Review Article

Effects of Root Canal Irrigants and Medicaments on Dentin and Vice Versa: A Review of Literature

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Received 23 August 2019 and Accepted 14 December 2019

Abstract

Microorganisms are very important in the initiation of pulpo-periapical pathosis. Due to the complexity of the root canal system, the mechanical instrumentation of the root canal system should be supplemented with proper canal irrigants and medicaments. It has been revealed that due to the interactions of canal irrigants and medicaments with dentin and contents of the canal system, the antimicrobial effect of canal irrigants and medicaments in canal (*ex vivo*) are different from that *in vitro*. Furthermore, root canal irrigants may decrease the fracture resistance of dentin. The purpose of this paper was to review the data on the interactions between root canal irrigants/medicaments and dentin/root canal contents.

Keywords: Calcium hydroxide, Chlorhexidine, Dentin, EDTA, Iodine compounds, MTA, MTAD, NaOCl

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Mohammadi Z, Kinoshita J, Manabe A, Kobayashi M, Shalavi S, Palazzi F, Yaripour S, Jafarzadeh H. Effects of Root Canal Irrigants and Medicaments on Dentin and Vice Versa: A Review of Literature. J Dent Mater Tech 2020; 9(1): 221. 229

Introduction

Microorganisms play an important role in initiation of pulpo-periapical pathosis (1-3). Elimination of microorganisms from an infected root canal system (RCS) is a complicated stage of endodontic treatment. Numerous measures such as usage of different mechanical instrumentation techniques, irrigation solutions, and intra-canal medicaments have been proposed to decrease the number of microorganisms from the RCS. Mechanical preparation alone cannot predictably result in bacteria-free RCS, which is not surprising given the complexity of the RCS. On the contrary, there is both clinical and *in vitro* documents indicating that mechanical preparation may leave significant areas of the RCS untouched (4-6). Any pulp tissue left in the RCS may serve as a nutrient source for microorganisms. This tissue may be digested by the bacteria up to two months, depending on whether the RCS is open to the oral environment or not (7). Furthermore, tissue remnants may impede the antibacterial effects of irrigants/medicaments. Therefore, some forms of medicaments and irrigants may be necessary to remove debris and tissue from the RCS and kill the remaining microbes. The aim of the present review was to collect data on the effects of root canal irrigation solutions and intra-canal medicaments on dentin by reviewing the related articles.

Dentin Chemistry

As the main hard tissue of the tooth structure, dentin consists of dentinal tubules surrounded by highly mineralized peri-tubular and inter-tubular dentin. Depending on the tooth location and age, its composition may show some variations. Furthermore, external irritation, such as caries, may affect dentin composition (8). Amongst the hard tissues of the tooth, dentin is chemically the closest tissue to the bone (9). Its inorganic components are composed of calcium phosphate compounds, mostly apatite. In addition, small amounts of potassium, sodium, magnesium, carbonate, chloride, and fluoride may be found in dentin (10). Type I collagen is its major organic component, although small amounts of type III and V have been found, too (9, 11). Type I collagen has higher proportions of hydroxylysine in dentin (11). As a result, it has more intra-molecular/intermolecular cross-links than type I collagen in bone. Cross-links increase the structural stability and strength of dentin collagen. They also contribute to making dentin collagen relatively insoluble in acid as compared with collagen from other sources. Consequently, acid etch of dentin removes the great part of mineral phase, whereas the collagen may remain almost intact (9, 11).

Inorganic component of dentin (Apatite)

Non-homogeneous apatite form most of the mineral content of dentin. Based on spectroscopy analysis, LeGeros (12) showed that the calcium content of dentin apatite is less than that of enamel, whereas the amount of carbonated apatite was slightly more in dentin than in enamel (13). Furthermore, according to Tsuda et al. (14), the concentration of HPO_{42-} is higher and the concentration of OH- is lower in dentin, comparing the enamel. Hydroxyapatite is surrounded by a layer of adsorbed ions and water. The hydration layer and exchange/adsorption of ions allow changes in the chemical microenvironment, reflecting pH and interaction with chemical compounds (10).

Buffering effect of dentin

Bone apatite is a major carbonate reservoir, and so providing buffering for all acid-base disturbances and maintaining its balance in the body (15-17). Considering a quite similar chemical composition between the bone and dentin, it can be expected that dentin can possess a corresponding buffering effect on bases and acids. According to Wang and Hume (18), the buffering efficacy of dentin against alkali is weaker but considerable. Dentin chips are able to keep the pH unchanged after the addition of NaOH or HCl. Inorganic apatite is the main responsible for the buffering effect of dentin. However, other inorganic and even organic components may also contribute to the buffering effect. (15, 17, 18).

Sodium hypochlorite (NaOCl)

NaOCl acts as a solvent for organic and fatty acids, by transforming them into fatty acid salts and glycerol reducing the surface tension of the solution (16). It neutralizes amino-acids forming water and salt. With the exit of hydroxyl ions, there is a reduction in pH. In contact with organic tissues, hypochlorous acid which is present in NaOCl solution, acts as a solvent and releases chlorine, which combined with the protein amino group, forms chloramines that interfere in cell metabolism. Hypochlorous acid and hypochlorite ions lead to amino-acid hydrolysis and degradation (19). As a strong oxidant, chlorine presents antibacterial actions inhibiting bacterial enzymes, thereby leading to the irreversible oxidation of sulphydryl group of bacterial enzymes (19, 20). Considering the physic-chemical properties of NaOCl when in contact with tissues, these reactions may be verified. Its antibacterial mechanism of action can verify its physic-chemical characteristics and reaction with tissues (16).

The antibacterial effectiveness of NaOCl, based on its pH is similar to the mechanism of calcium hydroxide (CH) (20). The high pH of NaOCl interferes in the integrity of cytoplasmic membrane with enzymatic inhibition, and phospholipid degradation. The amino-acid chloramination reaction forming chloramines interferes with cell metabolism. This enzyme inactivation may be observed in the reaction of chlorine with amino groups and oxidation of sulphydryl groups of bacterial enzymes (19, 20). Therefore, NaOCl presents antibacterial activity with acting on microbial enzymatic sites promoting inactivation originated by chloramination action. Dissolution of organic tissues can be verified in the saponification reaction when NaOCl degrades lipids (20).

Buffering effect of dentin on the antibacterial activity of NaOCl

The organic components of dentine alone accounted for 1.5% of the total buffering capacity (21, 22). The RCS milieu is mixture of a variety of inorganic and organic compounds. Hydroxyapatite which is the principal part of dentine is the major representative of the inorganic base. Difficulty in designing studies rendering reliable data is a great challenge for many years. Ultimately, Haapasalo et al. (23) introduced a new dentine powder for investigating the inhibitory effect of dentine on RCS irrigants and also medicaments. NaOCl which is a non-specific oxidizer, with amino-acids by chloramination and neutralization reactions, leading to amino-acids degradation (19, 20). An immuno-histochemical research showed that type I collagen and glycosaminoglycan lost their immune-reactivity after NaOCl usage when a dematerialized dentine was used (24). However, in intact dentine model, this was minimal, proposing that hydroxyapatite has a protective role by embedding

proteins such as collagen against the oxidation of NaOCl. Haapasalo et al. (23) have showed the inhibitory effect of dentine on the effectiveness of 1% NaOCl against *Enterococcus faecalis*. They also showed that killing all of the microorganisms required 24 hours of incubation with hypochlorite. However, after one hour of incubation, all of the microorganisms were still viable. It seems that dentine decreases or inhibits the antimicrobial effect of NaOCl.

Effect of NaOCl on the composition and structure of dentine

The impact of NaOCl on the dentine matrix is one of its side effects. Dentine is composed of 22% organic component specially type I collagen, which contributes to dentine mechanical properties. It can fragment long peptide chains and chlorinate protein terminal groups (25). Consequently, it affects dentine mechanical properties by the degradation of organic dentine materials (26). It has been shown that concentrated hypochlorite solutions may cause untoward effects on dentine biomechanical properties (27). A 2-hour dentine exposure to ≥3% NaOCl can reduces the elastic modulus and flexural strength of dentine (28, 29). Mountouris et al. (30) found that both 5% NaOCl solution and acid-etched coronal dentine surfaces reduced the organic matrix but did not affect carbonates and phosphates. Di Renzo et al. (31) showed that treatment with NaOCl using a photo-acoustic FTIRS technique, induced a slow and heterogeneous removal of its organic phase, leaving calcium hydroxyapatite and carbonate apatite unchanged. Another study showed that 5% NaOCl induced alterations in dentine collagen, whereas hydroxyapatite showed a protective role in stability of organic matrix. Marending et al. (26) found that NaOCl caused a concentration-dependent reduction of flexural strength and elastic modulus in dentine. The carbon and nitrogen content were significantly decreased.

Chlorhexidine (CHX)

Structure and mechanism of action

CHX is a synthetic cationic bis-biguanide. It is a positively charged hydrophobic/lipophilic molecule interacts with phospholipids on the bacterial cell membrane (32, 33). Its efficacy is due to the interaction of the positive charge of the molecule and the negatively charged phosphate groups on bacterial cell walls (34, 35). This enhances the cell wall permeability, which allows the CHX molecule to penetrate into the microorganism. The most common oral form of CHX is CHX gluconate which is water-soluble that dissociates and releases the positively charged CHX component at physiologic pH (32, 33, 36). At low concentration (0.2%), potassium and phosphorous can leak out of the cell (35).

Effect of CHX on dentin

CHX significantly decreases the micro-hardness of root dentin at 500 and 1000 µm from the pulpo-dentinal junction (37). Also, it has been shown that 2% CHX gel cannot adversely affect dentin micro-hardness when associated with the bleaching agents (38). Ari et al. (39) showed that all widely used irrigation solutions except for 0.2% CHX, significantly reduced the micro-hardness of root dentin. In this study, 3% H_2O_2 and 0.2% CHX gluconate had no effect on root dentin roughness. According to the results of this study, 0.2% CHX gluconate may be an appropriate irrigation solution in endodontic treatment because of its weak effect on the micro-hardness and roughness of dentin. In another study, 0.2% CHX was reported to have no great effect on dentin micro-hardness (40). Aslantas et al. (41) also studied the effects of endodontic irrigants on dentin micro-hardness in presence of surface-modifying agents (17% ethylenediaminetetraacetic acid (EDTA), REDTA, 2% CHX, 2% CHX with surface modifiers (CHX-Plus), 6% NaOCl, or 6% NaOCl with surface modifiers (Chlor-XTRA). They showed that EDTA, REDTA, NaOCl, and Chlor-XTRA significantly decreased dentin micro-hardness. Marcelino et al. (42) concluded that dentin micro-hardness was decreased after exposure to CHX, NaOCl, phosphoric acid, and sodium ascorbate. However, dentin flexural strength was not affected by the chemical agents. Kara Tuncer et al. (43) also showed that maleic acid significantly reduced dentin micro-hardness compared to EDTA+CHX, EDTA+NaOCl, QMix. Das et al. (44) showed that NaOCl+Q Mix were least detrimental to dentin micro-hardness comparing *Morinda citrifolia* juice and conventional irrigation solutions.

Modulating effect of dentine on CHX

Portenier et al. (45) evaluated the inhibition of the antibacterial effect of saturated CH solution, CHX acetate, and IKI by dentine, hydroxyapatite, and bovine serum albumin. They concluded that 0.05% CHX was inhibited by bovine serum albumin and slowed down by dentine. However, hydroxyapatite had little inhibitory effect on CHX. They also showed that inorganic hydroxyapatite had little or no inhibitory effect against CHX comparing dentine, whereas bovine serum albumin was the strongest inhibitor of CHX. Portenier et al. (46) also concluded that dentine pre-treated by citric acid or EDTA showed only slight inhibition dentine matrix, whereas killed microbial cells were the most effective inhibitors of CHX. Another study demonstrated that the presence of dentine or bovine serum albumin caused delay in killing of *Enterococcus faecalis* by CHX (47). The inhibitory effect of bovine serum albumin on antimicrobial effect of CHX was confirmed by Sassone et al. (48). It seems that dentine and its components (hydroxyapatite and collagen), killed microorganisms, and inflammatory exudates in the RCS can reduce or inhibit the antimicrobial activity of irrigants (such as CHX) and medicaments.

MTAD

BioPure (MTAD) (Dentsply, Tulsa Dental, Tulsa, OK, USA), introduced by Torabinejad and Johnson, is a mixture of 3% doxycycline, 4.25% citric acid, and a detergent (Polysorbate 80) (49).

MTAD can remove the smear layer (49) and affect *Enterococcus faecalis* (50-52). Shabahang et al. (51) compared the antimicrobial effect of a combination of 1.3% NaOCl as an irrigant and MTAD as a final rinse with that of 5.25% NaOCl. They concluded that the first combination is more effective in RCS disinfection than using 5.25% NaOCl alone. However, Tay et al. (53) found that when MTAD was applied to 1.3% NaOCl-irrigated dentine, its antibacterial substantivity was decreased.

Effect of MTAD on dentin

Machnick et al. (54) showed no significant difference in modulus of elasticity and flexural strength between dentin exposed to saline and MTAD. Dineshkumar et al. (55) evaluated the effect of 17% EDTA, MTAD, and 18% HEBP on dentin micro-hardness. MTAD showed the highest effect on dentin micro-hardness. On the other hand, Ulusoy and Gurgol (56) demonstrated that MTAD had the least effect on dentin micro-hardness. In another study, MTAD significantly reduced dentin micro-hardness (57).

Effect of dentin on MTAD

A recent study confirmed earlier findings about the inhibition of CHX activity by dentin. They also showed a corresponding inhibition of MTAD by dentin (58). Although MTAD killed *Enterococcus faecalis* within 5 minutes, the addition of dentin slowed down the killing process (58).

Iodine compounds

Iodine is bactericidal, virucidal, fungicidal, tuberculocidal, and sporicidal by attack the proteins. Aqueous iodine solutions are unstable, with molecular iodine being responsible for the antimicrobial activity. Iodophors are complexes of iodine and a solubilizing agent or carrier (59).

Buffering effect of dentin

The presence of dentin is responsible for inhibitory patterns on the activity of iodine solutions. Haapasalo et al. (34) demonstrated that dentin powder effectively abolished the effect of 0.2% IKI 0.4%, it took only 5 minutes to kill *Enterococcus faecalis*. Portenier et al. (45) showed that hydroxyapatite caused little or no inhibition, whilst the dentinal collagen matrix effectively inhibited 0.1% IKI 0.2%.

EDTA

EDTA which is a colorless, water-soluble solid is widely used as a chelating agent. It binds to metals through 4 carboxylate groups and 2 amine groups. It forms especially strong complexes with Mn, Cu, Fe, and Co. It is mostly synthesized from ethylenediamine, formaldehyde, sodium cyanide, and water. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity (60-62).

Effects on dentine micro-hardness

Chelators can decrease dentine micro-hardness, whereby the greatest differences are to be found in dentine immediately adjacent to the canal (63). The effect of the chelator is already apparent after 5 minutes. Cruz-Filho et al. (64) showed that EDTA and citric acid had the greatest effect on dentin micro-hardness. In another study, Ballal et al. (65) found no significant difference between EDTA and maleic acid in reduction of dentine micro-hardness.

Eldeniz et al. (66) assessed the effect of EDTA and citric acid on dentine micro-hardness and roughness and showed a significant difference in micro-hardness among the test groups, citric acid group being the least hard. In addition, Ari et al. (67) and Cruz-Filho et al. (68) confirmed the decrease of dentine micro-hardness after using EDTA. De-Deus et al. (69) assessed the effect of EDTA, EDTAC, and citric acid on dentine micro-hardness and found that micro-hardness decreased with the increasing time of the application of chelators.

CH

CH was originally introduced as a pulp capping agent (70, 71). It has low solubility in water, which decreases by temperature rise (71). The dissociation coefficient of CH permits a slow release of both hydroxyl ions and calcium. The low solubility is a suitable characteristic because a long period is necessary for CH to become soluble in tissue fluids when in contact with vital tissues (70, 72).

Buffering effect of dentine on CH

Haapasalo et al. (23) showed that dentine powder effectively abolished killing of *Enterococcus faecalis* by CH. On the other hand, in absence of dentine, saturated CH killed *Enterococcus faecalis* in a few minutes. Portenier et al. (45) demonstrated that hydroxyapatite had an effect similar to that of dentine on CH. The substantial effect of dentine on the antimicrobial effect of CH may be attributed to the buffering action of dentine against alkali (18). Buffering by dentine may be the main factor behind the decreased antimicrobial effect of CH. It is possible that outside the canal, CH is present at concentrations even below that level (23). Besides dentine, the remnants of necrotic pulp and inflammatory exudative fluid may affect the antimicrobial effect of disinfectants (8).

Effect of CH on dentine

Endodontic treatment of immature teeth with non-vital pulp is a great challenge. Apexification by CH may induce apical healing through induction of an apical barrier whilst at the same time; the high pH provides an antimicrobial activity. The flexural strength of dentine may depend on the link between the hydroxyapatite

crystals and the collagenous network. The organic matrix is composed of acid proteins and proteoglycans (73-75). These substances act as bonding agents between the hydroxyapatite crystals and collagen network (75). Rosenberg et al. (76) showed that the micro-tensile fracture strength of teeth and was decreased by almost 50% following 7-84 days. Another study showed this to be 32% (77). The results of another study indicated that the fracture strength of sheep dentine was decreased by 50% following CH treatment after one year (78). Kawamoto et al. (79) have also concluded that CH paste may significantly increase the elastic modulus of dentine. Grigoratos et al. (80) reported that CH may cause reduction in flexural strength of dentine. Andreasen et al. (75) concluded that the fracture strength of CH-filled immature teeth was halved in 1 year and attributed the frequent reports of the fractures of open apex teeth filled with CH to extended exposure periods. Doyon et al. (81) examined the resistance of dentine to CH and found that the fracture resistance of dentine was reduced after six months.

Mineral trioxide aggregate (MTA)

MTA is a mixture of refined Portland cement, bismuth oxide, and trace amounts of SiO_2, CaO, K_2SO_4, and Na_2SO_4 (82). MTA powder is mixed with supplied sterile water. Upon hydration, MTA materials form a colloidal gel that solidifies to a hard structure in approximately 3-4 hours, with moisture from the surrounding tissues (83). Hydrated MTA has an initial pH of 10.2, which rises to 12.5 about 3 hours after mixing (84). The compressive strength of MTA increases in the presence of moisture for up to 21 days, while MTA micro-hardness and hydration behavior adversely affected with exposure to the pH range of inflammatory condition as compared to physiologic environment (85).

MTA and susceptibility to root fracture

The most promising alternative to long-term CH therapy for induction of apexification is the use of MTA as an apical barrier or apical plug (83). However, the increase of root resistance to fracture remains a challenge (84, 85). Andreasen et al. (86) showed that when CH was kept in the canals of immature teeth for 1 month, followed by MTA filling, there was no significant reduction in the fracture strength within 100 days. Bortoluzzi et al. (87) showed that the combined use of MTA and metallic posts increased the resistance to the fracture. Hatibović-Kofman et al. (88) showed that fracture strength of teeth treated with CH or MTA decreased but not significantly over time. For the MTA-treated teeth, the fracture strengths were not significantly different between the untreated and CH-treated teeth after 15 days or 60 days. Tuna et al. (89) assessed the fracture resistance of immature teeth filled with BioAggregate (BA), MTA, and CH *in vitro* and showed that BA group exhibited the highest fracture resistance, while CH group showed the lowest amount of resistance. Aksel et al. (90) also showed that MTA increased the resistance of immature teeth to vertical fracture. Guven et al. (91) assessed fracture resistance of immature teeth filled with BA, MTA, and EndoSequence Root Repair Material. The results revealed that BA-filled teeth had higher fracture resistance than other groups after 24 months. Schmoldt et al. (92) showed that gutta-percha or MTA cannot increase the fracture resistance. Likewise, Sawyer et al. (93) showed that the flexural strength of dentin exposed to MTA Plus decreased after 3 months. Elnaghy and Elsaka (94) assessed the fracture resistance of immature teeth filled with BD and white MTA. They observed that after 1 year, there was no difference between these materials. The results of another study showed that one year after exposure to CH and MTA, the flexural strength of dentin reduced to 72% and 39%, respectively (95). Forghani et al. (96) showed that MTA increased the fracture resistance of dentin, while Portland cement had no effect on dentin fracture resistance.

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