

Review

Properties and applications of calcium hydroxide in endodontics and dental traumatology

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Abstract

Mohammadi Z, Dummer PMH. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *International Endodontic Journal*, **44**, 697–730, 2011.

Calcium hydroxide has been included within several materials and antimicrobial formulations that are used in a number of treatment modalities in endodontics. These include, inter-appointment intracanal medicaments, pulp-capping agents and root canal sealers. Calcium hydroxide formulations are also used during treatment of root perforations, root fractures and root resorption and have a role in dental traumatology, for example, following tooth avulsion and luxation injuries. The purpose of this paper is to review the properties and clinical applications of calcium hydroxide in endodontics and dental traumatology including its antibacterial activity, antifungal activity, effect on bacterial biofilms, the synergism between calcium hydroxide and other agents, its effects on the properties of dentine, the diffusion of hydroxyl ions through

dentine and its toxicity. Pure calcium hydroxide paste has a high pH (approximately 12.5–12.8) and is classified chemically as a strong base. Its main actions are achieved through the ionic dissociation of Ca^{2+} and OH^- ions and their effect on vital tissues, the induction of hard-tissue deposition and the antibacterial properties. The lethal effects of calcium hydroxide on bacterial cells are probably due to protein denaturation and damage to DNA and cytoplasmic membranes. It has a wide range of antimicrobial activity against common endodontic pathogens but is less effective against *Enterococcus faecalis* and *Candida albicans*. Calcium hydroxide is also an effective anti-endotoxin agent. However, its effect on microbial biofilms is controversial.

Keywords: antimicrobial, apexification, calcium hydroxide, dental traumatology, endodontics, root resorption, vital pulp therapy.

Received 11 November 2010; accepted 29 March 2011

Introduction

Materials and therapeutic agents containing calcium hydroxide are used extensively in a variety of treatment modalities within endodontics and dental traumatology. The main purpose of this article is to review the properties and clinical applications of calcium hydroxide in endodontics and dental traumatology including its antibacterial activity, antifungal activity, effect on

bacterial biofilms, the synergism between calcium hydroxide and other agents, its effects on the properties of dentine, the diffusion of hydroxyl ions through dentine, and its toxicity. The paper sets out initially to provide the background to the main clinical applications of calcium hydroxide ($\text{Ca}(\text{OH})_2$) and then focuses on its specific properties and more detailed uses.

Background

Root canal medicaments

Microorganisms are the cause of apical periodontitis (Kakehashi *et al.* 1965, Möller *et al.* 1981, Sundqvist

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1992) and their elimination from the root canal space during root canal treatment results in predictable healing of apical pathosis (Byström & Sundqvist 1981). Indeed, many studies have demonstrated that teeth with infected root canals at the time of canal filling have substantially poorer outcomes than root canals where no culturable microorganisms could be detected (Molander *et al.* 2007). Unfortunately, the complete elimination of bacteria by instrumentation alone is unlikely to occur (Byström & Sundqvist 1981, Wu *et al.* 2006). In addition, pulp tissue remnants may prevent microorganisms from being entombed (Haapasalo *et al.* 2007) as well as have a negative impact on the root filling in terms of its physical properties and adaptation to the canal walls (Wu *et al.* 2006). Thus, some form of irrigation and disinfection is necessary to kill and remove microorganisms, their by-products and residual tissue, as well as remove the smear layer and other debris from the canal system. Such chemical (therapeutic) treatments of the root canal can be arbitrarily divided into irrigants, canal rinses, and inter-appointment medicaments; calcium hydroxide is included in this latter group.

Endodontic sealers

Sealers are responsible for the principal functions of root fillings, which aim to prevent reinfection. That is, sealing the root canal system by entombing remaining bacteria and filling of irregularities in the prepared canal system (Ørstavik 2005). The rationale for the addition of calcium hydroxide to root canal sealers emanates from observations of liners and bases containing Ca(OH)_2 and their antibacterial and tissue repair abilities (Ørstavik 2005).

Immature teeth with open apices

The primary purpose of treating immature permanent teeth with saveable pulps is to maintain pulp health and allow root development to continue. Vital pulp therapies include indirect and direct pulp-capping, partial (superficial) pulpotomy and cervical pulpotomy. Traditionally, mechanically exposed, but otherwise healthy, pulps of permanent teeth have been capped with a wound dressing containing calcium hydroxide (Schuurs *et al.* 2000). In teeth with open apices and necrotic pulps, creating a barrier across the apical foramen is important to fill the root canal adequately. Historically, creation of a suitable environment for the formation of a calcified barrier involved cleaning and shaping the canal to remove bacteria and debris

followed by placement of a calcium hydroxide paste to fill the canal system for 6–24 months (Frank 1966).

Traumatology

Dental trauma involves damage to teeth and the supporting tissues. Intracanal medicaments containing calcium hydroxide are used to control internal resorption (Haapasalo & Endal 2006) as well as inflammatory apical root resorption (Majorana *et al.* 2003). Furthermore, the International Association of Dental Traumatology (2007) guidelines recommend that any tooth with a necrotic pulp associated with a luxation injury should be dressed with a calcium hydroxide medicament until the root canal is filled. For avulsion injuries, the use of calcium hydroxide medicament is recommended for up to 1 month (Kawashima *et al.* 2009).

Retrieval of literature

A Medline search was performed from 1971 to the end of 2009 and was limited to English-language papers. The keywords searched on Medline were 'calcium hydroxide AND endodontics (1943)', 'calcium hydroxide AND *Enterococcus faecalis* (134)', 'calcium hydroxide AND *Candida albicans* (51)', 'calcium hydroxide AND endotoxin (23)', 'calcium hydroxide AND dentine (986)', 'calcium hydroxide AND biofilm (17)', 'calcium hydroxide AND sodium hypochlorite (174)', 'calcium hydroxide AND chlorhexidine (145)', 'calcium hydroxide AND vital pulp therapy (121)', 'calcium hydroxide AND apexification (138)', 'calcium hydroxide AND root fracture (59)', 'calcium hydroxide AND root resorption (203)', 'calcium hydroxide AND perforation (32)' and 'calcium hydroxide AND avulsion (73)'. Then, the reference section of each of those articles was studied to find other suitable sources. The number of retrieved papers was presented in the parentheses.

Characteristics of calcium hydroxide

Chemical composition and activity

Calcium hydroxide was introduced to endodontics as a direct pulp-capping agent (Hermann 1920). It is a white odourless powder with the chemical formula Ca(OH)_2 and a molecular weight of 74.08 (Farhad & Mohammadi 2005). It has low solubility in water (around 1.2 g L^{-1} at 25°C), which decreases with a rise in temperature (Siqueira & Lopes 1999). It has been demonstrated that the dissociation coefficient of Ca(OH)_2 (0.17) controls the slow release of both

calcium and hydroxyl ions (Rehman *et al.* 1996). This low solubility is a useful clinical characteristic as an extended period is necessary before it becomes solubilized when in direct contact with fluids from vital tissues (Spångberg & Haapasalo 2002). The pure powder has a high pH (approximately 12.5–12.8) and is insoluble in alcohol (Farhad & Mohammadi 2005). The material is chemically classified as a strong base, its main actions come from the ionic dissociation of Ca^{2+} and OH^- ions and their effect on vital tissues, generating the induction of hard-tissue deposition and being antibacterial (Siqueira & Lopes 1999).

According to Rehman *et al.* (1996), Ca(OH)_2 dissociates into calcium and hydroxyl ions on contact with aqueous fluids. Estrela & Pesce (1996) analysed chemically the liberation of calcium and hydroxyl ions from Ca(OH)_2 pastes with vehicles of different acid–base and hydrosolubility characteristics in the connective tissues of dogs. Taking into account the molecular weight of calcium hydroxide (74.08), the percentage of hydroxyl ions is 45.89%, whilst 54.11% corresponds to the calcium ions (Estrela & Pesce 1996). Ca(OH)_2 in water has a thixotropic behaviour and will be fluid when agitated (Spångberg & Haapasalo 2002).

When Ca(OH)_2 is exposed to carbon dioxide (CO_2) or carbonate ions (CO_3^-) in biological tissue, its dissociation leads to the formation of calcium carbonate (CaCO_3) and an overall consumption of Ca^{2+} ions. However, it has been shown that after 30 days of exposure to carbon dioxide, six preparations of Ca(OH)_2 maintained a purportedly bactericidal pH within the root canal (Estrela & Pesce 1997). Estrela & Pesce (1997) analysed chemically the formation of calcium carbonate in the connective tissue of dogs and showed that when saline vehicles were used with Ca(OH)_2 in a paste, the rate of formation of calcium carbonate was practically unaltered. Estrela & Bammann (1999) evaluated the presence of calcium carbonate in samples of Ca(OH)_2 stored for 2 years in containers under varying conditions. They determined CaCO_3 by means of volumetric analysis of neutralization, using hydrochloric acid, and visualization with methyl orange and phenolphthalein. The level of Ca(OH)_2 converted into calcium carbonate ranged from $5 \pm 1\%$ to $11 \pm 1\%$ and was not sufficient to interfere with its properties.

In summary, calcium hydroxide is a white odourless powder and is chemically classified as a strong base; in contact with aqueous fluids, it dissociates into calcium and hydroxyl ions.

Mode of action

Depending on its application, the mode of action of Ca(OH)_2 may vary.

Antimicrobial activity

The antimicrobial activity of Ca(OH)_2 is related to the release of hydroxyl ions in an aqueous environment (Siqueira 2001). Hydroxyl ions are highly oxidant free radicals that show extreme reactivity with several biomolecules. This reactivity is high and indiscriminate, so this free radical rarely diffuses away from sites of generation (Siqueira & Lopes 1999). The lethal effects of hydroxyl ions on bacterial cells are probably due to the following mechanisms (Siqueira & Lopes 1999):

- damage to the bacterial cytoplasmic membrane;
- protein denaturation; and
- damage to the DNA.

Although scientific evidence suggests that these three mechanisms may occur, it is difficult to establish, in a chronological sense, which is the main mechanism involved in the death of bacterial cells after exposure to a strong base (Siqueira & Lopes 1999). Estrela *et al.* (1994) studied the biological effect of pH on the enzymatic activity of anaerobic bacteria and concluded that hydroxyl ions from Ca(OH)_2 developed their mechanism of action in the cytoplasmic membrane. This membrane is responsible for essential functions such as metabolism, cellular division and growth, and it takes part in the final stages of cellular wall formation, biosynthesis of lipids, transport of electrons and oxidative phosphorylation. Extracellular enzymes act on nutrients, carbohydrates, proteins and lipids that, through hydrolysis, favour digestion. Intracellular enzymes located in the cell favour respiratory activity of the cellular wall structure. The pH gradient of the cytoplasmic membrane is altered by the high concentration of hydroxyl ions from calcium hydroxide acting on the proteins of the membrane (protein denaturation). The high pH of Ca(OH)_2 alters the integrity of the cytoplasmic membrane through chemical injury to the organic components and transport of nutrients or by means of the destruction of phospholipids or unsaturated fatty acids of the cytoplasmic membrane, observed in the peroxidation process, which is a saponification reaction (Estrela *et al.* 1999).

Adjustment of intracellular pH is influenced by several cellular processes such as the following:

- cellular metabolism;

- alterations in shape, mobility, adjustment of transporters and polymerization of cytoskeleton components;
- activation of cellular proliferation and growth;
- conductivity and transport through the membrane; and
- isosmotic cellular volume.

Thus, many cellular functions can be affected by pH, including the enzymes that are essential for cellular metabolism (Putnam 1995). Estrela *et al.* (1998) found that bacterial enzymatic inactivation under extreme conditions of pH for a long period of time was irreversible.

In summary, the antimicrobial activity of $\text{Ca}(\text{OH})_2$ is related to the release of highly reactive hydroxyl ions in an aqueous environment, which mainly affects cytoplasmic membranes, proteins and DNA.

Mineralization activity

When used as a pulp-capping agent and in apexification cases, a calcified barrier may be induced by calcium hydroxide (Eda 1961). Because of the high pH of pure calcium hydroxide, a superficial layer of necrosis occurs in the pulp to a depth of up to 2 mm (Estrela & Holland 2009). Beyond this layer, only a mild inflammatory response is seen and, provided the operating field is kept free from bacteria when the material was placed, hard tissue may be formed (Estrela *et al.* 1995). However, commercial products containing $\text{Ca}(\text{OH})_2$ may not have such an alkaline pH.

The hydroxyl group is considered to be the most important component of $\text{Ca}(\text{OH})_2$ as it provides an alkaline environment, which encourages repair and active calcification. The alkaline pH induced not only neutralizes lactic acid from osteoclasts, thus preventing dissolution of the mineral components of dentine, but could also activate alkaline phosphatases that play an important role in hard-tissue formation (Estrela *et al.* 1995). The pH necessary for the activation of this enzyme varies from 8.6 to 10.3, according to the type and concentration of substratum, temperature and source of enzymes (Estrela *et al.* 1999). Alkaline phosphatase is a hydrolytic enzyme that acts by means of the liberation of inorganic phosphatase from the esters of phosphate. It can separate phosphoric esters, freeing phosphate ions, which then react with calcium ions from the bloodstream to form a precipitate, calcium phosphate, in the organic matrix. This precipitate is the molecular unit of hydroxyapatite (Seltzer & Bender 1975), which is believed to be intimately related to the process of mineralization.

$\text{Ca}(\text{OH})_2$ in direct contact with connective tissue gives rise to a zone of necrosis, altering the physico-chemical state of inter-cellular substance which, through rupture of glycoproteins, determines protein denaturation. The formation of mineralized tissue following contact between $\text{Ca}(\text{OH})_2$ and connective tissue has been observed from the 7th to the 10th day following application (Holland 1971). Holland (1971) also reported the existence of massive granulation in the superficial granulation zone interposed between the zone of necrosis and the deep granulation zone. These structures are composed of calcium salts and calcium-protein complexes and are birefringent to polarized light, reacting positively to chloramitic acid and to Van Kossa's method, proving that part of the calcium ions come from the protective material. Below the deep granulation zone is the proliferation cellular zone and the normal pulp. Holland *et al.* (1999) evaluated the reaction of rat subcutaneous connective tissue to the implantation of dentine tubes filled with $\text{Ca}(\text{OH})_2$. At the tube openings, there were Von Kossa-positive granules that were birefringent to polarized light. Next to these granulations, there was irregular tissue resembling a bridge that was Von-Kossa positive in the walls of dentinal tubules a structure highly birefringent to polarized light appeared as a layer at different depths.

In summary, the mineralizing action of $\text{Ca}(\text{OH})_2$ is directly influenced by its high pH. The alkaline pH not only neutralizes lactic acid from osteoclasts, but could also activate alkaline phosphatases, which play an important role in hard-tissue formation.

Effect of liquid vehicle

The vehicles mixed with $\text{Ca}(\text{OH})_2$ powder play an important role in the overall dissociation process because they determine the velocity of ionic 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 ionic dissociation. The high molecular weight of common vehicles minimizes the dispersion of $\text{Ca}(\text{OH})_2$ into the tissues and maintains the paste in the desired area for longer periods of time (Athanasiadis *et al.* 2007).

There are three main types of vehicles:

1. Water-soluble substances such as water, saline, anaesthetic solutions, carboxymethylcellulose, methylcellulose and Ringers solution.
2. Viscous vehicles such as glycerine, polyethylene-glycol (PEG) and propylene glycol.

3. Oil-based vehicles such as olive oil, silicone oil, camphor (the oil of camphorated parachlorophenol), some fatty acids (including oleic, linoleic, and isostearic acids), eugenol and metacresylacetate (Fava & Saunders 1999).

Ca(OH)₂ should be combined with a liquid vehicle because the delivery of dry Ca(OH)₂ powder alone is difficult, and fluid is required for the release of hydroxyl ions. 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 anaesthetic solutions have an acidic pH (between 4 and 5), they provide an adequate vehicle because Ca(OH)₂ is a strong base, which is affected minimally by acid (Athanasiadis *et al.* 2007).

The effects of glycerine and propylene glycol vehicles on the pH of Ca(OH)₂ preparations were investigated using conductivity testing by Safavi & Nakayama (2000). A range of 10–30% for a glycerine/water mixture and 10–40% for a propylene glycol/water mixture resulted in the greatest conductivity. They reported that a higher concentration of these vehicles may decrease the effectiveness of Ca(OH)₂ as a root canal medicament (Safavi & Nakayama 2000). Viscous vehicles are also water-soluble substances that release calcium and hydroxyl ions more slowly and for longer periods (Gomes *et al.* 2002). A viscous vehicle may remain within root canals for several months, and hence the number of appointments required to change the dressing will be reduced (Fava & Saunders 1999).

In addition to the type of vehicle used, the viscosity of the paste can influence antimicrobial activity, especially for Ca(OH)₂. Behnen *et al.* (2001) reported that thick mixtures of Ca(OH)₂ and water (1 g mL⁻¹ H₂O) resulted in a significant reduction in antibacterial activity against *E. faecalis* in dentine tubules compared to a thin mix and the commercial product Pulpdent paste (Pulpdent Corporation, Watertown, MA, USA).

Oily vehicles have restricted applications as they are difficult to remove and leave a residue on the canal walls. The difficulty of removing them from the canal walls will affect the adherence of sealer or other materials used to fill the canal (Fava & Saunders 1999); they are not recommended.

Polyethylene glycol (PEG) is one of the most commonly used vehicles in root canal medicaments, and it possesses an ideal array of properties including low toxicity, high solubility in aqueous solutions and low immunogenicity and antigenicity (Athanasiadis *et al.* 2007). Concentrated PEG 400 solutions have their own substantial antibacterial activity against

various pathogenic bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*, which is in addition to any other substances added to the PEG base as a medicament (Chirife *et al.* 1983). In a study by Camões *et al.* (2003) the pH in an aqueous medium was tested outside the roots of human teeth when various vehicles (aqueous or viscous) were used with Ca(OH)₂. They reported that vehicles with glycerine and PEG 400 had a tendency to acidification during the first 8 days (pH 6.85 to 6.4 – PEG 400) but then the pH returned to the levels of the other groups after 42 days (pH 7.1 – PEG 400).

In summary, the vehicle to which calcium hydroxide is added affects the physical and chemical properties of the compound and therefore its clinical applications. Compared with water-soluble agents, viscous and oily vehicles prolong the action of the calcium hydroxide but can have associated negative side effects.

Calcium hydroxide when used in medicaments during root canal treatment

Definition of a medicament

A medicament is an antimicrobial agent that is placed inside the root canal between treatment appointments in an attempt to destroy remaining microorganisms and prevent reinfection (Weine 2004). Thus, they may be utilized to kill bacteria, reduce inflammation (and thereby reduce pain), help eliminate apical exudate, control inflammatory root resorption and prevent contamination between appointments (Farhad & Mohammadi 2005). When intracanal medicaments were not used between appointments, bacterial numbers increased rapidly (Byström & Sundqvist 1981).

Anti-bacterial activity

Calcium hydroxide will exert an antibacterial effect in the root canal system as long as a high pH is maintained (Siqueira & Lopes 1999). In their *in vivo* study, Byström *et al.* (1985) reported that root canals treated with Ca(OH)₂ had fewer bacteria than those dressed with camphorated phenol or camphorated monochlorophenol (CMCP). They attributed this to the fact that Ca(OH)₂ could be packed into the root canal system allowing hydroxyl ions to be released over time. Stevens & Grossman (1983) also reported Ca(OH)₂ to be effective in preventing the growth of microorganisms but to a limited extent when compared to CMCP, stressing the necessity of direct contact to

achieve the optimum antibacterial effect. Sjögren *et al.* (1991) demonstrated that a 7-day application of a $\text{Ca}(\text{OH})_2$ medicament was sufficient to reduce canal bacteria to a level that gave a negative culture. Han *et al.* (2001) found that aqueous $\text{Ca}(\text{OH})_2$ paste and silicone oil-based $\text{Ca}(\text{OH})_2$ paste were effective in the elimination of *E. faecalis* in dentinal tubules. Shuping *et al.* (2000) showed that placement of $\text{Ca}(\text{OH})_2$ for at least 1 week rendered 92.5% of canals bacteria free.

Estrela *et al.* (2001) assessed two methods for determining the antimicrobial effectiveness of (i) $\text{Ca}(\text{OH})_2$ in saline, (ii) $\text{Ca}(\text{OH})_2$ in polyethylene glycol and (iii) $\text{Ca}(\text{OH})_2$ in CMCP. They concluded that both the direct exposure test and agar diffusion test (ADT) were useful in establishing the antimicrobial spectrum of $\text{Ca}(\text{OH})_2$ and for developing improved infection control protocols. A complete antimicrobial effect was observed after 48 h in both tests, irrespective of the $\text{Ca}(\text{OH})_2$ paste vehicle. Behnen *et al.* (2001) demonstrated that $\text{Ca}(\text{OH})_2$ decreased the numbers of *E. faecalis* at all depths within dentinal tubules up to 24 h and that less viscous preparations of $\text{Ca}(\text{OH})_2$ were more effective in the elimination of *E. faecalis* from dentinal tubules than viscous preparations.

In a study to evaluate the effect of electrophoretically activated $\text{Ca}(\text{OH})_2$ on bacterial viability in dentinal tubules, Lin *et al.* (2005) reported that treatment with electrophoresis was significantly more effective than pure $\text{Ca}(\text{OH})_2$ up to depths of 200–500 μm . Specimens treated with electrophoretically activated $\text{Ca}(\text{OH})_2$ revealed no viable bacteria in dentinal tubules to a depth of 500 μm from the root canal space within 7 days.

Portenier *et al.* (2005) concluded that *E. faecalis* cells in the exponential growth phase were the most sensitive to $\text{Ca}(\text{OH})_2$ and were killed within 3 s to 10 min. Cells in a stationary phase were more resistant, with living cells being recovered at 10 min. However, cells in a starvation phase were the most resistant and were not totally eliminated during the 10-min test period.

By contrast, several studies have attested to the ineffectiveness of $\text{Ca}(\text{OH})_2$ in eliminating bacterial cells. DiFiore *et al.* (1983) reported that $\text{Ca}(\text{OH})_2$ had no antibacterial effect as a paste or as the commercial preparation, Pulpdent, when used against *S. Sanguis*, findings that were confirmed by Siqueira *et al.* (1998). Haapasalo & Ørstavik (1987) reported that a $\text{Ca}(\text{OH})_2$ paste (Calasept; Speiko, Darmstadt, Germany) failed to eliminate, even superficially, *E. faecalis* in dentinal tubules. Safavi *et al.* (1990) demonstrated that

E. faecium remained viable in dentinal tubules after relatively extended periods of $\text{Ca}(\text{OH})_2$ /saline mixture treatment. Ørstavik & Haapasalo (1990) observed that $\text{Ca}(\text{OH})_2$ could take up to 10 days to disinfect dentinal tubules infected by facultative bacteria. Siqueira & Uzeda (1996) demonstrated that $\text{Ca}(\text{OH})_2$ mixed with saline was ineffective in eliminating *E. faecalis* and *E. faecium* inside dentinal tubules after 1 week of contact. Estrela *et al.* (1999) found that $\text{Ca}(\text{OH})_2$ in infected dentinal tubules had no antimicrobial effect on *S. faecalis*, *S. aureus*, *B. subtilis*, *P. aeruginosa* or on the bacterial mixture used throughout the experiment. Waltimo *et al.* (2005) found that a $\text{Ca}(\text{OH})_2$ dressing between appointments did not have the expected effect in terms of disinfection of the root canal system nor the treatment outcome. Weiger *et al.* (2002) concluded that the viability of *E. faecalis* in infected root dentine was not affected by $\text{Ca}(\text{OH})_2$. In a systematic review to assess the antibacterial efficacy of $\text{Ca}(\text{OH})_2$, Sathorn *et al.* (2007) evaluated eight clinical trials including 257 cases. They concluded that $\text{Ca}(\text{OH})_2$ had limited effectiveness in eliminating bacteria from human root canals when assessed by culture techniques.

In a polymerase chain reaction study (PCR), the effect of root filling with or without prior $\text{Ca}(\text{OH})_2$ or 2% chlorhexidine (CHX) on the persistence of bacterial DNA in infected dentinal tubules was evaluated (Cook *et al.* 2007). The report indicated that 2% CHX treatment followed by canal filling was more effective in removing the DNA of *E. faecalis* than placement of $\text{Ca}(\text{OH})_2$ or immediate canal filling. Using an agar diffusion method, Ballal *et al.* (2007) found that 2% CHX gel was a more effective medicament than $\text{Ca}(\text{OH})_2$ paste against *E. faecalis*. Krithikadatta *et al.* (2007) reported that, as an intracanal medicament, 2% CHX gel alone was more effective against *E. faecalis* when compared to $\text{Ca}(\text{OH})_2$. Lee *et al.* (2008) concluded that a polymeric CHX-controlled release device (PCRD) was significantly more effective in reducing intradentinal bacteria than $\text{Ca}(\text{OH})_2$.

In summary, although some clinical studies have supported the efficacy of calcium hydroxide as an intracanal medicament, other studies have questioned its efficacy and indicated CHX instead of calcium hydroxide.

Anti-endotoxin activity

Endotoxin, a part of the cell wall of all Gram-negative bacteria, is composed of polysaccharides, lipids and proteins and is referred to as lipopolysaccharide (LPS),

emphasizing its chemical structure (Westphal 1975, Rietschel & Brade 1992). Lipid A is the region of the endotoxin molecule responsible for its toxic effects. When free to act, endotoxins do not cause cell or tissue pathosis directly, but they stimulate competent cells to release chemical mediators (Leonardo *et al.* 2004). Macrophages are the main target of endotoxins (Leonardo *et al.* 2004), which, therefore, are not intrinsically toxic.

Endotoxin (LPS) is released during multiplication or bacterial death causing a series of biological effects (Barthel *et al.* 1997), which lead to an inflammatory reaction (Rietschel & Brade 1992) and periapical bone resorption (Stashenko 1990, Yamasaki *et al.* 1992). Endotoxins from vital or nonvital, whole or fragmented bacteria act on macrophages, neutrophils and fibroblasts, leading to the release of a large number of bioactive or cytokine chemical inflammatory mediators, such as tumour necrosis factor (TNF), interleukin-1 (IL-1), IL-5, IL-8, alpha-interferon and prostaglandins (Leonardo *et al.* 2004).

Currently, one of the concerns in endodontics is the treatment of teeth with necrotic pulps and periapical pathosis because post-treatment disease persists more often than in cases without periapical disease (Leonardo *et al.* 1993, Katebzadeh *et al.* 1999). In teeth with chronic periapical lesions, there is a greater prevalence of Gram-negative anaerobic bacteria disseminated throughout the root canal system (dental tubules, apical resorptive defects and cementum lacunae), including apical bacterial biofilm (Leonardo *et al.* 1993, Katebzadeh *et al.* 1999, Nelson-Filho *et al.* 2002, Trope *et al.* 1999). Because these areas are not reached by instrumentation, the use of a root canal medicament is recommended to aid in the elimination of these bacteria and thus increase the potential for clinical success (Leonardo *et al.* 1993, Katebzadeh *et al.* 1999, Nelson-Filho *et al.* 2002). Teeth with and without radiographic evidence of periapical disease could be considered as different pathological entities requiring different treatment regimens. Where bone loss has occurred, the use of a root canal medicament between treatment sessions is recommended by some (Leonardo *et al.* 2000a), because the success of treatment in cases with periapical pathosis is directly related to the elimination of bacteria, products and subproducts from the root canal system. The procedures and medicaments used in root canal treatment should not only lead to bacterial death, but also to the inactivation of bacterial endotoxin (Leonardo *et al.* 2004).

In a laboratory study, Safavi & Nichols (1993) evaluated the effect of Ca(OH)_2 on bacterial LPS and concluded that it hydrolysed the highly toxic lipid A molecule that is responsible for the damaging effects of endotoxin. In another study, they found that Ca(OH)_2 transformed lipid A into fatty acids and amino sugars, which are atoxic components (Safavi & Nichols 1994). These results were confirmed in studies by Barthel *et al.* (1997) and Olsen *et al.* (1999) who reported that Ca(OH)_2 detoxifies bacterial LPS *in vitro*.

Nelson-Filho *et al.* (2002) carried out an *in vivo* study to evaluate radiographically the effect of endotoxin plus Ca(OH)_2 on the periapical tissues of dog's teeth. They observed that endotoxin caused the formation of periapical lesions after 30 days and that Ca(OH)_2 inactivated bacterial LPS. Silva *et al.* (2002) analysed histopathologically periapical tissues of teeth in dogs in which the root canals were filled with bacterial LPS and Ca(OH)_2 . They reported that LPS caused the formation of periapical lesions and that Ca(OH)_2 detoxified this endotoxin *in vivo*. Tanomaru *et al.* (2003) evaluated the effect of biomechanical preparation using different irrigating solutions and a Ca(OH)_2 -based root canal dressing in a dog experimental tooth model containing endotoxin. Biomechanical preparation with only irrigating solutions did not inactivate the endotoxin; however, the same treatment associated with the use of the Ca(OH)_2 dressing was effective in the inactivation of the toxic effects of this endotoxin. Jiang *et al.* (2003) also evaluated the direct effects of LPS on osteoclastogenesis and the capacity of Ca(OH)_2 to inhibit the formation of osteoclasts stimulated by endotoxin. They reported that Ca(OH)_2 significantly reduced osteoclast differentiation. Buck *et al.* (2001) found that long-term Ca(OH)_2 as well as 30-min exposure to an alkaline mixture of CHX, ethanol and sodium hypochlorite did detoxify LPS molecules by hydrolysis of ester bonds in the fatty acid chains of the lipid A moiety.

In summary, endotoxin, a component of the cell wall of Gram-negative bacteria, plays a fundamental role in the genesis and maintenance of periapical lesions because of the induction of inflammation and bone resorption. Ca(OH)_2 inactivates endotoxin, *in vitro* and *in vivo*, and appears currently the only clinically effective medicament for inactivation of endotoxin.

A recent concern indirectly related to the use of Ca(OH)_2 as a medicament and the outcome of treatment has focused on the limitations of conventional radiographic techniques. Post-treatment apical periodontitis with bone loss may not result in a visible apical radiolucency on a conventional or digital film,

depending on the density and thickness of the overlying cortical bone, and the distance between the lesion and the cortical bone. When a bone lesion is within the cancellous bone and the overlying cortical bone is substantial, the bone lesion may not be visible radiographically (Stabholz *et al.* 1994, Ricucci & Bergenholtz 2003). Therefore, post-treatment apical periodontitis can be radiographically visible or invisible. Clinically, it has been reported that a large lesion of up to 8 mm in diameter can be present without radiolucency (Wu *et al.* 2006). Thus, it now appears that conventional radiographic techniques lack sufficient sensitivity to serve as a reliable means for diagnosing post-treatment health. Therefore, the absence of radiolucency does not prove that residual bacteria have been entombed in the canal system by the placement of a root filling and thus rendered harmless. It should be noted that cone-beam computed tomography (CBCT) provides higher detection rates than conventional and digital radiographs for visualization of periapical lesions (Scarfe *et al.* 2009).

Anti-fungal activity

Fungi constitute a small proportion of the oral microbiota and are largely restricted to *Candida albicans* (Siqueira & Sen 2004). *C. albicans* is the fungal species most commonly detected in the oral cavity of both healthy (Arendorf & Walker 1980, Lucas 1993) and medically compromised individuals (Dupont *et al.* 1992). The incidence of *C. albicans* in the oral cavity has been reported to be 30–45% in healthy adults (Arendorf & Walker 1980, Lucas 1993) and 95% in patients infected with human immunodeficiency virus (Dupont *et al.* 1992). Fungi have occasionally been found in primary root canal infections (Baumgartner *et al.* 2000, Lana *et al.* 2001), but they are more common in filled root canals in teeth that have become infected some time after treatment or in those that have not responded to treatment (Siqueira & Sen 2004). Overall, the occurrence of fungi reported in infected root canals varies between 1% and 17% (Waltimo *et al.* 2004). A large number of other yeasts have also been isolated from the oral cavity, including *C. glabrata*, *C. guilliermondii*, *C. parapsilosis*, *C. krusei*, *C. inconspicua*, *C. dubliniensis*, *C. tropicalis* and *Saccharomyces* species (Siqueira & Sen 2004).

Waltimo *et al.* (1999a) reported that *C. albicans* cells were highly resistant to Ca(OH)_2 and that all *Candida* species (*C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei* and *C. tropicalis*) were either equally high or had higher resistance to aqueous calcium hydroxide than did

E. faecalis (Waltimo *et al.* 1999b). Because *C. albicans* survives in a wide range of pH values, the alkalinity of saturated Ca(OH)_2 solution may not have any effect on *C. albicans*. In addition, Ca(OH)_2 pastes may provide the Ca^{2+} ions necessary for the growth and morphogenesis of *Candida*. These mechanisms may explain why Ca(OH)_2 has been found to be ineffective against *C. albicans* (Siqueira & Sen 2004).

Siqueira *et al.* (2001) investigated the antifungal ability of several medicaments against *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. parapsilosis* and *S. cerevisiae*. They reported that whereas the paste of Ca(OH)_2 in CPMC/glycerine had the most pronounced antifungal effects, Ca(OH)_2 in glycerine or CHX and CHX in detergent also had antifungal activity, but at a lower level than the paste of Ca(OH)_2 in CPMC/glycerine. In another study, Ferguson *et al.* (2002) evaluated the *in vitro* susceptibility of *C. albicans* to various irrigants and medicaments. The minimum inhibitory concentrations of NaOCl, hydrogen peroxide, CHX digluconate and aqueous Ca(OH)_2 were determined. Their results revealed that NaOCl, hydrogen peroxide and CHX digluconate were effective against *C. albicans*, even when diluted significantly. Furthermore, aqueous Ca(OH)_2 had no antifungal activity when maintained in direct contact with *C. albicans* cells, whereas Ca(OH)_2 paste and CPMC were effective antifungal agents.

The antifungal effectiveness of CPMC was also reported by Valera *et al.* (2001) who investigated the effectiveness of several intracanal medicaments on *C. albicans* harvested inside root canals, observing that CPMC was the most effective, followed by Ca(OH)_2 /CPMC paste. Siqueira *et al.* (2003) evaluated the effectiveness of four intracanal medicaments in disinfecting the root dentine of bovine teeth experimentally infected with *C. albicans*. Infected dentine cylinders were exposed to four different medicaments: Ca(OH)_2 /glycerine, Ca(OH)_2 /0.12% CHX digluconate, Ca(OH)_2 /CPMC/glycerine and 0.12% CHX digluconate/zinc oxide. Specimens were left in contact with the medicaments for 1 h, 2 and 7 days. The specimens treated with Ca(OH)_2 /CPMC/glycerine paste or with CHX/zinc oxide paste were completely disinfected after 1 h of exposure. Ca(OH)_2 /glycerine paste only consistently eliminated *C. albicans* infection after 7 days of exposure. Ca(OH)_2 mixed with CHX was ineffective in disinfecting dentine even after 1 week of exposure. Of the medicaments tested, the Ca(OH)_2 /CPMC/glycerine paste and CHX digluconate mixed with zinc oxide were the most effective in eliminating *C. albicans* cells from dentine specimens.

In summary, fungi have occasionally been found in primary root canal infections, but they appear to occur more often in filled root canals of teeth in which treatment has failed. *C. albicans* is by far the fungal species most commonly isolated from infected root canals. It seems that the combinations of $\text{Ca}(\text{OH})_2$ with camphorated paramonochlorophenol or CHX have the potential to be used as effective intracanal medicaments for cases in which fungal infection is suspected.

Activity against biofilms

The term biofilm was introduced to designate the thin-layered (sessile) condensations of microbes that may occur on various surface structures in nature (Svensater & Bergenholtz 2004). Free-floating bacteria existing in an aqueous environment, the so-called planktonic form of microorganisms, are a prerequisite for biofilm formation (Bowden & Hamilton 1998). Biofilms may thus become established on any organic or inorganic surface substrate where planktonic microorganisms prevail in a water-based solution (Stoodley *et al.* 2004). In a dental context, a well-known and extensively studied biofilm structure is established during the attachment of bacteria to teeth to form dental plaque (Svensater & Bergenholtz 2004). Here, bacteria in saliva (planktonic organisms) serve as the primary source of organisms for the organization of this specific biofilm (Bowden & Hamilton 1998). In endodontics, the biofilm concept was initially discussed mainly within the framework of bacteria on the root tips of teeth with necrotic and infected pulps or pulpless and infected root canal systems (Nair 1987, Nair *et al.* 2005). Such bacterial aggregations have been thought to be the cause of therapy-resistant apical periodontitis (Nair *et al.* 2005, Wu *et al.* 2006). Although not described in as much detail, bacterial condensations (that is, biofilms) on the walls of infected root canals have been observed (Svensater & Bergenholtz 2004). On the basis of transmission electron microscopy (TEM), Nair (1987) examined the root canal contents of 31 teeth, which had gross coronal caries and to which the periapical inflammatory lesion was attached upon extraction. In addition to his observations of the microstructure of the inflammatory tissue, he noted that the major bulk of the organisms existed as loose collections of cocci, rods, filaments and spirochetes. Whilst most of these organisms appeared suspended, in what was described as a moist canal space, dense aggregates were also observed sticking to the canal walls and forming layers of bacterial condensations.

Amorphous material filled the inter-bacterial spaces and was interpreted as an extracellular matrix of bacterial origin. When they occurred, the bacterial condensations had a palisade structure similar to the one for dental plaque on external tooth surfaces, suggesting similar mechanisms for bacterial attachment as those for dental plaque. Sen *et al.* (1995) examined untreated extracted teeth with apical periodontitis by scanning electron microscopy (SEM) and found that root canals were heavily infected with microorganisms being observed in all areas of the canal. Cocci and rods predominated and formed colonies on the root canal walls and also, to a varying degree, penetrated the dentinal tubules. Nair *et al.* (2005) found that even after instrumentation, irrigation and canal filling 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 filled teeth examined.

Anti-microbial agents have often been developed and optimized for their activity against fast-growing, dispersed populations containing a single microorganism (Gilbert *et al.* 1997, Svensater & Bergenholtz 2004). However, microbial communities grown in biofilms are remarkably difficult to eradicate with anti-microbial agents and microorganisms in mature biofilms can be notoriously resistant for reasons that have yet to be adequately explained (Nair 1987, Bowden & Hamilton 1998). There are reports revealing that microorganisms grown in biofilms could be 2-fold to 1000-fold more resistant than the corresponding planktonic form of the same organisms (Svensater & Bergenholtz 2004). Using scanning electron microscopy and scanning confocal laser microscopy, Distel *et al.* (2002) reported that despite intracanal dressing with $\text{Ca}(\text{OH})_2$, *E. faecalis* formed biofilms in root canals. In another study, Chai *et al.* (2007) reported that $\text{Ca}(\text{OH})_2$ was 100% effective in eliminating *E. faecalis* biofilm. Brandle *et al.* (2008) investigated the effects of growth condition (planktonic, mono- and multi-species biofilms) on the susceptibility of *E. faecalis*, *Streptococcus sobrinus*, *Candida albicans*, *Actinomyces naeslundii* and *Fusobacterium nucleatum* to alkaline stress. Findings demonstrated that planktonic microorganisms were most susceptible; only *E. faecalis* and *C. albicans* survived in saturated solution for 10 min, the latter also for 100 min. Dentine adhesion was the major factor in improving the resistance of *E. faecalis* and *A. naeslundii* to calcium hydroxide, whereas the multispecies context in a biofilm was the major factor in promoting resistance of *S. sobrinus* to the disinfectant. In contrast, the

C. albicans response to calcium hydroxide was not influenced by growth conditions.

In summary, the few studies conducted on the antimicrobial potential of $\text{Ca}(\text{OH})_2$ on biofilms have demonstrated inconsistent results. Further studies are required to elucidate the anti-biofilm efficacy of $\text{Ca}(\text{OH})_2$.

Clinical outcome studies on the use of $\text{Ca}(\text{OH})_2$ medicaments

One-visit root canal treatment offers potential advantages to both the dentist and patient (Ashkenaz 1984). In addition to being less time-consuming and accepted by patients (Sathorn *et al.* 2005), it prevents the potential contamination or recontamination of the root canal system between appointments (Ashkenaz 1984). Root canal treatment on teeth with vital pulps should ideally be completed in one session provided that the time available, operator's skills and anatomical conditions are all favourable (Ashkenaz 1979). On the other hand, root canal treatment in one session for teeth with necrotic pulps, whether associated with a periradicular lesion or not remains controversial (Siqueira 2001).

Two factors must be taken into account before deciding upon a one-visit treatment of teeth with necrotic pulps: the incidence of postoperative pain and the long-term outcome of the treatment (Mohammadi *et al.* 2006). Studies have found no difference in the incidence of postoperative pain between one- and multiple-visit root canal treatment (O'Keefe 1976, Mulhern *et al.* 1982, DiRenzo *et al.* 2002, Mohammadi *et al.* 2006). Sathorn *et al.* (2008) reviewed systematically 16 studies with sample size varying from 60 to 1012 cases. The prevalence of postoperative pain ranged from 3% to 58%. However, the heterogeneity amongst the studies was too great to conduct a meta-analysis and yield meaningful results. They concluded that compelling evidence indicating a significantly different prevalence of postoperative pain/flare-up of either single- or multiple-visit root canal treatment was lacking.

Therefore, the outcome of the root canal treatment should be the major factor taken into account when deciding the number of therapy sessions. Pekruhn (1986) reported that there were significantly fewer failures in the two-visit treatment group than in the one-visit treatment group, regardless of the pretreatment diagnosis. In a well-controlled clinical study, Sjögren *et al.* (1997) investigated the role of infection on the outcome of one-visit treatment after a follow-up

period of 5 years. Success was reported for 94% of the infected root canals associated with periradicular lesions that yielded negative culture at the time of canal filling, whereas in the samples that yielded positive culture prior to filling the success rate was 68%, thus stressing the need to have a negative culture before canal filling in infected cases. In another clinical study, Trope *et al.* (1999) evaluated radiographic healing of teeth with periradicular lesions treated in one or two visits. In the two-visit group, root canals were medicated with $\text{Ca}(\text{OH})_2$ for at least 1 week. After a 1-year follow-up, the additional disinfecting action of calcium hydroxide resulted in a 10% increase in healing rates. However, some of the teeth were not associated with preoperative periapical lesions and some cases treated over multiple visits had not been dressed with an inter-appointment calcium hydroxide medicament (the main biological purpose of multiple-visit treatment). Katebzadeh *et al.* (1999, 2000) compared periradicular repair radiographically and histologically after root canal treatment of infected canals of dogs performed in one or two sessions and reported better results for the two-visit treatment in which $\text{Ca}(\text{OH})_2$ was used as an intracanal disinfecting medicament for 1 week.

On the other hand, several studies have concluded that one-visit treatment was as effective as multiple-visit treatment or even more effective. Weiger *et al.* (2000) evaluated the influence of $\text{Ca}(\text{OH})_2$ as an inter-appointment dressing on the healing of periapical lesions associated with pulpless teeth. In both treatment groups, the likelihood that the root canal treatment yielded a success within an observation time of 5 years exceeded 90%. However, a statistically significant difference between the two treatment groups was not detected. Furthermore, the probability that complete periapical healing would take place increased continuously with the length of the observation period. Peters & Wesselink (2002) found no significant differences in healing of periapical radiolucency between teeth that were treated in one visit (without) and two visits with inclusion of $\text{Ca}(\text{OH})_2$ for 4 weeks. In a randomized clinical trial, Molander *et al.* (2007) assessed the 2-year clinical and radiographic outcome of one- and two-visit root canal treatment and found similar healing results. In a systematic review, Figini *et al.* (2008) investigated whether the effectiveness and frequency of short-term and long-term complications were different when root canal treatment was completed in one or multiple visits. No detectable difference in the effectiveness of root canal treatment in terms of

radiologic success between single and multiple visits was noted. In a randomized controlled clinical trial, Penesis *et al.* (2008) compared radiographic periapical healing after root canal treatment completed in one visit or two visits with an interim calcium hydroxide/CHX paste dressing and concluded that both treatment options exhibited equally favourable periapical healing at 12 months, with no statistically significant difference. In a systematic review, Sathorn *et al.* (2005) compared the healing rate (as measured by clinical and radiographic parameters) of single-visit root canal treatment without calcium hydroxide dressing to multiple-visit treatment with calcium hydroxide dressing for 1 week. Single-visit root canal treatments were marginally more effective than multiple visits, i.e. 6.3% higher healing rate. However, the difference in healing rate between these two treatment regimens was not statistically significant.

In summary, the incidence of postoperative pain and the long-term outcome of treatment must be taken into account before deciding upon a one-visit or a multi-visit treatment for teeth with necrotic pulps. There is no compelling evidence to suggest a difference between the regimens in terms of the prevalence of postoperative pain/flare-up. There is still considerable controversy concerning the effect of the number of treatment visits on the biological outcome, whilst some studies support two-visit treatment, other studies found that there was no significant difference between the two treatment modalities. It should be noted that some recent clinical trials and systematic reviews found similar healing results between one-visit and multiple-visit treatments. Clearly, it is important to analyse the individual reports included in systematic reviews and judge whether the results are applicable (generalizable) to general dental practice. In the majority of reports, the root canal treatments were carried out in hospital settings by specialist endodontists with the result that the conclusions of such studies may not be relevant to conditions prevailing in most general dental practices, where resources and clinical expertise are often less favourable.

Buffering effect of dentine on $\text{Ca}(\text{OH})_2$

The root canal milieu is a complex mixture of a variety of organic and inorganic components. Hydroxyapatite is the major representative of the inorganic components, whilst pulp tissue, microorganisms and inflammatory exudate, rich in proteins such as albumin (Haapasalo *et al.* 2007), are the major organic components. The relative importance of the various organic

and inorganic compounds in the inactivation of root canal disinfectants have been studied to a limited extent only (Haapasalo *et al.* 2000). Difficulties in designing experiments that will give reliable and comparable data have been some of the greatest challenges. Haapasalo *et al.* (2000) introduced a new dentine powder model for studying the inhibitory effect of dentine on various root canal irrigants and medicaments. They concluded that dentine powder effectively abolished the killing of *E. faecalis* by $\text{Ca}(\text{OH})_2$ (Haapasalo *et al.* 2000). On the other hand, in the positive control group (absence of dentine), saturated $\text{Ca}(\text{OH})_2$ killed *E. faecalis* cells in a few minutes, whereas with the dentine powder added, no reduction in the bacterial colony-forming units could be measured even after 24 h of incubation with $\text{Ca}(\text{OH})_2$. Hydroxyapatite had an effect similar to dentine on $\text{Ca}(\text{OH})_2$, preventing the killing of *E. faecalis* (Portenier *et al.* 2001). Initially, they used a high concentration of dentine (18% w/v); however, in another study they showed that even 1.8% dentine (w/v) totally prevented the killing of *E. faecalis* by a saturated $\text{Ca}(\text{OH})_2$ solution (Portenier *et al.* 2001).

The substantial effect of dentine on the antibacterial activity of $\text{Ca}(\text{OH})_2$ can be attributed to the buffering action of dentine against alkali (Wang & Hume 1988). $\text{Ca}(\text{OH})_2$ is used as a thick paste *in vivo*; however, its solubility is low and saturation is achieved in a relatively low concentration of hydroxyl ions. Both laboratory and *in vivo* studies have shown that buffering by dentine, particularly in the subsurface layers of the root canal walls, might be the main factor behind the reduced antibacterial effect of $\text{Ca}(\text{OH})_2$. It is possible that deeper in dentine (outside the main root canal), $\text{Ca}(\text{OH})_2$ is present as a saturated solution or at concentrations even below that level (Haapasalo *et al.* 2000). Besides dentine, remnants of necrotic pulp tissue as well as inflammatory exudate might affect the antibacterial potential of endodontic disinfectants (Haapasalo *et al.* 2007).

In summary, it seems that dentine, hydroxyapatite and remnants of necrotic pulp tissue as well as inflammatory exudate decrease the antibacterial potential of $\text{Ca}(\text{OH})_2$. In other words, $\text{Ca}(\text{OH})_2$ is likely to be effective under laboratory conditions but relatively ineffective as a medicament *in vivo*.

Synergism between $\text{Ca}(\text{OH})_2$ and sodium hypochlorite

Chemicals should be used to supplement mechanical cleansing of canals, and irrigation with sodium

hypochlorite and/or intracanal placement of $\text{Ca}(\text{OH})_2$ are used as therapeutic agents in an attempt to alter the properties of tissue remnants and microorganisms so as to facilitate their removal/killing (Yang *et al.* 1995).

The synergy between $\text{Ca}(\text{OH})_2$ and sodium hypochlorite is controversial. Hasselgren *et al.* (1988) studied dissolution of necrotic porcine muscle tissue and reported that a paste of $\text{Ca}(\text{OH})_2$ powder and water was capable of dissolving tissue after 12 days of exposure. Furthermore, they reported an enhancement of the tissue-dissolving capability of sodium hypochlorite when the tissue was pretreated with $\text{Ca}(\text{OH})_2$ for 30 min, 24 h and 7 days. In another study, Metzler & Montgomery (1989) demonstrated that long-term (7 days) pretreatment with Pulpdent paste (Watertown, MA, USA), a non-setting $\text{Ca}(\text{OH})_2$ paste, followed by sodium hypochlorite irrigation cleaned canal isthmuses in mandibular molars better than hand instrumentation alone. Yang *et al.* (1995) evaluated and compared the tissue-dissolving properties of $\text{Ca}(\text{OH})_2$ and NaOCl on bovine pulp tissue under both aerobic and anaerobic conditions. Results demonstrated that both agents partially dissolved pulp tissue and that the anaerobic environment did not alter the tissue-dissolving properties of $\text{Ca}(\text{OH})_2$ or NaOCl. Furthermore, both chemicals were equal and more effective than water (control group). Wadachi *et al.* (1998) evaluated the tissue-dissolving ability of NaOCl and $\text{Ca}(\text{OH})_2$ in a bovine tooth model and reported that the amount of debris was reduced remarkably in teeth treated with NaOCl for >30 s or $\text{Ca}(\text{OH})_2$ for 7 days. However, the combination of $\text{Ca}(\text{OH})_2$ and NaOCl was more effective than the separate treatments. On the other hand, some studies demonstrated that $\text{Ca}(\text{OH})_2$ was an ineffective solvent of pulpal tissue. For example, Morgan *et al.* (1991) reported that $\text{Ca}(\text{OH})_2$ as an irrigant resulted in only 10% weight loss of bovine pulp tissue compared with isotonic saline control.

In summary, the pretreatment of root canals with $\text{Ca}(\text{OH})_2$ enhances the tissue-dissolving capability of sodium hypochlorite, and this may confer an advantage to multiple-visit root canal treatment where NaOCl would be used following a period of $\text{Ca}(\text{OH})_2$ medication.

$\text{Ca}(\text{OH})_2$ and chlorhexidine

Chlorhexidine is a cationic biguanide whose optimal antimicrobial activity is achieved within a pH range of 5.5–7.0 (Athanasiadis *et al.* 2007). Therefore, it is likely that alkalizing the pH by adding $\text{Ca}(\text{OH})_2$ to

CHX will lead to precipitation of CHX molecules, thereby decreasing its effectiveness (Mohammadi & Abbott 2009). It has been demonstrated that the alkalinity of $\text{Ca}(\text{OH})_2$ when mixed with CHX remained unchanged (Haenni *et al.* 2003). Therefore, the usefulness of mixing $\text{Ca}(\text{OH})_2$ with CHX still remains unclear and controversial (Athanasiadis *et al.* 2007).

When used as an intracanal medicament, CHX was more effective than $\text{Ca}(\text{OH})_2$ in eliminating *E. faecalis* from inside dentinal tubules (Athanasiadis *et al.* 2007). In a study by Almyroudi *et al.* (2002), all of the CHX formulations used, including a CHX/ $\text{Ca}(\text{OH})_2$ 50 : 50 mix, were effective in eliminating *E. faecalis* from dentinal tubules with a 1% CHX gel working better than the other preparations. These findings were corroborated by Gomes *et al.* (2006) in bovine dentine and Schäfer & Bossmann (2005) in human dentine where 2% CHX gel had greater activity against *E. faecalis*, followed by CHX/ $\text{Ca}(\text{OH})_2$ and then $\text{Ca}(\text{OH})_2$ alone.

In a study using agar diffusion, Haenni *et al.* (2003) could not demonstrate any additional antibacterial effect by mixing $\text{Ca}(\text{OH})_2$ powder with 0.5% CHX and reported that CHX had a reduced antibacterial action. However, $\text{Ca}(\text{OH})_2$ did not lose its antibacterial properties in such a mixture. This may be because of the deprotonation of CHX at a pH >10, which reduces its solubility and alters its interaction with bacterial surfaces as a result of the altered charge of the molecules. In a laboratory study using human teeth, Ercan *et al.* (2006) reported that 2% CHX gel was the most effective agent against *E. faecalis* inside dentinal tubules, followed by a $\text{Ca}(\text{OH})_2$ /2% CHX mixture, whilst $\text{Ca}(\text{OH})_2$ alone was totally ineffective, even after 30 days. The 2% CHX gel was also significantly more effective than the $\text{Ca}(\text{OH})_2$ /2% CHX mixture against *C. albicans* at 7 days, although there was no significant difference at 15 and 30 days. $\text{Ca}(\text{OH})_2$ alone was completely ineffective against *C. albicans*. In another *in vivo* study using primary teeth, a 1% CHX-gluconate gel, both with and without $\text{Ca}(\text{OH})_2$, was more effective against *E. faecalis* than $\text{Ca}(\text{OH})_2$ alone over a 48-h period (Oncag *et al.* 2006).

Schäfer & Bossmann (2005) reported that 2% CHX-gluconate was significantly more effective against *E. faecalis* than $\text{Ca}(\text{OH})_2$ used alone or a mixture of the two. Although this was also confirmed by Lin *et al.* (2003), a study by Evans *et al.* (2003) using bovine dentine concluded that 2% CHX with $\text{Ca}(\text{OH})_2$ was more effective than $\text{Ca}(\text{OH})_2$ in water. In an animal study, Lindskog *et al.* (1998) reported that teeth

dressed with CHX for 4 weeks had reduced inflammatory reactions in the periodontium (both apically and marginally) and less root resorption. Waltimo *et al.* (1999a) reported that 0.5% CHX-acetate was more effective at killing *C. albicans* than saturated $\text{Ca}(\text{OH})_2$, whilst $\text{Ca}(\text{OH})_2$ combined with CHX was more effective than $\text{Ca}(\text{OH})_2$ used alone. The high pH of $\text{Ca}(\text{OH})_2$ was unaffected when combined with CHX in this study.

In summary, although the usefulness of mixing $\text{Ca}(\text{OH})_2$ with CHX remains unclear and controversial, it seems that by mixing $\text{Ca}(\text{OH})_2$ with CHX the antimicrobial activity of $\text{Ca}(\text{OH})_2$ is increased. In other words, the descending order of the antimicrobial activity of $\text{Ca}(\text{OH})_2$, CHX and their combination is as follows: CHX, $\text{Ca}(\text{OH})_2/\text{CHX}$ and $\text{Ca}(\text{OH})_2$.

Effect of $\text{Ca}(\text{OH})_2$ on dentine

Endodontic treatment of immature teeth with non-vital pulps is a challenge. Apexification by $\text{Ca}(\text{OH})_2$ (Granath 1987, Frank 1966, Heithersay 1975) was based on the concept of apical healing being promoted through the induction of an apical barrier whilst at the same time the high pH providing an antibacterial capability. The flexural strength of dentine might, in part, depend on an intimate link between two main components of dentine, the hydroxyapatite crystals and the collagenous network. The organic matrix is composed of acid proteins and proteoglycans containing phosphate and carboxylate groups (Andreasen *et al.* 2002). These substances may act as bonding agents between the collagen network and the hydroxyapatite crystals (Andreasen *et al.* 2002).

Rosenberg *et al.* (2007) measured the effect of $\text{Ca}(\text{OH})_2$ on the microtensile fracture strength (MTFS) of teeth and found that it was reduced by almost 50% following 7–84 days of application. A study of bovine dentine maintained in Petri dishes for 5 weeks concluded that $\text{Ca}(\text{OH})_2$ reduced fracture strength by 32% (White *et al.* 2002). Another study indicated that the fracture strength of sheep dentine was reduced by 50% following $\text{Ca}(\text{OH})_2$ treatment after 1 year (Andreasen *et al.* 1989). Recently, Kawamoto *et al.* (2008) reported that exposure to $\text{Ca}(\text{OH})_2$ paste significantly increased the mean elastic modulus of bovine dentine, thereby making it more prone to fracture.

Grigoratos *et al.* (2001) reported that treatment with $\text{Ca}(\text{OH})_2$ reduced the flexural strength of dentine. Andreasen *et al.* (2002) concluded that the fracture strength of $\text{Ca}(\text{OH})_2$ -filled immature teeth was halved in approximately 1 year and attributed the frequent

reports of fractures of immature teeth filled with $\text{Ca}(\text{OH})_2$ for extended periods to this factor. Doyon *et al.* (2005) examined the resistance of human root dentine to intracanal medication with $\text{Ca}(\text{OH})_2$ and found that the fracture resistance of dentine was decreased significantly after 6 months.

In summary, dentine exposed to $\text{Ca}(\text{OH})_2$ for an extended period (6 months to 1 year) results in reduced flexural strength and lower fracture resistance. Therefore, other treatment modalities such as the apical barrier technique using mineral trioxide aggregate (MTA) should be used to manage teeth with non-vital pulps and open apices, following a short period of $\text{Ca}(\text{OH})_2$ medication where indicated.

Diffusion of hydroxyl ions through dentine

For calcium hydroxide to act effectively as an intracanal medicament, hydroxyl ions must be able to diffuse through dentine. It might be expected that this would occur in a manner similar to water, because diffusion through dentine is primarily determined by molecular weight (Nerwich *et al.* 1993). Several studies have attempted to measure diffusion of hydroxyl ions through dentine using a variety of experimental approaches, including pH indicating solutions or papers (Tronstad *et al.* 1981), pH measurement of ground dentine (Wang & Hume 1988) and pH values of the surrounding medium (Fuss *et al.* 1989).

Tronstad *et al.* (1981) examined histological sections of monkey teeth 1 month following placement of a $\text{Ca}(\text{OH})_2$ canal dressing and, using indicator solutions, found that there was a pH gradient with high values around the canal dressing towards the peripheral dentine. The pH of cementum remained unchanged but in resorption areas, where cementum was not present, the increased pH extended to the dentine surface. In another study related to the action of $\text{Ca}(\text{OH})_2$ in cervical root resorption, Kehoe (1987) placed $\text{Ca}(\text{OH})_2$ in the cervical part of the root canals previously filled with bleaching agents and reported a pH reversal from a slightly acidic to a slightly alkaline level using pH electrodes and alkacid test papers.

Fuss *et al.* (1989) measured pH changes in distilled water surrounding teeth filled with $\text{Ca}(\text{OH})_2$ and found small changes in pH level up to 10 days. Wang & Hume (1988) measured hydroxyl ion diffusion across dentine between an occlusal cavity containing $\text{Ca}(\text{OH})_2$ and a saline-filled pulp chamber at 16 days using a pH meter. By taking ground dentine (subsequently mixed with saline) from various depths, they demonstrated a

gradient of pH values from the cavity layer decreasing to the middle and pulpal layers, indicating slow movement of hydroxyl ions through dentine. Nerwich *et al.* (1993) investigated pH change over a 4-week period after application of a $\text{Ca}(\text{OH})_2$ dressing and concluded that hydroxyl ions derived from $\text{Ca}(\text{OH})_2$ dressings diffused in a matter of hours into the inner root dentine but required 1–7 days to reach the outer root dentine and 2–3 weeks to reach peak levels. Hydroxyl ions diffused faster and reached higher levels cervically more than apically.

Gomes *et al.* (1996) reported diffusion of calcium ions from $\text{Ca}(\text{OH})_2$ paste through dentine. Esberard *et al.* (1996a) found that $\text{Ca}(\text{OH})_2$ -containing sealers, although suitable for use as root canal sealants, did not produce an alkaline pH at the root surface. However, in another study $\text{Ca}(\text{OH})_2$, as an intracanal medicament, maintained a high pH at the root surface for at least 120 days (Esberard *et al.* 1996b). Calt *et al.* (1999) demonstrated that, when non-setting $\text{Ca}(\text{OH})_2$ pastes were applied to the root canal, diffusion of Ca^{2+} without an increase in pH in the surrounding media occurred. Saif *et al.* (2008) indicated that a final canal rinse with 3 mL 17% EDTA and 10 mL 6% NaOCl before $\text{Ca}(\text{OH})_2$ placement allowed the greatest hydroxyl ion diffusion to the root surface.

In summary, it seems that diffusion of hydroxyl ions through dentine depends on the period of medication, diameter of dentinal tubules (cervical versus apical) and smear layer removal (patency of dentinal tubules). Furthermore, diffusion of hydroxyl ions through to areas of root resorption where pH is acidic has a positive effect on the progression of inflammatory root resorption.

Removal of $\text{Ca}(\text{OH})_2$ from canals

$\text{Ca}(\text{OH})_2$ placed as a medicament has to be removed before the canal is filled. Laboratory studies have revealed that remnants of $\text{Ca}(\text{OH})_2$ can hinder the penetration of sealers into the dentinal tubules (Calt & Serper 1999), hinder the bonding of resin sealers to dentine, increase the apical leakage of root fillings (Kim & Kim 2002) and potentially interact with zinc oxide eugenol sealers and make them brittle and granular (Margelos *et al.* 1997). Therefore, complete removal of $\text{Ca}(\text{OH})_2$ from the root canal before filling is recommended.

Lambrianidis *et al.* (1999) evaluated the effectiveness of removing $\text{Ca}(\text{OH})_2$ associated with several vehicles from the root canal including normal saline, 3%

sodium hypochlorite (NaOCl), 3% NaOCl + 17% EDTA as irrigants in combination with hand filing and found that 45% of the canal surface area remained covered with $\text{Ca}(\text{OH})_2$. They inferred that the amount of $\text{Ca}(\text{OH})_2$ powder in the paste did not affect removal, but the vehicle did. Margelos *et al.* (1997) revealed that using 15% EDTA or NaOCl alone as irrigants did not remove $\text{Ca}(\text{OH})_2$ from the root canal, but combining these two irrigants with hand instrumentation improved the effectiveness of removal.

Nandini *et al.* (2006) reported that the vehicle used to prepare $\text{Ca}(\text{OH})_2$ paste was important for its removal. Oil-based $\text{Ca}(\text{OH})_2$ paste was more difficult to remove than $\text{Ca}(\text{OH})_2$ powder mixed with distilled water. Both 17% EDTA and 10% citric acid were found to remove $\text{Ca}(\text{OH})_2$ powder mixed with distilled water, whereas 10% citric acid performed better than EDTA in removing an oil-based $\text{Ca}(\text{OH})_2$ paste. In another study, Lambrianidis *et al.* (2006) compared the removal efficiency of $\text{Ca}(\text{OH})_2/\text{CHX}$ gel, $\text{Ca}(\text{OH})_2/\text{CHX}$ solution and $\text{Ca}(\text{OH})_2/\text{saline}$ pastes using instrumentation with or without a patency file and irrigation with NaOCl and EDTA solutions. Remnants of medicaments were found in all canals regardless of the experimental material or use of patency filing. When examining the root canal as a whole, $\text{Ca}(\text{OH})_2/\text{CHX}$ gel paste was associated with significantly larger amounts of residue, whereas the $\text{Ca}(\text{OH})_2/\text{CHX}$ solution paste was associated with less residue than the other two groups with or without the use of patency filing. They also noted that the use of patency filing facilitated removal of more of the medicament in the apical third of straight canals (Lambrianidis *et al.* 2006).

Another method to remove remnants of $\text{Ca}(\text{OH})_2$ from the root canal involved the use of ultrasonic devices. Kenée *et al.* (2006) evaluated the amount of $\text{Ca}(\text{OH})_2$ remaining in canals after removal with various techniques including combinations of NaOCl with EDTA irrigation, hand filing, rotary instrumentation, or ultrasonics. Overall, no technique removed the $\text{Ca}(\text{OH})_2$ entirely. Rotary and ultrasonic techniques, whilst not different from each other, removed significantly more $\text{Ca}(\text{OH})_2$ than irrigant only techniques. van der Sluis *et al.* (2007) evaluated the capacity to remove a $\text{Ca}(\text{OH})_2$ paste from the root canal and the efficacy of $\text{Ca}(\text{OH})_2$ removal during passive ultrasonic irrigation using either sodium hypochlorite or water as an irrigant. Results demonstrated that passive ultrasonic irrigation with 2% NaOCl was more effective in removing $\text{Ca}(\text{OH})_2$ paste from artificial root canal grooves than syringe delivery of 2% NaOCl or water

as irrigant. Balvedi *et al.* (2010) found that neither syringe injection nor passive ultrasonic irrigation were efficient in removing inter-appointment intracanal medicaments.

In summary, it seems that complete removal of Ca(OH)_2 paste from the root canal walls is not achievable using routine techniques. However, the type of vehicle used, use of patency filing and combining EDTA and NaOCl with hand instrumentation improves the efficacy of Ca(OH)_2 paste removal. Furthermore, it seems that ultrasonic methods are more efficient in removing Ca(OH)_2 remnants than passive irrigation.

Toxicity of Ca(OH)_2 in medicaments

Early reports on the outcome of Ca(OH)_2 extruded into the periapical region concluded it was well tolerated and was resorbed (Martin & Crabb 1977). However, the periapical response to Ca(OH)_2 based on results from other reports seems to be equivocal.

Spångberg (1969) reported an inflammatory response with inhibited bone healing 2 weeks after the implantation of Ca(OH)_2 into guinea-pig bone; nevertheless, it was found to be one of the least irritating root-filling materials and was replaced by new bone within 12 weeks of placement.

However, Ca(OH)_2 has been reported to have a detrimental effect on periodontal tissues when used as an intracanal medicament during root canal treatment (Hauman & Love 2003). Blomlöf *et al.* (1988) observed that Ca(OH)_2 could negatively influence marginal soft tissue healing and suggested the completion of root canal treatment prior to the removal of cementum as might occur during periodontal therapy. Breault *et al.* (1995) reported that the use of Ca(OH)_2 demonstrated a decreased but not statistically significant inhibition of attached human gingival fibroblasts (HGF) and proposed that Ca(OH)_2 should be avoided as an interim medicament when trying to regenerate or establish new attachment in tissues adjacent to endodontically involved teeth. Contrary to these findings, Hammarström *et al.* (1986) demonstrated that Ca(OH)_2 did not affect the healing of replanted monkey teeth with intact cementum and only temporarily in those undergoing cemental repair. Similarly, Holland *et al.* (1998) observed that periodontal healing associated with infected root canals filled with Ca(OH)_2 was not hindered 6 months after experimental periodontal surgical injury in dogs. Barnhart *et al.* (2005) found that Ca(OH)_2 was well tolerated by HGF. Ribeiro *et al.*

(2004) found that Ca(OH)_2 did not promote DNA damage in mammalian cells. Pissiotis & Spangberg (1990) evaluated mandible bone reactions of guinea pigs to implants of hydroxyapatite, collagen, and Ca(OH)_2 , alone or in different combinations, over a period of 16 weeks. Findings revealed that no major inflammatory reactions occurred in any of the implant combinations. Hydroxyapatite was not resorbed over the examination periods, but Ca(OH)_2 and collagen implants were partially or totally resorbed and replaced by bony tissue. Wakabayashi *et al.* (1995) evaluated the effect of a Ca(OH)_2 paste dressing on uninstrumented root canal walls and found that it could dissolve the odontoblastic cell layer, but had little effect on predentine. Holland *et al.* (1999) reported that rat subcutaneous connective tissue reaction to Ca(OH)_2 and MTA inside the dentine tubes was desirable. They observed the formation of calcite granulations, birefringent to polarized light, near the lumen of dentinal tubule in Ca(OH)_2 samples. Under these granulations, a von Kossa-positive bridge of hard tissue was formed. In MTA samples, the same granulations was observed, but their number was less than the Ca(OH)_2 group. Furthermore, contrary to the Ca(OH)_2 group, the calcite granulations were in contact with MTA. This may be because of the similarity of the mechanism of action of MTA and Ca(OH)_2 ; the calcium oxide in the MTA powder is converted into Ca(OH)_2 when the paste is prepared with water. In contact with tissue fluids, this mixture would dissociate into calcium and hydroxyl ions. The calcium ions reacting with the carbonic gas of the tissues would originate the calcite granulations. Close to these granulations, there is accumulation of fibronectin, which allows cellular adhesion and differentiation. Guigand *et al.* (1999) confirmed the cytocompatibility of Ca(OH)_2 and a calcium oxide-based compound.

In summary, it seems that Ca(OH)_2 paste is well tolerated by bone and dental pulp tissues. However, its effect on the periodontal tissue is controversial.

Calcium hydroxide when used in sealers during root canal treatment

Sealers are responsible for the principal functions of a root filling, namely, sealing the root canal system, entombment of remaining bacteria and the filling of irregularities in the canal system (Ørstavik 2005). Several different chemical formulations have served as bases for root canal sealers and the success of Ca(OH)_2 as a pulp-capping agent and as an inter-appointment

medicament prompted its use in sealer cement formulations. Sealapex (SybronEndo, Orange County, CA, USA) and Apexit (Ivoclar Vivadent Inc., Schaan, Liechtenstein) are brand names of this type of material (Ørstavik 2005).

Leakage

Limkangwalmongkol *et al.* (1991) assessed the apical leakage of four root canal sealers when used with laterally compacted Gutta-percha using dye penetration and concluded that the distance dye penetrated the canals was as follows: Apexit (Ivoclar Vivadent), 1.67 mm; Sealapex (SybronEndo), 2.28 mm; Tubliseal (SybronEndo), 1.95 mm; AH26 (Dentsply de Trey, Konstanz, Germany), 0.82 mm; and Gutta-percha alone, 8.37 mm. Sleder *et al.* (1991) reported that Sealapex had a sealing ability comparable to Tubliseal. In a laboratory study, Siqueira *et al.* (1999) evaluated the coronal leakage of human saliva into root canals filled using lateral compaction of Gutta-percha and one or other of two $\text{Ca}(\text{OH})_2$ -based sealers and found that 35% of the Sealer 26 (Dentsply, Petropolis, Brazil) samples and 80% of the Sealapex samples were entirely recontaminated at 60 days. Using dye penetration methods, Ozata *et al.* (1999) compared the apical leakage of Ketac-Endo (ESPE GmbH & Co., Seefeld-Oberbay, Germany), Apexit (Ivoclar Vivadent) and Diaket (3M/ESPE, Minneapolis, MN, USA) and found that there was no significant difference between Apexit and Diaket. However, there was significantly more leakage with Ketac-Endo. Timpawat *et al.* (2001) concluded that coronal bacterial leakage of canals filled with a $\text{Ca}(\text{OH})_2$ -based sealer (Apexit) was significantly greater than those filled with a resin-based sealer (AH26). Economides *et al.* (2004) found that apical sealing ability of Fibrefill (a resin-based sealer) (Pentron, Wallingford, CT, USA) was significantly better than CRCS (Coltène Whaledent/Hygenic, Mahwah, NJ, USA). In another study Cobankara *et al.* (2006) concluded that the apical sealing ability of Sealapex was significantly better than three other sealers (Rocanal 2, La Maison Dentaire SA, Balzers, Switzerland; AH-Plus, Dentsply De Trey, and RC sealer, Sun Medical Co Ltd, Shiga, Japan) at 7, 14 and 21 days. Siqueira *et al.* (1999) found that during a 60-day period, Sealer 26 (Dentsply, Indústria e Comércio Ltda, Petrópolis, Brazil) resulted in significantly less leakage than Sealapex. Pommel *et al.* (2003) found that there was no statistically significant difference amongst AH26, Pulp Canal Sealer, and Ketac-Endo. In

a laboratory study, Cobankara *et al.* (2006) evaluated the apical seal obtained with four root canal sealers (Rocanal 2, Sealapex, AH-Plus, and RC Sealer) and reported that apical leakage associated with all sealers decreased gradually from 7 to 21 days. Sealapex had better apical sealing than the other sealers at 7, 14 and 21 days. RC Sealer, AH Plus and Rocanal 2 had similar apical leakage values at every period. It has been demonstrated that the long-lasting seal of these materials may, amongst other influencing factors, depend on their thickness and solubility (Wu *et al.* 1995).

Considering that the main purpose of using sealers is to fill gaps within the irregular root canal system, their solubility and disintegration should be as low as possible. On the other hand, to achieve favourable effects, $\text{Ca}(\text{OH})_2$ should dissociate into calcium and hydroxyl ions, which is in contrast to the philosophy of using sealers. Therefore, a major dilemma arises regarding both the long-term sealing ability and favourable biological properties of $\text{Ca}(\text{OH})_2$ -based sealers.

In summary, the sealing ability of $\text{Ca}(\text{OH})_2$ -based sealers compared to other sealers is ambiguous. This may be because of factors such as the method used to evaluate leakage and the often limited sample sizes included. However, it is clear that there is no superiority for $\text{Ca}(\text{OH})_2$ -based sealers over other groups of sealers.

Biocompatibility

There are five approaches to assess the biocompatibility of endodontic materials such as sealers: cytotoxic evaluation, genotoxicity, subcutaneous implants, intraosseous implants, usage tests and human studies (Hauman & Love 2003). Cytotoxicity is usually assessed on cells such as leucocytes, HeLa (human cervical carcinoma) cells and fibroblasts. Cell culture experiments are easier, more rapid and cheaper than other methods used to test biocompatibility. However, results of these tests cannot be extrapolated to the clinical situation (Hauman & Love 2003). Briseño & Willershausen (1992) assessed the cytotoxicity of four different calcium hydroxide-based root canal sealers (Sealapex), Apexit (Ivoclar Vivadent), CRCS (Coltène Whaledent) and Endoflas FS (Sanlor, Miami, FL, USA) on HGF. According to their findings, Endoflas FS induced a dramatic reduction in the protein synthesis potential of the fibroblasts in the 24-h group. In the 48-h group, Endoflas FS gave a slightly better response. Endoflas FS, however, had a significantly higher

cytotoxicity with respect to other sealers in both trials. Sealapex demonstrated a relatively low cytotoxicity after 3 days of culturing. Although CRCS had a slightly higher cytotoxicity during the initial phase of the experiments, a declining level of toxicity could be measured after 3 days of culturing. Apexit had a relatively high cytotoxicity in the initial phase, but an ascending incorporation rate of L-[14C] leucine in the fibroblasts could be distinguished after 3 days of culturing.

Leonardo *et al.* (2000a) evaluated the cytotoxicity of four Ca(OH)₂-based sealers and a zinc oxide–eugenol-based sealer (Fill Canal; TechNew, CampoGrande, RJ, Brazil) and found that the least cytotoxic sealer was Fill Canal, followed in increasing order of cytotoxicity by CRCS, Sealer 26 (Dentsply), Apexit and Sealapex. Boiesen & Brodin (1991) evaluated the neurotoxic effect of Sealapex and CRCS and found that both materials exhibited reversible and irreversible blocking of nerve conduction after 90 s and 5-min exposure. However, after 30 min of contact, the conduction of the compound action potential was irreversibly blocked for both materials.

Using HeLa cells, Miletić *et al.* (2000) reported that the toxicity of Apexit was significantly less than AH26, AH-Plus, and Diaket (3M ESPE AG, Seefeld, Germany). Schwarze *et al.* (2002) evaluated the cytotoxicity of several types of root canal sealers *in vitro* over the period of 1 year using immortalized 3T3 fibroblasts and primary human periodontal ligament fibroblasts. Results revealed that pronounced cytotoxic effects were only caused by N2-extracts in both cell cultures. Furthermore, significant cytotoxic alterations were also induced by 10-week eluates of Endomethasone (Spécialités Septodont, Saint Maur-des-Fossés, France); other investigated materials did not significantly alter cell metabolism. Eldeniz *et al.* (2007) assessed *ex vivo* the cytotoxic effects of eight root canal sealers RC Sealer (Sun Medical), Epiphany (Pentron, Wallingford, CT, USA), EndoREZ (Ultradent, South Jordan, UT, USA), GuttaFlow (Colthène Whaledent), Acroseal (Septodont, France), AH-Plus (Dentsply De Trey), RoekoSeal (Colthène Whaledent) and Apexit (Ivoclar Vivadent) using primary HGF and a mouse fibroblast cell line (L929). Results showed that resin-based (Epiphany and EndoREZ) and calcium hydroxide-based (Apexit and Acroseal) sealers were significantly more cytotoxic than other sealers. However, L929 cells were more sensitive to Apexit and EndoREZ than HGF cells. RC Sealer had mild cytotoxicity to HGF at both setting times. AH-Plus did not exert any cytotoxic effect to HGF

and aged specimens appeared to induce cellular proliferation. RoekoSeal and GuttaFlow also demonstrated mild cytotoxicity. GuttaFlow was slightly more cytotoxic to both cultures, especially when tested fresh.

In a study to evaluate genotoxicity of Ca(OH)₂-based and epoxy resin-based root canal sealers, Huang *et al.* (2002) found that a resin-based sealer produced greater DNA damage than a Ca(OH)₂-based sealer. In a laboratory study to assess cytotoxicity of Ca(OH)₂-based sealers, Beltes *et al.* (1995) reported that Sealapex was the most cytotoxic sealer, followed by CRCS, with Apexit being the least cytotoxic with the smallest decrease in cell density. In a study to assess tissue toxicity of Grossman's sealer, eucapercha, Endo-Fill, CRCS, Sealapex and Hypocal, they were injected into specific dorsal subdermal tissue sites of 12 guinea pigs (Yesilsoy *et al.* 1988). Sealapex and Endo-Fill had less severe inflammatory reactions than any of the other materials. Grossman's sealer, CRCS and Hypocal created principally severe inflammatory responses at both 6 and 15 days, but mild reactions at 80 days. Eucapercha created less severe inflammatory responses than Grossman's sealer, CRCS and Hypocal (Yesilsoy *et al.* 1988).

Mittal *et al.* (1995) evaluated the tissue toxicity of zinc oxide–eugenol, Tubliseal, Sealapex and Endoflas FS by injecting them into the subcutaneous connective tissue of the dorsal surface of rats and studying the tissue response histologically. According to their findings, Sealapex was associated with the least inflammatory reaction compared to the other sealers used, because it caused moderate inflammation at 48 h that became mild. Zinc oxide-eugenol, Tubliseal and Endoflas F.S. were highly toxic at 48 h and 7 days. This toxicity decreased gradually with time. No inflammatory reaction was seen at 3 months with any of the sealers.

Silva *et al.* (1997) evaluated the inflammatory response to Sealapex, CRCS, Apexit and Sealer 26 in the subcutaneous tissue and in the peritoneal cavity of Balb/c mice. The inflammatory response of subcutaneous tissue was analysed after 2, 4, 8 and 16 days. Intense neutrophilia was seen in response to all sealers during the initial periods. Differences amongst them related to the presence of necrosis and the number of inflammatory cells. In the intermediate phase, marked differentiation of cells of the mononucleate phagocytic system into macrophages, epithelioid cells and multinucleate giant cells were observed with Sealapex. This response was less intense with CRCS and Apexit. Tissue necrosis was observed only at tissue sealer interfaces

and only during the initial period with Sealapex but was seen throughout the experiment with all other sealers. The animals were injected in the peritoneal cavity with solutions containing the sealers and five mice from each group were killed 6 and 24 h, and 5 and 15 days later. During the initial periods (6 and 24 h), there was an intense migration of polymorphonuclear leucocytes to the peritoneal cavity in response to all sealers compared to the control. This migration was more intense for Sealer 26 and Apexit. An increase in mononucleate cell number was observed after 6 and 24 h and 5 days for all sealers and no differences were observed in relation to the control after 15 days.

Kolokouris *et al.* (1998) evaluated the *in vivo* biocompatibility of Apexit and Pulp Canal Sealer after implantation in rat connective tissue. Findings revealed that severe inflammatory reactions with differing extensions of necrosis were observed with Apexit on the 5th and 15th days. The intensity of the reaction had diminished by the 60th day, and this reduction continued progressively through to the 120th day. It was characterized by the presence of connective tissue with a few macrophages. Moderate to severe inflammation with confined areas of necrosis was observed in the Pulp Canal Sealer specimens on the 5th day. The intensity of the reaction diminished by the 15th, 60th and 120th days but remained greater than Apexit through long-term observation periods. Figueiredo *et al.* (2001) evaluated tissue response to four endodontic sealers (N-Rickert, AH-26, Fill Canal, and Sealer 26) placed in the oral mucosa of rabbits by either submucous injection or implant in polyethylene tubes. Findings demonstrated that there was no difference between the two methods of implantation. In addition, all sealers elicited some kind of inflammatory response; the most irritant was Fill canal, followed by N-Rickert and AH-26. Sealer 26 elicited a mild reaction only. Bernáth & Szabó (2003) evaluated the type and degree of inflammatory reaction initiated by four sealers (AH26, Apexit, Endomethasone and Grossman's sealers) by overfilling the root canals in the teeth of monkeys. The result of the treatment was evaluated after 6 months by histological assessment of the periapical tissues. In the group of root canals filled within the root, no inflammatory reaction was detected in specimens of Apexit and Grossman's sealers, but the other two sealers initiated different degrees of lymphocytic/plasmocytic tissue reactions. Endomethasone initiated a mild lymphocytic/plasmocytic reaction in three of the nine cases and AH26 caused mild lymphocytic/

plasmocytic infiltration in two of the seven cases. In the group with overfilled root canals, all four sealers initiated inflammatory reactions. The periapical tissue reactions of overfilled root canals were similar to reactions detected in cases filled within the canal. However, additional histological features developed in specimens of Endomethasone and AH26: Endomethasone initiated a foreign body-type granulomatous reaction around the sealer particles and AH26 particles were engulfed by macrophages. The overfilled root canals of Apexit and Grossman's sealers initiated only lymphocytic/plasmocytic reactions.

In summary, some controversies regarding the biocompatibility of Ca(OH)₂-based sealers could be attributed to the evaluation method. However, most studies concluded that the biocompatibility of Ca(OH)₂-based sealers were within an acceptable range compared to other root canal sealers.

Antibacterial activity

Microorganisms infecting the root canal dentine might adhere superficially to the dentinal wall or penetrate deeper into the dentinal tubules (Ando & Hoshino 1990, Peters *et al.* 2001). Superficially adhering bacteria might be expected to be killed more readily than those shielded in the depths of dentinal tubules, but microorganisms inside the dentinal tubules might also be challenged by antimicrobial components leaching from sealers. Therefore, antimicrobial testing of sealers should take into consideration these two effects based on the contact of the sealer with microorganisms (Kayaoglu *et al.* 2005). Two main methods have been used to study the antimicrobial effects of Ca(OH)₂-based sealers including ADT and direct contact tests (DCT).

In an agar diffusion study, Mickel & Wright (1999) reported that Roth (a zinc oxide-eugenol-based sealer) inhibited the growth of *Streptococcus anginosus* (milleri) more effectively than several Ca(OH)₂-based sealers (Sealapex, Apexit, and CRCS). In another agar diffusion study, Mickel *et al.* (2003) evaluated the antimicrobial activity of four root canal sealers on *E. faecalis*. A statistically significant difference was observed between all four groups of sealers. Roth 811 had the largest zone of inhibition (1.1 mm), followed by Sealapex (0.8 mm) and Kerr EWT (0.5 mm), whereas AH-Plus had no antimicrobial activity. Abdulkader *et al.* (1996) evaluated the effect of several sealers against *Capnocytophaga ochracea*, *Porphyromonas gingivalis* and *Peptostreptococcus micros* using ADT. Findings revealed that zones of bacterial growth inhibition in descending order were

as follows: Roth Sealer, Ketac-Endo, Tubliseal, Apexit and Sealapex. al-Khatib *et al.* (1990) assessed the antibacterial effect of Grossman's sealer, Tubliseal, Calciobiotic, Sealapex, Hypocal, eucapercha, Nogenol and AH26 against *Streptococcus mutans*, *Staphylococcus aureus* and *Bacteroides endodontalis* using ADT. Results demonstrated that Grossman's sealer had the greatest overall antibacterial activity. However, AH26 had the greatest activity against *B. endodontalis*, whilst the zinc oxide–eugenol-based sealers had more antimicrobial activity than either the calcium hydroxide-based sealers or eucapercha. Using ADT, Lai *et al.* (2001) found that the antibacterial activity of zinc oxide-based and resin-based sealers were more than Sealapex (a Ca(OH)_2 -based sealer).

The number of studies with DCT is fewer. Heling & Chandler (1996) as well as Saleh *et al.* (2004) have demonstrated that Sealapex as well as Apexit (two Ca(OH)_2 -based sealers) had less antibacterial efficacy than resin-based and zinc oxide-based sealers. Furthermore, these two studies demonstrated that the antimicrobial effect of Ca(OH)_2 -based sealers increased with time, probably as a result of disintegration of the sealer and an increase in the amount of hydroxyl ions over time. Furthermore, Kayaoglu *et al.* (2005) showed that the Ca(OH)_2 -based sealers, Sealapex and Apexit, were ineffective in killing bacteria in the short term (24 h). According to Cobankara *et al.* (2004), Ketac-Endo and AH-Plus were more potent bacterial growth inhibitors than Sealapex.

Duarte *et al.* (2000) evaluated the pH and calcium ion release of three root canal sealers, Sealapex, Sealer 26 and Apexit at 24 and 48 h, and 7 and 30 days after spatulation. Sealapex produced an alkaline pH and released significantly greater amounts of calcium, with even more pronounced results after 30 days. Furthermore, Sealapex had the highest calcium and hydroxyl release, especially after longer time intervals, whereas Sealer 26 had the highest release during the initial periods (i.e. during its setting period). Apexit had the least satisfactory results.

In summary, the antibacterial activity of Ca(OH)_2 -based sealers is lower than other similar materials, especially zinc oxide–eugenol-based and resin-based sealers.

Solubility

When considering the solubility of endodontic sealers, it should be noted that their solubility in specific solvents, such as chloroform, is a positive characteris-

tic, whereas their solubility in tissue fluids is negative characteristic.

Solubility in tissue fluids

Ca(OH)_2 -based sealers were introduced in an attempt to stimulate periapical healing with bone repair through the release of Ca(OH)_2 (Bergenholtz *et al.* 2003). According to Esberard *et al.* (1996a), Ca(OH)_2 -based sealers release OH^- and Ca^{2+} ions. These sealers evoke an increase in pH when placed in distilled water (48 h after setting) of 9.14 and 8.6; under the same conditions, pure Ca(OH)_2 paste increased the pH to 12.5. Sleder *et al.* (1991) demonstrated that Sealapex had no greater dissolution (based upon linear penetration) than Tubliseal at both 2 and 32 weeks and concluded that Sealapex could withstand long-term exposure to tissue fluids without significant leakage. Tronstad *et al.* (1988) assessed solubility of CRCS and Sealapex in dogs' teeth and found that CRCS was more stable than Sealapex. McMichen *et al.* (2003) reported that the solubility of Apexit in water was significantly more than AHPlus, Tubliseal EWT and Endion.

In summary, owing to the small number of studies, the solubility of Ca(OH)_2 -based sealers compared to other sealers in tissue fluids is not known.

Solubility in solvents

Removal of root canal filling materials from the root canal is a requirement for retreatment (Mandel & Friedman 1992). Various solvents for dissolving materials have been studied (Olsson *et al.* 1981, Barbosa *et al.* 1994). Chloroform is the most common solvent to remove root-filling materials including Gutta-perch and sealers (Wilcox 1995). Benzene and xylene, which are effective solvents, may be potential carcinogens (Lyng *et al.* 1997). Halothane, another solvent, is highly volatile (Keles & Köseoglu 2009). The high cost and volatility of halothane and its potential for inducing idiosyncratic hepatic necrosis make it a less desirable solvent (Keles & Köseoglu 2009). Whitworth & Boursin (2000) evaluated the effect of two volatile solvents (chloroform and halothane) on the solubility of root canal sealers (Ketac-Endo, TublisSeal EWT, Apexit, and AH-Plus). Ketac-Endo was the least soluble in chloroform and halothane, with less than 1% weight loss after 10-min exposure to either solvent. Apexit had low solubility with 11.6% and 14.19% weight loss after 10-min exposure to chloroform and halothane, respectively. The difference between solvents was not significant. Tubliseal EWT was significantly less soluble

in halothane than chloroform (5.19% and 62.5% weight loss after 10-min exposure, respectively). Its solubility in halothane was not significantly different from that of Apexit. AHPlus was significantly more soluble than all other materials in both chloroform and halothane (96% and 68% weight loss after 10-min exposure, respectively).

Schäfer & Zandbiglari (2002) reported that $\text{Ca}(\text{OH})_2$ -based sealers had greater solubility in chloroform than in eucalyptus oil. Keles & Köseoglu (2009) found that the solubility of a $\text{Ca}(\text{OH})_2$ -based sealer in NaOCl and EDTA was significantly greater than ZOE-based, silicone-based and resin-based sealers. However, its solubility was similar to polyketone. Martos *et al.* (2006) evaluated the solubility of $\text{Ca}(\text{OH})_2$ -based (Sealer 26), silicon-polydimethylsiloxane-based (RoekoSeal), and zinc oxide–eugenol based (Endofill and Intrafill) sealers in eucalyptol, xylol, orange oil and distilled water. Xylol and orange oil had similar effects, with significant solubilization of the cements tested. Endofill and Sealer 26 did not have any significant difference in solubilization at the two immersion times, whereas RoekoSeal and Intrafill had more pronounced solubility at 10 min. The lowest levels of solubilization occurred in RoekoSeal, Sealer 26, Endofill and Intrafill.

In summary, the solubility rate of $\text{Ca}(\text{OH})_2$ -based sealers compared to other sealers in solvents is still controversial.

Toxicity of $\text{Ca}(\text{OH})_2$ in sealers

Economides *et al.* (1995) reported that Sealapex (a $\text{Ca}(\text{OH})_2$ -based root canal sealer; SybronEndo) caused a moderate-to-severe inflammatory reaction, whereas CRCS (a $\text{Ca}(\text{OH})_2$ -based root canal sealer, Coltène-Whaledent) caused mild-to-moderate reactions in rat connective tissue. Kolokouris *et al.* (1998) evaluated the *in vivo* biocompatibility of Apexit (a $\text{Ca}(\text{OH})_2$ -based root canal sealer, Ivoclar Vivadent) and Pulp Canal Sealer (a zinc oxide–eugenol-based sealer, SybronEndo) after implantation in rat connective tissue at 5-, 15-, 60-, and 120-day intervals. Severe inflammatory reactions occurred with differing levels of necrosis with Apexit on the 5th and 15th days. The intensity of the reaction had diminished by the 60th day, and this reduction continued progressively to the 120th day. It was characterized by the presence of connective tissue with a few macrophages. Moderate-to-severe inflammation with confined areas of necrosis was observed in the Pulp Canal Sealer specimens on the 5th day. The intensity of the reaction diminished by the 15th, 60th

and 120th days, but remained marginally greater than Apexit through long-term observation periods.

Osorio *et al.* (1998) reported that CRCS was well tolerated by HGF and L929 cells. Leonardo *et al.* (2000b) found that the cytotoxicity of four $\text{Ca}(\text{OH})_2$ -based sealers [Sealapex, CRCS, Apexit, and Sealer 26 (Dentsply, Industria e Comercio Ltda)] was more pronounced than a zinc oxide–eugenol-based sealer (Fill Canal; Dermo Laboratorios, Rio de Janeiro, Brazil). Camps & About (2003) concluded that the high cytotoxicity of Sealapex did not decrease over time. Soares *et al.* (1990) found that overfilled canals containing $\text{Ca}(\text{OH})_2$ -based sealers caused chronic inflammatory reactions in the periapical tissues of dog's teeth.

In summary, although $\text{Ca}(\text{OH})_2$ paste is well tolerated by periapical tissues, it has a detrimental effect on periodontal tissues when used as an intracanal medicament. The biocompatibility of $\text{Ca}(\text{OH})_2$ -based sealers is controversial. Overall, because of their solubility, $\text{Ca}(\text{OH})_2$ -based sealers do not fulfil all the criteria of an ideal sealer. The antibacterial effects of calcium hydroxide in sealers are variable. Cytotoxicity appears to be milder than for other groups of sealers.

Clinical applications of calcium hydroxide when used as pulp-capping agents in vital pulp therapy

Abnormal root development on teeth undergoing root canal treatment will impact on the prognosis and thus tooth retention. Therefore, the primary purpose of treating immature permanent teeth should be, where possible, to maintain pulp vitality in order for root development to continue (apexogenesis). Such vital pulp therapy includes indirect and direct pulp-capping, partial (superficial) pulpotomy and cervical pulpotomy. There are long-term prognostic advantages of this treatment outcome over apexification. For example, the prognosis of superficial pulpotomy is 94–96% whereas the prognosis of apexification is 79–96% (Trope *et al.* 2002).

A number of materials have been advocated to induce normal root development with the most popular being $\text{Ca}(\text{OH})_2$. Zander (1939) was amongst the first to report on the use of a $\text{Ca}(\text{OH})_2$ material as a treatment for the exposed dental pulp and speculated that the success of $\text{Ca}(\text{OH})_2$ was related to its high alkalinity. According to Schröder & Granath (1971), the mechanism for the induction of dentinal bridge formation and repair under $\text{Ca}(\text{OH})_2$ was that it caused a superficial coagulation of the pulp tissue on which it was placed, initiated by damage to blood vessels. The

initial damage from Ca(OH)_2 occurs in the capillaries closest to the region of the capping or pulpotomy (Seltzer & Bender 1975). Because of its high pH, Ca(OH)_2 helps to maintain the immediate region in a state of alkalinity, which is necessary for bone and dentine formation. Under this region of Ca(OH)_2 -induced coagulation necrosis, which is saturated with calcium ions, cells from the underlying pulp tissue differentiate into odontoblast-like cells, which then begin to elaborate matrix (Farhad & Mohammadi 2005).

Pulp capping/pulpotomy

Many materials and drugs have been used as direct pulp-capping agents. One of the most effective and popular agents is Ca(OH)_2 (Farhad & Mohammadi 2005). Ca(OH)_2 can be used as a pulp protectant, but it should be used only where indicated and in a thin layer. Regular aqueous or methylcellulose Ca(OH)_2 fails as a base material (Farhad & Mohammadi 2005). It is biocompatible, but unfortunately has a low compressive strength that is not compatible with the condensation forces used when placing some definitive restoration, particularly amalgam. It should be noted that in the case of indirect pulp capping (IPC), Ca(OH)_2 is being used as an antibacterial agent and mild pulp stimulant to produce irritation dentine (Farhad & Mohammadi 2005). Warfvinge *et al.* (1987) reported that to achieve these two objectives Ca(OH)_2 paste in saline was much more effective than a commercial hard-setting Ca(OH)_2 cement (Life; SybronEndo). Another variation of a Ca(OH)_2 liner, Prisma VLC Dycal (LD Caulk Co., Milford, DE, USA), consists of Ca(OH)_2 and fillers of barium sulphate dispersed in a specially formulated urethane dimethylacrylate resin-containing initiators (camphorquinone) and activators. According to Stanley & Pameijer (1985) Prisma VLC Dycal has a number of advantages over regular water or methylcellulose-based Ca(OH)_2 : 'dramatically improved strength, essentially no solubility in acid, minimal solubility in water, control over working time, and reaching the maximum physical properties almost immediately'.

Numerous studies have demonstrated dentinal bridge formation in about 50–87% of cases treated with various Ca(OH)_2 formulations (Hargreaves & Goodis 2002). However, there is controversy concerning the application of Ca(OH)_2 in vital pulp therapy, particularly its caustic action. According to Meadow *et al.* (1984) pure Ca(OH)_2 necroses approximately 1.5 mm of the pulp tissue. The caustic action of the

high-pH formulations of Ca(OH)_2 reduce the size of the subjacent dental pulp by up to 0.7 mm (Schröder 1973, Cox *et al.* 1985, Heide 1991). To overcome these drawbacks, hard-setting Ca(OH)_2 formulations were introduced. Stanley & Lundy (1972) reported that hard-setting Ca(OH)_2 formulations did not necrose the superficial layer and found that the pulpal reactions to Dycal, Prisma VLC Dycal, Life and Nu-Cap were similar. However, in another study, they found that in contrast to regular Dycal, which caused a thickness of pulp mummification of 0.3–0.7 mm at the exposure site, Prisma VLC Dycal caused no inflammation (Stanley & Pameijer 1985).

Ca(OH)_2 dressings of Life and Dycal can dissolve clinically within 1–2 years (Stanley & Pameijer 1985, Cox *et al.* 1985). As the majority of dentine bridges under the materials appear to contain tunnels, about 50% of the pulps may become infected or become necrotic because of microleakage (Cox *et al.* 1985). Another problem with Dycal and Life is that they are degraded by etching and rinsing prior to restoration (Olmez *et al.* 1998). In newer products such as Prisma VLC Dycal, the Ca(OH)_2 is incorporated into urethane dimethylacrylate with initiators and accelerators by which they bind to dentine and have a higher resistance to acid dissolution (Pameijer & Stanley 1998).

Although suspensions of Ca(OH)_2 are highly alkaline, other compounds such as ammonium hydroxide with the same alkalinity cause liquefaction necrosis of the pulp when placed on exposed pulp tissue (Siqueira & Lopes 1999). The calcium ions delivered to the exposure site by the Ca(OH)_2 suspension are not utilized in the repair of the exposure. Pisanti & Sciaky (1964) demonstrated by way of radio-autographs that the calcium ions present in dentine bridges originated from the systemic circulation. The Ca(OH)_2 , which contained radioactive calcium, did not enter into the formation of the bridge.

Schröder & Granath (1972) examined the coronal surface structure of Ca(OH)_2 -induced bridges with both the light and scanning electron microscope and found tubular openings surrounded by collagen bundles similar to those found in normal predentine. It has been reported that saturated calcium and barium hydroxide completely inhibited alkaline phosphatase and lactic dehydrogenase activity, but Ca(OH)_2 preparations at lower pH levels were much less inhibitory (Seltzer & Bender 1975). Franz *et al.* (1984) evaluated dentinal bridges formed 4–15 weeks after capping deliberately exposed human pulps with a Pulpdent (a

Ca(OH)₂ paste) using scanning electron microscopy (SEM) as well as microradiographic techniques and found complete bridging and increasing thickness over longer post-treatment periods. Cross-sections of pulps treated for more than 6 weeks revealed a superior amorphous layer composed of tissue debris and Ca(OH)₂, a middle layer of a coarse meshwork of fibres identified as fibrodentine, and an inner layer containing tubular osteodentine (Franz *et al.* 1984).

Seltzer & Bender (1975) attributed two undesirable side effects to Ca(OH)₂ when used as a pulp-capping or pulpotomy agent: one is the possibility of eventual complete calcification of the tissue in the root canal. If this occurs, subsequent root canal treatment, if needed, becomes a difficult and often impossible. The second adverse effect is the persistence of induced inflammation, eventually causing internal resorption.

In summary, considering its alkalinity, biocompatibility and antimicrobial activity, it seems that Ca(OH)₂ is a suitable material for pulp capping and pulpotomy. However, its solubility in fluids is a problem that requires a good coronal seal.

Apexification

Apexification is defined as the process of creating an environment within the canal and periapical tissues after pulp death that allows a calcified barrier to form across the open apex of an immature root (Pitt Ford 2002). This calcified barrier consists of osteocementum or other bone-like tissue (Grossman 1988). Creation of a proper environment for formation of the calcified barrier involves cleaning and shaping of the canal to remove debris and bacteria, followed by placement of a suitable material to the apex (Pitt Ford 2002). Different materials have been used successfully, but the most favoured is a paste of Ca(OH)₂ and water; the addition of other medicaments to Ca(OH)₂ has no beneficial effect on apexification (Gutmann & Heaton 1981).

Thorough debridement to remove bacteria and necrotic tissue from the canal system is the primary factor responsible for apical closure. Ca(OH)₂ is used as a temporary canal filling material and has a bactericidal effect (Pitt Ford 2002). Although apexification had been attempted in the past, the technique was given impetus by the description of three cases by Frank (1966) who cleaned and irrigated canals and then sealed them with a paste of camphorated chlorophenol and Ca(OH)₂. Radiographic examination 3 and 6 months later revealed evidence of a root apex cap or barrier, following which the root canals were filled.

Actual root growth does not occur as a result of apexification, but radiographic evidence of a calcified mass at the root apex gives that impression (Grossman 1988).

Cvek (1972) observed apical root closure and bone healing following intracanal placement of Ca(OH)₂ in 50 of 55 maxillary incisors with immature roots. Furthermore, Binnie & Rowe (1973) dressed immature premolars in dogs with Ca(OH)₂ and distilled water and observed a minimal inflammatory response in the periapical tissues with continued root formation.

Although apexification occurs in association with many materials, it has been reported even after the removal of necrotic pulp tissue without the provision of a root filling (England & Best 1977). According to Kleier & Barr (1991), the usual time required to achieve apexification is 6–24 months (average 1 year ± 7 months). However, in one case it has been reported that 4 years of treatment was required for complete apexification (Grossman 1988).

There is disagreement in the literature on how often the canal should be refilled with Ca(OH)₂ paste to produce apexification, and the decision appears to be empirical. According to Tronstad *et al.* (1981) refilling every 3–6 months is favoured. Other reports favour refilling only if there is radiographic evidence of resorption of the paste (Cohen & Burns 2002). Chosack & Cleaton-Jones (1997) suggested that after initial root filling with Ca(OH)₂, there was nothing to be gained by its replacement either monthly or after 3 months for at least 6 months. If signs or symptoms of reinfection or pathosis occur at any time during the periodic recall of apexification cases, the canal is cleaned again and refilled with Ca(OH)₂ paste (England & Best 1977). If apexification is incomplete, the canal is refilled with the Ca(OH)₂ and the periodic recall continues (England & Best 1977).

From the histological perspective, the calcified tissue that forms over the apical foramen has been identified as an osteoid or cementoid material (Ham *et al.* 1972). According to Grossman (1988), the residual undamaged pulp tissue, if there is any, and the odontoblastic layer associated with the pulp tissue resume their matrix formation and subsequent calcification is guided by the reactivated Hertwig epithelial root sheath (HERS). Grossman (1988) also stressed that the HERS and the pulp tissue that were once damaged may explain why some of the apical formations appear atypical.

Pitt Ford (2002) concluded that the type of barrier that forms depends on the extent of pulp necrosis at the

start of treatment. Vital (but probably inflamed) pulp may be present at the root-end; following pulpectomy, canal preparation, irrigation and insertion of $\text{Ca}(\text{OH})_2$, some continued root formation may be expected from the surviving HERS. However, if there is severe inflammation (or even abscess formation) in the periapex (with or without sinus tract), the HERS has probably been destroyed completely.

In summary, $\text{Ca}(\text{OH})_2$ is the material of choice to create a calcified barrier at the root-end of teeth with necrotic pulps and immature 'open' apices. However, elimination of infection and necrotic pulp tissue, and the establishment of an effective coronal seal after placing the intracanal medicament appear to be more important than the type of intracanal medicament used. The more recent introduction of the MTA apical barrier technique may replace the use of $\text{Ca}(\text{OH})_2$ in this treatment modality.

Other clinical applications of calcium hydroxide when used in endodontic therapy

Canals with exudate

A perplexing condition to treat is the tooth with constant clear or reddish exudate associated with a large apical radiolucency. Such a tooth is often asymptomatic, but it may be tender to percussion or sensitive to digital pressure over the apex. If cultured, the drainage will not generally support bacterial growth (Weine 2004). When the pulp chamber is opened at the start of the appointment, a reddish discharge may appear, whereas at the succeeding appointment the exudate will be clear. If such a tooth is left opened under a rubber dam for 15–30 min, the exudate will stop; however, a similar condition will still be present at the next appointment even though canal preparation to an acceptable size has been achieved. This is referred to as a 'weeping canal' (Weine 2004).

According to Weine (2004), the best way to stop the exudate in such cases is to dry the canal with sterile paper points and to place $\text{Ca}(\text{OH})_2$ paste in the canal. The possible mechanism of action of $\text{Ca}(\text{OH})_2$ in these cases is related to its basic pH, which converts the acidic pH of periapical tissues to a more basic environment. Two other theories have also been proposed: (i) the calcifying potential of $\text{Ca}(\text{OH})_2$ may start to build up bone in the lesion and (ii) the caustic action of $\text{Ca}(\text{OH})_2$ cauterises residual chronically inflamed tissue (Weine 2004).

Horizontal root fractures

The use of $\text{Ca}(\text{OH})_2$ in teeth with horizontal root fractures was first recommended by Cvek (1974). He proposed that the canal at the level of the fracture line was comparable to the apical foramen of an immature tooth. Thus, he assumed that the repair would be similar to the apexification procedure employed for a tooth with an open apex (Cvek 1974). The benefits of root canal treatment with $\text{Ca}(\text{OH})_2$ occur probably because of its antibacterial effect and its ability to promote the formation of a hard-tissue barrier at the apical opening of the coronal fragment, thereby facilitating filling with Gutta-percha (Cvek *et al.* 2008). The frequency of healing (86%) in this treatment protocol is similar to that reported for treatment of immature teeth without a root fracture, i.e. healing after calcium hydroxide treatment and subsequent filling with Gutta-percha (Cvek 1972, 1992). This may also indicate that a tooth with a necrotic pulp in the coronal fragment should be regarded and treated as an immature tooth with a necrotic pulp. The chair-side time and the refilling of the canal with calcium hydroxide that are required to obtain these results may be seen as a drawback (Cvek *et al.* 2008). A better alternative treatment protocol is now available with use of MTA. In this situation, because the apical part of the coronal fragment resembles teeth with open apices, MTA can be used for optimal closure of the apical end of the coronal root segment (Kusgoz *et al.* 2009).

In summary, in the management of horizontal root fractures, the coronal segment is considered as an immature tooth with an open apex, and an apexification procedure is conducted. However, MTA can now be used for optimal closure of the apical end of the coronal root segment once canal infection has been eliminated.

Perforations

Root or furcation perforations can cause failure of root canal treatment, leading to tooth loss (Bramante & Berbert 1994). Several materials have been used to seal perforations, including $\text{Ca}(\text{OH})_2$. $\text{Ca}(\text{OH})_2$ has many benefits in this treatment modality including, easy manipulation, rapid resorption when extruded into the periodontium, promotion of the reorganization of periodontal tissues and induction of mineralized material (Bramante & Berbert 1994). It has been suggested that in such cases, the $\text{Ca}(\text{OH})_2$ should be renewed regularly (Heithersay 1975, Frank & Weine 1973).

It has been suggested that large apical perforations should be treated in a similar way as teeth with immature apices, i.e. with long-term Ca(OH)_2 treatment to achieve a hard-tissue barrier (Fuss & Trope 1996). Petersson *et al.* (1985) and Bogaerts (1997) stated that materials based on Ca(OH)_2 as a main ingredient were not suitable for crestal and furcation perforations because of the initial inflammatory response to these materials, which could lead to breakdown of the supporting tissues and subsequent pocket formation. ElDeeb *et al.* (1982) and Himel *et al.* (1985) expressed concerns about using Ca(OH)_2 in close proximity to the attachment apparatus because of the necrotizing properties of the material and the inflammatory reaction to it. On the other hand, the use of hard-setting Ca(OH)_2 to repair furcation perforations in monkey teeth did not appear to alter the pattern of healing, except to prevent ingrowth of granulation tissue into the instrumented root canal and was followed by a high rate of repair (Beavers *et al.* 1986).

In contact with tissue fluids, Ca(OH)_2 may be displaced (Schuurs *et al.* 2000) with the result that a reliable seal cannot be achieved; in such situations, a more conventional restorative material such as MTA is required. Pitt Ford *et al.* (1995) demonstrated that cementum was generated underneath MTA in most teeth with perforations, in contrast to the teeth whose furcation perforation sites were repaired with amalgam. Yildirim *et al.* (2005) compared the healing of furcation perforations repaired with MTA versus Super EBA in dogs' teeth. Their findings revealed cementum formation underneath all MTA specimens at the 6-month interval, whereas Super EBA samples had mild-to-severe inflammation around the repair material and no cementum formation during the same time interval. Main *et al.* (2004) reported clinical success of MTA in root perforation repairs.

In summary, Ca(OH)_2 has been suggested as a traditional agent to manage perforations, and its use is still indicated to control infection, arrest bleeding and as a temporary solution when insufficient time is available to perform a permanent repair. However, MTA now appears to be the material of choice for the permanent repair of perforations from both a conventional and surgical approach.

Root resorption

Root resorption can affect the cementum and/or dentine of the root (Trope 2002). On the basis of the site of origin of the resorption, it may be referred to as

internal, external or root-end resorption (Chivian 1991).

Ca(OH)_2 has an active influence on the local environment around a resorptive area by reducing osteoclastic activity and stimulating repair (Tronstad *et al.* 1981). This is directly related to the alkaline pH of Ca(OH)_2 , which permeates through the dentine. Hard-tissue resorption, with its enzymatic activity, takes place in an acidic pH Ca(OH) creates an alkaline environment in which the reaction is reversed and hard-tissue deposition can take place (Estrela & Holland 2009). The phenomenon of pH change towards the periphery is increased, especially where resorption has exposed dentine (Tronstad *et al.* 1981). Frank & Weine (1973) reported on a technique using a Ca(OH)_2 -camphorated monochlorophenol mixture for the non-surgical treatment of perforating internal resorption. In such situations, other similar techniques have been used that resulted in the deposition of a cementum-like or osteoid tissue.

Mineral trioxide aggregate is an alternative for Ca(OH)_2 in the management of internal root resorption. Successful surgical and non-surgical treatment of internal resorption using MTA in both primary and permanent teeth has been reported in several case reports (Hsien *et al.* 2003, Sari & Sonmez 2006, Silveira *et al.* 2009). Hsien *et al.* (2003) reported successful treatment of internal resorption in a maxillary central incisor using MTA with 1-year follow-up. Sari & Sonmez (2006) reported successful root filling of a primary second molar with MTA with an 18-month follow-up.

The initial treatment of choice for internal root resorption is to pack the canal and the resorption lacuna with Ca(OH)_2 paste. The Ca(OH)_2 will tend to necrotize remaining tissue in the lacuna, and the necrotic remnants are then removed by irrigation with sodium hypochlorite (Chivian 1991). When lateral resorption is noticed from the outset, pulp extirpation, debridement and Ca(OH)_2 therapy are preferred (Trope 2002, Haapasalo & Endal 2006). In addition, when lateral resorption reaches the dentine or perforates the root canal, the Ca(OH)_2 procedure should be attempted after canal debridement (Stewart 1975). According to Chivian (1991), Ca(OH)_2 should be placed into the resorptive defect at 3-month intervals until there is evidence of hard-tissue repair, confirmed by both radiographic and direct examination through the access cavity. When the physical barrier has been established, the defect can be filled with Gutta-percha or MTA.

The arrest of external root resorption related to necrotic pulps can be attributed exclusively to removal of necrotic tissue and antibacterial treatment of the root canal (Mohammadi *et al.* 2006). When external resorption occurs following luxation injuries pulp extirpation, debridement and Ca(OH)₂ therapy are necessary (Chivian 1991). In some situations when root resorption continues after the completion of active and retentive phases of orthodontic treatment, intentional pulp extirpation and Ca(OH)₂ is often successful in abating resorption (Gholston & Mattison 1983). Andreasen (1971) was able to arrest inflammatory root resorption in nine of ten cases using an intracanal Ca(OH)₂ dressing.

In summary, by creating an alkaline environment, Ca(OH)₂ inhibits osteoclast activity and stimulates hard-tissue deposition. However, MTA can be used to repair teeth during the management of internal root resorption.

Conclusions

Chemically, calcium hydroxide is classified as a strong base with a high pH (approximately 12.5–12.8). Its main properties come from the ionic dissociation of Ca²⁺ and OH⁻ ions and their effect on vital tissues, generating the induction of hard-tissue deposition and being antibacterial. Although some studies have confirmed its efficacy against endodontic bacteria, other studies have questioned its effectiveness. The effectiveness of Ca(OH)₂ against bacterial biofilms is uncertain and needs to be further elucidated. It seems that the combinations of Ca(OH)₂ with camphorated paramonochlorophenol or CHX have the potential to be used as effective intracanal medicaments for cases in which fungal infection is suspected. Ca(OH)₂ inactivates endotoxin, *in vitro* and *in vivo*, and appears currently the only clinically effective medicament for inactivation of endotoxin. The inhibitory effect of dentine, hydroxyapatite and remnants of necrotic pulp tissue as well as inflammatory exudate on the antibacterial potential of Ca(OH)₂ has been demonstrated. Synergistic effect between Ca(OH)₂ and NaOCl as well as between Ca(OH)₂ and CHX has been demonstrated. Six-months-to-one-year contact between Ca(OH)₂ and dentine results in reduced flexural strength and lower fracture resistance of dentine. Diffusion of hydroxyl ions through dentine depends on the diameter of dentinal tubules (cervical versus apical), smear layer removal (patency of dentinal tubules) and period of medication. Removing efficacy of Ca(OH)₂ paste from the root canal system seems to be

improved by using patency file, combining EDTA and NaOCl with hand instrumentation and the type of vehicle used. In addition, ultrasonic methods are more efficient in removing Ca(OH)₂ remnants than passive irrigation. Ca(OH)₂ paste is well tolerated by bone and dental pulp tissues. However, its effect on the periodontal tissue is controversial. The biocompatibility of Ca(OH)₂-based sealers is controversial and because of their solubility, they do not fulfil all the criteria of an ideal sealer. Furthermore, their antibacterial activity is variable, and their cytotoxicity appears to be milder than for other groups of sealers. Ca(OH)₂ is a suitable material for pulp capping and pulpotomy. However, its solubility in fluids is a problem that requires a good coronal seal. Ca(OH)₂ has been the material of choice to create a calcified barrier in non-vital open-apex teeth. However, MTA apical barrier technique may replace it. Ca(OH)₂ has been successfully used to manage perforations, horizontal root fracture and root resorption.

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