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Comparative Evaluation of The Effect of Cavity Disinfectants on The Micro Leakage of The Dentine Bonding System - An In Vitro Study.

KEYWORDS: Cavity disinfectants, Self etch adhesives, 2% Chlorhexidine gluconate, 0.1%Benzalkonium chloride, 3%Sodium hypochlorite, microleakage.

DR.NAVDHEERAJ PATTANAIK
M.D.S in Conservative and Endodontics; Department of Conservative and Endodontics; Senior Lecturer -Hi-Tech dental college and hospital: Bhubaneswar,Odisha

DR.MANOG CHANDAK
M.D.S in Conservative and Endodontics; HOD and Prof. Department of Conservtive and Endodontics, Sharad Pawar Dental college, Wardha,Maharastra

ABSTRACT *Aim of the study- This in vitro study was conducted with an aim to evaluate and compare the effect of three cavity disinfectants-Chlorhexidine gluconate-(Conspesis) ;Benzalkonium chloride –(Tubulicid Red);3% Sodium hypochlorite – (ChlorCid-V) on sealing ability of dentine bonding system(3M ESPE).*

Materials and Methods-85 Class V cavity preparations were prepared on the buccal surfaces of extracted maxillary premolars with occlusal margins at the enamel and gingival margins in cementum and then they were divided into 5 groups -A, B, C, D & E. In the experimental groups A, B, C cavities were treated with combinations of one of the three cavity disinfectants with Adeper Easy One, self -etch dentine bonding agent and whereas groups D & E act as negative and positive control groups. After the cavity preparations were restored with resin composite Filtek Z 25O (3M ESPE), specimens were thermocycled, then the samples were subjected to dry leakage tests using Stereomicroscope.

Results-After statistical analysis (Wilcoxon Signed Rank test, One way ANOVA test) it was concluded that no statistical significance between groups-A, B and D but there was statistically significant in groups C and E.

Conclusion-2% Chlorhexidine gluconate based and 0.1% Benzalkonium chloride based can be used as cavity disinfectants with 3M ESPE bond, without affecting the sealing ability of dentine bonding system. Whereas 3%Sodium hypochlorite based is should not be used with dentin bonding system, because it alters its sealing ability.

Introduction:
The primary goal of restorative dentistry is the restoration of oral function, esthetics, and lost tooth structure along with the preservation of oral health. There have been constant change and improvements in the health science, restorative dentistry has no difference so today dentists are searching for a restorative material which will chemically bond to the tooth structure to form a perfect seal capable of withstanding moist environment and temperature fluctuations.[1]

There has always been much speculation as to what happens to the bacteria sealed in the dental cavities under filling materials. From this speculation controversy has arisen over the need or value of cavity sterilization, the employment of germicidal filling materials, and the importance of removing every trace of carious dentin[2] .According to Brannstorm in 1971 bacterial growth has also been demonstrated in the dentinal tubules and he suggested that such bacteria may have existed on the walls of the cavity before restoration since the cavity were not filled under the aseptic conditions[3]. Whereas In 1972 Branstorm and Nyborg investigated the pulp irritation in the teeth restored with composite resin. They found the presence of many bacteria on the cavity walls beneath the restoration [4].

Kidd in 1976 stated that an inherent problem with any restoration is microleakage defined as the passage of bacteria, fluids, chemical substances, molecules and ions between the tooth and its restoration[5]. Many microleakage studies employ different tracers that may penetrate around restorative materials to varying extents because of their physical or chemical characteristics.

Till date no restorative material has consistently been shown to seal and adhere to dentin. The problems associated with microleakage can be magnified by incomplete sterilization of the preparation. Bacteria remnants during and after the cavity preparation pose one of the major problem in restorative dentistry and for these reasons elimination of the bacteria from the cavity surfaces is of major importance, and a disinfectant solution that eliminates these residual bacteria could be useful after cavity preparation. In 1989 Boston and Graver found few bacteria in the dentinal tubules after the removal of dye stained caries[6].

Studies by Turkun.M in 2004 have suggested that a number of antimicrobial solutions such as – Chlorohexidine, Sodium hypochlorite, Fluoride based solutions Benzalkonium chloride, EDTA, Hydrogen peroxide and Iodine solutions can be used as cavity disinfectant to eliminate the residual bacteria from the prepared cavity[7].

The application of disinfectants after cavity preparation and before tooth restoration is gaining acceptance as it eliminates potential risks due to bacterial activity. However, there is concern about the use of cavity disinfectants with dentin bonding agents, since they may alter the ability of the hydrophilic resin to seal the dentin[8]. It has been suggested that cavity disinfectants can improve the sealing ability of dentin bonding agents by remoistening the cavity, prior to placing a dentin-bonding agent that bonds to damp tooth structure.

Materials and methods-
Eighty five extracted human molars, free of cracks, caries and restorations on visual inspection, were used for the study. The teeth were scraped of any residual tissue tags and rinsed under running water for 15 minutes each. Later, they were cleaned with pumice and stored in normal saline at 40°C until use.

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Class V cavity preparations were prepared on the facial surfaces of each tooth, with a cylindrical diamond bur Dianfong SR-12, in a high speed handpiece utilizing water-spray coolant. Standardized preparations were obtained by making cavity preparations that were approximately 1 mm wide, 1 mm deep and 2 mm long, paralleling the cementoenamel junction (CEJ).The gingival half of the preparation was extended 0.5 mm below the CEJ. No bevels were used in the preparation. Cavosurface walls were then finished and polished.

Each preparation was rinsed with distilled water for 20 seconds and dried with compressed air for 20 seconds. The teeth were then randomly divided into five groups [Table 1] as follows:

Group A
consisted of twenty five teeth (25 cavity preparations) treated with chlorhexidine based cavity disinfectant solution followed by the application of a Self etch adhesive (Adeper Easy One, 3MESPE).

Group B
consisted of twenty five teeth (25 cavity preparations) treated with benzalkonium chloride based cavity disinfectant solution followed by the application of a Self etch adhesive (Adeper Easy One,3MESPE).

Group C
consisted of twenty five teeth (25 cavity preparations) treated with 3% sodium hypochlorite based cavity disinfectant solution followed by the application of a Self etch adhesive (Adeper Easy One,3MESPE).

Group D
(negative control) consisted of five teeth (5 cavity preparations) used without any cavity disinfecting solution treatment; however, a dentin bonding system (Adeper Easy One) was applied.

Group E
(positive control) consisted of five teeth (5 cavity preparations), used without either a cavity disinfecting solution treatment or a dentin bonding system.

Table (1)- The teeth were randomly divide into five groups-

Serial no.	Groups -	No. Of samples-	Cavity disinfectants-	Manufacturer-
1	Group A	25	Conspesis	Ultradent ,USA
2	Group B	25	Tubulicid red	Dental therapeutics AB, Sweden
3	Group C	25	Chlor Cid V	Ultradent ,USA
4	Group D	5	-	
5	Group E	5	-	

Figure (1): Armamentarium used.

In the respective test groups, cavity disinfectants were applied with a sterile brush applicator for 20 seconds; any excess disinfectant was removed by five seconds of light air drying, to prevent desiccation.

After cavity disinfection, the dentin bonding system (Adeper Easy One) was applied to the appropriate groups according to manufacturer's instructions. A layer of bonding resin was applied to the preparation with a brush, spread gently with air and cured for 20 seconds and then the cavity preparations were restored with a resin composite (Filtek Z250) by light curing for 60 seconds. The cavosurface margins were then finished with a finishing bur and 3M USA discs.

All the teeth were stored in distilled water for 24 hours, at 37°C, and subjected to 1,000 thermal cycles between water baths of 50°C and 550°C, with a dwell time of 30 seconds. The teeth were then subjected to dye leakage tests.

All the teeth to be subjected to dye-leakage tests were covered with two coats of nail varnish to within 1 mm of the tooth-restoration margin, after the root apices were sealed with modelling wax. The specimens were immersed in India ink, in separate sealable glass vials, at 37°C for 24 hours.

After staining the teeth were rinsed under running water for at least 5 minutes each in order to remove any residual stain and then each sample was sectioned mesiodistally and the radicular portion was cut 2mm below the CEJ using low speed diamond saw.

Microleakage was assessed for both occlusal and gingival margins using Stereomicroscope X 20 (ZEISS Stemi, DV4,Germany).

The depth of the penetration of stain i.e. dry leakage was scaled according to the following scale-

1- No leakage
2- Penetration less than one half of the length of occlusal /gingival wall
3- Penetration greater than one half of the length of occlusal /gingival
4- Penetration up to and along the axial wall

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5- Penetration within the pulp

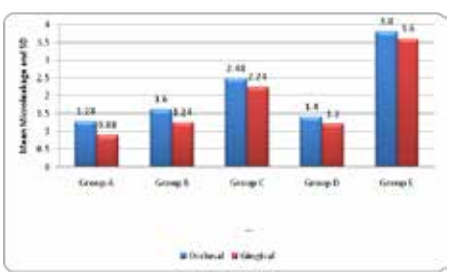
All the recording were recorded and subjected for the statistical analysis.

Results:

Table(2)- Comparison of microleakage in occlusal and gingival walls using Wilcoxon Signed Rank test.

		N	Mean	Std. Deviation	Z-value	p-value
Group A		25	1.28	0.79	1.85	0.06 NS,p>0.05
	Gingival	25	0.88	0.60		
Group B	Occlusal	25	1.60	0.91	1.78	0.07 NS,p>0.05
	Gingival	25	1.24	0.92		
Group C	Occlusal	25	2.48	1.04	1.01	0.31 NS,p>0.05
	Gingival	25	2.24	0.87		
Group D	Occlusal	5	1.40	0.89	0.44	0.65 NS,p>0.05
	Gingival	5	1.20	0.44		
Group E	Occlusal	5	3.80	0.44	1.00	0.31 NS,p>0.05
	Gingival	5	3.60	0.54		

In this present study when the mean values of microleakage in occlusal and gingival walls was compared in Groups A,B, C, D and E, the result shows that the in all the groups the value of microleakage between occlusal and gingival walls are stasticially insignificant (p>0.05).



Figure(2): Graph showing comparison of occlusal and gingival walls between the groups with positive and negative control.

This bar diagram illustrates that the microleakage in both occlusal and the gingival walls of Group A (Conspesis) and Group B(Tubulicid Red) are least followed by Group D (Negative control).Whereas group C (Chlorcid V) shows high value of microleakage followed with group E (Positive control).

Table (3) - Comparison of microleakage in occlusal walls using One way ANOVA.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Group A	25	1.28	0.79	0.15	0.95	1.60
Group B	25	1.60	0.91	0.18	1.22	1.97
Group C	25	2.48	1.04	0.20	2.04	2.91
Group D	5	1.40	0.89	0.40	0.28	2.51
Group E	5	3.80	0.44	0.20	3.24	4.35

In this present study the mean value of microleakage in occlusal walls of Groups A, B, C,D and E shows that the –the mean microleakage is least for Group A followed by Group B and Group D. The mean microleakage is highest for Groups C and E.

Table (4)- Comparison of microleakage in gingival walls using One way ANOVA.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Group I	25	0.88	0.60	0.12	0.63	1.12
Group II	25	1.24	0.92	0.18	0.85	1.62
Group III	25	2.24	0.87	0.17	1.87	2.60
Group IV	5	1.20	0.44	0.20	0.64	1.75
Group V	5	3.60	0.54	0.24	2.91	4.28

In this present study the mean value of microleakage in gingival walls of Groups A, B, C,D and E shows that the –the mean microleakage is least for Group A followed by Group B and Group D. The mean microleakage is highest for Groups C and E.

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Discussion-

Success in operative dentistry depends on total removal of the infected structure and achievement of a good seal [9]. It is clinically very important to enhance the adhesion between the dentine and the adhesive resin, because such improved adhesive strength not only leads to better retention of restorations but prevents marginal leakage thus reducing the chances of developing secondary caries [10].

In past it was suggested that dentin should be sterilized before the placement of any restorative material. Many chemicals, such as silver nitrate precipitated with eugenol, thymol, and potassium ferrocyanide, had been proposed for this purpose [6]. The rationale prevailing for this was that any residual microorganisms should be eliminated in order to prevent the potential propagation of caries. But it was found that that these chemicals are irritating to the pulp when applied to the dentin surface.

The integrity and durability of the marginal seal has always been of prime concern in the investigation of dental restorative materials performance. One of the key functions of a dental restoration is to seal the exposed dentin from the oral environment, to prevent pulpal damage and further decay. Therefore, the microleakage at the tooth restorative interface is a major concern influencing the clinical longevity of composite resin restorations (Gwinnett et al., 1995) [11].

Several factors can affect the integrity of the tooth-restoration interface and can contribute to microleakage. Among these are Polymerization shrinkage, Cavity configuration factor, Hydroscopic expansion, Light polymerization concepts and units, Thermal cycling and Occlusal stresses[12].

According to J C Meirs and J C Kresin (1996) chlorohexidine solutions have been found to be effective in reducing the levels of S.Mutans found in occlusal fissures and exposed root surfaces. The use of this product as cavity wash after tooth preparation and before the application of dentine bonding agents could help to reduce the potential for residual caries and post operative sensitivity. Chlorohexidine has got the potential to stabilize the smear layer turning it from a semi permeable loosely bound layer to more impermeable firmly bound layer thereby decreasing the microleakage[6]. Gultz et al (1995) stated that Consepsis solution did not adversely affect the sealing ability as it dissolve the smear layer and incorporate it into the primer, as they demineralize the dentin and envelop the collagen fibers and hydroxylapatite crystals [13]. The scanning electron microscopic observations of their study revealed the presence of resin –tags in the Consepsis treated group.

But According to Tulunoglu O etal Chlorhexidine solution had an adverse effect on Syntac and Prime & Bond and produced significantly higher microleakage when used with these bonding systems because of the varying thickness of the hybrid [14].

Brannstorm in 1982 stated that Tubulicid red contains (0.2% EDTA, 0.1% Benzalkonium chloride and 1% NaF-) removes the smear layer leaving the tubules plug undisturbed. The author stated that this solution could be used in combination with dentine bonding systems that bond micromechanically to intertubular dentine. The bonding agents could adhere to the intertubular dentine while the dentinal orifices remain closed by fluoridated smear plugs and thus prevents the influx of irritants to the pulp [15]. It accomplishes three goals in one treatment-cleansing, disinfection and impregnation.

According to R. Frankerberger et al one of the keys in the field of dentine adhesion s the observation of the hybrid layer, resulting from the resin-penetration into the acid-dimeneralised dentine. They stated that NaOCl pre treatment has detrimental effects on the dentine bonding performance of the dentine adhesive system and thus resulted in lower bond strength and worse marginal adaptation due to the unhindered free shrinkage towards the bonded area [16]. Haller et al stated that the application of 10% NaOCl considerably influences adhesive systems containing ethanol and acetone by interfering with the wettability of dentin surfaces [17].

Lai et al stated that the presence of the reactive residual free-radicals in dentin treated with sodium hypochlorite may compete with the propagating vinyl free-radicals generated during light activation of the adhesive. This results in premature chain termination and incomplete polymerization and can eventually leads to increase microleakage [18].

According to D.Felton, G.Bergenholtet and C.H Fox (1989) - GLUMA Dentin Bond is described as a dentin bonding agent that contains 5% w/w glutaraldehyde in a solution of 35% betahydroxyethyl methacrylate (Munksgaard and Asmussen, 1984). In their study they stated that GLUMA did not show inflammatory infiltrates at either time interval suggests that marginal leakage of bacterial components was either not occurring or did not influence the pulp[19]. Whereas According to Martin Brannstorm, Hilding Nyborg there was presence of high frequency of bacteria beneath the composite restorations without the application of cavity disinfectant and bonding agent. The main factors considered were chemical irritation caused by composite and poor adaptability of the material [20].

Therefore it can be concluded that the effect of the cavity disinfectants on the composite resin restorations appears to be material specific with regards to interaction with ability of various dentine bonding systems to seal the dentine.

Conclusion:

Under the limitations of the study, based on the results obtained and discussed and the following conclusions were drawn from the present study that 2% Chlorhexidine gluconate based and 0.1%Benzalkonium chloride based can be used as cavity disinfectants with 3M ESPE bond, without affecting the sealing ability of dentine bonding system. Whereas 3%Sodium hypochlorite based is not an appropriate disinfectant to be used with dentin bonding system, because it alters its sealing ability.

Clinical significance-

The application of disinfectants after cavity preparation and before the restoration is gaining acceptance. This study opens the perspective further research of the use of cavity disinfectants in dentistry as it behaves in the oral environment after cavity preparation. So it would prudent to research the performance of the cavity disinfectant in long term clinical study.

Limitation-

The present study was conducted in vitro condition whereas the vivo nature of cavity disinfectants affecting the sealing ability of dentine bonding system needs to be evaluated. In this study few cavity disinfectants were compared and evaluated while study related to the other cavity disinfectants should be taken into consideration.

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