

The Effect of Various Disinfecting Techniques on Extracted Teeth Used for in Vitro Studies: A Microleakage Evaluation

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ABSTRACT

Background: This in vitro study compares the effect of disinfecting solutions like formalin, sodium hypochlorite and autoclaving on extracted teeth for evaluating microleakage studies.

Materials and Methods: Sixty extracted human maxillary premolar teeth were taken and randomly divided into four groups (with different methods of disinfection) with 15 teeth each. Class V cavities were prepared on the labial surface and restored with Z100 restorative material. Finishing and polishing was done. Samples were then subjected to 500 cycles of thermo cycling and were immersed in methylene blue for 24 hours. The margins of restoration were evaluated for dye leakage using a stereo microscope at 10X magnification. Depth of penetration was marked and scored according to the depth. The data was subjected to nonparametric Kruskal Wallis one way analysis of variance. Inter-group comparisons were performed using the Mann Whitney test ($P < 0.05$).

Results: The authors found that the formalin group and sodium hypochlorite group show lower microleakage than the control group. The autoclave group show slightly higher mean microleakage.

Conclusion: In this study it was observed that the method used for disinfection of extracted teeth could influence the final outcome of microleakage studies giving false positive results.

Keywords: Autoclaving, Disinfection, Formalin, Microleakage, Sodium hypochlorite.

INTRODUCTION

Extracted human teeth are frequently used for preclinical work and for research purpose in dentistry. The pulp and periradicular tissues of these teeth are loaded with a number of viable bacteria and their toxins including Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV), which may act as potential sources of infectious diseases.¹ American Dental Association (ADA) and the Center for Disease Control (CDC) call for a

thorough removal of any organism capable of transmitting disease. The extracted teeth should be cleaned of visible blood and gross debris and maintained in a hydrated state in a well-constructed, closed container during transport. The container should be labeled with the biohazard symbol. Before being used for research purposes the teeth samples should be stored in 10% formalin for two weeks or autoclaved for 40 minutes at 121° C and 15 psi pressure.²

Received: June. 21, 2015; Accepted: Nov. 3, 2015

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Table 1: Score for dye penetration.

Score	Criteria
0	No evidence of dye penetration
1	Dye penetration along cavity wall up to one-third the cavity depth
2	Penetration greater than one-third, but less than two-thirds of cavity depth
3	Penetration greater than two-thirds of cavity depth, but not along the dentinal tubules
4	Penetration up to the cavity depth, and along the dentinal tubules

Table 2: Proportion of Study Groups According to Micro-Leakage Distribution Score.

Groups	Number of Readings	Score 0	Score 1	Score 2	Score 3	Score 4
Group 1	30	0 (0.0%)	15 (50.0%)	15 (50.0%)	0 (0.0%)	0 (0.0%)
Group 2	30	6(20.0%)	9 (30.0%)	13 (43.3%)	1 (3.33%)	1 (3.33%)
Group 3	30	1 (3.33%)	19 (63.3%)	10 (33.3%)	0 (0.0%)	0 (0.0%)
Group 4	30	1 (3.33%)	1 (3.33%)	4 (13.3%)	12 (40.0%)	12 (40.0%)

Table 3: Mean, 25, Median, 75 Percentile Value of Micro-Leakage Score.

Values	(Group 1)	(Group 2)	(Group 3)	(Group 4)
Mean	1.50	1.40	1.30	3.1*
Percentile				
25	1.00	1.00	1.00	3.00
50 (median)	1.50	1.50	1.00	3.00
75	2.00	2.00	2.00	4.00
P- value		0.627 ^{ab}	0.158 ^{ac}	0.000 ^{ad}

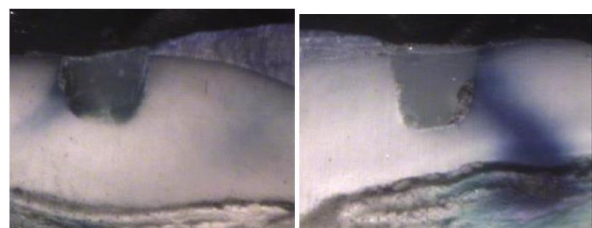
There are various methods available for sterilization / disinfection of extracted teeth such as Formalin, Sodium hypochlorite, Thymol, Alcohol, Antibiotics, Glutaraldehyde, Autoclaving, Dry heat, Distilled water, Normal saline or Freezing, 1:10 Household bleach, Ethylene oxide sterilization and Gamma radiation. Ethylene oxide has been found to be only 20 - 36% effective against *B. subtilis* spores in extracted teeth.³

J. M. White conducted an experiment on sterilization of extracted teeth and compared gamma irradiation with autoclaving, ethylene oxide, and dry heat. He concluded that Gamma radiation sterilizes teeth and endodontic filling material without altering the structure and function of dentin⁴. However, Gamma radiation requires expensive equipment which is not readily available.

Dominici et al. in their study stated that autoclaving extracted teeth for 40 minutes at 240°F

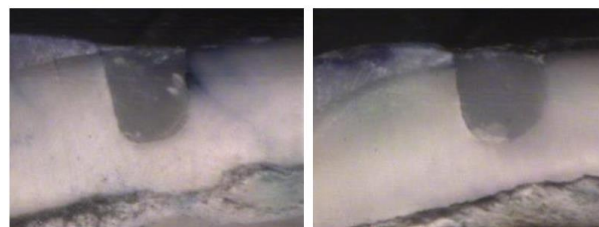
and 20 psi pressure or soaking them in 10% formalin for one week is 100% effective in preventing the growth of *B. stearothermophilus* spores, suggesting that any of these methods can effectively disinfect the teeth⁵

Loyayekar N and Bhat V in their study found that 10% formalin, 5.25% sodium hypochlorite and autoclaving were the most effective methods in disinfecting the extracted teeth⁶. There have been concerns regarding the effect of these disinfecting techniques on the tooth structure. If they alter the tooth structure, it may affect the microchemical bonding between the dental materials and dentin *per se*. Though literature review revealed many studies on efficacy of sterilization by using various methods, there was however no reference on the effect of these techniques on tooth structure and its effect on microleakage.



1

2

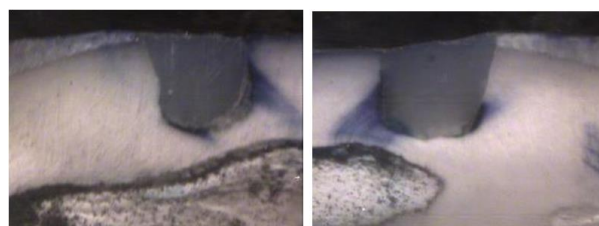


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Fig 1: Control group.



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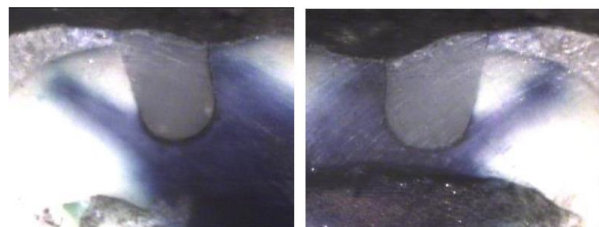
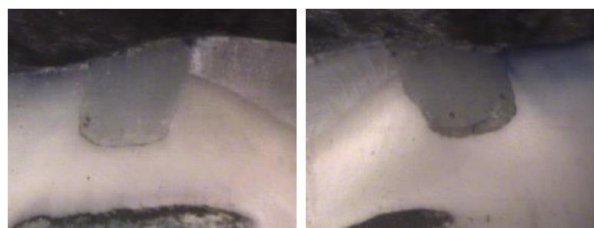


Fig 2: Formalin group.



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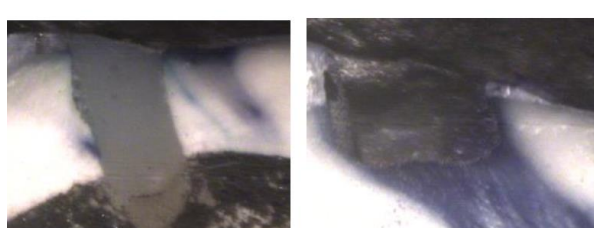
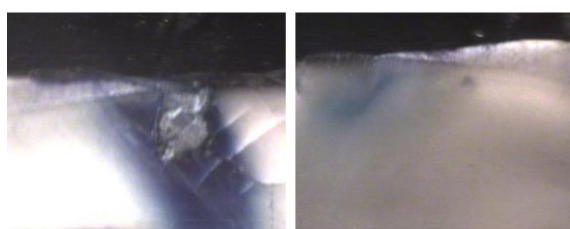


Fig 3: Sodium hypochlorite group.



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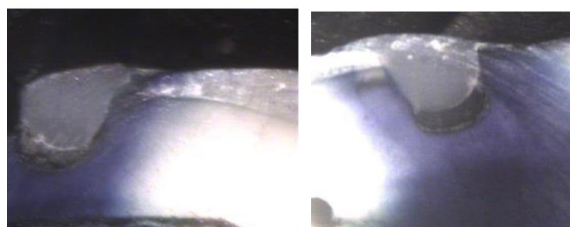


Fig 4: Autoclaved group.

Studies by Shetty S and Sureshchandra B et al have shown that use of sodium hypochlorite on cut dentinal tubules before bonding enhances the bond strength and reduces microleakage⁷. We hypothesized that if a similar agent is used as a disinfecting solution on extracted teeth before using them for analysis of microleakage, then the result thus obtained would be false positive or negative. With this theme we analyzed various disinfecting solutions and their effect on microleakage studies.

MATERIALS AND METHODS

Tooth material: Freshly extracted 60 human maxillary premolar teeth were obtained and kept in saline for 24 hours. Only teeth that were free of caries, cracks or previous restoration were selected. They were randomly divided into four groups of 15 teeth each.

Group 1: Control: The teeth were kept in saline throughout the procedure.

Group 2: The teeth were disinfected by placing them in 10% formalin (LOBA Chemie Pvt. Ltd., Mumbai, India) for two weeks.

Group 3: The teeth were disinfected by placing them in 5.25% freshly prepared sodium hypochlorite for two weeks.

Group 4: The extracted teeth were autoclaved at 121°C and 15 psi pressure for 40 minutes.

The teeth were then debrided of the soft tissue, calculus and bone. Any handling of teeth was done by wearing gloves, mask, and protective eyewear. Class V cavities were prepared at the cemento-enamel junction (CEJ) of the labial surface of all the specimens using number 245 carbide bur (SS White, USA). The depths of the cavities were kept at 0.5 mm into the dentin and the margins as butt joints. Preparation dimension of 1.5 mm (depth) 1 mm (occlusogingivally) × 4 mm (mesiodistally) was kept constant for all the teeth. During the procedure the teeth were held in moistened gauze so as to keep them from getting brittle.

After cleaning and drying the cavity, etching was done with Scotchbond Multipurpose (3M ESPE Dental products, St Paul MM, USA) for 15

seconds. The specimens were then rinsed and dried. A thin layer of adhesive resin, Adper Single Bond 2 (3M ESPE Dental products, St Paul MM, USA) was applied and photocured according to the manufacturer's instructions. Restoration was performed using Z 100, B2 shade (3M ESPE Dental products, St Paul MM, USA) restorative material and photocured using light-emitting diode (LED) curing light (Gnatus, Brazil) for 40 seconds. Finishing and polishing was performed using super snap disc (Shofu Inc. Japan) at slow speed (10,000 RPM) with no separate spray of water as per manufacturer's instructions. Teeth were then stored in saline at 37°C for 24 hours and later subjected to 500 cycles of thermocycling between 5 to 55°C with a dwell time of 30 seconds.

Microleakage Detection

The tooth surfaces of each sample were coated with two layers of nail varnish except on the restoration and 1 mm margin around it. The apical foramina of all the samples were blocked with sticky wax. These samples were then immersed in 1% methylene blue dye (pH = 7) (S.D. Fine Chemical Ltd, India) for 24 hours. The specimens were then washed and the nail varnish coating was stripped off using scalpel blade. Each sample was then sectioned bucco-lingually through the center of the restoration using a slow speed diamond wheel, under water cooling.

These samples were then examined under a stereo microscope (Vardhan Works Pvt Ltd, India) at 10X magnification. The depth of the dye penetration was scored as shown in Table 1. As every sample was sectioned along the midline of the restorations, two readings were obtained from each sample. Therefore, a total of 30 readings were obtained for each group. The data was then subjected to statistical analysis.

Statistical Analysis

To determine a statistically significant difference in the quantity of leakage at the restoration margin, the data was analyzed using nonparametric Kruskal Wallis one way analysis of variance. Inter-group comparisons were done using the Mann Whitney test. $P < 0.05$ was considered as significant. The statistical calculations were executed using SPSS 14.0 software.

RESULTS

The following scores were obtained for all four groups [Table 2]. The mean scores for Group 1, 2, 3 and 4 are 1.50, 1.40, 1.30, and 3.1 respectively. The median scores for Group 1, 2, 3 and 4 are 1.50, 1.50, 1.00, and 3.00 respectively [Table 3]. There was a significant difference among the median scores ($P = 0.00$). The microleakage in Group 2 was significantly lower than that in Group 1 ($P = 0.627$). The median microleakage in Group 3 was lower than that in Group 1 ($P = 0.158$) and also lower than Group 2. The median microleakage in Group 4 was higher than that in Group 1 and statistically significant ($P = 0.00$).

DISCUSSION

Disinfection/Sterilization is a crucial step that is to be performed before handling extracted teeth. It is also important that the disinfecting medium chosen provides complete sterilization of the sample but does not affect the properties of the tooth or interfere with the tests that are planned subsequent to it and does not affect the feel and cutting characteristics of the teeth.

Formalin:

Formalin, an electrophilic agent is composed of formaldehyde, methyl alcohol, and sodium acetate in water. It preserves tissues by cross-linking proteins, glycoproteins, nucleic acid, and polysaccharides to form insoluble methylene bridge products.⁸ Formalin in 10% concentration is the only disinfectant solution that penetrates to the pulp chamber of teeth and could be considered as an effective antimicrobial concentration. A minimum exposure time of two weeks is required for the complete disinfection of extracted teeth. Although formalin is the most effective disinfectant, it is a hazardous material and a potential carcinogen. Therefore, the container holding the teeth must be opened only under a fume hood and the health care personnel must use protective personal equipment.^{9, 10} Haller et al evaluated the effect of formalin on class V restoration microleakage and found a decrease over a period of four weeks.¹¹

George SW et al demonstrated that storage of samples in formalin resulted in a significant

decrease in apical microleakage compared to the nonfixed samples.¹² The mechanism leading to a decrease is uncertain, but may be due to an alteration of dentinal tissues by fixing the properties of formalin. Storage in formalin may have promoted the cross linking of collagenous proteins present in the pulp and dentin matrix, leading to accumulation of insoluble products along the canal wall after instrumentation. A formalin impregnated smear layer may have formed during cavity preparation which prevents apical microleakage.

Michael Pichardo et al postulated that stabilization of the collagen may prevent their collapse, allowing increased mechanical interlocking of the restorative material to the dentin. He also found a significant decrease in the microleakage in teeth stored in formalin.¹³ Our study also shows that teeth disinfected in formalin solution resulted in decreased microleakage. The mechanism of action is the same as mentioned by George SW et al¹² and Michael Pichardo et al¹³

Autoclaving:

Pantera and Schuster reported that autoclaving the extracted teeth for 40 minutes at 121°C and 15 psi pressure renders them free of viable microorganisms¹⁴. Although autoclaving of teeth may cause a slight reduction in dentin microhardness, it does not alter the physical properties sufficiently to compromise the strength of the tooth.¹⁵

Attam K, Talwar S reported that autoclaving the extracted teeth results in increased microleakage¹⁶. The reason can be disruption of collagen molecule by heat and pressure which can affect the ionic bond between the collagen and hydroxyapatite. This can alter the hybrid layer formation after resin penetration and thus increasing the microleakage values. In our study we also found that autoclaving the extracted teeth increases the microleakage. The mechanism of action was same as mentioned above by Attam K, Talwar S.¹⁶

Sodium Hypochlorite:

Loyayekar N and Bhat V reported that sodium hypochlorite storage for 7 days can

effectively disinfect the extracted teeth⁶. Sodium hypochlorite is a non specific proteolytic agent that effectively removes organic components at room temperature. It removes dentine's organic components and changes its chemical composition so that it becomes similar to etched enamel and achieves better composite resin bonding. Kasraei SH et al found decrease in microleakage after collagen removal using sodium hypochlorite in class V cavities at cemento-enamel junction.¹⁷

Mohammad SN et al investigated the effect of a phosphoric acid and sodium hypochlorite mixture as a dentin conditioner on shear bond strength and found improvement in the bond strength of an etch and rinse single bottle adhesive to dentin.¹⁸ There were however no studies to evaluate the effect of sodium hypochlorite on microleakage. In our study we found sodium hypochlorite samples show a decrease in microleakage. We hypothesize that this could be due to its deproteinizing action on enamel and dentin which alters the tooth structure that resulted in better penetration of composite resin and better bonding as reported by the studies which used sodium hypochlorite as a deproteinizing agent.⁷

CONCLUSION

Within the limitations of this study, the results indicate that:

- Selection of disinfecting technique is an important criterion to avoid error in assessment of in vitro microleakage studies.
- False negative results in studies using formalin, sodium hypochlorite and autoclaving may contribute to false assessment of microleakage studies using various restorative materials.

We propose that selection of disinfecting techniques could alter the final results in microleakage studies giving false negative results. This error in assessment could negate the results of any in vitro microleakage studies. On the basis of data collected and analyzed, we strongly recommend correct choice of disinfecting method before undertaking microleakage studies. Further studies have to be taken up to substantiate the proposed claim.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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