Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances

C. H. J. Hauman1 & R. M. Love2
1Departments of Oral Rehabilitation, and 2Stomatology, School of Dentistry, University of Otago, Dunedin, New Zealand

Abstract

Irrigation solutions and intracanal medicaments are used within the root canal to clean and aid in disinfecting the dentinal walls. Although these materials are intended to be contained within the root canal, they invariably contact the periapical tissues, either through inadvertent extrusion through the apex or leaching. This paper is a review on the methodology involved in biocompatibility testing followed by a discussion on biocompatibility of contemporary intracanal drugs and substances used in endodontics.

Keywords: biocompatibility, biomaterials, endodontics.

Received 8 February 2002; accepted 17 October 2002

Concepts of biocompatibility testing
Biocompatibility is defined as the ability of a material to function in a specific application in the presence of an appropriate host response (Williams 1987). According to EN 1441 (European Committee for Standardization 1996) biocompatible materials must be free of any risks.

Endodontic materials can be broadly categorized as those used to maintain pulp vitality and those used in pulp canal therapy for disinfection of the pulp space (irrigants and intracanal medicaments) and root canal filling (solid materials and sealers). Biocompatibility of these endodontic materials is characterized by many parameters such as genotoxicity, mutagenicity, carcinogenicity, cytotoxicity, histocompatibility or microbial effects. It is thus impossible to biologically characterize the materials by a single test method alone and their properties need to be investigated by a battery of various in vitro and in vivo tests in a structured approach.

Autian (1970) was the first to propose a structured approach as a concept consisting of three levels:
1 Nonspecific toxicity (cell cultures or small laboratory animals);
2 Specific toxicity (usage tests, e.g. in subhuman primates);
3 Clinical testing in humans.

According to Autian (1970) the term ‘nonspecific’ refers to test systems which do not reflect the application of a material in a clinical situation whilst the term ‘specific’ applies to the use of biological models simulating the actual clinical use of the material. The following sequence was adopted by the ISO (1984) in Technical Report 7405:
1 Initial tests (cytotoxicity, mutagenicity);
2 Secondary tests (sensitization, implantation tests, mucosal irritation);
3 Usage tests.

In both concepts, newly developed materials should be subjected to the three steps in the given sequence from the simple to the complicated test method, from in vitro to animal tests and from preclinical to clinical testing on humans.

Correspondence: Dr CHJ Hauman, Department of Oral Rehabilitation, School of Dentistry, University of Otago, PO Box 647, Dunedin, New Zealand(Tel.: +64 3 479 7118; fax: +64 3 479 5079; e-mail: tina.hauman@stonebow.otago.ac.nz).

Cell culture tests
The employment of in vitro tests offers the possibility of studying the effects of the release of material components on cell systems (Pertot et al. 1997). Cell culture studies have been used for more than 30 years for the investigation of cytotoxic reactions induced by endodontic materials (Rappaport et al. 1964, Keresztesi & Kellner 1966). Permanent cell lines, e.g. HeLa, 3T3 or L929 cells and primary/diploid human cells, mainly oral fibroblasts are used for these experiments. Primary cells are considered to be more relevant for biocompatibility studies than permanent cultures (Matsumoto et al. 1989, Al-Nazhan & Spångberg 1990). Various biological endpoints are used for these investigations. They include growth inhibition, determination of the effective dose 50 (ED50), membrane integrity, DNA, RNA or protein synthesis and/or the determination of alterations of cellular morphology by light or electron microscopy (Matsumoto et al. 1989, Al-Nazhan & Spångberg 1990, McNamara et al. 1992, Barbosa et al. 1994, Beltes et al. 1995).

Assays have been developed to investigate the influence of dentine on the cytotoxicity of sealers or isolated sealer components, such as the pulp chamber test or the application of a layer of dentine chips mimicking an apical dentine plug (Hanks et al. 1989, Meryon & Brook 1990, Schmalz et al. 1994, Schmalz & Schweikl 1994).

Genotoxicity

In vitro test systems for genotoxicity can be differentiated (Hel et al. 1996) into prokaryotic (e.g. Ames test, umu test) and eukaryotic assays (e.g. DNA synthesis inhibition test (DIT)). Since various dental or endodontic materials are highly cytotoxic, it is a basic requirement for genotoxicity tests to easily quantify cytotoxicity simultaneously. Moreover, it must be considered that various endodontic filling materials reveal a strong antibacterial activity as has been described by Orstavik (1988) and by Stea et al. (1994). A combination of prokaryotic and eukaryotic tests, e.g. the bacterial umu test (Oda et al. 1985) with the eukaryotic DIT (Painter 1977) supplemented by an in vivo assay (e.g. alkaline filter elution (AFE) assay) (Hel et al. 1996), is thus necessary to gain more reliable results with respect to the genotoxicity of endodontic materials.

Usage tests
Specific in vivo toxicity tests involve the use of the test material for root-canal therapy in animals, predominantly dogs (Soares et al. 1990, Sonat et al. 1990, Suzuki et al. 1995, Torabinejad et al. 1995) or monkeys (Torabinejad et al. 1997). In such studies, root canals are either filled to the cemento–dental junction or are deliberately overfilled to determine the reaction of the periapical tissue (Sonat et al. 1990). Due to ethical considerations these tests are rarely performed in humans (Lambjerg-Hansen 1987).
Although in vivo tests are helpful in understanding the complex interactions between the host and host tissue, the use of animals faces ethical problems and is under public discussion. Furthermore, these tests are expensive, time consuming and are difficult to control (Schmalz 1997).

**Human studies**

Finally, retrospective or preferably controlled prospective clinical studies in humans are necessary to determine long-term biocompatibility of permanent endodontic materials. It must be emphasized that all studies, including well-performed prospective clinical trials, only yield a statistical approximation of the biocompatibility of an oral or endodontic material. Thus, materials rated with a good biocompatibility may cause adverse reactions in a number of patients.

**Irrigants and intracanal medicaments**

**Sodium hypochlorite**

A 0.5% sodium hypochlorite (NaOCl) solution, also known as Dakin’s solution (1% sodium hypochlorite diluted with 1% sodium bicarbonate), was successfully used as a wound disinfectant during World War I (Dakin 1915). Sodium hypochlorite is an effective antimicrobial against endodontic flora (Byström & Sundqvist 1983) with some tissue-dissolving properties (Rosenfeld et al. 1978, Hand et al. 1978, Walker & del Rio 1991) and is the most commonly used irrigation fluid for root-canal preparation. The antimicrobial efficacy of the solution is due to its ability to oxidize and hydrolyse cell proteins and, to some extent, osmotically draw fluids out of cells due to its hypertonicity (Paschley et al. 1985). Sodium hypochlorite has a pH of approximately 11–12 and when hypochlorite contacts tissue proteins, nitrogen, formaldehyde and acetaldehyde are formed within a short time and peptide links are broken resulting in dissolution of the proteins (Engfelt 1922). During the process, hydrogen in the amino groups (–HN) is replaced by chlorine (–NCl) thereby forming chloramine, which plays an important role in antimicrobial effectiveness. Necrotic tissue and pus are thus dissolved and the antimicrobial agent can reach and clean the infected areas better. An increase in temperature of the solution significantly improves the antimicrobial and tissue-dissolving effects of sodium hypochlorite. As a consequence to these properties, NaOCl is highly toxic at high concentrations (Spångberg et al. 1973) and tends to induce tissue irritation on contact (Dakin 1915).

The minimum amount of a household bleach (3.12–5.25% NaOCl) to cause a caustic burn in dog oesophagus was found to be 10 mL over a 5-min period (Yarington 1970). Results from this study suggest that provided the time of contact with sodium hypochlorite is minimal, it does not have the same untoward effect on the mucosal surface as is evidenced when it is introduced interstitially. In a similar study, Weeks & Ravitch (1971) observed severe oedema with areas of haemorrhage, ulceration, necrosis and stricture formation in cat oesophagus when undiluted household bleach was placed on the mucosal surface. Paschley et al. (1985) demonstrated the cytotoxicity of NaOCl using three independent biological models. They found that a concentration as low as 1 : 1000 (v/v) NaOCl in saline caused complete haemolysis of red blood cells in vitro. As the solution used in this study was isotonic and thus excluded an osmotic pressure gradient, the observed haemolysis and loss of cellular protein was due to the oxidizing effects of NaOCl on the cell membrane. Undiluted and 1 : 10 (v/v) dilutions produced moderate to severe irritation of rabbit eyes whilst intradermal injections of undiluted, 1 : 2, 1 : 4 and 1 : 10 (v/v) dilutions of NaOCl caused skin ulcers. Kozol et al. (1988) proved Dakin’s solution to be detrimental to neutrophil chemotaxis and toxic to fibroblasts and endothelial cells.

Heggars et al. (1991) examined wound healing relative to irrigation and bactericidal properties of NaOCl in in vitro and in vivo models. They concluded that 0.025% NaOCl was the safest concentration to use because it was bactericidal but not tissue-toxic.

Different concentrations of NaOCl (e.g. 0.5, 1, 2.5 or 5.25%) are currently used as root-canal irrigants. Clinical tests showed that sodium hypochlorite at 0.5 or 5% concentration has similar clinical efficiency in supporting mechanical debridement of the root canal (Cvek et al. 1976a, Byström & Sundqvist 1985). As the proteolytic effect is dependent on the amount of free available chlorine that is used up during the process by reacting with inorganic reducing substances, frequent irrigation with a lower concentration may achieve as much of a proteolytic effect as the use of a higher concentration. Therefore, an adequate concentration of NaOCl to be used for endodontic irrigation may be 0.5–1.0% with the pH close to neutral, as a pH close to neutral has optimal antimicrobial effectiveness with minimal tissue irritating effect (Dakin 1915).

Most complications of the use of sodium hypochlorite appear to be the result of its accidental injection beyond...
the root apex which can cause violent tissue reactions characterized by pain, swelling, haemorrhage, and in some cases the development of secondary infection and paraesthesia (Reeh & Messer 1989, Becking 1991, Ehrich et al. 1993). Hypersensitivity reactions to sodium hypochlorite have also been reported (Kaufman & Keila 1989, Caliskan et al. 1994, Dandakis et al. 2000, Hulsman & Hahn 2000). A great deal of care should therefore be exercised when using sodium hypochlorite during endodontic irrigation. Ehrich et al. (1993) suggested that a clinician should check, both clinically and radiographically, for immature apices, root resorption, apical perforations or any other conditions that may result in larger than normal volumes of irrigant to be extruded from the root-canal system into the surrounding tissue. Irrigation should be performed slowly with gentle movement of the needle to ensure that it is not binding in the canal. In an in vitro study by Brown et al. (1995), the use of a reservoir of irrigation fluid in the coronal access cavity and carried into the root canal during filing resulted in significantly less apical extrusion of irrigation solution than with deep delivery with an irrigation needle.

**Ethylene diamine tetaacetic acid**

The disodium salt of ethylene diamine tetraacetic acid (EDTA) is generally accepted as the most effective chelating agent and lubricant (if in the correct vehicle e.g. RC-Prep, Premier Dental Products Co., Pennsylvania, USA) in current endodontic practice (Nygard-Ostby 1957, von der Fehr & Nygaard-Ostby 1963, Koulaouzidou et al. 1999). It is used in endodontic therapy to enhance the chemomechanical enlargement of canals (Walton & Torabinejad 1996), to remove smear layer (Meryon et al. 1987) and to clean and aid in disinfecting the dentinal walls (Yoshida et al. 1995). The solution most frequently used is the 15% disodium EDTA salt in a neutral solution. RC-Prep consists of 10% urea peroxide, glycol and 15% EDTA. Koulaouzidou et al. (1999) evaluated the cytotoxic effects of different concentrations of neutral and alkaline EDTA and sodium hypochlorite solutions using an established mouse skin fibroblast cell line: L929. Both neutral and alkaline EDTA showed moderate-to-severe cytotoxicity in a concentration-dependent manner. In addition, EDTA has been shown to inhibit the substrate adherence capacity of macrophages as well as the binding of vasoactive peptide to macrophage membranes in vitro (Segura et al. 1996, 1997). These results suggest that leakage of EDTA to periapical tissues during root-canal preparation may inhibit macrophage function, and thus alter the inflammatory response in periapical lesions. EDTA has been shown to have weak antibacterial and antifungal properties (Yoshida et al. 1995, Gultz et al. 1999, Heling et al. 1999, Steinberg et al. 1999).

**Chlorhexidine**

Chlorhexidine is a cationic bisbiguanide with optimal antimicrobial action ranging from pH 5.5 to 7.0. It is active against a wide range of microorganisms, such as Gram-positive and Gram-negative bacteria, bacterial spores, lipophilic virus, yeast and dermatophytes. It seems to act by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components (Leonardo et al. 1999). It is bacteriostatic at low concentrations, bactericidal at high concentrations and adsorbs to dental tissue and mucous membrane resulting in its prolonged gradual release at therapeutic levels (substantivity) (Jeansonne & White 1994, White et al. 1997). Chlorhexidine gluconate was found to be an effective antimicrobial agent when used as an endodontic irrigation solution and when used as an intracanal antimicrobial dressing further reduction of remaining bacteria within the root-canal system were seen (Delany et al. 1982). Studies have suggested that chlorhexidine gel has potential as an intracanal medicament (Ferraz et al. 2001) and as a root-canal irrigant (Siqueira & Uzeda 1997). Results from a study by Sanchez et al. (1988) on the cytotoxic effect of chlorhexidine on canine embryonic fibroblasts and *Staphylococcus aureus* showed that bactericidal concentrations of chlorhexidine were lethal to canine embryonic fibroblasts whilst noncytotoxic concentrations allowed significant bacterial survival. In a study by Tatnall et al. (1990) the cytotoxic effects of chlorhexidine, hydrogen peroxide and sodium hypochlorite were examined on cultured human fibroblasts, basal keratinocytes and a transformed keratinocyte line (SVK 14 cells). At concentrations recommended for wound cleansing all agents produced 100% killing of all cell types. Comparison of the ED$_{50}$ concentration for each agent on all cell types produced a ranking order of toxicity showing chlorhexidine to be the least toxic antiseptic agent.

When the antimicrobial activity of similar concentrations of chlorhexidine and sodium hypochlorite was compared in vitro, both compounds were found to be equally effective as antibacterial agents (Vahdaty et al. 1993). The authors commented on the relative lack of toxicity of chlorhexidine and suggested that it may be a useful substitute in those patients who are allergic to
suggests that at least 1 day is required before full antimicrobial effect is produced (Safavi et al. 1990). Pure calcium hydroxide paste causes a complete inactivation of various types of microorganisms within 12–72 h dependent on the bacterial strain (Stuart et al. 1991, Estrela et al. 1998). In vivo studies showed that a 3-month, 1-month and 7-day intracanal dressing of calcium hydroxide following chemomechanical debridement efficiently eliminated most bacteria from infected root canals (Cvek et al. 1976b, Byström et al. 1985, Sjögren et al. 1991). Calcium hydroxide also hydrolyses the lipid moiety of Gram-negative bacterial lipopolysaccharides (LPS) (Safavi & Nichols 1993, Safavi & Nichols 1994) and has been shown to eliminate LPS ability to stimulate TNF-α production in peripheral blood monocytes (Barthel et al. 1997), thereby possibly reducing the local inflammatory response.

Calcium hydroxide introduced into the periapical region appears to be well tolerated and is subsequently resorbed (Martin & Crabb 1977). However, the periapical response to calcium hydroxide based on results from previous studies seems to be equivocal. Although an inflammatory response with inhibited bone healing was observed 2 weeks after the implantation of calcium hydroxide into guinea-pig bone, it was found to be one of the least irritating root filling materials and was replaced by new bone within 12 weeks after placement (Spängberg 1969). Supporting these findings, Cvek (1972) observed apical root closure and bone healing following intracanal placement of calcium hydroxide in 50 of 55 maxillary incisors with immature roots. Furthermore, Binnie & Rowe (1973) dressed immature premolars in dogs with calcium hydroxide and distilled water and observed a minimal inflammatory response in the periapical tissues with continued root formation.

Calcium hydroxide has been reported to have a detrimental effect on periodontal tissues when used as an intracanal medicament during routine endodontic therapy. Blomlöf et al. (1988) observed that calcium hydroxide could negatively influence marginal soft tissue healing and suggested the completion of endodontic therapy prior to the removal of cementum as might occur during periodontal therapy. Breault et al. (1995) reported that the use of calcium hydroxide demonstrated an increased but not statistical significant inhibition of attached human gingival fibroblasts and proposed that calcium hydroxide 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 calcium hydroxide 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 calcium hydroxide was not hindered 6 months after experimental periodontal surgical injury in dogs.

**Antibiotics and anti-inflammatory drugs**

Ledermix (Lederle™ Pharmaceuticals, Wolfrathausen, Germany) setting cement is used as a base or liner, whilst nonsetting Ledermix paste is used as an endodontic intracanal medicament. Ledermix is a therapeutic agent with two active components: a corticosteroid; triamcinolone (1%) and a bacteriostatic broad-spectrum antibiotic; demethylichlortetracycline (3.21% demeclocycline). Originally the manufacturer (Lederle™) intended the corticosteroid to be the active ingredient, the antibiotic was added to prevent overgrowth of microorganisms following the impairment of the immune defence by the corticosteroid and not for disinfection of the root canal. The choice of antibiotic was based on the availability of the manufacturer’s brand of tetracycline and not on its effectiveness in eliminating intraradicular bacteria (unpublished data).

In a study by Barker & Lockett (1971), Ledermix was ineffective in eliminating Streptococcus viridans in root canals of dogs. Abbott et al. (1988) investigated the distance and concentration of infiltration of Ledermix in dentinal tubules of teeth dressed with the medicament by using absorption spectrophotometry. They estimated that demeclocycline attained its highest concentration in the dentine adjacent to the root canal within the first day of application with the initial rate of release about 10 times that of the release rate after 1 week. A similar phenomenon was observed in the peripheral dentine. These results suggest that demeclocycline may be effective against bacteria within the first few days after Ledermix placement but the effect would not be of long duration. It has been reported that Gram-positive microorganisms are more susceptible to lower concentrations of tetracycline than are Gram-negative ones (Heling & Pecht 1991). As Gram-negative species dominate established endodontic infections (Sundqvist 1994) the efficacy of Ledermix in an endodontic application may thus be questionable.

Ledermix has been found to be safe to periapical tissues by Barker & Lockett (1972), who observed a normal histological appearance of the periapical tissues 3 months after the application of Ledermix in dog root canals. Contrary to their findings Tepel et al. (1994) found an infiltrate of inflammatory cells in the periapical tissue after the use of Ledermix in rats with experimentally induced apical periodontitis. The periapical lesions in this study were also notably worse after root-canal dressing with Ledermix than in untreated teeth with periapical periodontitis, therefore suggesting that a combination of a corticosteroid and an antibiotic appear to impair healing.

In an in vitro study by Taylor et al. (1989), Ledermix was found to kill mouse fibroblasts at a concentration of \(10^{-3}\) mg mL\(^{-1}\) and above. It killed S. mutans at about the same concentration at which it killed the mammalian cells, but required a 1000-fold greater concentration to kill Lactobacillus casei. The other active component of Ledermix, triamcinolone, has been reported to be approximately five times more potent in suppressing inflammation than cortisol on an effect/weight basis (Fauci et al. 1976). Shortly after the introduction of Ledermix, opposition arose to its use due to a perceived risk of systemic side-effects to the corticosteroid (Klotz et al. 1965). De Deus & Han (1967) reported that hydrocortisone applied directly to the dental pulp in hamsters could be detected in other organ tissues within 2 min. Seltzer (1988) expressed concern that the intracanal use of corticosteroids, which have an effect on inflammatory cells and protein synthesis, may interfere with phagocytosis with resultant impaired and delayed tissue repair.

Abbott (1992) calculated the highest possible amount of Ledermix that can be used as an intracanal dressing, analysed the release and diffusion characteristics of Ledermix, and together with comparisons with known endogenous levels of corticosteroids, suggested that the intradental use of Ledermix paste and Ledermix cement is unlikely to result in any systemic side-effects.

*In vitro* and *in vivo* tests showed that Ledermix inactivates clastic cells associated with root resorption and that the inhibitory effect on clastic cells was due to the action of triamcinolone (Suda et al. 1983, Hammarström et al. 1986, Pierce & Lindskog 1987, Pierce et al. 1988). This property may be of use in inhibiting inflammatory root resorption subsequent to dental injuries (Trope 2002).

**Phenol and phenol-derivatives**

Phenols or phenol-derivatives, such as para-monochlorophenol and cresol, used to be the most commonly used inter-appointment intracanal medicaments. They were often mixed with camphor to form camphorated solutions to release phenol at a slower rate and make these compounds somewhat less caustic. Cresol is often mixed
with formaldehyde to give formocresol. All of these compounds coagulate cell contents indiscriminately and will cause tissue necrosis on contact. These compounds have been proven to be tissue irritating and highly toxic (Engström & Spångberg 1969, Spångberg et al. 1973, Spångberg et al. 1979) and to have limited antimicrobial effectiveness (Byström et al. 1985). The combination of high toxicity and limited clinical effectiveness exclude the phenol-based compounds from the recommended list of contemporary intracanal antibacterial medicaments. It is, however, still frequently used, at very low concentrations (1 : 5 dilution of Buckley’s formula containing 19% formaldehyde and 35% cresol) (King et al. 2002), during pulpotomy procedures in children.

**Conclusion**

Research shows that intracanal drugs and substances can have deleterious effects on vital tissue. Although these substances are meant to only contact nonvital dentine during use, they often come into contact with the periapical tissues. It is thus important to consider biocompatibility when choosing an endodontic irrigant or intracanal medicament.

**References**


© 2003 Blackwell Publishing Ltd


