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Issues Of Biocompatibility Associated With Commonly Used Endodontic Irrigants: A Review

[1] Shweta Verma (MDS)
[2] Munish Goel (MDS)
[3] Shikha Bala (MDS)
[4] Mahender Singh (BDS)

[1] Senior Lecturer
[2] Professor and Head
[3] Associate Professor
Dept. of Conservative Dentistry & Endodontics
Himachal Dental College, Sundernagar.
[4] Medical Officer
FRU, Baldwara, Mandi, Himachal Pradesh.

Address For Correspondence:
Dr. Shweta Verma,
Deptt. of Conservative Dentistry & Endodontics,
Himachal Dental College, Sundernagar, H.P.
Phone No. 94184-76681
E-mail : drshweta.verma83@yahoo.com
Fax no. 01907-226267

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Abstract
The root canal anatomy always presents itself with surprises. It is highly variable and full of complexities and for successful outcome it needs to be respected in each and every step of treatment. The step of biomechanical preparation involves cleaning and shaping of the root canal, thereby emphasising that even with best of instruments and techniques there remain areas which cannot be prepared mechanically and have to be only cleaned using irrigants. Since these irrigants often come in contact with living tissues their biocompatibility is of utmost concern. This article presents an elaborated review of biological effects of irrigants routinely used in endodontics.

Key Words
irrigants, cytotoxicity and biocompatibility.

Introduction

The use of Endodontic materials goes around saving the pulp vitality, disinfecting the pulp and then finally filling the root canal three dimensionally (solid materials and sealers) to prevent re-infection. The concern while using wide variety of materials in endodontics is their biological effects. 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). This definition implies the interaction between a host, a material and an expected function of a material.

Adverse reactions to various materials are assessed in terms of genotoxicity, mutagenicity, carcinogenicity, cytotoxicity, histocompatibility or microbial effects, thus making it impossible to say a material safe on basis of one single test. 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); do not reflect the application of a material clinical situation.
2. Specific toxicity (usage tests, e.g. in subhuman primates).
3. Clinical testing in humans.

Permanent cell lines, e.g. HeLa, 3T3 or L929 cells and primary/diploid human cells, are used for cell culture tests. Primary cells are considered to be more relevant for biocompatibility studies than permanent cultures [1,2]. They are assessed for growth inhibition, determination of the elective dose 50 (ED50), membrane integrity, DNA, RNA or protein synthesis and/or the determination of alterations of cellular morphology by light or electron microscopy [1,2,3,4,5].

Since the microorganisms may persist even after the successful endodontic treatment, it would be best if an endodontic material has biocompatible as well as antibacterial properties. Antimicrobial activity of materials to endodontic pathogens is normally measured using simple tests, e.g. agar diffusion and agar dilution tests. Endodontic materials with strong antibacterial activity have frequently been found to induce strong adverse effects during and after treatment and were also found to be cytotoxic and even mutagenic[6].

In vivo nonspecific tissue reactions caused by endodontic materials are normally investigated by histological studies following the implantation of the test material into various tissues of animals either directly injected or implanted within Teflon, silicone or polyethylene tubes into various tissues, such as the subcutaneous connective tissue, muscle or bone of rats, rabbits, guinea pigs, hamsters and ferrets [7,8,9,10,11,12,13].

Specific in vivo toxicity tests involve the use of the test material for root-canal therapy in animals, predominantly dogs [14,15,16,17] or monkeys. Due to ethical considerations these tests are rarely performed in humans.[18].

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.

One of the goals of root canal treatment is to eliminate bacteria, bacterial products and debris from the root canal system. Most bacteria found in the canal space may be removed by the mechanical action of endodontic instruments. However, in several situations, due to the

complex anatomy of the root canal system, organic residues and bacteria lodged deep inside the dentinal tubules cannot be reached even after careful mechanical instrumentation. In these cases, the use of irrigating solutions is essential to ensure bacterial minimization and elimination of organic tissue remnants. Numerous products are currently used as endodontic irrigants, such as sodium hypochlorite (NaOCl), chlorhexidine gluconate, calcium hydroxide and saline. It is highly desirable that the chemical agents selected as endodontic irrigants possess favorable properties, such as antimicrobial activity and dissolution of organic tissues, assist in root canal system debridement and induce a favorable reaction in the periapical tissues.

Because root canal irrigants and medications can come in contact with periradicular tissues, in addition to having good antibacterial ability, they also should be biocompatible. The following is the detail account of biocompatibility issues of routinely used endodontic irrigants.

Sodium Hypochlorite

Sodium hypochlorite is an effective and most commonly used irrigation fluid in endodontics with anti-microbial effects[19] and some tissue-dissolving properties [20,21]. The antimicrobial efficacy of the solution is due to its ability to oxidize and hydrolyze cell proteins and, to some extent, osmotically draw fluids out of cells due to its hyper tonicity [22].

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. 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.

It has been found to be highly toxic when used in higher concentrations. In a study demonstrating the cytotoxicity of NaOCl using three independent biological models, it was found that a concentration as low as 1:1000 NaOCl in saline caused complete haemolysis of red blood cells in vitro[22]. Undiluted and 1 : 10 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. It has been proved that Dakin's solution is detrimental to neutrophil chemotaxis and toxic to fibroblasts and endothelial cells [23]. A study examined wound healing relative to irrigation and bactericidal properties of NaOCl in in-vitro and in vivo models. It was concluded that 0.025% NaOCl was the safest concentration to use because it was bactericidal but not tissue-toxic[24]. 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 [25,26]. 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 it has optimal antimicrobial effectiveness with minimal tissue irritating effect [27].

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 [28,29].

Hypersensitivity reactions to sodium hypochlorite have also been reported [30,31,32]. A great deal of care should therefore be exercised when using sodium hypochlorite during endodontic irrigation. **Ehrich et al. (1993)[19]** 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.

Ethylene Diamine Tetra-acetic Acid

The disodium salt of ethylene diamine tetra-acetic acid (EDTA) is generally used as the most effective chelating agent to enhance the chemo-mechanical enlargement of canals, to remove smear layer [33] and to clean and aid in disinfecting the dentinal walls [34].

In a study which 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, it was found that both neutral and alkaline EDTA showed moderate-to-severe cytotoxicity in a concentration dependent manner[35]. 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 [36,37]. 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 [34,38,39,40].

Chlorhexidine

Chlorhexidine is a broad-spectrum antimicrobial agent that has been shown to be active against vegetative bacteria and mycobacteria. It has moderate activity against fungi and viruses, and inhibits spore germination. It has been shown to be most effective against gram-positive cocci, while less active against gram-positive and gram-negative rods. The antibacterial efficacy of chlorhexidine is comparable with that of NaOCl and it is effective against strains of bacteria re-sistant to calcium hydroxide, such as gram-positive *Enterococcus faecalis*. The concentration often used in endodontic therapy is 2% chlorhexidine.

In several studies, chlorhexidine as an irrigant has been shown to lower the number of post irrigation positive bacterial cultures, as well as the number

of colony-forming units remaining in positive cultures. Because of its cationic properties, chlorhexidine can bind to surfaces covered with acidic proteins, such as the hydroxyapatite component of dentin, and be released at therapeutic levels, a phenomenon known as substantivity. This can occur in 48 hours to 72 hours after instrumentation.

One of the often cited reasons for using chlorhexidine as a canal irrigant is its perceived minimal toxicity to host tissues. While chlorhexidine does not appear to cause any long-term damage to host tissues, it still may cause an inflammatory response in these tissues if expressed beyond the root canal. In a study 0.12% chlorhexidine was injected into the subcutaneous tissues of the backs of guinea pigs to help assess short-term toxic effects. After histologic examination, they found a mild inflammatory response after 2 hours, moderate inflammatory response after 2 days, and foreign body granuloma formation at 2 weeks, which resolved over time. However, this study was performed using a lower concentration of chlorhexidine than is often used in endodontic therapy. To help evaluate the inflammatory response of 2% chlorhexidine, in a separate study 0.5% NaOCl, 2% chlorhexidine digluconate, and phosphate-buffered saline were injected into the peritoneal cavity of mice. This study found that the number of inflammatory cells resulting from 2% chlorhexidine injection was similar to the phosphate-buffered saline control at all times tested, while the 0.5% NaOCl injection resulted in a significantly larger number of inflammatory cells. The researchers concluded that 2% chlorhexidine was biocompatible. It was also reported that, chlorhexidine in concentrations of 0.5% and 1% induced large foci of coagulative necrosis associated with an inflammatory infiltrate mainly composed of neutrophils and mononuclear cells, along with interstitial dermal and subcutaneous edema. The concentration of 0.25% chlorhexidine caused only small foci of tissue necrosis, while 0.125% chlorhexidine appeared to cause no necrosis. This study also examined the effect of chlorhexidine on tissue healing by testing different concentrations of chlorhexidine on cultured L929 fibroblasts. They found that at lower concentrations, chlorhexidine induced apoptosis of the fibroblasts and, at higher concentrations, induced necrosis and increased expression of heat-shock protein 70, an indicator of cellular stress. These findings seem to indicate that a 2% concentration of chlorhexidine, as commonly used in endodontic therapy, may have toxic effects on host tissues if expressed beyond the confines of the root canal and may impair healing [41].

Another toxicity concern with the use of chlorhexidine is the formation of para-chloranaline (PCA), which is an aromatic amine. When studied in rats, rabbits, and cats, the primary toxic effect was methemogloblin formation. In humans, accidental occupational exposure to PCA produced symptoms of increased methemoglobin and sulfhaemoglobin levels, cyanosis, the development of anemia, and systemic changes from anoxia. While chlorhexidine may spontaneously hydrolyze to PCA over time, it undergoes a chemical reaction when combined with NaOCl and forms a precipitate that contains PCA. Water or alcohol can be used as an irrigant to flush NaOCl from the canal before chlorhexidine is used, thus minimizing PCA formation. Ethylenedia minetetraacetic acid (EDTA) may also be an appropriate substance to flush the remaining NaOCl out of a canal, as the combination of chlorhexidine and EDTA does not result in a chemical reaction. The white precipitate that is formed from the combination of EDTA and chlorhexidine has been shown to be a salt containing no PCA.

It is important to note that various symptoms of immediate hypersensitivity, including anaphylactic reactions, have been reported after topical treatment with chlorhexidine [42, 43]. The toxic potency of chlorhexidine is dependent on the length of exposure and the composition of the exposure medium. It has been found that chlorhexidine rapidly disrupts the cell membrane of both crevicular and peripheral blood neutrophils at concentrations above 0.005% within 5 min, indicating that its inhibitory effect on neutrophil function is mostly due to its lytic properties [44].

MTAD

It is an endodontic irrigant, which represents an innovative approach in simultaneous removal of endodontic smear layer and complete disinfection of root canals. It has been shown to be clinically effective and biocompatible, with potential antibacterial substantivity. It is available as powder-Liquid system. Part A is liquid and is supplied in syringes (5ml, 20ml-single, multiple doses). It contains 4.25% citric acid and 0.5% polysorbate 80 detergent (Tween 80). Its low pH 2.15 contributes to its role as a calcium chelator, thereby causing root surface demineralization and thus helps in the removal of smear layer.

Tween 80 (polyoxyethylene sorbitan monooleate), is a detergent present in MTAD and is a non-ionic surfactant, it helps in reducing the surface tension of distilled water, NaOCl and EDTA, thereby enhancing the flow and penetration of irrigating solutions like MTAD deeper into the dentinal tubules. It has a pH of 7.0 and is a biologically acceptable material.

Part B is powder supplied in bottles (single, multiple doses-150mgs, 600mgs). It contains Doxycycline hyclate which is a broad spectrum antibiotic effective against a wide range of microorganisms. It is bacteriostatic and shows the property of substantivity and anticollagenase activity.

There was a study which evaluated eugenol, 3% hydrogen peroxide, 5.25% NaOCl, REDTA Aqueous Irrigant, Peridex, Pulpdent paste, and MTAD for their cytotxicity. Four concentrations of NaOCl (5.25%, 2.63%, 1.31% and 0.66%) were evaluated for cytotoxicity. Each experiment was conducted using six cultures for each group. L929 fibroblasts were grown on cell culture plates and were placed in contact with various concentrations of test irrigants and medications. The cytotoxicity of these materials was evaluated 24 hours after incubation using MTT assay[45].

Consequently, the MTT method is considered a sensitive index to evaluate the cytotoxicity of dental materials. The advantage of this method is simplicity, rapidity, and repeatability, and it does not require radioisotopes. MTT is a water-soluble, tetrazolium salt yielding a yellowish solution when prepared in media or salt solution. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes in living cells. The test results reflect not only the

cell number but also the cell metabolic level.

Based on the test results, it seems that MTAD is less cytotoxic than eugenol, 3% H2O2, Ca(OH)2 paste, 5.25% NaOCl, Peridex, and EDTA. The results show MTAD is more cytotoxic than 2.63%, 1.31%, and 0.66% NaOCl. Eugenol is almost 100 times more toxic than 5.25% NaOCl. It also is more cytotoxic than MTAD, and 3% H2O2 is almost 50 times more toxic than MTAD.

Clinical studies are in progress to determine the safety and efficacy of MTAD as a final irrigant to remove the smear layer and disinfect root canals.

Conclusion

The question of whether results from in vitro experiments can be applied to the clinical situation remains to be investigated. There is evidence that in vitro methods adequately measure cytotoxicity and therefore could reasonably be used as a screening tool to evaluate biocompatibility of test materials.

Research shows that intracanal drugs and irrigation 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.

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