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Comparison of enamel demineralization around orthodontic brackets bonded with conventional etching, self-etch primer and antimicrobial monomer containing self-etch primer

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ABSTRACT

To compare enamel demineralization around orthodontic brackets bonded with conventional etching, self-etch primer and antimicrobial monomer containing self-etch primer. Result: The results of Scanning electron microscope analysis inferred that control sample Transbond XT showed 84.6% of deep penetration and Transbond Plus self-etch primer and Clearfil Protect Bond showed 86.7% and 100% of shallow penetration respectively. Microhardness tests results showed antibacterial monomer-containing adhesive was significantly more efficient than the self-etching primer and conventional adhesive system, reducing enamel demineralization in almost all evaluations. Conclusion: Clearfil Protect bond was found to be better in reducing demineralisation around orthodontic bracket followed by Transbond plus self-etch primer compared to conventional Transbond XT.

Keywords— *White spot lesion, Enamel demineralisation, Clearfil protect bond, Transbond XT, Transbond plus self-etch primer*

1. BACKGROUND

Bonding is an integral part of fixed orthodontic treatment. [1] Phosphoric acid-etching causes dissolution of inter-prismatic material in the enamel, producing a roughened and porous layer. [2,3] This irreversible damage is later manifested as *white spot lesion* after completion of orthodontic treatment. Conventional etching has various disadvantages that it is a strong acidic conditioner, prolonged etching time and it has uncontrolled demineralisation of enamel surface due to which, there is loss of surface enamel and subsurface enamel weakening, leading to detachment or fracture of enamel surface. In order to prevent the occurrence of demineralisation, the use of fluoride regimens such as combining a dentifrice (1100 ppm fluoride) and a mouth rinse (0.05% sodium fluoride), have shown to reduce or prevent white spot lesions in orthodontic patients. [4] Unfortunately, a compliance rate of only 13% was obtained from patients asked to decrease their caries risk with a daily fluoride mouth rinse. [5] Transbond Plus Self Etching Primer (3M Unitek, Monrovia, Calif) was developed especially for orthodontic bonding which includes methacrylated phosphoric acid esters, which will both etch and prime the enamel surface before bonding. [6] The advantages of self-etching primer allow adequate etching and priming of enamel and dentin in only one step. [4] Manufacturers claim that it has a conservative etch pattern than conventional etching thereby reducing enamel dissolution. The self-etching adhesive system, Clearfil Protect Bond, employing antibacterial primer is claimed to release fluoride and prevent the occurrence of demineralisation around brackets ultimately leading to the prevention of occurrence of white spot lesions. [7] The antibacterial adhesive system contains monomer, *12-methacryloyloxy dodecyl pyridinium bromide* (MDPB), which has strong bactericidal activity against oral bacteria. [8] Therefore, the present study was done to evaluate the effect of Transbond SEP and antibacterial monomer containing SEP in reducing enamel demineralization around orthodontic brackets and also to compare with the conventional etching system.

2. METHOD

20 orthodontic patients from the Department of Orthodontics and Dentofacial Orthopedics scheduled to have four premolars extracted for orthodontic reasons were selected. This study was organized as a parallel group - split-mouth design with Group 1 consisting of 7 patients receiving primer A (Transbond Plus self-etch) with control primer X (Transbond XT primer) and the other Group 2 receiving primer B (Clearfil Protect Bond) with control primer X, brackets were left for 30 days and then extracted.

Group 1 (Primer A- SEP), was gently rubbed onto the surface for approximately three seconds with the disposable applicator supplied with the system. Then, a moisture free air source was used to deliver a gentle burst of air to the enamel. The bracket was bonded within 15 seconds of priming with Transbond XT adhesive paste as in a group. Group 2 (Primer B - Clearfil Protect Bond), all teeth were etched similar to Group 1 for 15 seconds. The self-etching primer containing the antibacterial monomer Clearfil Protect Bond was applied to the etched surfaces for 20 seconds and sprayed with a mild air stream to evaporate the solvent. Then Clearfil Protect Bond was applied, gently air dried, and light cured for 10 seconds. After these steps, a thin layer of Transbond XT adhesive paste was applied to the base of the bracket and immediately pressed into the adhesive on the tooth surface. The Control side was etched with a 37% phosphoric acid gel and then washed with water and dried with compressed oil-free air. A layer of Transbond XT - primer was applied on the tooth. Transbond XT paste was applied to the base of the bracket and pressed firmly onto the tooth. Excess adhesive was removed and the adhesive was light-cured positioning the light guide of an - Koden visible light curing unit on each interproximal area for 10 seconds. For the testing procedure, after 30 days the brackets were removed; the teeth were extracted and stored in a refrigerator in flasks containing gauze dampened with 2% formaldehyde, pH 7 until the analysis. The extracted premolars were divided equally and subjected to two set of tests - Microhardness test (MHT) and Scanning electron microscopy (SEM) examination. Prior to testing the roots was removed 2 mm apical to the cemento-enamel junction (CEJ), and the crowns were Hemi sectioned vertically into mesial and distal halves with a 15 HC (large) wafering blade on a low speed saw (Isomet, Buehler, Lake Bluff III), leaving a gingival portion and an incisal portion. The teeth will be embedded in self-curing epoxy resin, leaving the cut face exposed. The half-crown sections will be polished with 3 grades of abrasive paper disks (320, 600 and 1200 grit); final polishing was done with a 1-micrometre diamond spray and a polishing cloth disk.

Evaluation of Micro-hardness (Vickers cross-sectional microhardness analysis)-Operator who was blinded from the group allocation will carry out the micro-hardness analysis. A microhardness tester (HWMMT-XLE, Shimadzu, and Kyoto, Japan) under 2 N load was used for micro-hardness analysis. Indentations were done in each half-crown and values of micro-hardness numbers found in the 2 half-crowns were averaged. Forty-eight indentations were made in each half-crown from 8 positions and 6 depths according to the definitions of Pascotto et al. On the buccal surface, indentations were made under the bracket. In the occlusal and cervical regions, indentations were made at the edge (0) of the bracket and at 100 and 200 mm from it. Indentations were also made in the middle third of the lingual surface of each half-crown, as another control. In all these positions, 5 indentations were made at 10, 20, 30, 50, 70, and 90 mm from the external surface of the enamel. The values of micro-hardness numbers found in the 2 half-crowns were averaged.

Evaluation under SEM (Scanning Electron Microscopy)- was done to observe the adhesive penetration into the enamel, mounted specimens were subjected to demineralization cycles which will promote complete dissolution of the dental structures. The specimens were placed on Aluminum stubs and evaluated under a scanning electron microscope (SEM-JSM-5310, JEOL, Tokyo, Japan).
To standardize the microscopic observations, the microphotographs of the composite resin replicas were made at 1000x and 3500x for each enamel surface in the centre of the samples. The lowest magnifications showed the uniformity of the etch pattern of the enamel, whereas the highest magnifications demonstrate morphological characteristics of resin tags penetrating both substrates. The samples were evaluated double blind by three different calibrated examiners that gave comparative scores according to the following:

Score 0- without penetration
Score 1- shallow penetration
Score 2- deep penetration

Characteristics microphotographs of each score were used previously to perform intra- and inter-examiner calibration.

Statistical analysis was tabulated and analysed using the Statistical Package for Social Sciences Version 23.0. Descriptive statistical measures were expressed as percentages, mean and standard deviations. Inferential statistics were established using analysis of variance (ANOVA). Cohen's Kappa statistics were used to measure inter-rater observation Differences and associations were expressed as statistically significant at $P< 0.05$.

3. RESULTS

3.1 Scanning Electron Microscopic results

The null hypothesis was rejected. The Chi square-test indicated that the adhesive penetration when the Clearfil protect bond (CPB) was used it was significantly lower ($P<0.01$) as compared to Transbond conventional XT primer and Transbond self-etch primer with conventional adhesive system respectively. The Cohens kappa determined the inter-rater agreement (Table 1&2) and the Kappa value of 0.60, implied good agreement between the examiners. Transbond XT showed almost 85.7% of deep penetration as compared to SEP which showed only 7.1% and Clearfil Protect Bond almost nil. It was observed that even though self-etching primer consistently showed an unsubstantial homogenous pattern (figure 2) as a conventional primer (XT) (figure 1), it still proved to be enhanced as seen in the microphotographs of the scanning-electron-microscopic observations compared to CPB samples (figure 3). The intact enamel surfaces etched with different acids had different surface morphologies. A characteristic and uniform etch pattern was observed in the resin samples of the phosphoric acid/Transbond XT primer group, revealing increased roughness and resin tags penetrating the demineralized enamel surface. The use of CPB produced a uniform etch pattern that was more conservative and less destructive to the enamel surface (figure 3) and it is to be noted that only the surface region revealed resin tag formation whereas in conventional primer -XT and in the self-etching primer samples there was uniform and deep penetration. Consequently, a regular resin tags distribution was observed, which showed less magnitude when compared with the control group.

3.2 Microhardness test results

ANOVA of the data showed statistically significant effects for the factors adhesive type, position, and depth, and for the interactions adhesive type*depth, adhesive type*position, position*depth, and adhesive type*depth*- position ($P <0.05$). Descriptive statistics and multiple comparisons of micro-hardness for antibacterial monomer-containing, self-etching primer and conventional adhesive systems at different depths from the enamel surface are presented in Table 3. The interaction between adhesive type and depth showed significant differences at depths of 10µm, 20µm, and 30 µm from the enamel surface. Less lesion depth was found in enamel around the brackets bonded with antibacterial monomer-containing adhesive in comparison with the conventional system. (P<0.05) Table 3 and 4 describes the individual primers - Transbond XT, Transbond Plus SEP and Clearfiil Protect Bond in their respective position and depth. Post Hoc analysis is applied individually to determine the significant depth and penetration at their respective significant position. Less lesion depth was found in enamel around the brackets bonded with antibacterial monomer-containing adhesive in comparison with the conventional system. The ANOVA- one-way analysis on all the three primers comparing the depth and position revealed significant at 10µm,20µm and 30µm and CPB showed highest mean in all the three depths (297.26, 309.65 and 319.17 respectively) indicating less demineralisation and caries. Furthermore, Tukey Post hoc revealed that CPB is better than Transbond XT (Mean of 26-31.6) in all the three depths and so was Transbond plus SEP (Mean of 13-21) but CPB compared to other two showed the highest mean difference implying its efficacy over the others. (P<0.05) The null hypothesis that the antibacterial monomer- containing adhesive suggested for bonding brackets can significantly reduce the overall amount of demineralization around orthodontic brackets could not be rejected.

4. DISCUSSION

The advantage of this in-vivo study was that the development of the caries lesions was studied in vital teeth and it required minimal patient cooperation and no special diet. The protected enamel surface allowed the accumulation of thick plaque and no other site was at risk of caries with this procedure. The results of the Scanning electron microscopic analysis revealed that enamel etching with phosphoric acid created an etch pattern characterized by a deep and uniform demineralization area as shown in Figure 1. Dissolution of prism cores and boundary regions can be observed, however, the conditioning of enamel surface was not uniform along the unground surfaces. The use of phosphoric acid on enamel has been associated with an increase in the superficial roughness. **Cehreli and Altay** [9] observed in their study that regardless of treatment time, etching with 37% phosphoric acid results in irreversible damage of the enamel surface. Currently, there is an increasing preference for milder etching procedures. Compared with phosphoric acid, Transbond Plus SEP produced a uniform and more conservative etch pattern, with regular adhesive penetration and a less aggressive enamel demineralization as shown in Figure 2 and according to the results obtained in our study, 92.8% of the samples showed shallow penetration. The etching patterns ranged from mild demineralization to an aspect of surfaces etched with phosphoric acid. The resin tags were shorter than those observed in the control group. **Pashley and Tay (2001)** [10,11] reported that the efficacy of self-etching primers in intact enamel does not depend solely upon their etching aggressiveness, but also on the monomeric composition of each material. The prism cores and boundaries were etched by phosphoric acids, causing dissolution of both inter and intraprismatic areas. The predominant etching pattern had the peripheral region of prisms removed and prism cores relatively unaffected. SEM observations indicated that only shallow pits were produced after some self-etching treatments in intact enamel. The Clearfil protect bond (CPB) samples evaluated under SEM showed negligible deep penetration of enamel and the surfaces were predominantly un-etched (Figure 3). Because of the action of CPB and the monomer MDPB, the surfaces are left intact and fewer resin tags were formed. Also owing to the 30-day period of testing the demineralisation efficacy, the tooth showed minimal demineralisation effect of the intact enamel as compared to extensively demineralised teeth treated with Transbond XT control samples. As described by Imazato et al, [12] the efficacy of MDP (methacryloyloxydodecyl- pyridinium -bromide) monomer on the acid-etched teeth could be the reason why the demineralised areas on the teeth were minimal as compared to control group and self-etching primer samples. It is to be noted that Kappa score could not be applied when the examiners tested Clearfil protect bond due to the fact that all the results were indicating shallow penetration.

Cross-sectional microhardness test was preferred to evaluate demineralization and caries because a strong correlation coefficient (r = 0.91) was found between enamel microhardness and the percentage of mineral loss in the caries lesions. The hardness values of enamel under 2 controls located under the bracket and at the lingual surface, bonded by 2 types of adhesives were used to evaluate the effect of etching and individual enamel hardness. The findings of the present study showed that the hardness values were similar, indicating that demineralization was due to caries and not to the effect of etching. At the depth of 50, 70 and 90 microns there was no significant result obtained showing no difference due to no effect produced by etching and therefore it is not included in the post-hoc analysis. Significant differences were found between the 3 adhesive systems at depths of 10, 20, and 30 microns from the enamel surface. Shallower lesion depths were found in the enamel around the brackets bonded with the antibacterial monomer-containing adhesive compared with the conventional adhesive system. The lesion-depth results were higher than those of the study by **Pascotto et al** [13] but lower than the report of **de Moura et al**. [14] **O'Reilly and Featherstone** [15] explained the difference in enamel hardness under the brackets by the etching technique. They found mineral losses of 3% to 8% directly under the brackets with etching. **Tancan Uysal et al** [16] had done a similar study comparing the Clearfil protect bond against the conventional Transbond XT and hence proved that the latter was better in preventing demineralisation of enamel. The limitation of this study is that it was cross-sectional and hence, gives no indication of the sequence of events and the duration of exposure to the oral environment was for 30 days. A 30-day experimental period was used because measurable demineralization can be observed around orthodontic appliances 1 month after bonding. The hardness values of enamel under 2 internal controls (under the bracket and at the lingual surface) bonded by 2 types of adhesives were used to evaluate the effect of etching and individual enamel hardness. Another limitation is that the sample was drawn from a pool of patients from one centre and that may prejudice the findings. Some authors have emphasized that, with the use of an antibacterial monomer-containing adhesive, the clinician needs to perform an additional step during the bonding procedure as compared with conventional systems and a most importantly self-etching primer having the advantage of fewer steps.

5. CONCLUSION

Based on the Scanning electron microscopic test, Clearfil Protect Bond showed the least demineralization around orthodontic brackets followed by Transbond Plus SEP ™. Transbond XT ™ control group showed that highest demineralization around orthodontic brackets. Microhardness test revealed that the Clearfil protect bond significantly shows least mineral loss among all the surfaces measured followed by Transbond plus SEP and the Transbond XT showed highest mineral loss amongst all the positions and depth measured. This cariostatic effect was localized at the area around the brackets and was significant after 30 days.

6. FIGURE LEGENDS

1. Table 1: Showing distribution of various types of primer and its effect on the study participants the null hypothesis was rejected. The Chi square-test indicated that the adhesive penetration when the MDPB was used was significantly lower (P .001) as compared to SEP and XT when phosphoric acid and the primer were used separately with a conventional adhesive system.
2. Table 2: Showing association of type of primer and this effect on the tooth of study participants Transbond XT showed almost 85.7% of deep penetration as compared to SEP which showed only 7.1% and CPB almost nil.
It was observed that even though self-etching primer consistently showed an unsubstantial homogenous pattern (figure 2) as a conventional primer (XT) (figure 1), it still proved to be enhanced as seen in the microphotographs of the scanning-electron-microscopic observations compared to MDPB samples. The intact enamel surfaces etched with different acids had different surface morphologies. A characteristic and uniform etch pattern was observed in the resin samples of the phosphoric acid/Transbond XT primer group, revealing increased roughness and resin tags penetrating the demineralized enamel surface. The use of MDPB produced a uniform etch pattern that was more conservative and less destructive to the enamel surface (figure 3) and it is to be noted that only the surface region revealed resin tag formation whereas in conventional primer -XT and in the self-etching primer samples there was uniform and deep penetration (figure 1,2). Consequently, a regular resin tags distribution was observed, which showed less magnitude when compared with the control group.
3. Table 3: Post Hoc Test – XT, SEP and CPB primers on study participants. Descriptive statistics and multiple comparisons of micro hardness for antibacterial monomer-containing, self-etching primer and conventional adhesive systems at different depths from the enamel surface are presented. The interaction between adhesive type and depth showed significant differences at depths of 10, 20, and 30 mm from the enamel surface. Less lesion depth was found in enamel around the brackets bonded with antibacterial monomer-containing adhesive in comparison with the conventional system. ($P<0.05$)
4. Table 4: Showing the difference between various types of primers, position and depth on study participants. The ANOVA- one-way analysis on all the three primers comparing the depth and position revealed significant at 10,20 and 30 microns and CPB showed highest mean in all the three depths (297.26, 309.65 and 319.17 respectively) indicating less demineralisation and caries. Furthermore, Tukey Post hoc revealed that CPB is better than Transbond XT (Mean of 26-31.6) in all the three depths and so was Transbond plus SEP (Mean of 13-21) but CPB compared to other two showed the highest mean difference implying its efficacy over the others. ($P<0.05$) The null hypothesis that the antibacterial monomer- containing adhesive suggested for bonding brackets can significantly reduce the overall amount of demineralization around orthodontic brackets could not be rejected.
5. Figure 1: Scanning electron microscopic micro-photographs of conventional primer Transbond XT.
6. Figure 2: Scanning electron microscopic micro-photographs of Self-etch primer Transbond pluys SEP.
7. Figure 3: Scanning electron microscopic micro-photographs of an antibiotic monomer containing primer Clearfil Protect Bond.

7. DECLARATION

- **Ethics approval and consent to participate:** This original article has been approved and cleared by the institutional ethical committee meeting conducted at JSS Dental College & Hospital, Mysuru on 19th January 2017.
- **Consent for publication**: Not applicable
- **Availability of data and material**: The datasets generated and/or analysed during the current study are not publicly available as a part of institutional norms due to data manipulation and reproducibility but are available from the corresponding author on reasonable request.
- **Competing interests**: Nil
- **Funding**: Not applicable
- **Authors' contributions**: SN and RN formed the concept, design and definition of intellectual content. SN conducted the elaborate literature search, data acquisition. Analysed and interpreted the patient's data regarding the scanning electron microscopic test and Microhardness test results and prepared the manuscript. RN edited and reviewed the manuscript. All authors read and approved the final manuscript.
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APPENDIX

List of abbreviations

CPB : Clearfil Protect Bond
SEP : Self Etch Primer
SEM : Scanning Electron Microscope
MDPB : *12-methacryloyloxydodecylpyridinium bromide*
CEJ : Cemento Enamel Junction
MHT : Microhardness Test
ANOVA : Analysis Of Variance

Tables

Table 1: Showing distribution of various types of primer and its effect on the study participants

Examiners	Category	Type of primer		
		XT	SEP	CPB
Examiner 1	**Shallow**	02 (14.2)	13(92.8)	14 (100)
	Deep	12 (85.7)	01 (7.1)	0
Examiner 2	**Shallow**	01 (7.1)	12 (85.7)	14 (100)
	Deep	13 (92.8)	02 (14.2)	0
Examiner 3	**Shallow**	02 (14.2)	13 (92.8)	14 (100)
	Deep	12 (85.7)	01 (7.1)	0

Note: Numbers within brackets indicate row percentages for individual observations

The above table showing three examiners who separately examined and gave their results of the study samples with all the three types of primers. Examiner 1 gave result of XT with shallow for 02 (14.2 %) samples, SEP 13 (92.8%), CPB 14 (100%) and deep for 12 (85.7%), SEP 01 (7.1%) and none for CPB.

Examiner 2 gave result of XT with shallow for 01 (7.1 %) samples, SEP 12 (85.7%), CPB 14 (100%) and deep for 13 (92.8%), SEP 02 (14.2%) and none for CPB.

Examiner 3 gave result of XT with shallow for 02 (14.2 %) samples, SEP 13 (92.8%), CPB 14 (100%) and deep for 12 (85.7%), SEP 01 (7.1%) and none for CPB.

Table 2: Showing association of type of primer and this effect on the tooth of study participants

S. No.	Type of primer	Effect		Total	Chi square	P value
		Shallow	Deep			
1	**XT**	2 (15.4)	11 (84.6)	13	25.971	**0.001***
2	**SEP**	13 (86.7)	2 (13.3)	15		
3	**CPB**	14 (100)	0	14		
Total		29	13	42		

Note: Numbers within brackets indicate row percentages for individual observations
****Statistically significant p <0.050***

Among the study participants, it was found that there was an association of various types of primers and its effect on the tooth which was statistically significant.

Table 3: Post Hoc Test – XT, SEP and CPB primers on study participants

Depth	Position		Mean difference	P value	95% Confidence Internal	
					Lower bound	Upper bound
10 µ	Occlusal +200 µ	Cervical -200 µ	28.726	**0.001***	11.28	46.18
	Occlusal +200 µ	Lingual	39.755	**0.001***	22.30	57.21
	Occlusal +100 µ	Under the bracket	21.099	**0.001***	7.57	42.47
	Occlusal +100 µ	Cervical -100 µ	25.019	**0.001***	21.90	56.80
	Occlusal +100 µ	Cervical -200 µ	39.348	**0.001***	32.93	67.83
	Occlusal +100 µ	Lingual	50.376	**0.001***	32.93	67.83
	Under the bracket	Cervical -200 µ	18.249	**0.035***	.80	35.70
	Under the bracket	Lingual	29.277	**0.001***	11.83	46.73
	Cervical -100 µ	Lingual	25.357	**0.001***	7.91	42.81
20 µ	Occlusal +200 µ	Cervical -200 µ	26.100	**0.007***	4.84	47.36
	Occlusal +200 µ	Lingual	37.048	**0.001***	15.79	58.31
	Occlusal +100 µ	Under the bracket	25.743	**0.008***	4.48	47.01
	Occlusal +100 µ	Cervical -100 µ	30.348	**0.001***	9.09	51.61
	Occlusal +100 µ	Cervical -200 µ	37.200	**0.001***	15.94	58.46
	Occlusal +100 µ	Lingual	48.148	**0.001***	26.89	69.41
	Under the bracket	Lingual	22.405	**0.033***	1.14	43.67
30 µ	Occlusal +100 µ	Lingual	43.224	**0.010***	6.93	79.52

***Statistically significant p <0.050*, Tukey post-hoc test**

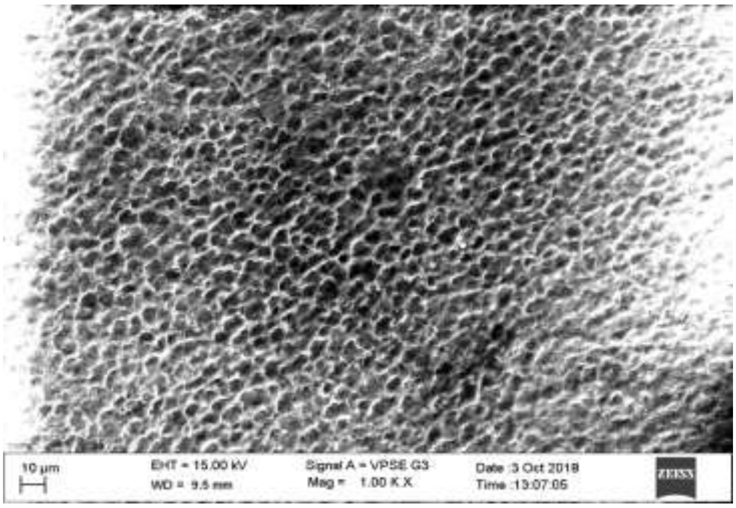
The variables in the present study were the depth of all the three which were tested between various types of primers and its positions and found a statistically significant difference between 10 µ, 20 µ, 30 µ and the position of the brackets. Tukey post hoc test was done among the variables which were significant, which showed that the CPB had a higher value of 31.6 than XT and SEP at 10 µ, 20 µ and 30 µ depth whereas, +0 µ, -0 µ, 50 µ, 70 µ and 90 µ did not show any statistically significant difference between the depth, position and type of primer, therefore they were not mentioned in the above table.

Table 4: Showing the difference between various types of primers, position and depth on study participants

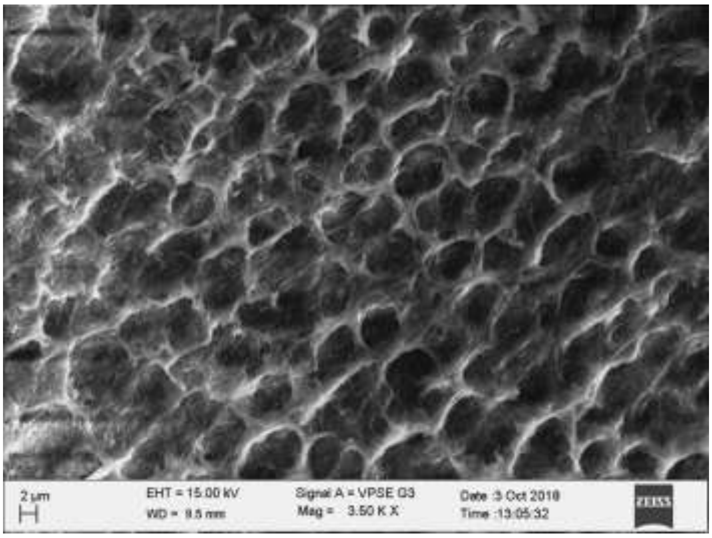
Depth	Primer Category	Mean	Std. Deviation	F	P value
10µ	**XT**	271.06	23.859	13.244	**0.001***
	SEP	284.06	21.718		
	MDPB	**297.26**	24.317		
20µ	**XT**	277.99	19.566	16.696	**0.001***
	SEP	297.20	16.267		
	MDPB	**309.65**	35.664		
30µ	**XT**	288.73	17.914	6.259	**0.003***
	SEP	310.23	52.967		
	MDPB	**319.17**	42.441		

Statistically significant p <0.050

The variables in the present study were the depth of all the three which were tested between various types of primers and its positions and found a statistically significant difference between 10 µ, 20 µ, 30 µ and the position of the brackets. Tukey post hoc test was done among the variables which were significant, which showed that the CPB had a higher value of 31.6 than XT and SEP at 10 µ, 20 µ and 30 µ depth whereas, +0 µ, -0 µ, 50 µ, 70 µ and 90 µ did not show any statistically significant difference between the depth, position and type of primer, therefore they were not mentioned in the above table.

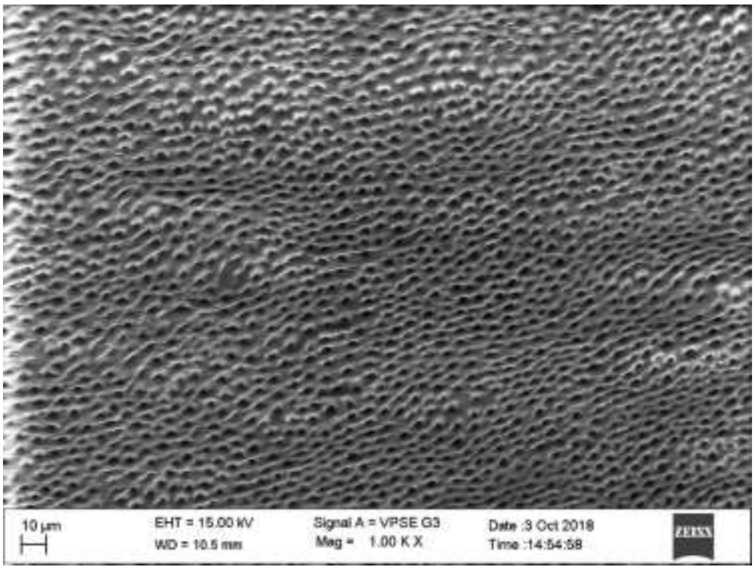


(A) 1000X

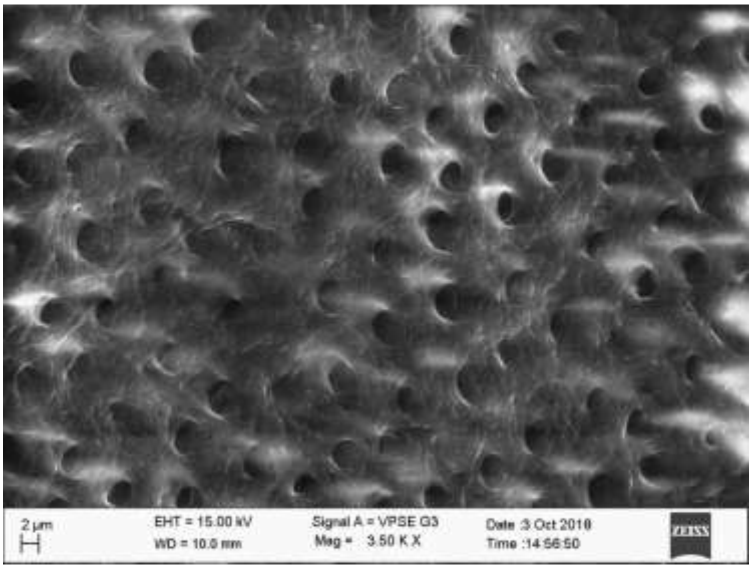


(B) 3500X

Fig. 1: (A) Enamel-etch pattern obtained after phosphoric acid treatment. Uniform formation and deep penetration of resin tags are clearly noted (scanning electron microscopy [SEM] 1000). (B) Higher magnification of resin-tag formation (SEM 3500).

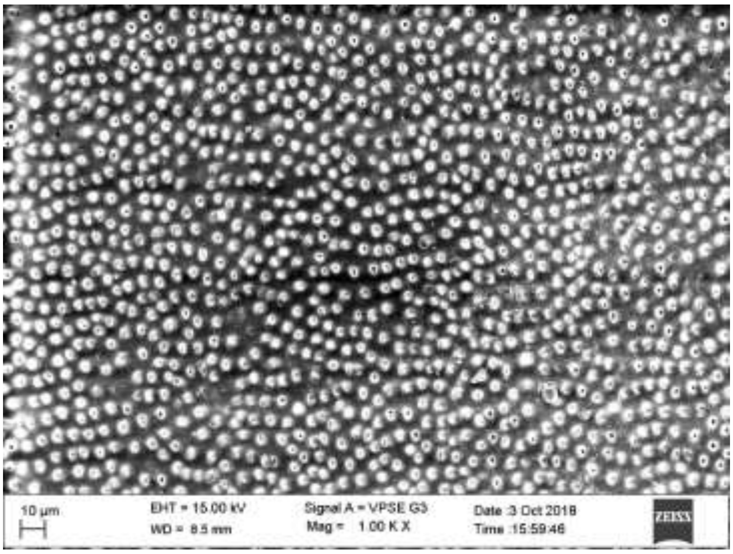


(A) 1000X

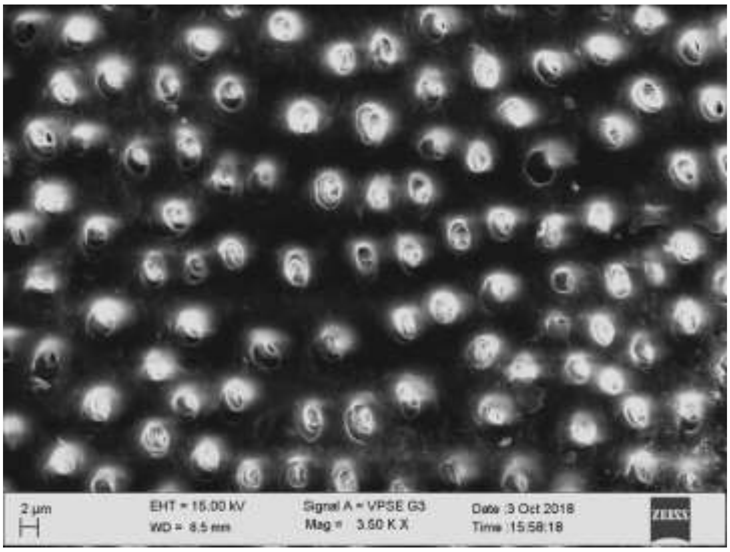


(B) 3500X

Fig. 2: (A) Resin sample of enamel conditioned with Transbond Plus self-etching primer. The etch pattern was shallower than that observed in Figure 1A, (scanning electron microscopy [SEM] 1000). (B) The resin tags were short and regularly formed (SEM 3500).



(A) 1000X



(B) 3500 X

Fig. 3: (A) Resin sample of enamel conditioned with antibiotic containing primer (MDPB). The etch pattern was shallower than that observed in Figure 1A and 2A, (scanning electron microscopy [SEM] 1000). (B) The resin tags were seen mostly in the surface of the enamel and were less penetrating. (SEM 3500).

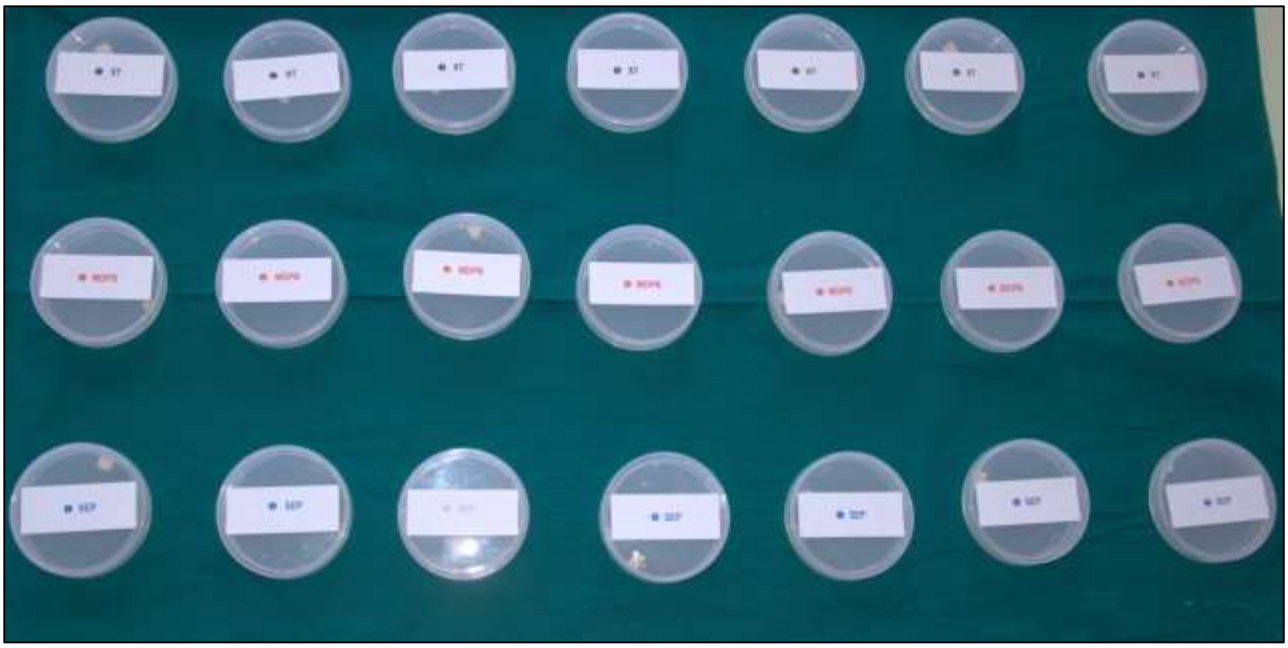


Fig. 4: Teeth samples

Fig. 5: Etchant

Fig. 6: Transbond XT ™

Fig. 7: Control Primer: Transbond XT ™

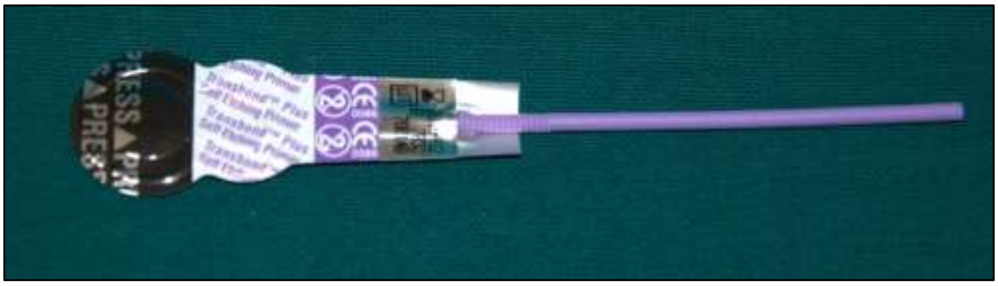


Fig. 8: Transbond PLUS Self Etching Primer™

Fig. 9: Clearfil Protect Bond (Kuraray Okayama, Japan)

Fig. 10: Mounted teeth sample

Fig. 11: Scanning Electron Microscope

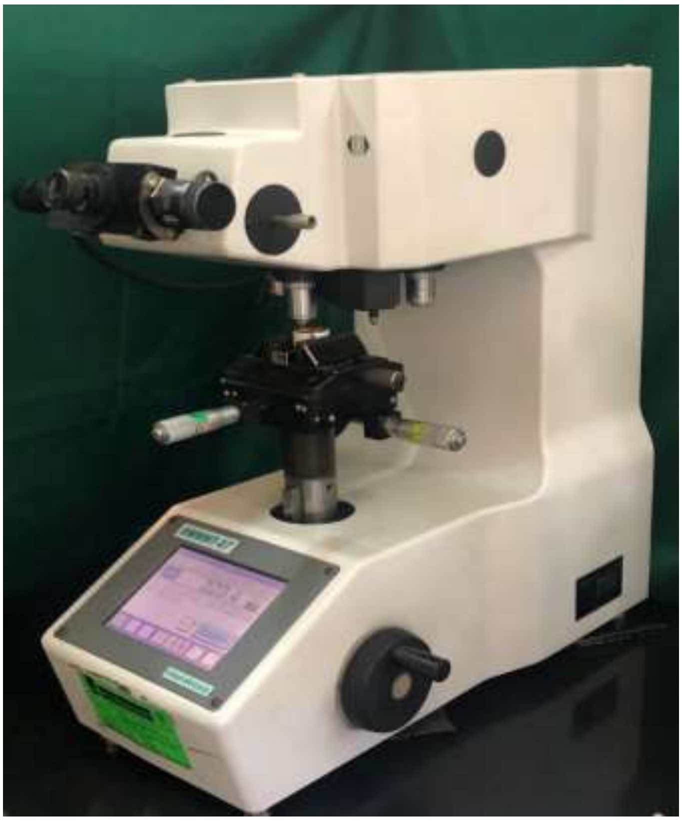


Fig. 12: Microhardness tester - HWMMT-XLE, Shimadzu, and Kyoto, Japan

