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Evaluation of demineralisation adjacent to bracket bonded with conventional orthodontic composite, resin modified GIC and nano-ceramic restorative composite

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ARTICLE INFO

Article history:
Available online 1 September 2017

Keywords:
Demineralization
Transbond XT†
Filtek Z 350◆
Fuji-Ortho-LC

ABSTRACT

Context: White spot lesions form within 4 weeks of bracket placement and 96% patients show its prevalence during fixed treatment. Less than optimal esthetic result is achieved after orthodontic treatment due to demineralization of enamel adjacent to fixed orthodontic appliances in patients with inadequate oral hygiene.

Aims: An invitro evaluation of areas of demineralization, before and after subjecting the teeth to caries producing solution for 30 days for three adhesive resins, Transbond XT†, Fuji-Ortho-LC ⋆ and Filtek Z 350◆.

Settings and design: 180 sound premolars were divided into 2 broad groups of 90 each. Control group were exposed to artificial saliva solution for 30 days, whereas, experimental group were exposed to both artificial saliva (for 11 h) and caries producing solution (for 1 h) at the rate of 2 cycles per day for a period of 30 days. These groups were further subdivided into six subgroups of 30 teeth each. After 30 days, for both groups, teeth cusps were ground flat and longitudinal bucco-lingual sections were made with a series 1000 Deluxe Hard tissue microtome, photographed under polarized light microscopy and Motic Image plus software 3.2 was used to measure amount and depth of demineralisation. Depth of demineralization lesion was obtained as an average from three sites within the lesion (gingival, middle and occlusal).

Results: Analysis of variance (two-way ANOVA) and Tukey's HSD post hoc test indicated significant differences among these groups and materials. Teeth bonded with conventional composite (Transbond XT™† 3M), nanofilled restorative material (Filtek Z 350™◆ 3M) showed more lesion depth when compared with RMGI cement (Fuji-Ortho-LC™ ⋆). RMGIC groups had smaller mean lesion depths when compared with composite resin groups. Materials from experimental group showed increased depth of demineralization ($p < 0.0000$) when compared with control group.

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http://dx.doi.org/10.1016/j.jpfa.2017.08.003

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Conclusions: Area of demineralization in experimental group increased significantly when compared with control group. Least amount of demineralization was seen with RMGIC (Fuji-Ortho-LC ⁎) and highest with conventional orthodontic composite (Transbond XT†).

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1. Introduction

Decalcification of teeth bonded with fixed appliances has been a major problem for which orthodontists are very commonly blamed for.[1] Rough resin surfaces encourage rapid attachment of bacteria and promote gingival and periodontal disease.[2] Plaque accumulates in association with these bonded brackets within 24 h.[2] Studies have shown that 75% of all orthodontic patients develop demineralization/white spot formation on labial surfaces during fixed appliance therapy within 4 weeks of bracket placement.[3]

Etching of enamel surface with phosphoric acid (H_3PO_4) before placement of orthodontic attachments predisposes under covered enamel to caries.[4] Hence areas of enamel show tissue loss[5] and dissolution of enamel[6] due to mineral loss/demineralization, lesion formation[3] and progression of dental caries.

Factors like microbial organisms (particularly *Streptoccus mutans*), salivary factors (like pH, flow rate and buffer capacity), oral hygiene and diet cause enamel demineralization. Low pH of saliva and its buffering capacity contribute to counter the acid produced in plaque which otherwise would cause to colonize the aciduric bacteria.[7]

Non-compliant methods such as fluoride varnishes and bonding agents like glass ionomer cement (GIC) have shown to decrease enamel demineralization both in vitro and in clinical trials[8] as they chemically bond enamel and leach fluoride. Remineralization occurs when there is incorporation of fluoride into enamel structure.[9] Slow release of fluoride from cements reduces plaque formation.[10] GICs release fluoride over a long term and at much higher levels than fluoride releasing composites. But they are weaker than composite resins.[11] Under artificial cariogenic conditions, GICs have poor bond strength and greater bond failure rates than composite resins.[12] In an attempt to provide greater fluoride release and adequate bond strength, combinations of GICs and composite resins have been developed. Resin modified GIC is one such combination that autoset by acid base reaction of GIC.[13]

It was envisaged that subjecting the teeth to demineralizing solution, affects substrate upon which the brackets are bonded. Hence, shear bond strength of materials could reduce, leading to bracket bond failures. Besides, materials by virtue of their properties affect demineralization of enamel per se which led to aims and objectives of this study.

2. Aims and objectives of the study

To comparatively evaluate area of demineralization adjacent to brackets bonded with Transbond XT†, Filtek Z 350◆ and Fuji-Ortho-LC⁎ before and after subjecting materials to demineralizing solution.

3. Materials and methods

Premolars were collected and stored in saline solution at 37 °C till the 27th day when the requirement of 180 premolars was fulfilled. Only freshly atraumatically extracted, caries free, human first permanent premolars which had similar mesio-distal and labiolingual lengths were included in the study to eliminate bias occurring due to differences in surface area available for interaction between host and environment causing demineralization. All the teeth which met the inclusion criteria were subjected to Laser fluorescence via DIAGNOdent pen to determine the presence of white spot lesions before the start of experiment. Premolars were then scaled and dried thoroughly before bonding. Bonding procedure was carried out using a conventional orthodontic composite (Transbond XT†), nano-ceramic restorative material (Filtek Z 350◆) and resin modified GIC (Fuji-Ortho-LC⁎) according to manufacturer's instructions. Prior to acid etching and bonding, a masking tape was used to cover occlusal area adjacent to brackets to isolate these areas from acid etching procedure.

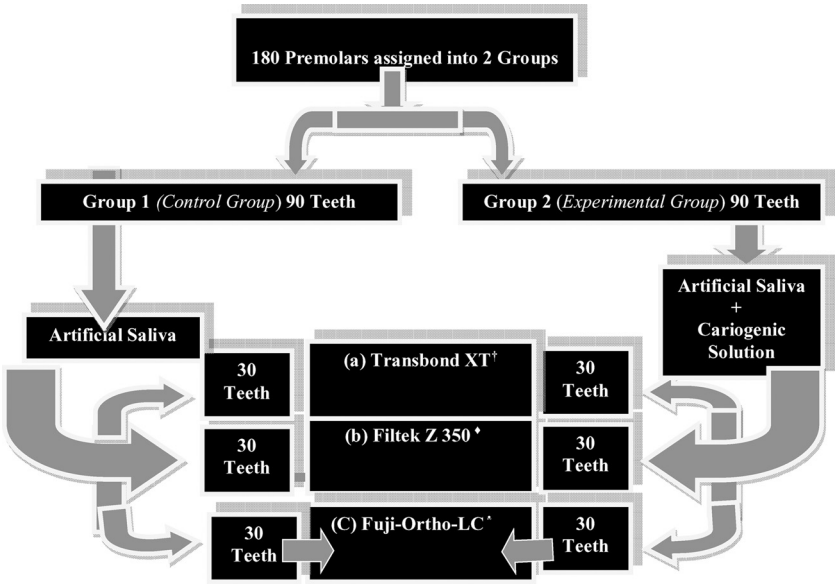
Acid resistant varnish (Nail polish) was used to paint rest of tooth surfaces. 180 teeth were randomly assigned to 2 groups of 90 each; groups were then subdivided into 3 subgroups (Schemes 1 and 2).

Group 1 (*Control group*) – Artificial saliva solution group; *30 teeth in each subgroup*
- Subgroup (a): conventional orthodontic composite (Transbond XT†)
- Subgroup (b): nano-ceramic restorative material (Filtek Z 350◆)
- Subgroup (c): resin modified glass ionomer cement (Fuji-Ortho-LC⁎)

Group 2 (*Experimental group*) – Caries producing solution group; *30 teeth in each subgroup*
- Subgroup (a): Transbond XT†
- Subgroup (b): Filtek Z 350◆
- Subgroup (c): Fuji-Ortho-LC⁎

† = 3M UNITEK; ◆ = 3M ESPE; ⁎ = GC ORTHO.

Teeth in group 1 and group 2 were mounted on a standardized acrylic block and bonded with appropriate material according to Protocol 1. Teeth of group 1 were immersed in artificial saliva and group 2 teeth were alternately cycled in artificial saliva and cariogenic solution as per Protocol 2. Teeth were sectioned and evaluated for area of demineralization quantitatively as per Protocol 3.



Scheme 1 – Depicting distribution of sample size.

4. Protocol 1: bonding procedure (Figs. 1a–g and 2a–c)

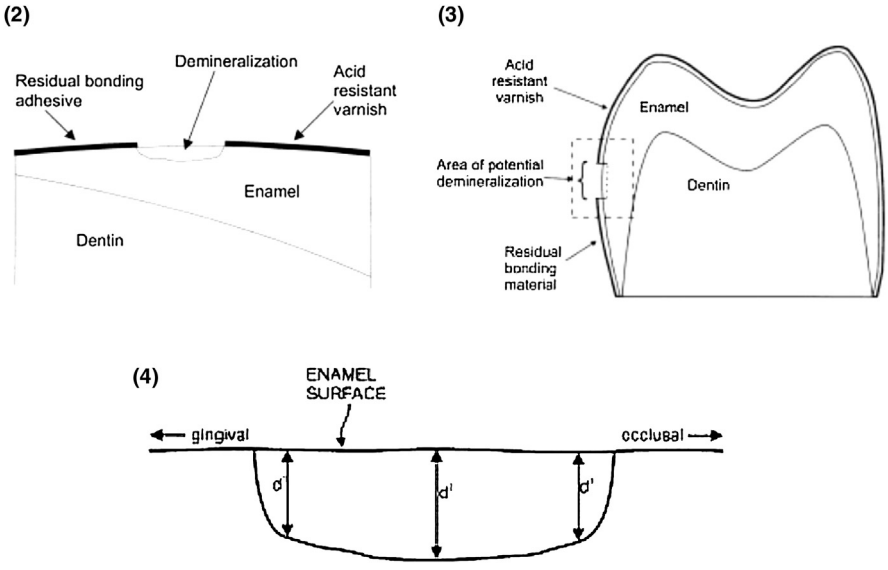
Buccal crown surface of each tooth was rinsed and dried after a 15-s polish with fluoride-free pumice slurry. Stainless steel premolar brackets (0.022″ × 0.028″ slot, MBT prescription, 3M Unitek) were bonded utilizing procedure outlined for each group. Bracket bases had an average surface area of 9.00 mm^2.

4.1. *Subgroup 'a' (1a and 2a)*

Buccal enamel surface was etched with 37% phosphoric acid for 30 s, rinsed for 15 s, and dried with oil and moisture-free air until enamel had a faint white appearance. Transbond XT† primer was applied as a thin film to etched surface and light cured for 10 s. Transbond XT† adhesive paste was applied to bracket base and bracket was positioned on tooth and pressed firmly with an instrument to expel excess adhesive after which excess bonding resin was removed using a sharp scaler. Material was light cured as per manufacturer's protocol that is, 20 s from incisal and 20 s from gingival end.

4.2. *Subgroup 'b' (1b and 2b)*

Buccal enamel surface was etched with 37% phosphoric acid for 30 s, rinsed for 15 s, and dried with oil and moisture-free air until enamel had a faint white appearance. Transbond XT† primer was applied as a thin film to etched surface and light cured for 10 s. Filtek Z 350♦ adhesive pastes was applied to



Schemes 2–4 – Schematic diagrams illustrating measurements of demineralized areas.



Fig. 1 – (a and b) Instruments used for bonding in the study. (c and d) Premolar brackets used in the study. (e and f) Composite material used in the study. (g) GIC used in the study.



Fig. 2 – (a–c) Bonding procedure used in the study. (d and e) Placement of teeth in saliva and artificial caries solution respectively.

bracket base and bracket was positioned on tooth and pressed firmly with an instrument to expel excess adhesive after which excess bonding resin was removed using a sharp scaler. Then, adhesive was light cured as per manufacturer's protocol for Filtek Z 350◆ that is, 20 s from incisal and 20 s from gingival end.

4.3. *Subgroup 'c' (1c and 2c)*

Buccal enamel surface was etched with 37% phosphoric acid for 30 s, rinsed for 15 s, and dried with oil and moisture-free air until enamel had a faint white appearance. Fuji-Ortho-LC* paste was applied to bracket base and bracket was positioned on tooth and pressed firmly with an instrument to expel excess adhesive after which excess bonding resin was removed using a sharp scaler. Then, adhesive was light cured for 40 s.

5. Protocol 2: immersion in artificial saliva and cariogenic solution (Fig. 2d and e)

Small hole was drilled near apex of each tooth and dental floss was fed through the hole to facilitate suspension of teeth in different solutions.

Group 1 was immersed in artificial saliva solution of neutral pH 20 mmol/L $NaHCO_3$, 3 mmol/L NaH_2PO_4 and 1 mmol/L $CaCl_2$ at room temperature.

Teeth in group 2 were stored in artificial saliva solution for 12 h before subjecting them to artificial caries producing solution which consisted of 2.2 mmol/L of Ca^{2+}, 2.2 mmol/L of PO^{4-}, 50 mmol/L of acetic acid at pH of 4.4.

Teeth in group 2 were cycled between saliva and caries solution twice daily for 30 days. After every 11 h in artificial saliva, these were removed and placed in caries producing

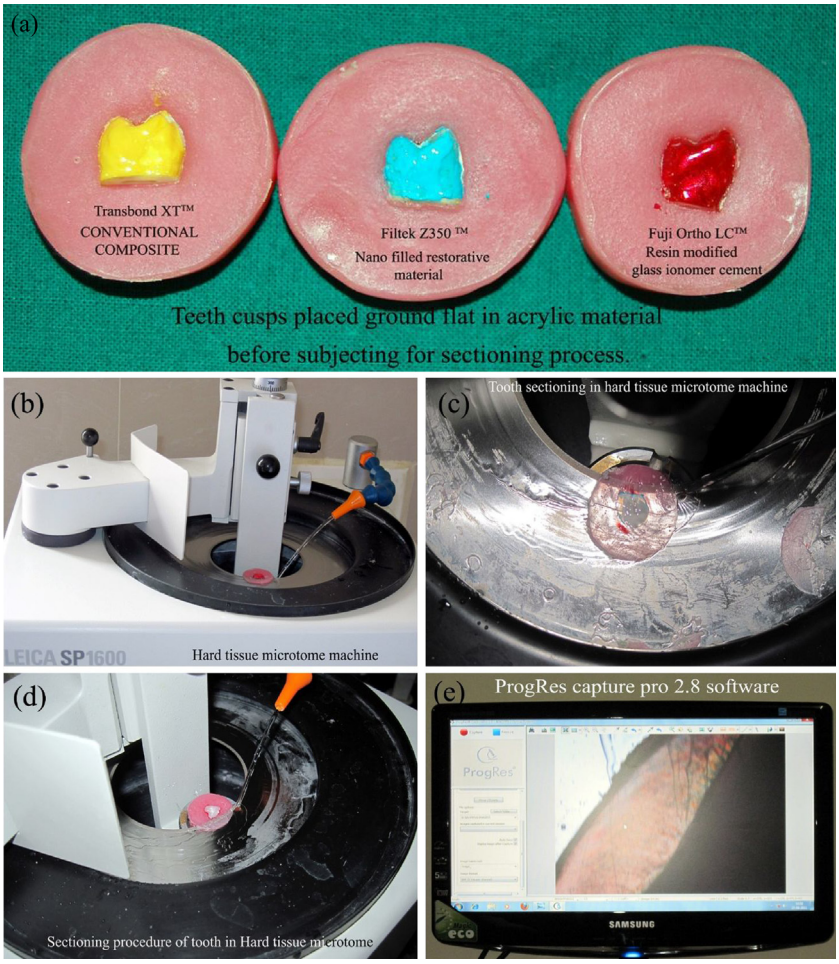


Fig. 3 – (a) Teeth embedded in acrylic. (b–e) Tooth sectioning process.

solution for 1 h and after that they were put back into artificial saliva. Artificial saliva solution was changed twice weekly.

6. Protocol 3: Estimation of demineralized lesions (Figs. 3a–e and 4)

After debonding with a Universal Testing Machine (HOUNSFIELD H5KS-0195), teeth cusps were ground flat and longitudinal bucco-lingual sections were made for both groups with a series 1000 Deluxe Hard tissue microtome (Leica SP 1600) to yield sections 100–140 μm thick. Acrylic slices containing tooth embedded in it were placed in acetone solution for 30 min. Acetone made acrylic soft and thus softened acrylic was separated from tooth with help of paint brush. Further reduction in thickness to 90–100 μm was accomplished by hand grinding on a polishing board with progressively finer grades of aluminum powder. Tooth slices were then placed in alcohol solution for 30 min to remove any residual acrylic within the tooth. Teeth were then placed in a mixture of xylene and alcohol. Residual acrylic debris was cleaned up using a soft brush. Teeth were placed in xylene for a proper fixation.

Sections were then mounted on glass slide using DPX and photographed under polarized microscope (ProgRes C5, Capture Pro 2.8 Microscope) at 20× magnification. Motic Image plus software (3.2 Version) was used to measure amount and depth of demineralization adjacent to brackets. Lesion depth for each section was averaged out of 3 sites within the lesion (gingival, middle and occlusal) (Schemes 3 and 4). Resulting averages of all sections from each tooth were obtained to give an overall depth for each tooth in terms of micrometers (μm) (Fig. 5).

Descriptive statistics including mean and standard deviation values were calculated for each group of teeth.

Two-way ANOVA: was used to determine comparison between materials (Transbond XT†, Filtek Z 350◆, Fuji-Ortho-LC⋆) and groups (artificial saliva and artificial saliva + caries producing solution).

Tukey's HSD Post hoc test: was used to determine pair wise comparison of materials (Transbond XT†, Filtek Z 350◆, Fuji-Ortho-LC⋆) and groups (artificial saliva and artificial saliva + caries producing solution).

Comparison of interaction effects of groups (artificial saliva and artificial saliva + caries producing solution) and materials (Transbond XT†, Filtek Z 350◆, Fuji-Ortho-LC⋆) was calculated using same test.

Significance for all statistical tests was predetermined at $p \leq 0.05$.



Fig. 4 – Polarized light microscope used in the study.

7. Results

During sectioning, 30% of samples of teeth fractured. However, multiple sections of same teeth were prepared to make up to sample size of 180. Sectioning process inadvertently removed enamel surfaces over demineralized lesion. When this occurred, a line was drawn to connect intact enamel borders and allow measuring of demineralized depths thus, arbitrarizing the values evaluated for demineralization quantitatively.

It was observed that teeth not immersed in demineralizing solution also showed demineralization which could probably be attributed to bonding procedure. However, after these teeth were immersed in demineralization solution, amount of demineralizing seen increased and was statistically significant (Tables 1–4).

Amongst the materials used, Transbond XT† (20.70 ± 1.23; 31.26 ± 1.04) showed highest amount of demineralization followed by Filtek Z 350◆ (10.68 ± 0.71; 20.22 ± 0.72) and Fuji-Ortho-LC⁎ (6.97 ± 0.57; 10.24 ± 0.45). Differences were statistically significant.

8. Discussion

Sensitivity of material to moisture and development of white spot lesions surrounding the orthodontic brackets led to development of GIC as an adhesive material. Fluoride releasing GIC when used for bonding orthodontic brackets showed decreased demineralization but bond strength remained questionable. Miguel et al.,[14] Miller et al.,[11] Bishara et al.[15] and Lippitz et al.[16] showed decreased bond strength when GIC's were used for bonding. This led to development of light cured resin reinforced glass ionomers.[17] Regular orthodontic composite Transbond XT†, a nano-composite (Filtek Z 350◆), and a resin modified GIC (Fuji-Ortho-LC⁎), though evaluated for its individual properties, were not studied together to the best of our knowledge. Therefore, an attempt was made through this study to evaluate the same.

Iatrogenically caused white spot lesion leads to poor esthetics and, in severe cases, needs restorative treatment. Decalcification is associated with areas where plaque accumulations occur in orthodontic patient.[18] As per Gorelick et al.,[5] no difference in prevalence of white spot (demineralized) lesions in banded or bonded teeth was found.[5] Presence of adhesive flash around a bonded orthodontic attachment predisposes to plaque; therefore, excess adhesive was cleared away before the curing cycle.[2]

Preventive measures to minimize damage and reduce decalcification during fixed orthodontic treatment include; patient selection, dietary education, intensive oral hygiene instruction and monitoring. At each visit, oral hygiene and dietary education should be reinforced. At every adjustment appointment, inspect the teeth to avoid missing early demineralized spots. When patients use daily 0.05% sodium fluoride rinse, occurrence of caries decreases.[5] Professional cleaning of teeth is recommended if patients cannot achieve satisfactory oral hygiene. In severe cases and as a last resort, early removal of appliance is required to confine the damage.[19]

Methods recommended include use of fluoride paste and adjunct fluoride mouthwashes (0.05% sodium fluoride daily rinse or 0.2% sodium fluoride weekly rinse) to help remineralization and reduce unsightly markings. For more severe cases, treatment involving an acid/pumice micro-abrasion technique has been advocated, but should be performed at least 3 months after debonding to allow initial remineralization.[19]

This experimental study was designed to simulate commonly encountered situation in clinical orthodontics. To simulate eating two meals per day, teeth in group 2 were subjected to a one hour of caries challenge twice daily. This time coincides with time interval required for recovery of plaque pH after meal. Plaque pH has shown to dip below a critical level after food consumption; during this time, teeth experience an increased susceptibility to demineralization from acidic plaque.[20] Periodic acid challenge results in demineralization whereas surface layer must remain intact for remineralization to take place. As long as surface layer remains intact and saliva is saturated with calcium and phosphate, acid buffering agents such as bicarbonate and phosphate ions diffuse into plaque, neutralize acids, and

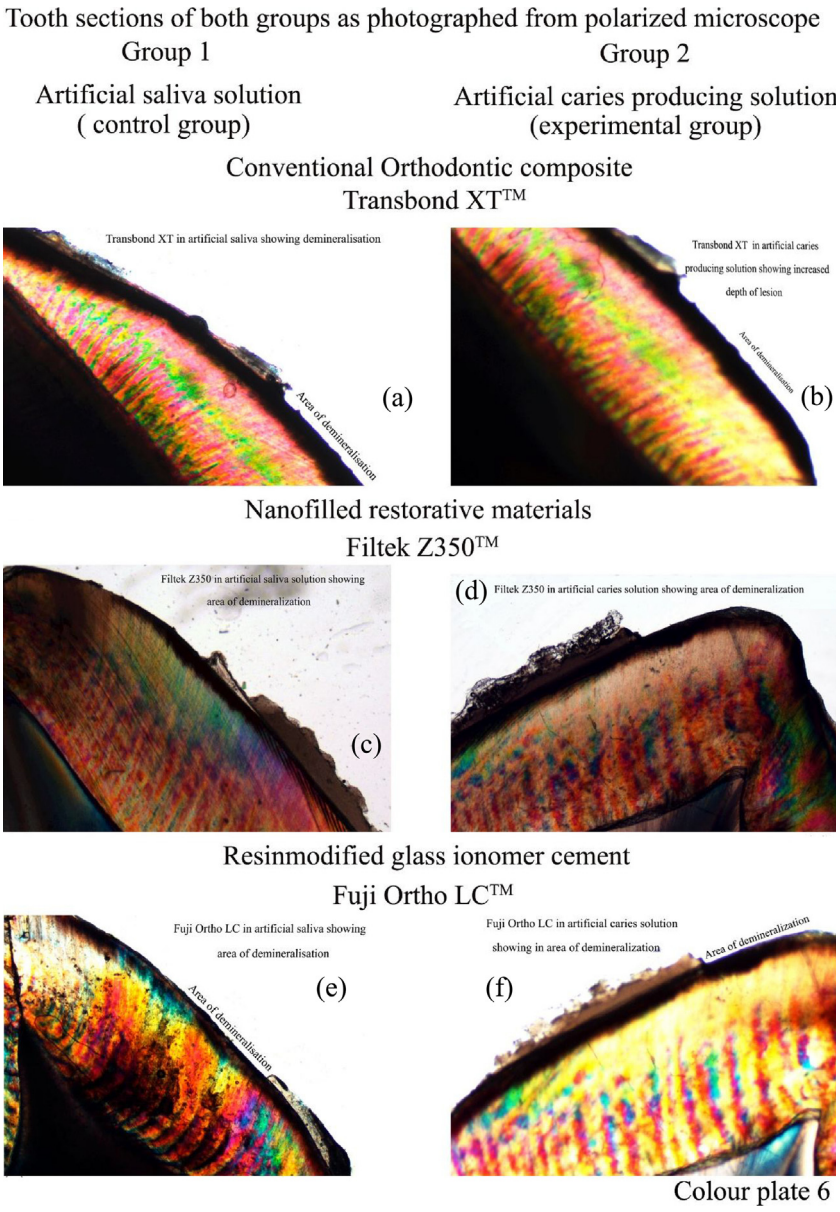


Fig. 5 – Tooth sections of both groups as visible in light microscope.

remineralize the enamel lesion.[21] In this study, artificial saliva provided calcium and phosphate ions for remineralization to occur for both groups. Fluoride from resin modified GIC (Fuji-Ortho-LC⋆) is claimed to alter the ratio between demineralization and remineralization, thus restricting progression of lesions.[22] Demineralization was evaluated after sectioning the tooth on a hard tissue microtome as proposed by Vorhies et al.,[22] Schmit et al.,[8] Paschos et al.[23] and Todd et al.[21]

Teeth bonded to brackets with resin modified GIC (Fuji-Ortho-LC⋆) had significantly smaller enamel lesion depths

Table 1 – Total depth in (μm) of various materials before and after subjecting it to demineralization solution (Mean ± SD in μm).

Subgroup	Materials	Group 1 Mean depth of lesion of teeth in artificial saliva	Group 2 Mean depth of lesion of teeth after subjecting to demineralization solution
a	Transbond XT†	20.70 ± 1.23	31.15 ± 1.04
b	Filtek Z 350◆	10.68 ± 0.71	20.22 ± 0.72
c	Fuji-Ortho-LC⋆	6.97 ± 0.57	10.24 ± 0.45

Table 2 – Comparison between materials (Transbond XT†, Filtek Z 350◆, Fuji-Ortho-LC) and groups (artificial saliva and artificial saliva + caries producing solution) with respect to total depth of demineralization by two-way ANOVA.

SV	DF	SS	MSS	F-value	p-value
Main effects					
Groups	1	2706.2236	2706.2236	3864.2245	0.0000*
Materials	2	9130.3223	4565.1611	6518.6066	0.0000*
Two-way interactions					
Groups × materials	2	456.4353	228.2177	325.8726	0.0000*
Error	174	121.8570	0.7003		
Total	179	12414.8382			

* $p < 0.05$.

Table 3 – Pair wise comparison of materials (Transbond XT†, Filtek Z 350◆, Fuji-Ortho-LC) with respect to Total depth of demineralization by Tukey's HSD post hoc procedure.

Materials	Transbond XT†	Filtek Z 350◆	Fuji-Ortho-LC*
Mean	25.93	15.45	8.60
Transbond XT†	–		
Filtek Z 350◆	0.0000*	–	
Fuji-Ortho-LC*	0.0000*	0.0000*	–

* $p < 0.05$.

Table 4 – Comparison of interaction effects of groups (artificial saliva and artificial saliva + caries producing solution) and materials (Transbond XT†, Filtek Z 350◆, Fuji-Ortho-LC) with respect to total depth of demineralization by Tukey's HSD post hoc procedures.

Interactions	TXT of group 1	FZ350 of group 1	Fuji of group 1	TXT of group 2	FZ350 of group 2	Fuji of group 2
Mean	20.70	10.69	6.97	31.15	20.22	10.24
TXT of group 1	–					
FZ350 of group 1	0.0000*	–				
Fuji of group 1	0.0000*	0.0000*	–			
TXT of group 2	0.0000*	0.0000*	0.0000*	–		
FZ350 of group 2	0.2263	0.0000*	0.0000*	0.0000*	–	
Fuji of group 2	0.0000*	0.3225	0.0000*	0.0000*	0.0000*	–

* $p < 0.05$.
TXT = Transbond XT†; FZ350 = Filtek Z 350◆; Fuji = Fuji-Ortho-LC*.

than teeth bonded with traditional orthodontic composite (Transbond XT†) and nano-ceramic restorative composite (Filtek Z 350◆). Conventional orthodontic composite showed greatest area of demineralization zone (Transbond XT† – 10.45 μm) followed by nano-ceramic restorative composite (Filtek Z 350◆ – 9.54 μm) and resin modified GIC (Fuji-Ortho-LC* – 3.27 μm).

When materials were compared by two-way ANOVA (Table 3) individually & in both groups, statistically there was significant difference between them (groups 1 and 2). Group 2 showed greater amount of demineralization when compared to group 1 (Table 4).

Decreased depth of demineralization was statistically significant and confirmed in resin modified GIC (Fuji-Ortho-LC*). This study is probably evidence that protective effect of fluoride released from resin modified GIC (Fuji-Ortho-LC*) decreased area of demineralization. Therefore hypothesize that resin modified GIC (Fuji-Ortho-LC*), when used to cement orthodontic brackets to human enamel, can reduce depth of enamel demineralization lesion adjacent to bracket when compared to nano-ceramic restorative composite (Filtek Z 350◆) followed by conventional composite (Transbond XT†). Vorhies et al.,[22] Schmit et al.,[8] Paschos et al.[23] and Todd et al.[21] supported this hypothesis by showing enamel lesion reduction using similar research methods for resin modified GIC (Fuji-Ortho-LC*).

Remineralization of demineralized enamel lesions in this study could not have occurred after surface layer of enamel was lost. Before sectioning the teeth, visual inspection did not show that any teeth had lost their enamel surface layer. When considering numerous tooth sections without enamel surface layers, it is probable that surface layers were intact before sectioning and sectioning process itself caused loss of delicate surface layers and subsurface lesion areas. This conclusion is substantiated by fact that, in a single tooth, some sections had intact enamel surfaces and demineralized lesions while adjacent sections had lost enamel surface and subsurface lesion and these findings were supported by Schmit et al.[8]

In literature, no data is available about fluoride release pertaining to conventional composite (Transbond XT†) and

nano-ceramic restorative composite (Filtek Z 350◆). Antibacterial properties of bonding system were not studied, as regimen we used for creating subsurface demineralization was based on pH cycling.

9. Conclusion

Transbond XT† and Filtek Z 350◆ had greatest depth of enamel demineralization lesion when compared to Fuji-Ortho-LC•.

Variables present in this study are innumerable with regard to demineralization with reference to methodology used. However, for want of better methodology, same was adhered to. Further research, utilizing a different methodology which is non invasive would be beneficial. Hence, results of this study could not be extrapolated to material success with respect to demineralization.

Clinical conditions significantly differ from an in vitro setting. Moreover, heat and humidity conditions of oral cavity are highly variable. Owing to differences in in vivo and in vitro conditions, further clinical research is suggested.

Conflicts of interest

The authors have none to declare.

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