been any previous root canal filling and when there has been a previous root filling – but further research is required.
- Further research is required regarding the use of intracanal medicaments combined with the effects of dentine on single and multiple species biofilms.

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