International Journal Dental and Medical Sciences Research
Volume 5, Issue 3, May - June 2023 pp 651-660 www.ijdmsrjournal.com ISSN: 2582-6018

Comparative Evaluation of Caries protective Efficacy of Seventh Generation Dentin bonding agents on Demineralisation of Root surface: An Invitro study.

Dr. Gaini Mounika, Dr P. Karunakar, Dr Raji Solomon

Date of Submission: 10-06-2023 Date of Acceptance: 25-06-2023

Aim: To evaluate the effectiveness of the seventh generation dentin bonding agents on demineralization of root surface.

Materials and Methods: The root surface of 70 freshly extracted caries-free human teeth was thoroughly cleaned and polished, thereby removing the cementum. The root surfaces were coated with acid-resistant nail polish and 2 mm × 3 mm rectangular window was prepared on the buccal surface. The samples were then divided randomly into three experimental groups; Group 1(Adper Easy One Bond), Group 2(Opti Bond All in One), and Group 3(Gluma Self etch) which were further divided into two subgroups (A and B) with 10 samples each in which second layer of adhesive was cured without air thinning and with air thinning respectively. Subsequently, all specimens were demineralized for 6 days with acidified gel (hydroxyl ethylcellulose, pH 4.8, 37°C).Each tooth samples were sliced (80 ± 20 µm) by Hard Tissue microtome and examined for caries-like lesions (demineralized area) under a polarized microscope. Data were analyzed using one-way ANOVA, Student's t test.

Results: All control group specimen exhibited lesions with a mean depth of 86.13µm, while the difference between subgroup 1A and 2A (2.9µm), between subgroup 1A and 3A (0.36µm) and between subgroup 2A and 3A (3.26µm) were found to be statistically nonsignificant. In Group 1–3, the lesion depth was reduced significantly, subgroup A results were better thanB.Minimum demineralization depth (38.5µm) was found for Subgroup 3B and intra- group differences were found statistically significant (P< 0.001).Maximum difference in demineralization was found between control and Group 3(49.49µm) and minimum between Groups1 and 3 (7.85 µm) which was statistically significant (P < 0.001).

Conclusion:- It can be concluded that among seventh generation self etch adhesive systems Gluma Self etch adhesive with desensitizer provided the least demineralization surface and also the adhesive with first layer cured with air thinning and second layer cured without air thinning performed best result when compared with adhesive without air thinning but was not statistically significant .

Keywords:- Cementum, demineralization, dentine bonding agent, self-etch, Artificial Caries .

I. INTRODUCTION

Due to increase in life expectancy, awareness of dental health treatment modalities and availability of dental care has been a rapid increase in elderly population with proper dentition, especially in developed countries.

Although enamel, cementum and dentin all develop carious lesions in roughly the same manner there are significant differences between enamel and the other two tissues. While early enamel lesions are white, root surface lesions in cementum or dentin are lite brown or yellow. The color probably arises from extrinsic stain materials, and it is possible that very early, actively forming lesions are colourless[1].

Though root cementum is rich in collagen, less rigidity and more permeability of cementum makes it more prone to acid attack. Thus, slight decrease in pH of the plaque fluid may lead to demineralization of the root's hard tissues and initiates root caries. Root caries is a disease that is expressed as a soft, progressive lesion found on a tooth root surface that has lost its connective tissue

International Journal Dental and Medical Sciences Research
Volume 5, Issue 3, May - June 2023 pp 651-660 www.ijdmsrjournal.com ISSN: 2582-6018

attachment and thereby has become exposed to the environment of the oral cavity.

According to data from the third National Health and Nutrition Examination Survey 1999 to 2004, the prevalence of root caries among dentate adults between the ages of 50 and 64 was 30.8%, compared to 10.4% among adults ages 20 to 34[2].

Dental adhesive technology has evolved in the past decades towards complex formulations with simplified clinical procedures. The demand for reduced technique-sensitivity,shorter clinical application time and less incidence of post-operative sensitivity have made self-etch adhesive systems a promising approach when compared to the etch-and-rinse systems.

The concept of self-etching, self-priming adhesives is to simultaneously etch and prime dentin at the same extent, integrating the smear layer into the adhesive interface. No separate etching step minimizes danger of overetching and desiccation of dentin. Recently, coating of tooth root surface with adhesives was investigated as a preventive treatment against root caries, as it provides a strong physical barrier with the formation of a hybridized layer.Currently, self-etch adhesives are widely used to treat root caries, as they show reliable bonding to dentin.[3]

In the present study, three self etch adhesives namely, Opti Bond All in one , Adper Easy Bond , Gluma Self Etch were used and assessed for their caries protective effect on root surface area near the cementoenamel junction using a Polarised Microscope.

SPECIMEN PREPARATION

This Study utilised 70 freshly extracted caries free human single rooted teeth which were collected from the Department of oral and maxillofacial surgery Panineeya institute of dental sciences and Research centre.The Extracted teeth were cleaned and stored in saline solution 0.9%. This is a preferred procedure as it does not influence the physical properties of dentin.

The root surfaces 1mm apical to the cemento enamel junction were thoroughly polished using the sof flex disc there by removing cementum. The root surfaces were coated with acid resistant nail polish and 2 mm × 3 mm rectangular window was prepared on buccal surface. The samples were then divided randomly into three experimental groups;

Group 1 (n = 20) – Seventh Generation Self Etch – (Adper Easy Bond) subgroup IA (n= 10) without air thinning
2A (n=10) with air thinning

Group 2 (n =20) - Seventh Generation Self Etch - (Opti Bond All in One), Sub group IA (n= 10) without air thinning
2A (n=10) with air thinning

Group 3 (n = 20) - Seventh Generation Self Etch - (Gluma Self Etch)
Sub groups IA (n= 10) without air thinning
2A (n=10) with air thinning

Control Group (n = 10)

In Subgroup A of each group, adhesive will be applied on the experimental surface of the tooth and light cured for 10 seconds with air thinning. After this, in the same manner the second layer of adhesive is applied and light cured for 10 seconds without air thinning. In Subgroup B of each group, adhesive was applied on the experimental surface of the tooth and light cured for 10 seconds with air thinning. After this, in the same manner, second layer of adhesive was applied and light cured for 10 seconds with air thinning. Specimens were demineralized for 6 days with acidified gel (hydroxyl ethyl cellulose [HEC], pH 4.8, 37°C).

Samples of each sub group is placed separately into glass vials with readily available 10 ml solution of 0.9% sodium chloride solution, for a maximum of 8 days before the start of the experiment.

In this study the following specimen, materials, instruments, and equipments were used.

S.NO	MATERIALS/ EQUIPMENTS	PURPOSE OF USE	MANUFACTURER / BATCH NO :
1.	Freshly extracted single rooted human teeth	Specimen	

2.	Saline 0.9%	For Storage of Specimens	
3.	No.245 Diamond shaped Bur	For Cavity Preparation	Deccan Dental Depot Hyd .
4.	Airotor handpiece	For Cavity preparation	NSK, Confident Dental, System
5.	Hydroxy ethyl cellulose	For artificial Caries	
6.	Ph Meter	To determine the caries	
7.	Gluma Self etch	Bonding Agent	Heraeus Kulzer
8.	Adper Easy	Bonding Agent	3M ESPE
9.	Opti Bond all in one	Bonding Agent	Kerr
10.	LED curing light	Curing of Bonding agent	Ivoclar Vivadent
11.	Polarised Microscope	To Evaluate the Demineralisation of root surface	Oral pathology dept Panineeya institute of Dental Sciences Hyderabad.
12.	Hard Tissue Microtome	For Sectioning of Specimens	Sri Ram chandra dental college,Chennai.

Miscellaneous : -
Bonding agent, Applicator tips.

TABLE 2: List of the dentine bonding agents, their composition, and manufacturers.

Bonding Agent	Composition	Manufacturer
Gluma Self-etch	Acetone/water-based formulation of light activated methylacrylate resins Ethanol, water and hydroxypropyl cellulose. Containing potassium fluoride, polyethylene glycol dimethacrylate ,methacrylate	Heareus Kulzer

International Journal Dental and Medical Sciences Research
Volume 5, Issue 3, May - June 2023 pp 651-660 www.ijdmsrjournal.com ISSN: 2582-6018

Adper Easy Bond	BisGMA, HEMA, dimethacrylates, ethanol, water, Novel photoinitiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids	3M ESPE
Opti Bond All in One	Glycerolphosphate dimethacrylate. Co-monomers including mono- and difunctional methacrylate monomers.	Kerr

SPECIMEN PREPARATION FOR POLARISED MICROSCOPE

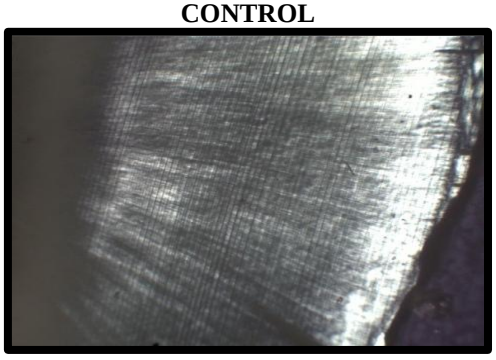
Each slices were grounded to sizes 80 µm (±20 µm) using Hard tissue microtome. Each section is then immersed in water and examined for caries like lesions (demineralized area) with a polarized microscope.

Lesion depths were measured using the microscope eyepiece reticle, which was calibrated by placing a 10 micron/unit graticule on the microscope stage. In lesions with irregular advancing fronts, only the deepest measurement was recorded.

II. RESULTS

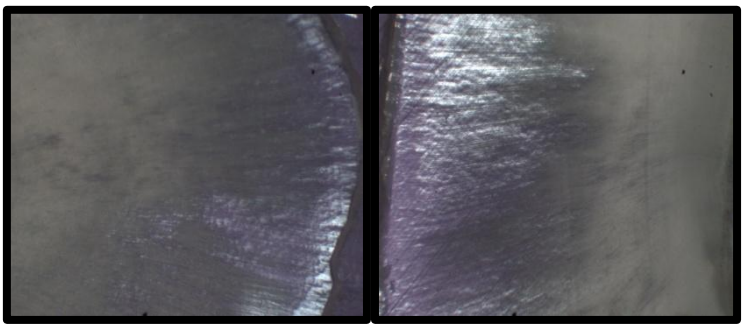
Photomicrograph showing Demineralization

CONTROL



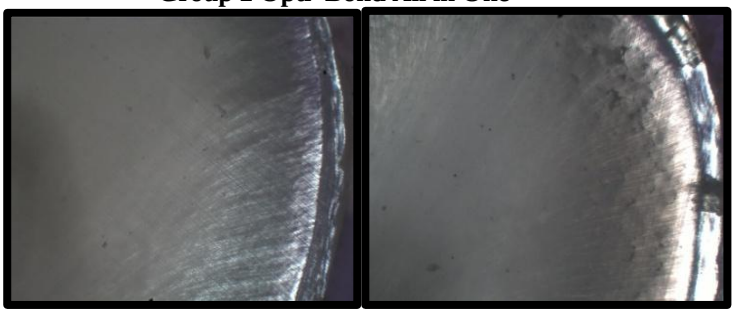
GROUP 1 (Adper Easy Bond)

International Journal Dental and Medical Sciences Research
Volume 5, Issue 3, May - June 2023 pp 651-660 www.ijdmsrjournal.com ISSN: 2582-6018



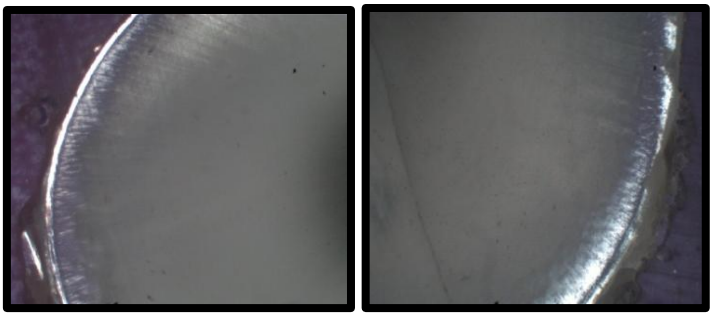
Subgroup 1A Subgroup 1B

Group 2 Opti Bond All in One



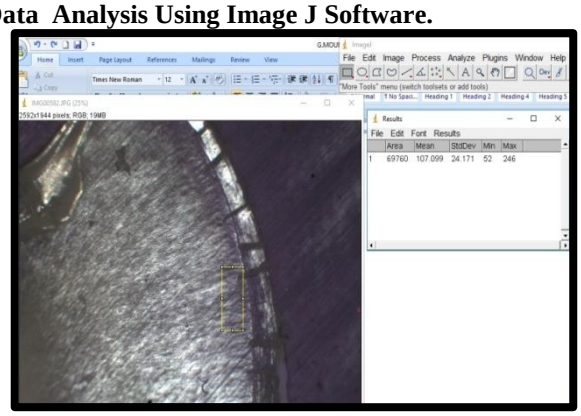
Subgroup 2A Subgroup 2B

Group 3 - Gluma Self Etch



Subgroup 3A Subgroup 3B

Deminearalisation Depth Data Analysis Using Image J Software.



Using Image J Software depth of demineralised zone analysis done by using the ROI in polygonal form .

Statistical Analysis

The statistical analysis was done using one way ANOVA, Student's t test.

International Journal Dental and Medical Sciences Research
Volume 5, Issue 3, May - June 2023 pp 651-660 www.ijdmsrjournal.com ISSN: 2582-6018

Table 3:- Demineralization depth Values between Subgroups A of each group (in µm).

S.No	Control (n=10)	Subgroup 1A(n=10)	Subgroup 2A(n=10)	Subgroup 3A (n=10)
1	86.2	33	33.5	41.5
2	88.8	38.5	43.5	31
3	92.4	35.5	38.7	34.9
4	67.5	33.5	35	32
5	87.5	36	34.9	33.2
6	87.9	38.7	41.2	36.1
7	83.3	34	38.5	33.4
8	91.3	33.8	37.5	34.2
9	86.7	34.2	38.5	35.5
10	89.8	34.2	39.1	36

Table 4:- Demineralization depth Values between Subgroups B of each group (in µm).

S.No	Control(n=10)	Subgroup 1B (n=10)	Subgroup 2B (n=10)	Subgroup 3B (n=10)
1	86.2	47.2	44.5	32.3
2	88.8	61.5	60.5	49.5
3	92.4	53.2	50.6	38.9
4	67.5	53.5	54.6	36.2
5	87.5	54.6	55.3	47.5
6	87.9	56.2	51.1	38.9
7	83.3	51.5	49.3	33.6
8	91.3	52.6	48.5	34.7
9	86.7	54.2	47.2	36.1
10	89.8	53.9	46.3	37.4

International Journal Dental and Medical Sciences Research
Volume 5, Issue 3, May - June 2023 pp 651-660 www.ijdmsrjournal.com ISSN: 2582-6018

TABLE 4: Comparison of demineralization depth between Subgroups A of each group (in µm) .

SUMMARY

Groups	Count	Sum	Average	Variance	Standard Deviation
control	10	861.3	86.13	49.7	7.1
subgroup 1A	10	351.4	35.14	4.1	2.0
subgroup 2A	10	380.4	38.04	9.1	3.0
subgroup 3A	10	347.8	34.78	8.4	2.9

TABLE 5: Comparison of demineralization depth between Subgroups B of each group (in µm) .

SUMMARY

Groups	Count	Sum	Average	Variance	Standard Deviation
control	10	861.3	86.1	49.7	7.1
subgroup 1B	10	538.4	53.8	13.0	3.6
subgroup 2B	10	507.9	50.8	23.3	4.8
subgroup 3B	10	385.1	38.5	32.4	5.7

Table 6: Comparison of demineralization depth between the groups (in µm).

Comparison between the groups	Mean Difference
Control versus Group 1	41.64
Control versus Group 2	41.72
Control versus Group 3	49.49
Group 1 versus Group 2	0.07
Group 1 versus Group 3	7.85
Group 2 versus Group 3	7.77

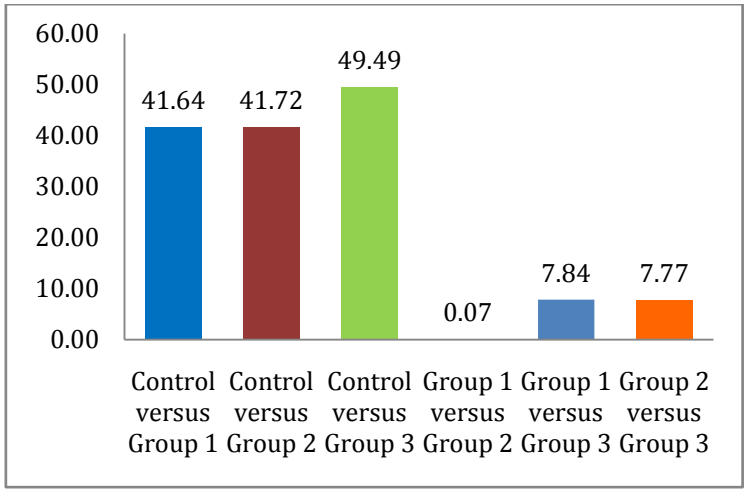


Table 6 depicts that maximum difference in demineralization was found between control and Group 3 (49.49µm) and minimum between Groups 1 and 3 (7.85 µm) which was statistically significant (P < 0.001).

III. DISCUSSION

International Journal Dental and Medical Sciences Research
Volume 5, Issue 3, May - June 2023 pp 651-660 www.ijdmsrjournal.com ISSN: 2582-6018

Cementum is the first layer to be encountered when the root surface is exposed to the oral environment. However, the cementum formation exists as a superficial layer on the root and is easily peeled off by intensive root planing during the treatment of periodontal diseases or by tooth brushing.

Therefore, the peel-off vulnerability of the cementum layer causes the underlying dentin to be prone to exposure, hence increasing the risk of root caries formation. A concomitant development is the high incidence of root caries in the middle-aged and elderly, due to gingival recession and exposure of susceptible root surfaces of the retained dentition.[1-8]

The etiology of root caries formation appears to be similar to that of enamel caries. A susceptible tooth surface (exposed root), acidogenic microflora and fermentable carbohydrates are all needed to produce caries. The bacterial organisms implicated in root caries include Streptococcus mutans, lactobacilli, and some species of Actinomyces[9].

It has been reported that dentin bonding systems, provided strong physical barrier with the formation of a hybrid layer, are well poised to be an effective preventive option against root caries[4].

With increase in Advancements a variety of dentin bonding agents are available. The concept of self-etching approach was created approximately 20 years ago, however, the first and second generations of bonding agents can be considered self-etch materials because no acid etching/rinsing or conditioning step were used[10].

In this study, seventh generation dentin bonding agents were used which developed in late 2002 (self etch) i.e., all in one adhesive systems that in single component adhesives, acidic monomers, hydrophilic monomers, water and/or organic solvents are combined into one solution. The advantage of these self etching primer systems is that they do not etch very far into the dentin beneath smear layers. This avoids removal of smear plugs in the tubules and seems to be responsible for the lack of postoperative sensitivity associated with these technique-insensitive adhesive systems. The shallow etch ensures good resin infiltration. Even through the hybrid layer is thin, resin dentin bond strengths are very high.

The bonding mechanism of self-etch adhesive systems has been intensely investigated and two-fold bonding mechanisms; micro-mechanical interlocking and chemical bonding were described, which seems to be advantageous in terms of restoration durability. The micro-mechanical bonding contributes to provide strength against mechanical stress, while the chemical interaction reduces hydrolytic degradation, keeping the marginal sealing of restorations for a longer period.

The self etching mechanism of these systems is based on acidic monomers that are able to partially demineralize and infiltrate the dental hard tissues[5]. Unlike etch and rinse systems, they use the smear layer as a bonding substrate, by incorporating it into the hybrid layer. As a result of this postoperative sensitivity, a potential risk related to incomplete resin infiltration, is supposed to be limited.

In this Study Gluma, Adper, and Opti bond bottle/all-in-one adhesive systems, which were in essence water- and solvent-based adhesives. As such, manufacturers of the latter adhesives recommended strong air-blowing before curing to remove both water and solvent in the adhesives, resulting in the formation of a thin adhesive layer.

In this study, two coats of dentin bonding agents was applied on the experimental surface of each tooth as for some all - in - one adhesives. Application of the all - in - one adhesive in multiple layers may result in higher bond strengths and better infiltration into the hybrid layer[11].One manufacturer recommended rubbing the adhesive continuously for 15 s, followed by the application of a second coat after gentle air drying and curing the first coat. This second coat prevents the formation of dry spots on the dentin surface.

The single application produces a porous and fibrous appearance, which is supposed to be over etched. In the second application, the additional supply of adhesive resin may improve the infiltration of resin monomers into the intertubular demineralised dentin which appears dense. Gentle air blowing for 1–2 s with oil free

compressor from a distance of approximately 20 cm was followed.[12]

In clinical applications, two mandatory prerequisites for coating materials are durability and wear resistance. In a study by Kaneshiro et al. on the prevention of root surface demineralization, an experimental coating system was investigated by being applied as an appropriately thick layer[8,9] .

To investigate tooth demineralization in vivo and in vitro, several methods have been employed, such as hardness test, microradiography, contact microradiography energy dispersive spectroscopy and electron probe microanalysis.

In the present study, the teeth were sectioned using a mechanized hard tissue microtome (LEICA SP 1600) to obtain buccolingual longitudinal midsections of 100 micron thickness.

Hard tissue microtome has been used as it gave the uniform thickness of the specimens and the effect of root surface coating on prevention of dentin demineralization was evaluated using Polarised Microscope.

Polarized light microscope was used to assess the lesion depth because the histological features of dentin and enamel can be visualized better due to its birefringence property. In Wefel study they analysed that the positive birefringence of dentin seen under polarized light microscopy is increased during demineralization, and, therefore, no stark contrast is produced between demineralized and sound dentin[10].

The depth of demineralised dentin was analysed using Image J SOFTWARE . Image J software, a Javabased image processing program used to interpret the lesion depth.

In this study the control group with no application of bonding agents has done in order to differentiate the histological pattern (Depth of demineralised zone) with other groups.

Statistical analysis in this study showed that Subgroups A (first layer cured with air thinning and second layer cured without air thinning) undergone less demineralization compared to Subgroup B (both first and second layer cured with air thinning). The above results can be because shorter drying time attributes to higher bond strength[11,12].

Dr. John Kanca and Gwinnet in 1992 who introduced the concept of wetting phenomenon , explained that the complete drying of the dentin with air causes the collagen to collapse which would prevent the monomers from penetrating the nanochannels formed by dissolution of hydroxyapatite crystals between collagen fibres.Dentin collagen collapses easily upon excessive air drying which results in closing of micropores in the exposed intertubular collagen[13-14]. This might in turn lead to incomplete impregnation of adhesive resin into the underlying dentin and further increase in demineralization

Statistical analysis in the current study showed that there was no significant difference between Group 2 (Opti Bond) and Group 3 (Gluma Self etch), which undergone least demineralization. Gluma Self etch and Opti Bond All in one both have pH > 2 which makes them “mild” self etch adhesives, whereas Adper Easy one has pH < 2 which makes it “intermediary strong” self etch adhesive. “Mild” self etching appears most promising, especially with regard to bond stability.

In general, self-etching adhesives can be classified as ultra-mild (pH.2.5), yielding demineralization of only a few hundreds of nanometers; mild (pH;2), with demineralization of about 1micron intermediately strong (pH=1-2), with demineralization about 1-2 micro m and strong (pH < 1), with demineralization greater than 2 micro m.

Acidity of self etching monomers can be buffered by the mineral components of smear layer. This implies that more the acidic the adhesive, more will be the penetration, resulting in more demineralization[12].So Adper Easy one group being more acidic (pH < 2) resulted in more acidic demineralization of dentin subsurface.

The interfacial structure of highly acidic self-etching adhesives resembles that of etch-and-rinse systems, with the difference that the dissolved mineral phase is not rinsed away. These calcium phosphates are known to be very unstable, greatly contributing to weakening the interface. “Mild” self-etching partially removes the smear layer, forming a thin hybrid layer. It has the great advantage of leaving substantial amount of hydroxyapatite-crystals around collagen fibrils, which may establish chemical bond with specific carboxylic or phosphate groups of functional monomers. Hence in this study results obtained with mild self etch adhesives of (Gluma bond) and

(Opti Bond All in one)showed the minimum depth of demineralisation zone than other adhesive group (Adper Easy One).

In the light of bonding durability, mild self etch adhesives (Gluma Self etch) have unique property that all hydroxyapatite are not removed from the interaction zone, and much calcium is available for additional chemical interaction with specific adhesive monomers. Hence, the bonds are stable, even in the aqueous environment, and the mechanism is supposed to prolong the clinical lifetime of the restorations[13].

Gluma in Self etch provides antimicrobial effect in dentinal tubules reduces the risk of pulpitis. Collagen coagulates in the dentinal tubules reducing sensitivity. Reduction of production of MMP's decreases bond degradation. Glutaraldehyde, popularized by the name brand Gluma Desensitizer by Kulzer, has become well-known as an additive to desensitize.

Gluma has been used as a desensitizing agent since 1991 and is a combination of 5% glutaraldehyde and 35% hydroxyethyl methacrylate (HEMA). MMPs, which degrade Type I collagen, can slowly degrade the collagen fibrils at the interface of the dentin with the resin.[13,14.]Gluma self etch adhesive also contain the potassium fluoride which prevent the secondary caries formation due to the continuous, sustained release of fluoride ions.

In clinical applications, two mandatory prerequisites for coating materials are durability and wear resistance. In a study by Kaneshiro et al. on the prevention of root surface demineralization, an experimental coating system was investigated by being applied as an appropriately thick layer[15].

IV. CONCLUSION:

Depth of Demineralised zone on experimental root surface specimen assessed with Gluma Self Etch showed the minimum value than Adper Easy and Opti Bond All in One adhesive, which were statistically significant ($P < 0.001$).

Therefore, future studies should be undertaken to investigate if differences in surface coating thickness would result in tandem differences in the durability and wear resistance of the coating layer. The study demonstrated that prevention of dentin demineralization using surface coatings was material-dependent.

It is the scope of future studies to determine which type of lesions can be managed by less invasive treatments.

REFERENCES

[1]. Nakabayashi N, Pashley DH. Hybridization of dental Tissues. Che. 3, 1, 2.

[2]. Chi DL, Shyue C. Managing caries risk in adults. Dimen Dent Hyg. 2014;12:36-40.

[3]. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. J Dent Res 1992;71:1530-40.

[4]. McIntyre JM, Featherstone JD, Fu J. Studies of dental root surface caries.Comparison of natural and artificial root caries lesions. Australian Dental Journal. 2000 Mar;45(1):24-30.

[5]. Imazato S, Walls AW, Kuramoto A, Ebisu S. Penetration of an antibacterial dentine-bonding system into demineralized human root dentine in vitro. European journal of oral sciences. 2002 Apr;110(2):168-74.

[6]. Doi J, Itota T, Yoshiyama M, Tay FR, Pashley DH. Bonding to root caries by a self-etching adhesive system containing MDPB. American journal of dentistry. 2004 Apr;17(2):89-93.

[7]. Yoshiyama M, Nishitani Y, Itota T, Tay FR, Carvalho RM, Pashley DH. Bonding ability of adhesive resins to caries-affected and caries-infected dentin. Journal of Applied Oral Science. 2004 Sep;12(3):171-6.

[8]. Shinohara MS, Yamauti M, Inoue G, Nikaido T, Tagami J, Giannini M, Goes MF. Evaluation of antibacterial and fluoride-releasing adhesive system on dentin—microtensile bond strength and acid-base challenge. Dental materials journal. 2006;25(3):545-52.

[9]. Yamamoto K, Takeuchi O, Zennyuu K, Fukui M, Suzuki K, Hatsuoka Y, Shiraishi M, Inoue M. Effect that air thinning of bonding resin has on the bond strength in three self-etching primer

adhesive systems. Journal of Osaka Dental University. 2006;40(1):13-7.

[10]. Kurokawa H, Miyazaki M, Takamizawa T, Rikuta A, Tsubota K, Uekusa S. One-year clinical evaluation of five single-step self-etch adhesive systems in non-carious cervical lesions. Dental materials journal. 2007;26(1):14-20.

[11]. Espejo LC, Simionato MR, Barroso LP, Netto NG, Luz MA.Evaluation of three different adhesive systems using a bacterial method to develop secondary caries in vitro. Am J Dent. 2010 Apr 1;23(2):93-7.

[12]. ChiDL,ShyueC. Managing caries risk in adults. Dimen Dent Hyg. 2014;12:36-40.

[13]. Yamamoto K, Takeuchi O, Zennyuu K, Fukui M, Suzuki K, Hatsuoka Y, Shiraishi M, Inoue M. Effect that air thinning of bonding resin has on the bond strength in three self-etching primer adhesive systems. Journal of Osaka Dental University. 2006;40(1):13-7.

[14]. Espejo LC, Simionato MR, Barroso LP, Netto NG, Luz MA. Evaluation of three different adhesive systems using a bacterial method to develop secondary caries in vitro. Am J Dent. 2010 Apr 1;23(2):93-7.