ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)
IJDSIR : Dental Publication Service
Available Online at: www.ijdsir.com
Volume – 2, Issue – 4, July – August - 2019, Page No. : 265 - 270

Evaluation of Antimicrobial Property of an Orthodontic Adhesive Combined With Gluteraldehyde at Different Concentrations: an In Vitro Study

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Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Introduction: The bonding of brackets induces a continual accumulation and retention of plaque that may be influenced by design and surface characteristics of both orthodontic attachments and composite resulting in enamel decalcification appearing as white lesions at the end of the treatment. The present aim of the study is to evaluate the antimicrobial property of 2%,5%,10% gluteraldehyde added to Transbond-XT (3M Dental products, Monrovia, California) against streptococcus mutans.

Materials & Methods: Streptococcus mutans cultured in Brain Heart Infusion (BHI) broth.These microbial suspensions are used to inoculate the agar diffusion test plates and performthe adhesion assays. Gluteraldehyde added in various concentrations group A-2%, B- 5.0%, and C -10.0%w/w to the light cure bracket adhesive, Transbond XT (Unitek 3M, Monrovia,Calif). The substances to be tested were deposited on sterile (Whatman No.1) filter paper disks of 5 mm in diameter and1.5 mm thickness. The culture plates are incubated for 48hrs and the diameter of inhibition halos were measured with a digital calliper.

Results: The resulted showed that Group C (10%gluteraldehyde) produced halo ring with highest diameter than Group A&B and Group A produced the halo ring with the least diameter.

Conclusion: The increase in concentration of gluteraldehyde showed a stastitically significant increase in antimicrobial property.

Keywords : Gluteraldehyde, *s.mutans*, Orthodontic adhesive

Introduction

The bonding of brackets using acid etching and composite resin though considered major advancement in orthodontic practice; it induces a continual accumulation of plaque and increases the level of streptococci& lactobacilli.[1,2,3,4,5] This increase is evident after the second week of placement and comes to normal once the appliance is removed.[2] The high incidence of streptococci favoured by low pH (<4.5) results in enamel decalcification appearing as white lesions around the brackets at the end of the treatment.

The adhesive resins used for bracket bonding contribute to demineralization as they produce rough surfaces that are favourable for bacterial colonization.The orthodontic adhesive used for bracket bonding also produces 10µ gaps at the enamel-bracket interface favouring bacterial colonization. In spite of the recent advances in orthodontic materials & techniques, the incidence of enamel decalcification is still evident compromising the aesthetics. With this objective, self-etching adhesives with supposedly

antibacterial properties have been introduced into the market. One such material is Transbond plus Self Etching Primer (TSEP, 3M Unitek, Monrovia, California, USA), a self-etching fluoride-releasing orthodontic adhesive.But due to the acidic environment around the brackets, the fluoride is found to be ineffective in remineralization. There are also other self-etching adhesives used in conservative dentistry such as iBondGluma Inside (iBond, HeraeusKulzer GmbH). iBond contains glutaraldehyde, allowing the material to act as a desensitizer as well reducing or eliminating bacterial levels in cavity preparations (Felton *et al.*, 1989). Clearfil Protect Bond (CPB, Kuraray MedicalInc., Okayama, Japan), a more advanced version of the self-etchingadhesive Clearfil SE Bond (CSB, Kuraray Medical Inc.), differs from its predecessor in that it contains the antibacterial monomer MDPB in its primer and sodium fluoride in the bonding.So it indicates the importance of the role of antimicrobial agent in the bonding systems as the orthodontic treatment outcome is much more concerned towards the esthetics.

Previous studies have attributed the antimicrobial property of self-etching primer to their low pH when compared to conventional acid etching with phosphoric acid. Nevertheless which is the origin in the inhibitory effect towards *s.mutans*, (either low pH, fluoride release, antimicrobial agent in the material) it is an advantage to the bonding system. But no study has been reported with adding an antimicrobial agent to the light cure composite material itself.

So, the present study is designed with the objective of evaluating the antimicrobial property of gluteraldehyde added in different proportions to thecommercially available light cure orthodontic adhesive Transbond XT® (3M Unitek Dental Products, Monrovia, California).

Materials & Methods

Adhesive

The light cure adhesive commonly used and commercially available in the market Transbond XT® (3M Unitek Dental Products, Monrovia, California) is used.

Bacteria

The antimicrobial property of adhesive is tested against *s.mutans*, the most commonly found bacteria around orthodontic brackets.

Bacterial culture

A sample was taken using a sterile swab around the bracket in the anterior region of a randomly selected patient, who is undergoing fixed orthodontic treatment from the Department of Orthodontics, Navodaya Dental College. Then the swab was taken to Department of Microbiology, Navodaya Medical College using a transport medium of thiogycolate broth. The swab with bacterial colonies was transferred onto a nutrient agar plate and allowed to incubate for 24 hrs. Meanwhile the Brain Heart Infusion (BHI) broth, the selective broth for culture of s.mutans was prepared and stored in an aseptic condition.

After 24hrs, the nutrient agar plate was removed from the incubator and the bacterial colonies were gram stained for the confirmation of gram positive s.mutans bacteria. The gram positive colony of s.mutans were selected with a sterile swab and transferred to BHI broth and allowed to

incubate for 4hrsat 37 degree C in a jar with a microaerophilic atmosphere enriched with 5 percent CO2, where it reached the stationary growth phase point. Mc Farland standardization was used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. After 4hrs, these microbial suspensions were used to inoculate the agar test plates that were used to assess the antimicrobial efficacy.

Agent preparation

According to the manufacturer instructions, 40ml,20ml,10ml of distilled water was added to commercially available Gluteraldehyde solution 25% (Merck specialities private limited, Mumbai) to make it to a concentration of 2%,5%,10% respectively.

Adhesive preparation

The orthodontic adhesive to be tested was divided into 3 groups A,B,C to receive 2%,5%,10% gluteraldehyde respectively. By using a mixing pad and sterile plastic spatula the adhesive was mixed with gluteraldehyde solution to achieve smooth consistency.

Antimicrobial test

The autoclavable plastic culture plates were loaded with 20ml of BHI agar (prepared according to the manufacturer instructions) and the microbial suspension of s.mutans from the BHI broth was inoculated on these plates using a sterile swab.

The substances to be tested were deposited on sterile (Whatman No.1) filter paper disks of 5 mm in diameter and 1.5 mm thickness. Each disk received (in aseptic conditions) adhesive of each group and adhesives were polymerized on the agar plates with a halogen light-curing unit (Unicorn Denmart, India.) for the duration indicated by the manufacturer (20 seconds). Each agar plate received 3 filter paper discs of three groups of adhesive to be tested. Once the polymerization was done the agar plates were incubated for 24 hrs.

After taking out from the incubator, the diameter of halo rings (zone of inhibition) was measured using a digital calliper (RSK^{TM}, China). The procedure was repeated for 5 times (3agar plates at each time) i.e. 15 filter paper discs were checked for each group of adhesive.

Statistical Analysis

The mean and standard deviation of diameter of halo rings in each group was analysed statistically using one way ANOVA test. The existence of significant mean differences between halo sizes produced by each adhesive was evaluated using LSD test ($p<0.05$)

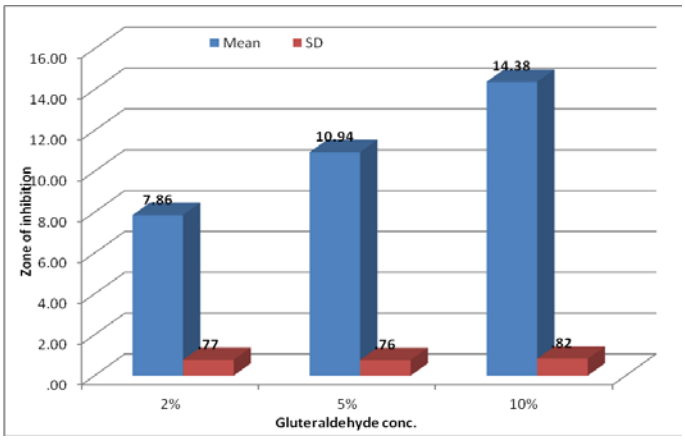
Results

Diameter of halo rings in millimeters

Group – A (2% Gluteraldehyde)	Group – B (5% Gluteraldehyde)	Group – C (10% Gluteraldehyde)
7.14	10.06	14.18
6.37	10.28	12.72
8.26	9.58	13.67
9.12	11.66	13.78
7.17	12.02	15.14
7.25	10.09	14.07
8.02	11.06	14.78
6.96	10.68	13.48
8.08	10.67	14.78
7.83	11.03	13.96
7.88	12.03	16.15
8.12	10.63	14.78
8.02	11.25	15.03
9.03	12.02	14.62
8.65	11.03	14.62

Descriptive statistics of variables—

Gluteraldehyde	N	Range	Minimum	Maximum	Mean	Std. Deviation	Std. Error
2%	15	2.75	6.37	9.12	7.86	.77	.20
5%	15	2.45	9.58	12.03	10.94	.76	.20
10%	15	3.43	12.72	16.15	14.38	.82	.21



Comparison of mean and SD of 2%,5% and 10%

(By one way ANOVA)

Gluteraldehyde	N	Mean	Std. Deviation	F	Sig.	Inference
2%	15	7.86	.77	258.492	.000	Highly significant
5%	15	10.94	.76			
10%	15	14.38	.82			

LSD test to see the mean difference in each group is significant or not

Group	Mean difference	P
Gluteraldehyde (2%-5%)	-3.08*	0.0001
Gluteraldehyde (2%-10%)	-6.52*	0.0001
Gluteraldehyde (5%-10%)	-3.44*	0.0001

*indicates that the mean difference is significant at 0.05 level.

The resulted showed that Group C (10%gluteraldehyde) produced halo ring with highest diameter than Group A&B and Group A produced the halo ring with the least diameter. When compared Group A& C the mean difference was more with $p<0.05$ than with Group A&B or Group B&C.

Discussion

The antibacterial action of adhesive systems is affected by the materials' inherent properties such as pH, viscosity, diffusion capacity, the presence of antibacterial agents and factors related to the dentinal substrate (thickness and permeability).[7,8,9,10,11,12,13,14] The self-etching adhesives have an acidic pH, that has been considered a key factor for bacterial inhibition,[7,9] although there are several recent studies [8,15,16] that did not find a significant relation between the acidity of self-etching adhesives and their antibacterial effects. So the interest of role of antimicrobial agent came into the scenario.

Some dental materials containing glutaraldehyde have been shown to be effective against *Streptococus, Lactobacillus,*and *Actinomyces,* a result of infiltration into dentinal tubules, which depends on the glutaraldehyde released by the cured materials.[17,18] As glutaraldehyde does not polymerize within the resin matrix, its antibacterial effect persists after polymerization of the resin because the remaining free molecules diffuse into the surrounding environment. So the results showed halo rings in each group and the diameter of the halo rings was increased as the concentration of the antimicrobial agent was increased.

The formation of halo rings by gluteraldehyde can be attributed to the bacteriostatic nature and its permeability into cell wall or membrane by causing alkylation or denaturation as free antimicrobial agents do. And it was shown in the previous studies that there was no role of adhesive alone without an antimicrobial agent in the formation of halo ring or the zone of inhibition. Recent studies by Jacobo et al[19] in 2014 on antibacterial properties and microbial colonization susceptibility of four self-etching adhesives concluded that Clearfil Protect

Bond, CPB and iBond produced clear growth inhibition halo rings and it was stated that the antimicrobial property of iBond may be attributed to its gluteraldehyde content. Another study by Shafiei F and Memarpour M[20] in 2012 suggested that the combination of fluoride and antimicrobial agent was suggested for efficacy of orthodontic adhesive. Tahani et al[21] in 2006 conducted a study on Antimicrobial properties of an orthodontic adhesive combined with cetylpyridinium chloride concluded that addition of cetylpyridinium chloride imparted long term antimicrobial activity to the adhesive without altering the tensile strength.

The ability of modified adhesives to release adequate amounts of gluteraldehyde over a long period is an important characteristic for clinical benefits. In this in-vitro study, an initial burst of gluteraldehyde was released from the modified adhesive discs, but the duration of release of gluteraldehyde from the resin matrix was not determined. And also minimum clinically significant amount of gluteraldehyde released from the modified discs was not determined. However, the pattern of release might offer several clinical advantages in orthodontic treatment, such as slow and continuous release of gluteraldehyde over a prolonged period, and release from the modifiedadhesive site specific to the area most susceptible to plaque accumulation and enamel decalcification, adjacent to bonded orthodontic brackets and independent of patient compliance.

Though the antimicrobial property of orthodontic adhesive has to be improved, but the antimicrobial agent should not be compromised in terms of shear bond strength. The shear bond strength of modified adhesive was also not determined in this study. Though the modified adhesive with antimicrobial agent has advantage over conventional adhesive in terms of antimicrobial property, the maximum safe level for long term clinical exposure is yet unknown. Thus this topic warrants further research in the ray of belief that orthodontic adhesives with antimicrobial additives can be developed into clinically and commercially useful products.

Conclusion

The following conclusions can be drawn from this in vitro study

1. Group-C had shown highest antimicrobial efficacy followed by Group- B&A.
2. Increase in concentration of gluteraldehyde increases the antimicrobial property.
3. Though statistically significant results achieved, bond strength has to be evaluated
4. The duration of release of gluteraldehyde from resin matrix is still a matter of interest.

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