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

Ravi Vaiyapuri¹, SaravanaPriyanSoundappan², AnuRadha.R.S³, AndamuthuSivakumar.A⁴, JambaiSampathkumar Sivakumar⁵, ChittrarasuMathimaraiselvan⁶

¹(Professor and Head of the Department) Conservative Dentistry and Endodontics
Vivekananda Dental College for Women, India
²(Reader) Department of Conservative Dentistry and Endodontics
Vivekananda Dental college for women, Country: India
³(Post Graduate Student) Department of Conservative Dentistry and Endodontics
Vivekananda Dental college for women, India
⁴(Professor) Department of Conservative Dentistry and Endodontics
Vivekananda Dental college for women, India
⁵(Reader) Department of Conservative Dentistry and Endodontics
Vivekananda 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 bioapatite and as a transient phase in biomineralization.

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.[9] ACP acts as a precursor to bioapatite 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.](image)

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 phosphopeptides and amorphous calcium phosphate (CPP-ACP) remineralization agent daily for 4 minutes for a period of one month (Fig-2).
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.

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

Fig 2: Application of remineralization agent for Group III

Fig 3: Preparation of teeth samples.

Fig 4: Resin built up on teeth samples.
Comparative Evaluation of Shear Bond Strength on Enamel after Demineralization

bond gets failed (Fig-5). The force required to dislodge the composite cylinder was recorded in Newton and converted to mega Pascal (MPa) using the formula: \[ Mpa = \frac{\text{peak load in failure area (Newton)}}{\text{bonding area (mm}^2)} \]

\[ \text{Fig 5: Prepared teeth samples under universal testing machine} \]

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)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Shear Bond Strength</th>
<th>ANOVA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0.159, 1.230, 0.747, 0.313</td>
<td>1.521</td>
<td>0.233</td>
</tr>
<tr>
<td>Demineralization</td>
<td>12</td>
<td>0.143, 1.319, 0.877, 0.314</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remineralization</td>
<td>12</td>
<td>0.443, 0.917, 0.686, 0.173</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Shear Bond Strength</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0.7467, 0.31101</td>
<td>1.021</td>
<td>0.318</td>
</tr>
<tr>
<td>Demineralization</td>
<td>12</td>
<td>0.8768, 0.31300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>0.7467, 0.31101</td>
<td>0.589</td>
<td>0.562</td>
</tr>
<tr>
<td>Remineralization</td>
<td>12</td>
<td>0.6861, 0.17303</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demineralization</td>
<td>12</td>
<td>0.8768, 0.31300</td>
<td>1.847</td>
<td>0.078</td>
</tr>
<tr>
<td>Remineralization</td>
<td>12</td>
<td>0.6861, 0.17303</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

\[ \text{Fig-6: Shear bond strength of all the groups} \]

DOI: 10.9790/0853-1905141217 www.iosrjournal.org 15 | Page
Comparative Evaluation of Shear Bond Strength on Enamel after Demineralization

<table>
<thead>
<tr>
<th>One-Sample Kolmogorov-Smirnov Test</th>
<th>Shear Bond Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>36.000</td>
</tr>
<tr>
<td>Normal Parameters (a, b)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.770</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.278</td>
</tr>
<tr>
<td>Absolute</td>
<td>0.076</td>
</tr>
<tr>
<td>Positive</td>
<td>0.066</td>
</tr>
<tr>
<td>Negative</td>
<td>-0.076</td>
</tr>
<tr>
<td>Kolmogorov-Smirnov Z</td>
<td>0.458</td>
</tr>
<tr>
<td>Asymp. Sig. (2-tailed)</td>
<td>0.985</td>
</tr>
</tbody>
</table>

Table-3: One-sample Kolmogorov-Smirnov Test

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 Ca²⁺ and PO₄³⁻. Aerated drinks containing acids with a pH of 2.52 (carbonic acid, citric / phosphoric acid) cause dental erosion.

As saliva has the ability to remineralize tooth using calcium and phosphates as a bio-available mineral, the action of salivary phosphoproteins (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.

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.

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 metastable solution.

ACP acts as a precursor to bioapatite and as a transient phase in bio-mineralization. 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.

The mechanism of CPP-ACP is that, it has the ability to localize ACP at the tooth structure, increasing the level of calcium phosphate in plaque and forms a calcium phosphates reservoir, the free calcium and phosphate...
ion gets buffered, thereby helping to maintain a state of super-saturation with respect to tooth enamel and thereby it decreases enamel demineralization and enhances enamel remineralization.\[11]\]

CPP-ACP can protect enamel surface from erosion and increases its wear resistance and helps better clinical management in tooth wear situations.\[5, 6]\] CPP-ACP causes 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 enamel surface 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 remineralization process. 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 debride ment, 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 treated teeth.

References:


DOI: 10.9790/0853-1905141217 www.iosrjournal.org