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Effect of enamel deproteinization on fluorosed and non fluorosed enamel bonding with resin modified glass ionomer cement -A vitro comparative study

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ABSTRACT

Objective: To determine the effect of deproteinization using 5.25% sodium hypochlorite (NaOCl) prior to acid etching on shear bond strength of orthodontic brackets bonded to fluorosed teeth and normal teeth using RMGIC.

Materials and methods: twenty freshly extracted human mandibular first premolars with TFI 4 were selected and divided into two groups of 10 each. All the teeth were deproteinized with 5.25% NaOCl prior to acid etching with 37% phosphoric acid and were bonded with RMGIC. Samples were then subjected to shear bond test by Instron Universal Testing machine. The sample from each group were selected for the SEM study (prior to bonding) to analyze the etching patterns achieved. Data was checked for normality by Shapiro Wilk Test, to compare the two groups unpaired t test was used. P value was predetermined at ≤ 0.05.

Results: Mean shear bond strength of group I was 12.53 ± 4.14 MPa. The S BS of Group II was lower than Group I and the difference was statistically significant (P = 0.000). On SEM the etching pattern was more of type 1 & 2 in Group II.
Conclusions: NaOCL significantly increases the shear bond strength of brackets bonded to normal teeth.

Key words: Fluorosed, deproteinization, sodium hypochlorite (NaOCl).

INTRODUCTION

Occurrence of white spot lesions (WSLs)is an important concern associated with fixed orthodontic treatment . Using advanced detection techniques it is observed that 97% of all subjects receiving fixed orthodontic treatment were affected with WSLs. As oral hygiene becomes more difficult in patients with fixed orthodontic appliances, the decalcification of the enamel surface adjacent to these appliances is prevalent which is manifested as a white spot lesion. These white spot lesions if left untreated, may progress to produce carious cavitations, and may also present esthetic problems. Thus, the prevention, diagnosis, and treatment of white spot lesions during orthodontic treatment is crucial to minimize tooth decay as well as tooth discoloration that could compromise the esthetics of the smile[1].

To overcome the problem of white spot lesions, manufacturers have attempted to increase the fluoride release levels of orthodontic adhesives as fluoride does protect enamel from developing WSLs. Though Glass Ionomer Cements have been shown to release fluoride over a long-term, they have poor bond strength, in the range of 2.37 to 5.5 MPa. In an attempt to provide greater fluoride release and adequate bond strength, combinations of glass ionomer cements and composite resins have been developed resulting in resin-modified glass ionomer cement (RMGIC) .

Orthodontists are aware that RMGICs provide a sustained fluoride release following bonding(for as long the bracket is maintained on the enamel) still they have been reluctant to use resin-modified glass ionomer cement (RMGIC) as a routine bracket adhesive because of shear bond strength (SBS) issues. Considering the unique advantage of RMGIC in reducing

white spot lesions in fixed orthodontic patients research is on to look for various methods to increase bond strength of RMGIC so as to enable the routine use of RMGIC in orthodontic bonding.

Espinosa et al in 2008 through his SEM study showed that the use of 5.25% sodium hypochlorite (NaOCl) for 1 minute prior to acid etching improves both the quantity and quality of the etched surface and thus he suggested that this method has the potential to be effectively used to optimize adhesion and improve bond strength. This process referred to as deproteinization doubles the enamel' s retentive surface to 94.47% and also results in an increase in the type 1 and 2 etched enamel which have significantly greater retentive capabilities than the usual type 3 etch pattern thus significantly improving the retention[2,3]. Enamel deproteinization thus has the potential to emerge as a cost effective, non-invasive and convenient method to increase bond strength. We tried to find out whether Enamel deproteinization can provide a clinically acceptable SBS of brackets bonded using Fuji Ortho LC a floride releasing RMGIC so as to make routine use of RMGIC as a orthodontic bonding material possible[3,4].

As large parts of India are endemic for fluorosis we also tried to find out if enamel deproteinization can be used to provide a clinically acceptable SBS of brackets bonded to flourosed teeth using Fuji Ortho LC[5,6]. Though the patients who exhibit fluorosis before orthodontic treatment are less likely to develop new white spot lesions during treatment as compared with patients with no pre-treatment fluorosis, the risk of developing new white spot lesions is always there. The AR (Absolute Risk) of developing white spot lesions during orthodontic treatment was 15% in patients with fluorosis and 26% in those without fluorosis.[(15)]NaOCl has been shown to be an effective protein denaturant and as the protein content of fluorosed enamel is significantly higher as compared to normal enamel we also tried to explore whether Enamel deproteinization can increase the bond strength of RMGIC on flourosed teeth to clinically acceptable levels.[6]

Hence this study was carried out to examine the effect of deproteinization with 5.25% NaOCl on the shear bond strength of brackets bonded using RMGIC to normal and fluorosed teeth and also to study the surface topography of deproteinized fluorosed teeth by scanning electron microscopy.

AIMS AND OBJECTIVES

The main aim of the study was to evaluate the effect of deproteinization with 5.25% sodium hypochlorite on

1. Shear bond strength of brackets bonded on normal teeth using RMGIC.
2. Shear bond strength of orthodontic brackets bonded to fluorosed teeth using RMGIC.

MATERIALS AND METHOD

The present in-vitro study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics Post Graduate Institute of Dental Sciences, Rohtak, (Haryana), Central Institute of Plastic Engineering and Technology (CIPET) Murthal, (Haryana) & Advanced Instrumentation & Research Facility (AIRF) Centre, Jawaharlal Nehru University, New Delhi.

Sample size calculation: Sample size was calculated for an effect size of 0.7^{9} at 95% of CI (Confidence Interval) and 99% power resulting in a final sample sizeof 20 teeth .

Teeth: Twenty teeth were used in this study and were selected from a sample of approximately two hundred mandibular first premolars which were extracted for orthodontic reasons from patients visiting the department of Orthodontics, PGIDS Rohtak for fixed orthodontic treatment. Teeth with caries, visible defects, obvious damage or abrasion and other deformities were excluded from the study. All the extracted teeth were thoroughly washed in tap water immediately after extraction to remove blood, debris and adherent tissues and the surface dried and twenty flourosed teeth were selected. The classification of fluorosed teeth was made by the consensus of two investigators (MV and RS) using the modified Thylstrup and Fejerskov index. Specimens were then stored at room temperature (for a period varying between min of 3 days to a max of 1 month.) in distilled water solution of 0.1% thymol (w/v) for disinfection and to inhibit bacterial growth. The samples of 10flourosedteeth were completed before the normal teeth pointing towards the endemic problem of fluorosis in this area.

As soon as the required sample was complete teeth were divided into two groups Group I Ten Normal i.e Non Flourosed teeth

Group II Ten Flourosed teeth.

METHODOLOGY

The following procedure was performed prior to bonding in each group:

1. The facial surface of each tooth was cleaned with nonflouride oil-free pumice paste placed in a prophy cup attached to a slow-speed hand piece.
2. The tooth was rinsed thoroughly with water and dried with an oil-free air spray.

3M Unitek, Pre-adjusted Edgewise mid- sized mandibular first premolar 0.022'' slot brackets without hooks (9.08 mm^2 surface area) were then bonded to all tooth samples (figure-1)**.**

Fig-1: Pre-adjusted Edgewise maxillary first premolar bracket with 0.022'' slot (Gemini, 3M Unitek, Monrovia, CA) and bracket base mesh.

The brackets were bonded according to the following protocol:

Group I Bracket bonded to normal teeth using RMGIC after deproteinization with 5.25% sodium hypochlorite (figure-2)

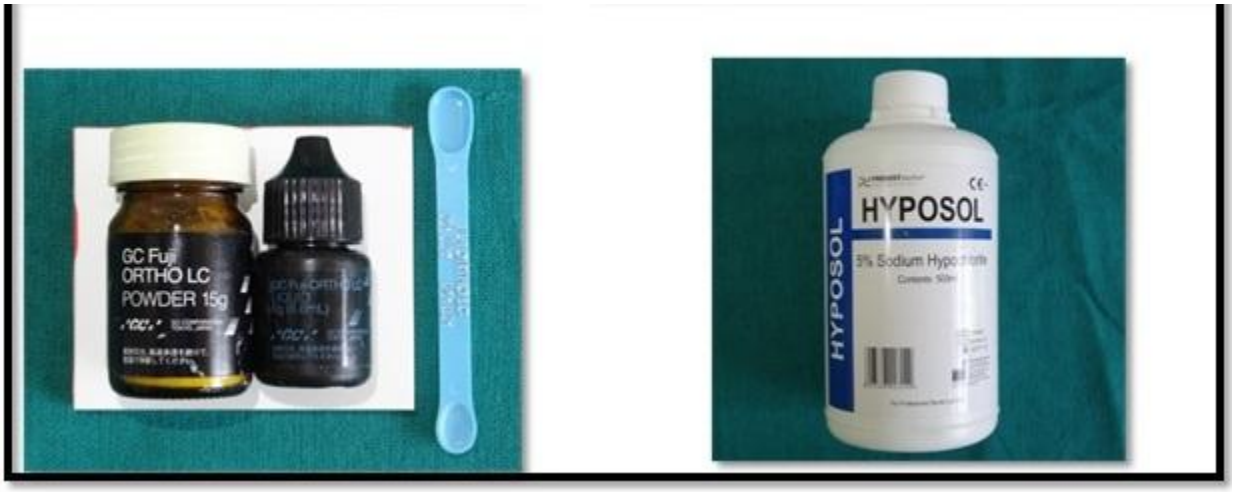

Figure-2: a) RMGIC ; Fuji ortho LC , b) Sodium Hypochlorite (Prevest Den Pro)

The normal teeth were dried after polishing with pumice, enamel was deproteinized with 5.25 per cent sodium hypochlorite with the use microbrush for 60 seconds, washed with water and dried with air, etched with 37% phosphoric acid for 30 seconds, and then washed with water for 10 seconds and dried with oil free compressed air. The brackets were bonded with RMGIC (Fuji Ortho LC; GC America)

R.M.G.I.C powder and liquid were mixed according to manufacturer's recommendation for 20-25 seconds and a small amount was applied on the bracket mesh covering the entire base of the bracket, without bubbles or voids. The bracket was held and carried to the tooth surface with a bracket holder. The bracket was then placed on the tooth surface using sufficient force to produce a "flash" of excess adhesive around the bracket to ensure a uniform thickness of adhesive. According to

the manufacturer, the working time of cement mix was 3 minute and 45 seconds hence two brackets were easily placed with each mix. The excess adhesive was removed with a sharp scaler. The bracket was light cured for 40 seconds (10 seconds on each surface of bracket) with LED light cure.

Group II Bracket bonded to fluorosed teeth using RMGIC after deproteinization with 5.25% sodium hypochlorite.(figure-3)

Figure-3: Etching pattern seen with 37% phosphoric acid on fluorosed teeth under SEM; A Type 2; B Type 1; C Unetched surface.

The fluorosed teeth were dried after polishing with pumice, enamel was deproteinized with 5.25 per cent sodium hypochlorite with the help of microbrush for 60 seconds, washed with water and dried with air, etched with 37% phosphoric acid for 60 seconds, and then washed with water for 10 seconds and dried with oil free compressed air. The brackets were bonded with RMGIC.

After bonding each tooth was then embedded in a cold cure acrylic resin cylindrical block. A jig was used to align the buccal surface of each tooth parallel to the base of cylinder. (Fig. 5)

Preparation of blocks

We used 10 ml syringes to prepare acrylic blocks. Syringes were cut from the nozzle side at the level of 1ml marking to make opening for pouring of acrylic into it. Cut edges were smoothened with help of bur and discs and finally finished with sand paper. These syringe tubes were then ready to prepare blocks. Piston of syringes was removed but rubber stops left to create equal length of blocks. Then the rubber stops were placed on 5ml mark. These appear as cylinder with one end open for pouring of liquid and powder into it and another end with rubber stops at 5ml mark to create equal length of acrylic blocks.[7] Then we placed these syringes below the teeth mounted on jig with the stable base of syringes on the floor and placed tooth in center of the open end of syringe tube. Acrylic powder and liquid were poured in the open end and filled up to CEJ of mounted tooth. **(figure-5)**

Retrieval of sample blocks

After setting of poured acrylic samples were dismounted from jig by cutting of ligature of bracketed tooth and recovered from plastic syringe by pushing of block from the piston side by help of piston. Damaged syringes at the time of retrieval were discarded and were replaced by new one. Intact syringes can be used again for next sample.

Specimen storage:
Two minutes after bonding, the test samples were stored in distilled water at room temperature for 7days. They were subjected to debonding procedure after 7 days of bonding.

SEM Study.
A qualitative study was carried out to observe with Scanning Electron Microscope (SEM) the type of etch pattern with and without the use of NaOCl before etching. 10 samples of fluorosed teeth were cleaned, and randomly divided into 2 groups (control and experimental),with premolars in each group. The buccal surfaces of the premolars in the experimentalgroupweredeproteinizedwith5.25%NaOClfor1 minute followed by rinsing, drying, and acid etching for 60 seconds, washed with water for 10 seconds, dried with oil free compressed air. Samples were prepared according to standard protocol to observe under SEM (Carl Zeiss SEM EVO 40) which was operated on an accelerating potential of 20 kv and then etching patterns were observed. The same protocol was used in the control group, except that NaOCl was not used. The teeth were prepared for observation at 2500 X magnification.(figure-4)

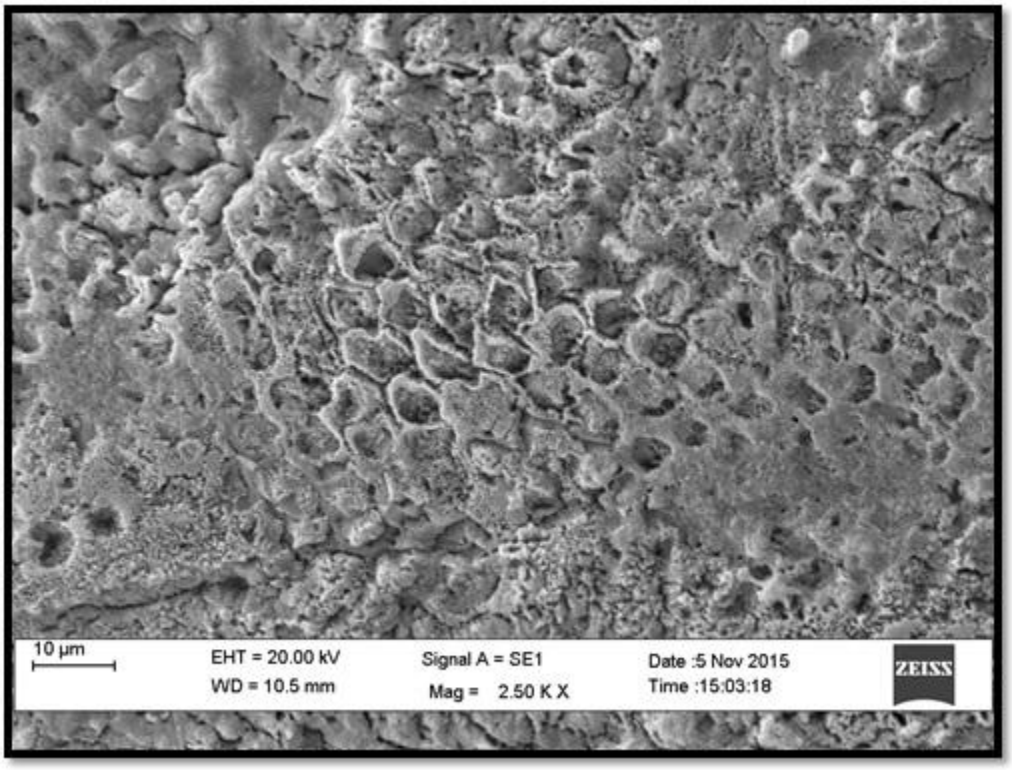

Figure-4: Etching pattern seen with deproteinization (5.25 % hypochlorite) followed by 37% phosphoric acid on fluorosed teeth under SEM showing uniform Type 1 & 2 etching pattern with no unetched areas

Debonding procedure (Fig. 5)

After bracket bonding, the teeth were stored in distilled water at room temperature until they were submitted to the shear test. A Universal Test Machine with a load cell of 500N (Shimadzu Autograph AG-IS) was used, operating at a speed of 0.5mm/ minute. Each specimen was loaded into the universal testing machine with the long axis of the specimen being parallel to the direction of the applied force. For shear testing, the acrylic block was fixed to the metal framework with a central circular opening, which in turn was secured in the lower jaw with the long axis of the tooth and the bracket base parallel to the direction of the shear force applied. A loop was made using 020" stainless steel wire and the ends of the wire were gripped in acrylic block (to securethe stainless steel wire), which in turn was fixed to the upper jaw. Loop was engaged under wings of bracket on which shear force is to be applied. The specimens were stressed in an occlusogingival direction with a uniform cross head speed of 0.5 mm/min. The maximum force necessary to debond or initiate bracket failure was recorded in Newtons(figure-5) The shear bond strength in mega pascals (MPa)was computed as a ratio of force in Newtons to the surface area of the bracket (9.08 mm^2), as informed by the manufacturer.[8,9]

Figure-5: A) Inostron machine; B) Assembly with holding samples

Statistical procedure

Shapiro-Wilk test was used to determine the normalcy of the data. Then mean and standard deviation of the shear bond strength values were calculated for the samples of the two groups. Each group has twenty sample teeth. Unpaired t-test was used to compare the mean debonding force (Newton) among the two groups. Significance for all statistical testswas predetermined at $P \leq .05$. The statistical analysis was made with the statistical program IBM SPSS 20.0 for Windows.

Adhesive remnant index

After debonding, all teeth and brackets in the test groups were analyzed using a light stereomicroscope (Model no. RSM -9 RADICAL) at 10x magnification to determine the failure interface. Any adhesive remaining on surface of teeth after debonding was assessed and scored according to the modified adhesive remnant index[10],[11].

RESULTS

Shear bond strength

All the values recorded from UTM were subjected to Kolmogorov- Smirnov and Shapiro- wilk test.(table-2). The descriptive statistics, including mean, standard deviation, standard error and P value for the 2 adhesive systems, are presented in[2](Table 3).Further independent –t test was applied (Table-4,5).

The mean SBS for the brackets bonded using RMGIC after deproteinization in group Ii.e non flourosed teeth was (11.28 ± 4.73Mpa) which is higher than the normally acceptable bond strength for successful orthodontic bonding (TABLE-3). The use of NaOCl (sodium hypochlorite) prior to acid etching produced clinically acceptable bond strength of brackets bonded to normal teeth using RMGIC.

The mean SBS for the brackets bonded using RMGIC after deproteinization in group II i.e flourosed teeth was (12.53 ± 4.14Mpa) which falls short of the normally acceptable bond strength for successful orthodontic bonding. The use of NaOCl (sodium hypochlorite) prior to acid etching did not produce clinically acceptable bond strength of brackets bonded to flourosed teeth using RMGIC. **Table(5)** showed that there was a mean difference of 4.68MPa between group I and group II. Group I (Bracket bonded to normal teeth using RMGIC after deproteinization with 5.25% sodium hypochlorite) showed higher mean S.B.S. values than group II (Bracket bonded to fluorosed teeth using RMGIC after deproteinization with 5.25% sodium hypochlorite). There was statistically significant difference in the mean S.B.S. between group I and group II. ($p = .014$ TABLE-5)

Table-1: Recorded value of SBS of flourosed and non flourosed

RMGIC Normal	13.07	14.27	5.87	10.5	6.88	8.96	11.9	22.30	7.6	11.51
RMGIC Flourosed	8.9	7.88	4.32	4.54	6.83	5.10	12.21	4.4	4.18	7.78

Table-2; descriptive statics and results of the shear bond strength of two groups tested.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Normal	.165	10	.200*	.892	10	.177
Flurosed	.216	10	.200*	.868	10	.095

Table-3; Group mean and standard deviation of SBS values, and statistical analysis.

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
1	10	11.2880	4.73716	1.49802
2	10	6.6050	2.63556	.83344
		.	.	.
		.	.	.

Table-4: Independent Samples Test intragroup SBS value, and statistical analysis

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Normal	Equal variances assumed	.014	4.68300	1.71426
	Equal variances not assumed	.016	4.68300	1.71426

Table-5: comparison of SBS values and p value and statistical analysis.

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Normal	Equal variances assumed	1.202	.287	2.732	18
	Equal variances not assumed			2.732	14.084

SEM Study (Fig. 4)

Comparison of the enamel surface so deproteinized teeth shows that the enamel conditioned with NaOCl produced a qualitatively rougher enamel surface than the enamel in which NaOCl was not used. The SEM images from the deproteinized group (using NaOCl) show a better etch pattern (types 1 and 2) than the non deproteinized group (type 3 etch pattern).

DISCUSSION

Clinicians are aware that enamel white spot lesions (WSLs) frequently occur in patients undergoing orthodontic treatment and that they are caused by the accumulation of plaque around the brackets .Many orthodontic patients do not adequately comply with oral hygiene protocols which may include fluoridated mouth rinses . In addition ,orthodontic patients may not keep their regularly scheduled dental appointments during their dental appointments during their orthodontic treatment. Monthly in office applications of highly concentrated fluoride varnish does result in a reduction in enamel demineralization but there is a limitation on the frequency of exposures that the patient will receive due to the costs to the patient and the clinicians chair time .Thus, orthodontists might very well be putting patients at risk of developing WSLs by using non fluoride releasing composite resins to bond brackets .

It has been shown that RMGICs provide a sustained fluoride release thus bonding orthodontic brackets with RMGIC can prevent the development of white spot lesions. If RMGICs were routinely used to bond brackets, the incidence of WSLs might dramatically decrease. However orthodontists have been reluctant to use rein modified glass ionomer as a routine bracket adhesive because of shear bond strength issues .The perception is that composite resins provide greater bracket bond strength than RMGIC .

Espinosa et al showed that wetting and / or conditioning the enamel surface with 5.25%sodium hypochlorite (NaOCl) for 1 minute before acid etching increased the quality of the etching pattern because NaOCl eliminated the organic matter from the enamel surface thus allowing more effective etching by 37 % phosphoric acid.[2,3,4] This results in more type 1 and 2 etching patterns which results in an increase in the SBS. If SBS can be increased with NaOCl , then RMGIC could be routinely used to bond brackets instead of composite resins thereby possibly reducing the incidence of WSls due to the fluoride releasing property of RMGICs.

The main objective of the present study was to determine whether deproteinization by application of sodium hypochlorite (NaOCl), for one minute before etching, increases SBS of brackets bonded to normal and fluorosed teeth using RMGIC resin thus promoting its use for orthodontic bonding .This can then provide a useful alternative to composite resins for orthodontic bonding while providing the unique advantage of its anti cariogenic properties. The findings indicate that it does!. When normal teeth were subjected to deproteinization prior to acid etching and brackets were bonded with RMGIC (Group I) the mean bond strength comes out to be 11.27 ± 4.74MPa. which is well above the clinically acceptable value of Reynolds.[11] these findings are in concordance with the study of T. Bahia et al.[10]R. Justus et al evaluated two contemporary adhesive systems used to bond orthodontic brackets --- a RMGIC, Fuji Ortho LC and a composite, Transbond XT. The main objective of the study was to determine whether NaOCl, applied for 1 minute before etching, increases bracket SBS. As a result deproteinization with NaOCl prior to acid etching increased the SBS in both experimental groups, with RMGIC group showing statistically significant difference. T. Bahia et al.[10] tested the effect of enamel deproteinization on bracket bonding with conventional (Transbond XT) and RMGIC (Fuji Ortho LC). The mean shear bond strength of brackets bonded to normal teeth using RMGIC in his study was 9.86 ± 2.90MPa which is in accordance with our findings.

According to Julien, et al (2013) the absolute risk of developing white spot lesions during orthodontic treatment was 15% in patients with fluorosis and 26% in those without fluorosis.[12] As several parts of India are endemic for fluorosis with Haryana state being one of them this leads us to the second part of our study namely to test the feasibility of use RMGIC as one of the adhesives to bond metallic brackets to fluorosed teeth. This work was carried out in the hope that it will provide the clinicians a successful alternative for orthodontic bonding which will diminish the incidence of WSLs by using NaOCl in combination with Flouride releasing RMGIC .

CONCLUSIONS

When 5.25% NaOCl is used to deproteinize the enamel surface ,brackets bonded to normal teeth with RMGIC can achieve SBS much higher than clinically acceptable bond strength.

Enamel deproteinization with 5.25% NaOCl cannot produce clinically acceptable bond strength in brackets bonded to Flourosed teeth using RMGIC.

NaOCl in combination with Fluoride releasing RMGIC can be used as an successful adhesive for orthodontic bonding to normal teeth.

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