



Research Article

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Diagnostic Efficacy of Sodium Hypochlorite Versus Chlorhexidine on the Microhardness of Root Canal Dentin: An In Vitro Study.

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Introduction

The primary objective of endodontic treatment is to achieve a clean environment in the root canal to minimize any risk which leads to unsuccessful treatment outcomes.[1] Irrigation is the most important aspect of the root canal preparation as it helps to clean the complex root canal system by supplementing the mechanical preparation of canals.[2] A number of irrigants have been used during the root canal preparation to minimize necrotic tissue, bacteria and residual debris, as well as smear layer formed during mechanical preparation of canals.[3]

Commonly used as root canal irrigants are proteolytic enzymes, alkaline solutions (sodium hypochlorite, sodium hydroxide, urea), acids (citric and phosphoric), chelating agents (ethylene diaminetetraacetic acid), oxidative agents (hydrogen peroxide), normal saline and local anesthetic solutions.[4]

Diakin firstly used 0.5% sodium hypochlorite solution for wound disinfection. In 1936, Alfred Walker introduced its application in dentistry as root canal irrigant.[5] NaOCl used in the concentration ranging from 0.5% to 6.15%.[6] It acts by releasing free chlorine which helps to dissolve the necrotic and vital tissue by breaking the proteins into amino acids.[7] It is inexpensive, easily available, has low viscosity and has a favorable shelf life.[8] It is well known for its action against biofilm by dissolving the bacterial cells and organic matrix.[9]

Chlorhexidine, another commonly used antimicrobial root canal irrigant known for its substantivity due to absorption and slow release from hydroxyapatite and biocompatibility. It was first developed in 1940s by Imperial Chemical Industries in England, marketed as an antiseptic in 1950s and first reviewed by Schroeder in 1969 for its activity against plaque inhibition.[10]. The efficacy of CHX is due to the interaction of positive charge on its molecule and negative charge on the phosphate groups of microbial cell walls which increases the permeability and causes leakage of the intracellular components of microorganisms.[11]] At low concentration (0.2%), it has bacteriostatic effect as low molecular weight substances such as phosphorous and potassium leaches out while at high concentration (2%), it precipitates the cytoplasmic contents resulting in cell death, hence bactericidal action.[12] Because of its biocompatibility and antimicrobial action, it is recommended in cases such as open apex, root perforation, root resorption and enlarged apical foramen.[13] Since, there is no single irrigant that has all the required properties to disinfect the root canal. So, different chemical solutions are used in combination to effectively remove organic as well as inorganic material from the infected canal.[14]

Human dentin is a unique structure composed of 70% inorganic component, 20% organic and 10% water. It constitutes the bulk of the tooth which helps to absorb the mechanical loads. The structure of dentin comprises of dentinal tubules filled with dentinal fluid and cytoplasmic processes, which is prone to biological and structural alterations. According to various studies, microorganisms are capable of invading the dentinal tubules as deep as 200-500µm. So, root canal irrigants should be able to penetrate the dentinal tubules to remove the bacteria and their byproducts.[15]

However, these chemical agents are capable of altering the proportion of organic and inorganic components of root canal dentin, changing the structural properties of dentin such as permeability, solubility and microhardness.[16]

The organic part of dentin is composed mainly of collagen fibers which plays vital role in the mechanical strength of dentin.[17] Sodium hypochlorite causes denaturation of collagen and oxidation of the of organic matrix which affects the mechanical properties of root dentin.[6]

Mircohardness is considered as the resistance to the local deformation and indirect evidence of mineral changes in root dentin. The change in hardness of dentin may adversely affect its adhesive properties, sealing ability and chances of causing root fracture by decreasing the strength of root.[12]

This study aims to evaluate the effect of 5% sodium hypochlorite and 2% chlorhexidine individually and in combination on the microhardness of root dentin.

Material and Method

The present in vitro study was conducted in the Department of Conservative Dentistry and Endodontics. 80 single rooted teeth were selected for the study (Figure1). The teeth were collected from the Department of Oral and Maxillofacial Surgery. Selected teeth were thoroughly cleaned using an ultrasonic scaler to remove the soft tissue debris or any calculus deposition and were stored in 10% formalin solution until utilization. The crowns of the specimens were decoronated transversally at the cement-enamel junction (CEJ) using diamond disc in high speed straight handpiece. (Figure 2)

The roots were then sectioned longitudinally with a diamond disc to obtain two equal halves and embedded horizontally in acrylic resin blocks, leaving the dentin surface exposed to facilitate manipulation. All the samples were then ground polished with sandpaper under distilled water to remove any surface scratches.

After preparation, all the teeth were randomly allocated into four groups, each containing 20 specimens according to the irrigant used. (Figure 3)

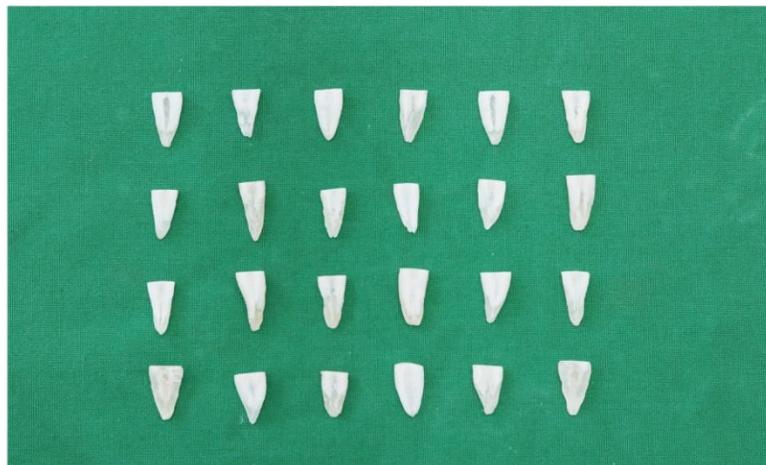
Group 1: Samples treated with 5% sodium hypochlorite for 5 minutes

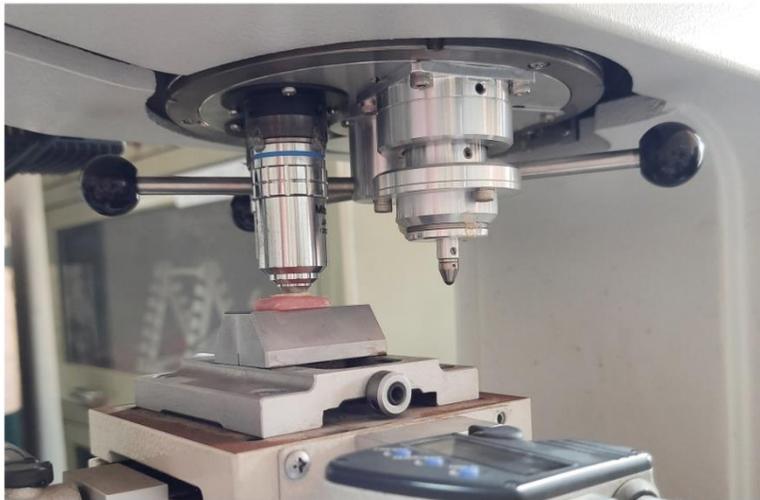
Group 2: Samples treated with 2% chlorhexidine for 5 minutes

Group 3: Samples treated with 5% sodium hypochlorite for 5 minutes followed by 2% chlorhexidine for 5 minutes.

Group 4: Samples treated with normal saline for 5 minutes (control group).

Following the sample treatment, the surfaces were dried and the microhardness of the root dentin was determined in each specimen with Vickers hardness tester.(Figure 4) The indentation was made on the dentin approximately 0.5mm from the root canal space at mid root level. Each measurement was carried out using 200 gram of load for 15 seconds, oriented perpendicular to the root surface. The diagonal lengths of indentations were determined by scaled micrometer and the measurements were converted into Vickers number. (Figure 5)





Result

The readings obtained from the study were tabulated and were statistically analyzed using One Way ANOVA (Analysis of Variance) (Table 1). Among the tested groups NaOCl decreased the microhardness to maximum and the combination of NaOCl and CHX increased the microhardness.

Groups	Minimum	Maximum	Mean	Std. Deviation	Std. Error
Sodium hypochlorite	44.50	63.10	54.03	5.46	1.22
Chlorhexidine	67.90	91.60	78.38	6.66	1.49
Sodium Hypochlorite and Chlorhexidine	80.0	102.10	91.66	5.81	1.30
Normal saline	73.10	91.00	81.52	5.24	1.17
ANOVA test (Fvalue)	150.32				
p value	0.000*				

Table 1: Comparison of microhardness among the experimental groups

	Sum of Squares	df	Mean Square	F	ANOVA P VALUE
Between Groups	15263.912	3	5087.971	150.327	0.000
Within Groups	2572.295	76	33.846		
Total	17836.207	79			

Discussion

Microorganism's plays an unequivocal role in the contamination of root canal system and they are unique from other oral infections as they survive in close environment in root canal which is surrounded by hard tissue all around.[18] Endodontic infection occurs when there is caries or traumatic injury to the tooth structure, leading to the ingress of microbes and their byproducts to the apical foramen and periradicular tissues. Several endodontic microorganisms are capable to encroach deep into the lateral and accessory canals, dentinal tubules and pulp space.[19]

Irrigation has a pivot role in the endodontic treatment. During canal preparation, irrigants influences the removal of remaining tissues, microorganisms and dentin fragments through a pumping action.[20] In addition, irrigants prevent hard and soft tissue remnants from accumulating and compressing at the apical end of the root canal and inhibit the leakage of these irritants toward the periapical area.[21] Since, there is no particular solution that is capable of removing all the irritants and smear layer from the root canal, ideal irrigation is achievable by using a blend of two or more appropriate irrigating solutions through a certain sequence.[14]

Massoud et al[22] suggested that after using the chemical solutions in the root canal, there is alteration in the properties of dentin such as permeability, solubility and microhardness and also, proportion of organic and inorganic components of root canals. Microhardness studied as indirect evidence of mineral changes in root canal dentin, such changes may affect the adhesive properties of dentin and strength of root and can cause root fracture.[16]

Sodium hypochlorite is known as the gold standard endodontic irrigant as it is has tissue-dissolving capabilities and an effective antimicrobial agent. Because of the low viscosity, it is easily introduced into the canal, easily available, an acceptable shelf life and inexpensive. With increasing concentration, the antibacterial and tissue dissolution action of hypochlorite also increases, but this is accompanied by an increase in toxicity.[5]

Chlorhexidine is used as a successful chemical substance for disinfection and to clean the root canal during endodontic procedure. It has been utilized as an irrigant or intracanal medicament owing to its biocompatibility, substantivity, lubricating property and rheological action.[14] It does not have unfavorable characteristics like sodium hypochlorite such as taste, odor and severe irritation of periapical tissues, but it is not capable to dissolve the tissue and replace it. Chlorhexidine has the ability to penetrate through microbial cell walls and membrane, cytoplasm, the bacterium and terminates them. This substance can coagulate the intercellular components in high densities.[11] The antimicrobial function of CHX against root pathogens is present in three densities 0.2%, 1%, and 2%available in gel and liquid form.[20]

Some irrigants will mechanically detach the organic, inorganic and hard tissues and chemically dissolve them while others have antimicrobial action. Therefore, blend of irrigants are used in groups during root canal treatment to get sterile canal space.[3]

It has been suggested that CHX is used as an irrigant and intracanal medicament because of its lower toxicity, comparable antibacterial efficacy and has the advantage of having substantivity when compared with NaOCl. But, it cannot be used as a replacement irrigant of NaOCl, because CHX lacks the tissue dissolution capabilities. Hence, it can be used as a supplemental final irrigation step after NaOCl and EDTA irrigation.[22]

Single rooted tooth were selected for the study. Torabinejad M et al[23] in an in vitro study stated that the absence of complex anatomies and ramifications in experimental studies allows better penetration and action of irrigating solutions.

The longitudinal section of the sample was preferred in the present study which is in accordance with Filho C et al[24], who stated that longitudinal sections show accurate presentation of clinical situations. Also, the irrigants can initially contact the superficial dentin in canal lumen which measures the microhardness of outermost dentin.

The experimental sample was stored in 10% formalin until used. The protocol was followed according to the Centers for Disease Control and Prevention and Milani AS[25] they suggested that 10% formalin does not alter the microhardness of enamel and dentin and can be used for the disinfection of extracted teeth.

According to various studies, micro-hardness decreases when tested from the superficial to deep dentin, because the number of dentinal tubules increases towards the pulp, providing minimal resistance to micro-hardness testing indenter. This is in accordance to Pashley D et al[26], who suggested that there is an inverse correlation existed between micro-hardness of dentin and tubular density.

Camargo CH et al[27] explained that the diameter and number of dentinal tubules are crucial for the penetration of irrigating solutions. Therefore, mid root region was selected for testing as the density of tubules decreases near the central lumen which provides better infiltration of irrigants. Also, the dentin surface is uniform in this area to minimize the effect of the structural variations.

The predominant factors that govern the action of an irrigant are contact time and concentration. An ideal contact time for an irrigant is required for the removal of smear layer and its antibacterial action.[28] The samples were irrigated with solutions for a constant period of 5 minutes in the present study. According to various studies, it has been suggested that irrigants have deleterious effects on root dentin if applied for longer durations.

Ulusoy[29] and Sayin[30] agreed with the fact that the use of root canal irrigants in their microhardness tests for 5 minutes is more realistic, thus simulating clinical practice. Goldberg S et al[17] also added

that the irrigation period has a crucial effect on the microhardness of dentin and irrigation for 5 minutes did not lead to significant change in the properties of dentin.

In the present study, micropipette was used to deliver the irrigant. According to the manufacturer guidelines to dispense the required volume accurately and precisely in micro liter range micropipette is the best equipment. Das A et al[28] and Pimenta JA et al[5] in their study used automated micropipette to deliver the standard volume of irrigant into the root canal of tooth.

In the present study, Vickers is used over knoop due to suitability and practicality for the evaluation of deep dentinal alterations as knoop tester is used for superficial dentin at 0.1mm rather than deep dentin as proposed by Kandil et al[31] Also, Salazar and Gasga et al[32] suggested that Vickers indenter is more useful than Knoop in tooth hardness studies because the indentation produced on a non-flat and square shape surface and the difference in hardness of enamel and dentin, is easily detected.

In the present study, 200 grams of indentation load was applied as suggested by Chuenarrom C et al[33] who stated that high load produces a large impression that is easy to measure the indentation diagonal.

The results showed that NaOCl reduced the mechanical properties of dentin. Birajdar A et al[34] explained that NaOCl disintegrate long peptide chains and chlorinate protein terminal groups, which result in the breakdown of N- chloramines into other species, degrading the organic components of dentin thus, affecting the mechanical properties of root dentin. Also, Kinney et al[35] stated that the decrease in hardness by NaOCl is caused by decrease in stiffness of intertubular dentin matrix caused by heterogeneous distribution of the mineral phase within the collagen matrix. Also Bakr DK et al[16]in his study quoted that NaOCl significantly change the Ca/P ratio of root dentin affecting the hardness profile of dentinal structure. Thus, it could be the reason of reduced microhardness in the samples treated with NaOCl.

Birajdar A et al[34] studied that Chlorhexidine has direct effect on components of dentin structure disrupting the links between collagen fibers and hydroxyapatite crystals causing decrease in dentin microhardness. Whereas, Kulkarni S et al[36] stated that 2% CHX increases the microhardness of dentin due to better preservation of the collagen fibrils of the dentinal surface as it decreases the release of matrix metalloproteinases by protein inhibition action, thus increases dentin strength. Thus, it could be the reason for the increase in the microhardness of dentin for the samples treated with CHX in the present study.

No single irrigant is helpful in the disinfection of infected root canal. Every irrigant has different mechanism of action and beneficiary concerns. For example, if NaOCl removes the organic tissue from the canal then CHX is popular for its substantivity action.[14] So, to acquire the advantages of both the irrigants, combination of NaOCl and CHX was attempted in the present study.

The results obtained in the present study may vary substantially from clinical situation. In the present study, large amount of irrigant was used which came in close contact with flat dentinal surface because of the single rooted tooth chosen. This may not be same in vivo conditions where ramifications and multiple root canals are present. Also, the extent to which these irrigants reach may vary. So, many more in vivo studies need to be performed to reach to the conclusion.

It may be concluded from the above study in spite of the limitations that no single irrigant is able to clean the infected root canal. Every single irrigant has its own positives and negatives. So, one should try combinations to get the benefits of each irrigant to obtain successful treatment outcome.

Conclusion

The purpose of present study was to evaluate the alteration in dentinal properties following different irrigating solutions. The readings obtained from the study concluded that:

1. Sodium hypochlorite significantly reduced the microhardness of root dentin followed by chlorhexidine, normal saline and sodium hypochlorite + chlorhexidine.
2. Also, chlorhexidine had more microhardness value than sodium hypochlorite but lesser than normal saline and combination of sodium hypochlorite and chlorhexidine.
3. Normal saline and sodium hypochlorite and chlorhexidine in combination showed maximum increase in the microhardness when compared to other groups.

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