IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 19, Issue 5 Ser.14 (May. 2020), PP 12-17
www.iosrjournals.org

Comparative Evaluation of Shear Bond Strength on Enamel after Demineralization and Remineralization - an *in vitro* Study

Ravi Vaiyapuri[1], SaravanaPriyanSoundappan[2], AnuRadha.R.S[3], AndamuthuSivakumar.A[4], JambaiSampathkumar Sivakumar[5],ChittrarasuMathimaraiselvan[6]

[1](Professor and Head of the Department) Conservative Dentistry and Endodontics
Vivekanandha Dental College for Women, India
[2](Reader)Department of Conservative Dentistry and Endodontics
Vivekanandha Dental college for women, Country: India
[3](Post Graduate Student) Department of Conservative Dentistry and Endodontics
Vivekanandha Dental college for women ,India
[4](Professor)Department of Conservative Dentistry and Endodontics
Vivekanandha Dental college for women, India
[5](Reader) Department of Conservative Dentistry and Endodontics
Vivekanandha Dental college for women, India
[6](Senior Lecturer)Department of Conservative Dentistry and Endodontics
Vivekanadha Dental college for women, India
Coressponding Author: AnuRadha.R.S (Post Graduate Student)
Department of Conservative Dentistry and Endodontics

Abstract
INTRODUCTION:*Remineralization is the process whereby calcium and phosphate ions are supplied from a source external to the tooth. Casein has the ability to stabilize calcium and phosphate ions by releasing small sequences of peptides (CPPs). ACP is pH responsive, with increasing pH, the level of bound ACP increases, ACP acts as a precursor to bio-apatite and as a transient phase in bio-mineralization.*
AIM AND OBJECTIVE*: To establish the shear bond strength on enamel following exposure to an aerated drink at various time intervals with and without application of remineralization agent.*
MATERIALS AND METHODS:*36 therapeutically extracted human maxillary premolars were selected. Prepared samples were randomly divided into three groups: Control group I (n=12), Demineralized group II (n=12) and Remineralized group III (n=12). Group II & III were treated with chilled carbonated soft drink for the observation period of 1 month as 5 minutes twice per week.Then specimens in Group III alone were treated with (CPP-ACP) remineralization agent daily for 4 minutes during the observation period of 1 month. Acid etching treatment, adhesive bonding and composite resin build up was done with (Filtek Z 250.X7;3M ESPE) on labial surface of all the specimens by conventional technique. Then all the specimens were tested for shear bond strength under universal testing machine.*
RESULT:*The obtained result showed statistically significant increases in bond strength in Demineralized group II rather than the Remineralized group III*
CONCLUSION:*The residual CPP-ACP molecules tends to occlude the porosities of the enamel surface even after thorough debridement.This inversely affects the bond strength.*
CLINICAL SIGNIFICANCE: *Proper diet history and oral measures of the patients should be recorded to determine the need of using remineralization agents to enhance the bonding of resin to the natural tooth.*
Key Words: *CPP-ACP, Remineralization, Demineralization, Bio-mineralization*

Date of Submission: 13-05-2020 Date of Acceptance: 25-05-2020

I. Introduction:

Tooth enamel is highly mineralized and it is the hardest tissue of the body. The loss of enamel may occur due to many reasons such as caries, tooth wear, and trauma.[1] Natural demineralization of tooth at an early stage is reversed by saliva, which contains calcium ions, phosphate ions, buffering agents, fluoride, and other substances.[2] In addition to dental caries, acidic beverages also cause demineralization of tooth enamel leading to tooth wear in the form of erosion. Dental erosion is the loss of dental hard tissues by chemical process without the involvement of cariogenic bacteria.[3]

Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain.[3] A newer concept for remineralization is with the use of milk and milk products, which appears to have a protective effect on the tooth.[4]The casein phosphopeptides (CPPs) are produced from the cryptic digest of casein, aggregated with calcium phosphate and purified through ultrafiltration. Casein has the ability to stabilize calcium and phosphate ions by releasing small sequences of peptides (CPPs) through partial enzymatic digestion that led to the development of a remineralization technology based on casein phosphopeptide and stabilized amorphous calcium phosphate complexes (CPP- ACP). It is pH responsive, i.e. with increasing pH, the level of bound ACP increases, stabilizing free calcium and phosphate and thus providing an anti- calculus action. Thus, making resistance to acid dissolution.[3]ACP acts as a precursor to bio-apatite and as a transient phase in bio-mineralization.[5]

The bond strength of adhesive materials is directly related to minerals in tooth structures. The acidic treatment (from carbonated beverages or any other source) results in loss of minerals from enamel and alters the surface properties compromising the bond strength.[1]

II. Aim And Objective:

To establish the shear bond strength of enamel following exposure to an aerated drink at various time intervals with and without application of remineralization agent.The null hypothesis would be the increase in the shear bond strength in the remineralized group.

III. Material And Methods:

ETHICAL CLEARANCE:

A detailed protocol explaining the purpose and procedures of the study was submitted to the Institutional Review Board, Vivekanandha Dental College for Women and the approval for the study were obtained.

INCLUSION CRITERIA:

Intact human maxillary and mandibular pre-molar teeth extracted for orthodontic purpose were included.

EXCLUSION CRITERIA:

Carious, restored / developmentally malformed teeth were excluded for this study.

IV. Methodology:

SAMPLE PREPARATION:-

36 therapeutically extracted human maxillary pre-molars were collected and cleaned of debris ultrasonically and polished for 30 seconds using non-fluoridated and oil-free pumice slurry. Then teeth were washed using deionized water to remove any residual debris or tissue remnant. Then the samples were stored in artificial saliva until further experimentation.Prepared samples were randomly divided into three groups,

Group I (n=12): Control group
Group II (n=12): Demineralized group
Group III (n=12): Remineralized group
All specimens in Groups were stored in artificial saliva for one month, (Fig-1A)

Fig 1: (A) Teeth samples stored in artificial saliva, (B) whereas group II and III were treated with aerated drink.

Group II and Group III specimens were treated with chilled carbonated soft drink for 5 minutes twice per week for one month (Fig-1B). Only in Group III, specimens were treated with application of casein phophopeptides and amorphous calcium phosphate (CPP-ACP) remineralization agent daily for 4 minutes for a period of one month (Fig-2).

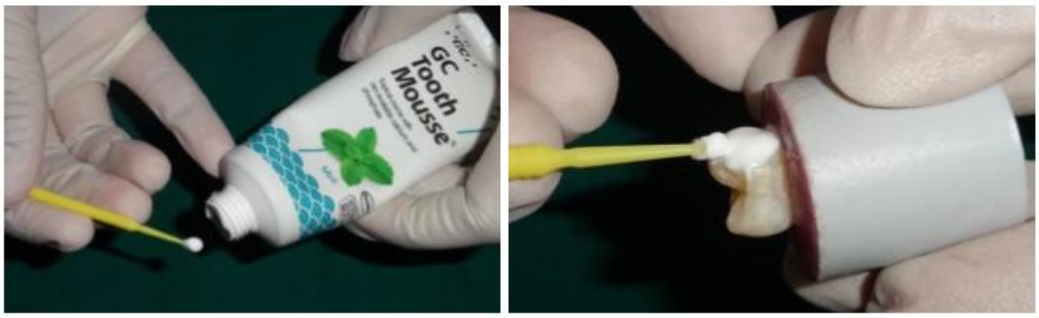


Fig 2: Application of remineralization agent for Group III

ACID ETCHING TREATMENT AND BONDING:

Conventional acid etching technique following the manufacturer's instructions was used. The labial enamel surface of all specimens was etched using 37% ortho-phosphoric acid (Total Etching) for 15 seconds followed by washing and air drying. The adhesive bonding agent (Adper Single Bond 2, 3M ESPE) was applied and cured for 10 seconds using a light curing unit. An attachment was built (4 mm in diameter and 3mm in height) on etched enamel(Fig-3, 4). Then the prepared specimens were measured for the shear bonding strength.

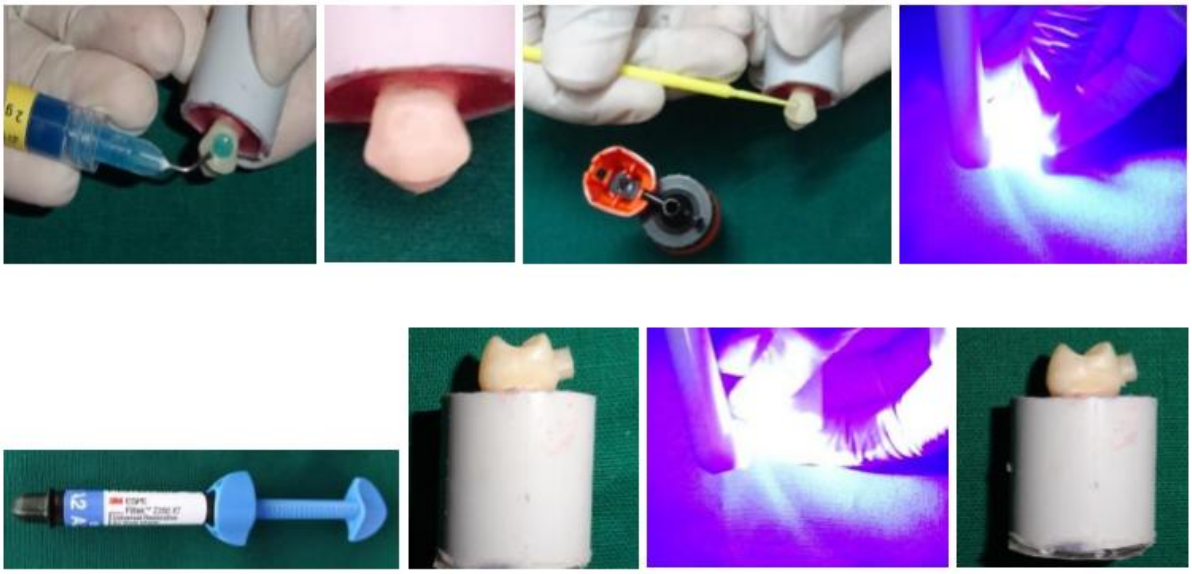


Fig 3: Preparation of teeth samples.

Fig 4: Resin built up on teeth samples.

SHEAR BOND STRENGTH MEASUREMENT:

Shear bond strength was measured using a steel parallel blade (1 mm in diameter) mounted on a universal testing machine at a crosshead speed of 0.5 mm/min and on the composite enamel interface until the

bond gets failed (Fig-5).The force required to dislodge the composite cylinderwas recorded in Newton and converted to mega Pascal (MPa) using the formula:[1,5]

$$Mpa = \frac{peak\ load\ in\ failure\ area\ (Newton)}{bonding\ area\ (\text{mm}^2)}$$

Fig 5: Prepared teeth samples under universal testing machine

V. Result:

The obtained result was statistically analysed using one way ANOVA test (Table-1, 2) and Kolmogorov-Smirnov test using the software SPSS 10.0(Table-3, Graph-1), shows that there is increase in shear bond strength in teeth samples treated only with demineralization agent rather than teeth samples treated with demineralization agent followed by remineralization agent.(Fig-6)

Group	N	Shear Bond Strength				ANOVA	P
		Min	Max	Mean	SD		
Control	12	0.159	1.230	0.747	0.311	1.521	0.233
Demineralization	12	0.143	1.319	0.877	0.313		
Remineralization	12	0.443	0.917	0.686	0.173		

Table-1: Statistical analysis by One-way ANOVA test

Group	N	Shear Bond Strength		T	P
		Mean	SD		
Control	12	0.7467	0.31101	1.021	0.318
Demineralization	12	0.8768	0.31300		
Control	12	0.7467	0.31101	0.589	0.562
Remineralization	12	0.6861	0.17303		
Demineralization	12	0.8768	0.31300	1.847	0.078
Remineralization	12	0.6861	0.17303		

Table-2: Statistical analysis by One-way ANOVA test

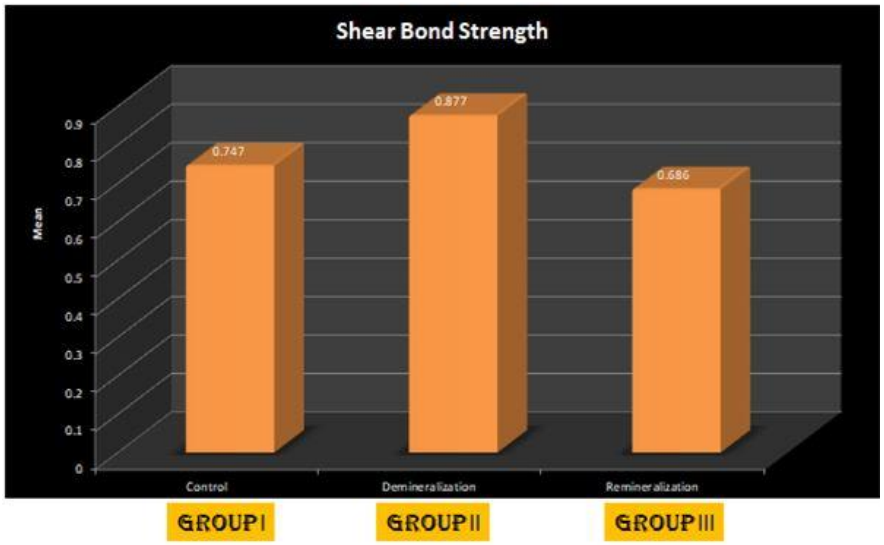
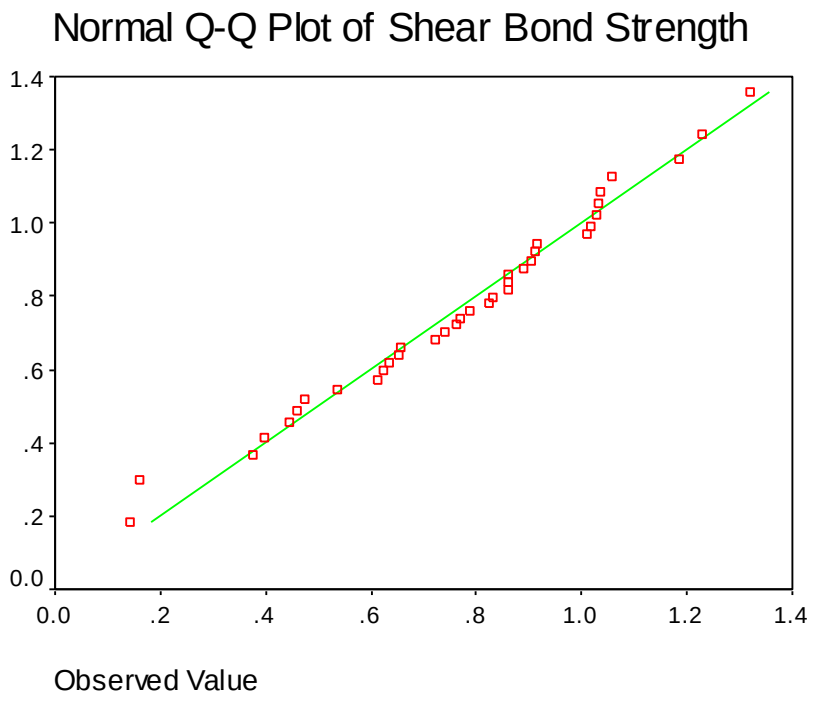


Fig-6: Shear bond strength of all the groups

One-Sample Kolmogorov-Smirnov Test		
		Shear Bond Strength
N		36.000
Normal Parameters(a,b)	Mean	0.770
	Std. Deviation	0.278
Most Extreme Differences	Absolute	0.076
	Positive	0.066
	Negative	-0.076
Kolmogorov-Smirnov Z		0.458
Asymp. Sig. (2-tailed)		0.985
a. Test distribution is Normal.		

Table-3: One- sample Kolmogorov-Smirnov Test



Test distribution is Normal – Q-Q Plot
Graph-1: Normal Q-Q Plot of Shear Bond Strength

VI. Discussion:

The structural integrity of the dental hard tissue shows the hierarchy and dynamic balance between demineralization and remineralization. Saliva has the ability to remineralize tooth using bio-available Ca^{2+} and Po_4^{3-} .Aerated drinks containing acidswith a pH of 2.52 (carbonic acid, citric / phosphoric acid) cause dental erosion.

As salivahas the ability to remineralize tooth using calcium and phosphates as a bio- available mineral, the action of salivary phosphor- proteins (statherin) causes precipitation of calcium and phosphorus salt. These acids are next stronger to battery acids with a pH of 2.52 and strong enough to demineralize enamel.[1]

Casein has the ability to stabilize calcium and phosphate ions by releasing small sequences of peptides (CPPs) through partial enzymic digestion that led to the development of a remineralization technology based on CPP-ACP. It is pH responsive.[3]

The CPP- ACP is a sticky protein maintains the amorphous state by binding the calcium and phosphorus ions. CPP stabilizes the nanoclusters of amorphous calcium/phosphate in the meta- stable solution.[1]

ACP acts as a precursor to bio-apatite and as a transient phase in bio-mineralization.[5] The bond strength of adhesive materials is directly related to the minerals in tooth structure. The acidic treatment (from carbonated beverages or any other source) results in loss of minerals from enamel and alters the surface properties compromising the bond strength.[1]

The mechanism of CPP–ACP is that, it has the ability to localize ACP at the tooth structure, increasing the level of calcium phosphatein plaque andforms a calcium phosphatereservoir, the free calcium and phosphate

ion get buffered, thereby helping to maintain a state of super-saturation with respect to tooth enamel and thereby it decreases enamel demineralization and enhances enamelremineralization.[11]

CPP-ACP can protect enamel surface from erosion and increases its wear resistance and helps better clinical management in tooth wear situations.[5]CPP-ACPcauses an increase in the level of calcium and phosphate ions in supra gingival plaque by bonding its CPP part to saliva pellicle and *Streptococcus mutans*bacterial surface. Its existence in plaque biofilm can lead to subsurface enamel lesion's remineralization, and thus they would be more resistant to acid attacks in the future.[7,8]

CPP-ACP can produce a hypermineralized enamelsurface by depositing calcium and phosphate, thus the weak acidity of the self-etch adhesives cannot remove enriched surface layer and adhesive penetration is adversely affected. This phenomenon can explain the more adhesive failures.[5, 9, 10]

The present study shows there is increase in shear bond strength in demineralized teeth (Group II) when compared to other two groups. The shear bond strength in control group (Group I) seems to be higher than the remineralized group (Group III) suggesting the possible hypermineralization of the enamel surface due to the application of CPP-ACP paste. Hence the null hypothesis is disproven.

In the literature of review, studies done with CPP-ACP paste in regards to shear bond strength where self-etch adhesive have shown poor bond strength and in this study, we have used total etch which also shown decrease in the shear bond strength. So, the etching time for the tooth treated with CPP-ACP paste should be modified.

Limitations for this study are that; this is an*in vitro* study and the experimental condition does not completely reflect the dynamic conditions of oral environment. A number of factors such as raise and fall in temperature and pH, food interaction, presence of microorganism which may interferes the remineralizationprocess. This study has provided the baseline results hinting that demineralization treatment before adhesive restorations has the potentials to improve the bond strength. However, further clinical studies are required to validate this condition.

VII. Conclusion:

Even after thorough debridement,the residual CPP-ACP molecules tend to occlude the porosities of the enamel surface. Thus, it inversely affects the bond strength.

Although remineralization using CPP-ACP is accepted, it seems to have detrimental bond strength with the tooth enamel.So, the etching might play a vital role when CPP-ACP based pastes are used.

Thus, further studies are recommended to obtain more detailed information on this topic with regards to etching time in CPP-ACP paste treatedteeth.

References:

[1]. Abbas Rizvi, Muhammad S. Zafar, Yasser Al- Wasifi, WamiqFareed, ZohaibKhurshid .Role of enamel deminerlization and remineralization on microtensile bond strength of resin composite. 2016 European Journal of Dentistry.

[2]. NamrataPatil, ShantanuChoudhari, SadanandKulkarni, Saurabh R Joshi. Comparative evaluation of remineralizing potential of three agents on artificially demineralized human enamel: An in vitro study. Journal of Conservative Dentistry | Mar-Apr 2013 | Vol 16 | Issue 2

[3]. ShashiPrabhaTyagi, ParidhiGarg, Dakshita Joy Sinha, UdaiPratap Singh. An update on remineralizing agents. Journal of Interdisciplinary Dentistry / Sep-Dec 2013 / Vol-3 / Issue-3

[4]. JayanthJayarajan, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPE on enamel remineralization – An in vitro study using scanning electron microscope and DIAGNOdent. Indian Journal of Dental Research, 22(1), 2011.

[5]. NiloofarShadman, ShahramFarzinEbrahimi, Maryam AziziShoul, HastiSattari. In vitro evaluation of casein phosphopeptide-amorphous calciumphosphate effect on the shear bond strength of dental adhesives to enamel. Dental Research Journal / March 2015 / Vol 12 / Issue 2 167

[6]. Tehrani MH, Ghafournia M, Samimi P, Savabi O, Parisay I, Askari N, et al. Effect of casein phosphopeptide-amorphous calcium phosphate and acidulated phosphate fluoride gel on erosive enamel wear. Dent Res J (Isfahan) 2011;8:S64-70.

[7]. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate.J Dent Res 2008;87:344-8.

[8]. Poggio C, Lombardini M, Dagna A, Chiesa M, Bianchi S. Protective effect on enamel demineralization of a CPP-ACP paste: An AFM in vitro study. J Dent 2009;37:949-54.

[9]. Adebayo OA, Burrow MF, Tyas MJ. Effects of conditionersonmicroshear bond strength to enamel after carbamide peroxide bleaching and/or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) treatment. J Dent 2007;35:862-70.

[10]. Margvelashvili M, Goracci C, Beloica M, Papacchini F, Ferrari M. In vitro evaluation of bonding effectiveness to dentin of all-in-one adhesives. J Dent 2010;38:106-12.

[11]. Divyapriya GK, Puja C Yavagal, Veeresh DJ. Casein phosphopeptide–amorphous calcium phosphate in dentistry: An update. International Journal of Oral Health Sciences;2016;6(1):18-25.

AnuRadha.R.S, et. al. "Comparative Evaluation of Shear Bond Strength on Enamel after Demineralization and Remineralization - an in vitro Study."*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(5), 2020, pp. 12-17.