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SHEAR BOND STRENGTH OF BRCKETS BONDED TO REMINERALISED ENAMEL USING DIFFERENT REMINERALIZING AGENTS

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ABSTRACT

Aim: to assess the effect of three different remineralizing agents on the shear bond strength (SBS) of orthodontic brackets bonded to remineralized enamel.

Materials and Methods: 32 human premolars were allocated into four groups (n=8), covered with nail varnish except labial enamel left uncoated, demineralization protocol was performed for all groups, then remineralizing regime were applied as follow (group I) demineralization only, (group II) casein phosphopeptide amorphous calcium phosphate [CPP-ACP], (group III) Novamin, (group IV) Self-assembling peptide P11-4 (SAP-P11-4). After bracket bonding, shear bond strength was assessed. Adhesive remnant index (ARI) was detected using stereomicroscope. Kruskal-wallis test followed by Dunn's post hoc test ($P<0.05$) was carried out for SBS, Shapiro-Wilk 'test and Levene's test were used to test normality and homogeneity respectively and on-way ANOVA test ($P<0.05$) for ARI evaluation.

Results: SAP P11-4 group showed significant low SBS results than the other groups while CPP-ACP and Novamin group showed significantly higher bond strength than the other two groups.

Conclusion. Pretreatment of demineralized enamel surface with CPP-ACP and Novamin before bracket cementation significantly increase shear bond strength.

KEYWORDS: Remineralizing agents, demineralized enamel, dental brackets, bonding agents.

INTRODUCTION

Disproportion between the enamel demineralization and remineralization process resulted in dental caries[1]. White spot lesions (WSLs) is the first clinical sign of dental caries which is a reversible demineralization of enamel[2]. Many teenage patients indicated for Orthodontic treatment are suffering already from WSLs which means that, they may need to undergo WSLs treatment before starting their orthodontic treatment [3].

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Also, WSLs around orthodontic brackets are a common complication of orthodontic treatment due to increased difficulty in maintaining oral hygiene in the presence of orthodontic appliances [4-5]. In addition,

Brackets re-bonding is a common need during orthodontic therapy because of the high de-bonding rates of orthodontic brackets [6].

Shear bond strength will be reduced if orthodontic brackets were bonded to enamel with initial carious lesion at the start of the treatment, or if re-bonding is required during the therapy [7-8].

So that, it is advisable to repair any enamel WSLs before bonding brackets or during orthodontic treatment [2, 9-10].

The traditional method for treating WSLs is to remineralize incipient lesions with the use of fluoride[9,11]. Recently different remineralizing agents have been developed to remineralize the incipient lesions, among of these are: -

Casein phosphopeptide-amorphous calcium, phosphate (CPP-ACP) gels which is a recent remineralizing agent[12] Casein is a phospho protein derived from Cow milk and can stabilize amorphous Calcium phosphate [13] that can release high concentration of Calcium and phosphate ions, those ions can freely diffuse to enamel subsurface lesions to promote remineralization [14]

Bioactive glass is another remineralizing agents which composed of Sio_2, Na_2O, CaO and P_2O_5 developed by Professor Larry Hench which is commercially produced in the form of Novamin^{Tm} [14-15]. When microscopic particles of Novamin^{Tm} are exposed to saliva, they release mineral ions forming hydroxy carbonate apatite, the essential constituent of teeth [6 14, 16]. In addition, Novamin have demonstrated a significant antimicrobial effect against caries pathogens specially S-mutans and S-sanguis. [16].

Self-assembling Peptide P 11-4 (SAP P11-4) is a recently introduced remineralizing agent for stabilizing WSLs [17]. Within seconds of application of this peptide to the WSLs, 3D matrix of hierarchical structure is formed from tapes and ribbons followed by fibrils and fibers during the following 24 hours which enables new hydroxyapatite crystal formation that can grow to a convenient length [15,17-20]

The present study aimed to investigate the influence of two remineralizing agents (CPP-ACP and Novamin) versus the recently introduced biomimetic remineralizing agent (SAP P11-4) on the shear bond strength of metal brackets to demineralized enamel surfaces.

MATERIALS AND METHODS

The materials used in this study were illustrated in table 1. Ethics Committee of October 6 university approved this study with approval no RECO6U/ 5–2021. After the patient's informed consent was attained, 32 premolars freshly extracted for orthodontic purpose were stored in distilled water including 0. 1 % thymol to prevent bacterial growth until used for no more than three months, the stored premolars were free of any carious or non-carious lesions. Sample size calculation was performed using G*Power version 3.1.9.7

Any debris affecting the premolars were washed out using low speed hand-piece and pumice, then all the surfaces of the premolars were covered with nail polish except the labial surface to standardize the demineralization area. The tested premolars were embedded, till 2 mm below the cemento-enamel junction, in a plastic mold filled with self cured acrylic resin .The plastic mold was sectioned from a l0.CC plastic syringe.

All the tested samples were subjected to demineralization/ remineralization protocol as follow:

Demineralization / remineralization protocol.

The samples were kept in the demineralizing solution for 22 hours then washed with deionized water and kept in the remineralizing solution for 2 hours. These cycles were repeated over a period of 14 days using a new solutions every day (2). The composition of the demineralization and remineralization solutions were shown in table 2.

TABLE (I) Materials used in the study.

Materials	Composition	Manufacture and lot no
CPP- ACP (Casine tooth mouse)	H_2O, Glycerol, EPP ACP . D. Sorbitol, CMC_Na, propylene. glycol, Silicon dioxide, itanium dioxide, Xylitol, phosphoric acid, Naf, Sodium saccharin, ethyle p-hydroxybenzoate, propyl -p- hydroxybenzoat butyl p-hydroxybenzoate	GC Europe NV Interleuvenlean 33 B-3001 leuveu Lot. 170911E
Novamin paste (Nupro sensodyne Paste)	Glycerin, Pumice, 15% Calcium sodium phosphosilicate (Novamin), Sodium silicat, titanium dioxide, methyl Salicylate, H_2O, sodium Carboxymethylcellulose, Sodium Saccharin, flavor	Dentsply professional Dentsply international 130 smile way yor, PA17404 Lot. 17121801
SAP P11-4 (Curodont Repair)	Self assembling peptid P11-4 , Ca2 and po4	Mectron Deutschlond vertriebs. Gmbtl, cologne, Germany
Assure Plus bonding agent	Bis GMA, ethanol 10 MDP phosphate Monomer	Reliance orthodontic Products, Itasca, IL, USA Lot 191223
Transbond XT light cure adhesive composite	Silane treated quartz, bis GMA, bis EMA, silane treated Silica, diphenyl iodonium hexa -fluorophosphate	3M unitek, Monrovia, CA, USA. Lot(17) 220328

TABLE (2) Composition of demineralization, remineralization and artificial saliva solutions.

Demineralization Solution	3 mmol /L,calcium, 3 mmol/ L phosphate, 50ml/ L acetic acid and 0.308g ammnonium a cetate. PH adjusted to 4-5 with sodium hydroxide (2)
Remineralization Solution	1.54 mmol / Lcalcium, 1.54 mmol/ Lphosphate, 20 mmol/ L acetic acid and 0.308g amunonium a cetate, adjusted to PH 7.0 with potassium hydrxide (2)
Artificial saliva	22.1 mmol/L hydrogen Carbonate, 16.1 mmol/L potassium, 14.1 mmol/ L sodium, 2.6 mmol/L hydrogen phosphate 0.8 mmol /L boric acid, 0.7 mmol/ L calcium, 0.4 mmol/ L thiocyanate and 0.2 mmol/ L magnesium. With PH 7.4 – 7.5(5).

After demineralization/ remineralization protocol the samples were randomly divided into four equal groups (n=8) according to the applied remineralizing agent.

Group I. (control). Receive no remineralizing agent

Group II. CPP-ACP gel was applied for 5 min to the labial surfaces according to the manufacturer instruction then rinsed with deionized water and retained in artificial saliva (table) 2. This cycles was repeated over 14 days and changing the artificial saliva every day (7).

Group III. Novmine paste were applied over the samples for 5 minutes using a low speed polishing brush according to the manufacturer instruction, the samples were rinsed using water spray until all white residue were removed then retained in artificial saliva. This cycles was repeated for 14 days and changing the artificial saliva every day.

Group IV. The stick of SAP P11-4 was pushed in its solution then with its wetted sponge the solution were applied for 5 minutes according to the manufacturer instruction to the labial surfaces of the samples then retained in artificial saliva. This cycles were repeated for 14 days and changing the artificial saliva every day.

Bonding procedures.

The labial surfaces of the samples were etched with 37% phosphoric acid gel for 15 seconds (SDI super etch) and rinsed for 5 seconds with water then air dried for l0 seconds, one coat of Assure plus adhesive were applied to each samples, lightly dry with air then light cured for 10 seconds. the composite transbond XT (3M unitek) were placed on stainless steel bracket base (LEONE S.p.a) and pressed lightly in the middle of the labial surfaces, then the excess composite was removed with dental explorer and light cured using LED light curing unite (3M, ESPE Elipar S10, Germany) for 15 seconds.

Shear Bond Strength test (SBS)

Instron universal testing machine (model 3345 England) at a constant crosshead speed of a 1 mm/min was used to stress the border brackets in the different groups until failure occurred. The force needed to remove the brackets was measured in newton and the SBS was then calculated by dividing the force values by the bracket base area (11 mm) using computer software Blue Hill universal Instron England.

Adhesive Remnant Index (ARI).

The debonded area was examined using a Stereomicroscope (MA 100 NIKON, Japan) at 10x magnification to assess the adhesive remnant index. The ARI Scores ranged from 0 to 3 where 0= no adhesive left on the enamel Surface, 1 = less than half of the adhesive left on the enamel surface, 2= more than half of the adhesive left on the enamel, surface 3 = all adhesive left on the enamel surface with the distinct impression of the bracket mesh. The scores were calculate twice by the same operator.

Statistical analysis

Ordinal data was represented as frequency and percentage values and were analyzed using Kruskal-Wallis test followed by Dunn's post hoc test with Bonfferroni correction. Numerical data was represented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Homogeneity of variances was tested using Levene's test. One-way ANOVA test followed by Tukey's post hoc test was used to analyze intergroup comparisons. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows*.

RESULTS

Shapiro-Wilk's test showed normally distributed data ($p>0.05$) and Levene's test revealed homogeneity of variances ($p>0.05$).

Descriptive statistics for shear bond strength values were presented in table (3) and figure (1). The highest value was found in (CPP-ACP) group (24.72 ± 5.67), followed by (Novamin) (19.73 ± 4.60), then the control group (18.73,5.29), while the lowest value was found in the (SAP P11-4) group (12.69 ± 3.55).Intergroup comparison of shear bond strength using post hoc pairwise test in table (4) showed value of the (SAP P11-4) group to be significantly lower than values of (CPP-ACP) , (Novamin), and control groups ($p<0.001$). Although they doing better, there is no significant difference between (CPP-ACP) and (Novamin) groups versus control group.

Results of ARI comparison presented in table (5) showed that there was no significant difference in failure scores distribution between different groups ($p=0.247$). Majority of the control group, Novamin and SAP P11-4 samples had score 1(50.0%). While 5 samples of CPP-ACP group (62.5%) had score (2). Figure 2 showed some representative samples

* R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

TABLE (3) Descriptive statistics for shear bond strength (MPa)

Treatment	Mean	95% CI		SD	Median	IQR
		Lower	Upper			
Control	18.73	15.06	22.40	5.29	19.02	7.95
CPP-ACP	24.72	20.79	28.65	5.67	24.31	8.40
Novamin	19.73	16.54	22.92	4.60	18.87	6.63
SAP P11-4	12.69	10.23	15.15	3.55	12.50	4.06

95% CI= 95% confidence interval for the mean; SD= standard deviation; IQR= interquartile range.

TABLE (4) Intergroup comparison of shear bond strength (MPa).

Shear bond strength (MPa) (Mean± SD)				P-value
Control	CPP-ACP	Novamin	SAP P11-4	
18.73±5.29^{AB}	24.72±5.67^{A}	19.73±4.60^{A}	12.69±4.60^{B}	< 0.001*

Different superscript letters a statistically significant difference within the same horizontal row; * significant) p<0.05)

TABLE (5) Intergroup comparison of ARI scores

Score		Control	CPP-ACP	Novamin	SAP P11-4	p-value
0	N	2	1	0	2	0.247
	%	25.0%	12.5%	0.0%	25.0%	
1	N	4	4	3	4	
	%	50.0%	50.0%	37.5%	50.0%	
2	N	2	3	5	2	
	%	25.0%	37.5%	62.5%	25.0%	
3	N	0	0	0	0	
	%	0.0%	0.0%	0.0%	0.0%	

Different superscript letters indicate a statistically significant difference within the same horizontal row; *significant (p<0.05)

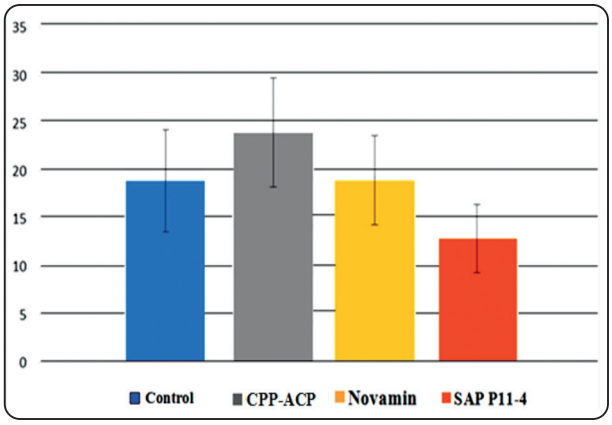

Fig. (1) Bar chart showing mean and standard deviation values shear bond strength in different groups.

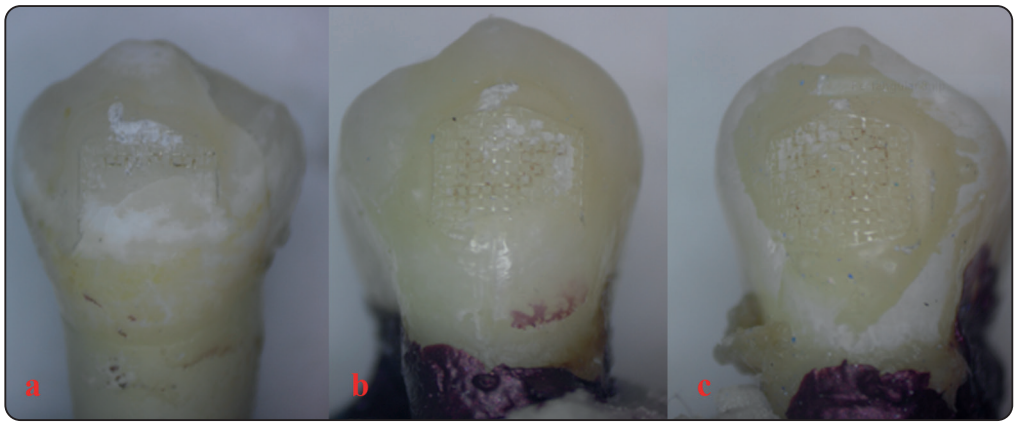

Fig. (2) Bar chart showing mean and standard deviation values shear bond strength in different groups.

DISCUSSION

Many patients indicated for orthodontic treatment are suffering already from WSLs which is also expected during the treatment as a results of food accumulation and difficulties in oral hygiene measurements around the brackets which may leads to bracket bonding failure and treatment delays[3]. The treatment of WSls at the beginning and during orthodontic treatment using remineralizing agents has become an area of interest in orthodontic treatment to avoid the unwanted extension of treatment time resulting from bracket bonding failure[2-3]. It was found that, bond strength of composite restorations increased significantly after application of remineralizing agents to demineralized dentin and this enhancement in bond strength was directly related to the degree of remineralization [21]. This study aimed to compare the SBS values of two reminenalizing agents of (CPP-ACP and Novamin) and one biomimetic remineralizing agent (SAP P11-4). The results revealed that CPP-ACP and Novamin treatment significantly improve the SBS of brackets bonded to remineralized enamel in comparison to the SAP P11-4 group which revealed significant reduction in SBS in comparison to all groups even the demineralized enamel.

Self-assembling peptide P11-4 is one of the recently commercially available materials used for enamel regeneration. When it is applied to the enamel carious lesions it will stimulate formation of biomimetic scaffolds within the lesions formed from fibrils and fibers organized into hierarchically structures within 24 hours due to specific non covalent interaction that supporting the natural remineralization energetic by saliva and promotes de novo nucleation of hydroxyapatite nano crystals[19,22- 24].

The lowest performance of SAP, p11-4 in our study may be explained by the longer time needed for remineralization to occur as showed by study conducted by Jablonski-Momeni and Heinzel-Gutenbrunner, they reported 8-12 weeks is needed for complete remineralization to occur when they tested the remineralization efficiency of P11-4 in chemically induced artificial carious lesion[25]. In the same line Silvertown et al evaluated the efficacy of self assembling peptide p11-4 to regenerate enamel in natural early Caries lesion and they reported significant remineralizing effect after 50 days of storage in artificial saliva [26] Also combined application of self assembling peptide p11-4 and fluoride varnish was recommended by many authors to provide prober enamel remineralization [27-28].

Furthermore, several studies concluded that enamel regeneration with SAP, p11-4 does not looks like the prismatic construction of the natural enamel, it produce amorphous layer of nonprismatic hydroxyapatite crystals [26, 29-30] which may explain the low bond strength of the brackets

to the regenerated enamel with SAP P11-4 in our study because bonding to nonprismatic enamel is questionable [31]. This results are in partial agreement with recent study reported no significant influence was identified on bonding of a metal bracket to the enamel surface treated with SAP P11-4 either directly before or with a 24h remineralization period in between [32].

In comparison to demineralized enamel, many authors reported significantly higher enamel remineralization after application of CPP-ACP[2,12,33-34] and Novamin[12,33]. Study by Manoharan et al[12], supported the results of the present study, they concluded that both CPP- ACPP and Novamin were able to highly remineralize the demineralized enamel lesions. Furthermore our results are consistent with other studies[35-36] the authors concluded that, bond strength of brackets significantly increased to demineralized enamel after application of CPP-ACP and the other tested materials were not able to act the same.

On the other hand, the results of CPP-ACP group of our study are disagreed with the results of study conducted by Gulec and Goymen[2], they demonstrated that, lower SBS values to demineralized enamel after application of both CPP-ACP and resin infiltrant.

Also, the higher SBS value in this study was inconsistent with study conducted by triwardhani et al [9] who had lower, SBS values when bonding Metal brackets to CPP- ACP pretreated demineralized enamel surface. The difference could be attributed to the RMGIC based adhesive used in their study which differ from resin based adhesive used in our study.

The results of ARI in this study couldn't detect any significant difference among the tested groups. Most values were one and two, meaning that cohesive failure was dominant which were compatible with many other studies [2, 9, 31] values of 4Mpa [37] has previously been reported as acceptable for the clinical applications of brackets. The lowest SBS value recorded in our study was 10.23Mpa which founded in one sample of SAP P11-4 group. Hence, clinical Sufficiency is therefor given for all the tested groups and that's way we could not detect any significant difference in ARI results for the different groups.

CONCLUSIONS

Within the limitations of this study, the following can be reported.

1- CPP-ACP and Novamin application significantly improve bond strength of brackets to demineralized enamel

2- SAP P11-4 application to demineralized enamel resulted in lower SBS values than other groups.

3- All the tested groups showed comparable results regarding the ARI values.

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Conflicts of interest:

There are no conflicts of interest

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