Comparative Assessment of *In Vitro* Effect of Three Fluoride Releasing Agents on Enamel Demineralization around Orthodontic Brackets

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ABSTRACT

Background: White spot lesion considered as irreversible tooth demineralization presenting challenge to orthodontists during treatment schedules, fluoride was the most successfully used measure to overcome this challenge.
Materials and method: A total of forty sound human permanent premolars were used in the present study and categorized into four groups, in one group the teeth were bonded with stainless steel brackets using Resin-modified glass ionomer cement (RMGIC) and the other three groups the teeth were bonded with light cured composite Resilience® (Ortho technology Co., USA). Group A; Acidulated phosphate fluoride (APF) topical gel (Mfg by DEEPAK PRODUCTS, INC, USA), fluoride ion 1.23% applied on examine area for four minute. Group B; RMGIC (GC Fuji Ortho LC, GC Corporation/Japan) used as bracket adhesive. Group C; Stannous and sodium phosphate fluoride gel yielding 0.72% fluoride ion (Mfg. for: dental resources DS-8) (0.4% Stannous fluoride, 1% sodium fluoride), was applied daily through the experimental study. D; the control group represents the conventional bonding procedure with no preventive method. The entire labial surfaces except 2 mm gingival to the bracket were isolated by acid resistance varnish. All the teeth were subjected individually during 30 days in to acid challenge cycle. After longitudinal sectioning of the teeth by using a hard-tissue microtome, the depth of the artificial lesion was estimated by taking the average of three penetration depths at the lesion centre under stereomicroscope. Also the enamel surface was classified according to acid etch pattern. Comparisons of the average caries penetration of the groups were submitted to ANOVA and LSD tests. The statistical significance level was set at $p \leq 0.05$.
Results: The results revealed that there were statistically significant differences among the tested groups. With different caries reduction abilities, APF group showed 14%, RMGIC Group 49%, group Stannous and sodium phosphate fluoride 39% depth reduction compared to the control group.
Conclusions: While all the groups showed caries reduction by different fluoride agents used in this study, the less average lesion depth was found at group B making the RMGIG the best caries fighting fluoride measure.
Key words: Demineralization, RMGIC, APF, stannous fluoride. (J Bagh Coll Dentistry 2016; 28(2):126-133).

INTRODUCTION

Despite the revolutionary events in dental and orthodontic materials, instruments and techniques yet, the white spot lesion (WSL) still presents a major drastic effect associated with orthodontic treatment. Previous studies showed that orthodontic attachments increase the plaque accumulation and adherence in oral cavity. [(1)] *Streptococcus mutans* and *Lactobacillus* aggregate in high concentrations in the mouth in the presence of orthodontic fixed appliances. [(2)] These and other cariogenic bacteria ferment carbohydrates to produce organic acids. These acids can lead with time to the dissolution of calcium and phosphate ions from the enamel surfaces. This process of demineralization may lead to WSL and even cavitation as little as 4 weeks. [(3)] This irreversible state of the tooth surfaces loss jeopardizing the successful outcome of orthodontic treatment.

Fluoride is stated to be the most important agent to prevent demineralization and inhibit lesion progression. [(4)] Fluoride ion prevents plaque activity and adhesion by blocking enzyme system. Also fluoride ions in dental plaque replace the hydroxide ion in the crystalline lattice of enamel surface to form a more acid resistant structure called fluorapatite. The remineralization of a preexisting WSL requires ten calcium ions and six phosphate ions with every two fluoride ions to form one fluorapatite unit. [(5)] Fluoride can be used topically (fluoridated tooth paste, gel, mouth rinse, and varnish) or incorporated into cements, elastomeric ligature, or chains. [(6)]

Although the preventive measures like toothpastes and mouth rinses are effective, they had not been entirely successful since they need patient cooperation. [(7)] Therefore, during the last years studies are being made to develop methods that do not require patient compliance [(8)], fluoridated varnishes, gels, bracket bonding materials can be utilized.

Many studies have stated that fluoride gels during orthodontic treatment can provide additional preventive actions when brackets have been bonded with composite resin cement. [(6)]

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Uysal et al [9] tried sodium fluoride containing topical gel that were applied for five minutes only once on the enamel surfaces and was significantly able to raise the enamel microhardness and prevention of dissolution.

Professionally applied 1.23% APF gel was introduced in the 1960s; it contains fluoride (12,300 ppm fluoride) in the form of sodium fluoride at pH 3. [10] This preventive agent was developed in response to elaborated investigations to accelerate enamel fluorapatite formation, inhibit decalcification, and increase fluoride incorporation to the enamel surface. Fluoride uptake by enamel surface can be increased by pH decreasing less than 4, this can achieved by acidulating fluoride solution, however, acidic solutions prepared from hydrochloric or acetic acid produced enamel demineralization. Therefore, they were replaced by phosphoric acid because it did not cause demineralization. [11]

Stannous fluoride gels 0.4% could be described as home non-prescription drugs, it will react with enamel to form tin fluoro phosphate complex that coat enamel surface and dentinal tubules, they provide some bacteriostatic activity, making them preferable in patients needing caries control, plaque reduction and sensitivity reduction. [12,13] Boyd [14] recommended SnF gel as a more effective preventive agent than NaF rinse or fluoridated tooth paste alone because of its dual action on decalcification and gingivitis prevention, he found 29% decalcification reduction in adolescent orthodontic patient using 0.4% SnF gel compared to 0.05% NaF rinse. Stannous fluoride can be used alone or in combination with other flouride containing solution as antierosive agent. [15,16] Tin would precipitate in the outermost enamel layers and become more acid resistant surface , a complex of demineralization and reprecipitation process modifies the uppermost layers of enamel and increasing its defense ability against acid. The scanning electron microscope (SEM) images support the theory that SnF_2, like TiF_4, may work through the formation of a protective surface layer, limiting or delaying the direct contact of the acid with the enamel mineral. [16]

Fluoride-releasing orthodontic adhesives were developed to exhibit an additional source of fluoride near the brackets and permit a regular release of topical fluoride. In the late 1980s, GIC were proposed as a substitute to the more popular used composite material for bracket bonding [17], they can offer regular exposure of fluoride over several months, and the possible development of a modified, less cariogenic microflora. [18] Fluoride-releasing GIC and RMGIC have been shown to exert some cariostatic effects in both prospective and longitudinal clinical trials. [19 20] Also; GIC has the ability to absorb fluoride from topical fluoride applications. This feature allows it to act as a long-term fluoride releasing agent. [21] Because of recent improvements in the fluoride-releasing capabilities and the shear bond strength (SBS) of RMGIC, it has been suggested that these bonding material should play a greater role in attachment bonding in the future. [22] The preventive effect of adhesives adjacent to brackets has been investigated in vitro [7, 23] and in vivo [19, 24] by quantifying the demineralization depths and the mineral losses with various evaluation methods.

This study was conducted to compare the acid resistance potential of three fluoride releasing measures being used against demineralization around orthodontic brackets, RMGIC as bracket bonding material, acidulated phosphate fluoride gel and Stannous and sodium phosphate fluoride gel.

MATERIAL AND METHODS

Teeth collection:

A total of forty sound human premolars were collected and stored in water containing 0.2% thymol. The collected teeth examined with magnifying lens, the buccal enamel surface of the teeth had no developmental defects, cracks, caries, or white spots. The teeth had not been exposured to any pretreatment with chemical agents such as hydrogen peroxide; the remaining visible soft tissue was debrided with razor blade. The teeth were cleaned and cleaned with a pumising paste for 10 seconds. Each tooth was then rinsed with tap water for 30 seconds and dried with oil free air for 10 seconds.

Teeth grouping:

The teeth were divided randomly into four groups containing 10 teeth each according to the caries prevention method:

Group A; Acidulated phosphate fluoride (APF) topical gel (DEEPAK PRODUCTS, INC, USA), concentration of fluoride ion 1.23%.

Group B; Resin-modified glass ionomer cement RMGIC (GC Fuji Ortho LC, GC Corporation/Japan) used as bracket adhesive.

Group C; Stannous and sodium phosphate fluoride gel yielding 0.72% fluoride ion (dental resources DS-8)(0.4% Stannous fluoride,1% sodium fluoride.

Group D; the control group represents the conventional bonding procedure with no preventive measure.

Bonding procedure:

All the teeth were bonded with Edgewise premolar metal Stainless-steel (Bionic) brackets (Ortho technology Co., USA). The bonding procedure was done using light cured composite Resilience®(Ortho technology Co., USA) for all groups except Group II was bonded using resin-modified glass ionomer cement RMGIC (GC Fuji Ortho LC, GC Corporation/Japan). After polishing and dryness of the buccal surface of the teeth, adhesive tape was placed on buccal surface of tooth at a level of base of bracket to leave only the exposed enamel received the acid etching and adhesive material and prevent any excess of the bonding agent and composite from invading the reading area. Conventional etching was performed with 37% phosphoric acid for 30 seconds followed by rinsing for 30 seconds and drying for 10 seconds and bonding layer were applied and air dried. Immediately after applying the adhesive (composite or RMGIC) to the bracket base, excess was removed with a probe. Flash Max 2 light cure unit (CSM dental Aps, Denmark) was used to cure the two types of adhesives. This super LED has an optical out-put well above 4.000 mW/cm^2. Six seconds; three seconds from mesial and three seconds from distal sides illuminated the adhesives with a minimum separation distance of 1-2 mm.

Demineralization procedure:

Each crown surface of all groups was painted with acid-resistant varnish, leaving an exposed 2 mm space along the gingival side of the bracket base which already was covered with adhesive tape so that only the exposed enamel would be attacked by acid, after the removal of the tape the varnish was left to set overnight , to induce caries like lesion on enamel surface of all the examined teeth a daily procedure of pH cycling was followed including a demineralization period of 7 hours and a remineralization period of 17 hours.

Each crown was immersed individually in 10 mL of demineralization solution consisted of (0.075M/L acetic acid,1.0 m M/L calcium chloride, 2.0 M/L m potassium phosphate) at ph 4.3 for 7 hours at 37°C. Specimens were then removed from the demineralization solution, rinsed with deionized water, and immersed individually in 10 mL of the remineralization solution at 37°C overnight (17 hours) to simulate the remineralizing stage of the caries process.[7] The remineralizing solution consist of (150 m M/L potassium chloride, 1.5 m M/L calcium nitrate 0.9m M/L potassium phosphate) buffer at pH 7.0. Each solution was changed and cycling system was repeated daily during the 30-day process. Teeth in group A and C were fluoridated according to the manufacturing instruction, for group A acidulated phosphate fluoride gel was applied with disposable brush for 4 minutes only once before the demineralizing-remineralizing cycling regimen while for group C teeth were dried after rinsing with air for 10 second then Stannous and sodium phosphate fluoride gel was applied with a disposable brush for one minute in between the demineralization and remineralization procedures every day, both fluoridated gel were removed after the application time with a piece of cotton and absorbent towel then wait for 30 minutes before return to demineralization-remineralization sequence.

Demineralization resistance test

After completing the demineralization procedure, each tooth was washed with distal water for 10 minutes and then the root embedded in acrylic resin block with exposed crown at a level of cement-enamel junction. The brackets were removed with bracket removal plier, then each tooth was immersed in 0.5 % methylene blue solution [25] for a whole day separately in plastic container, then the teeth were washed under running water for a 10 min, air dried and prepared for ground sectioning.

To test the acid resistance of the treated specimens, ground sections of approximately 100 µm of thickness were made in a coronal-apical direction perpendicular to the cusp edge so that each tooth was sectioned longitudinally by using low speed saw with of a hard-tissue microtome with water coolant. The sections carefully washed and isolated in labeled petri dishes then placed on glass slab. All ground longitudinal sections were examined under stereomicroscope with maximum illumination.

The enamel surface was classified by an experienced investigator, according to Ibrahim et al into:[26]

Type I - Preferential dissolution of the prism cores resulting in a honey-comb-like appearance;
Type II - Preferential dissolution of the prism peripheries creating a cobblestone-like appearance;
Type III - A mixture of type I and type II patterns;
Type IV - Pitted enamel surfaces as well as structures that look like unfinished puzzles, maps or networks;
Type V - Flat, smooth surfaces.

Measurement of lesion depth in testing groups was performed by calculating the average of three depths (d1, d2, d3) values of 100 µm apart located at the centre of artificial caries lesion with the aid of graduated ruler, as shown in fig1.

ANOVA test was performed to identify the presence of statistically significant differences for all group of this study. LSD test was performed to identify the differences between each paired group.

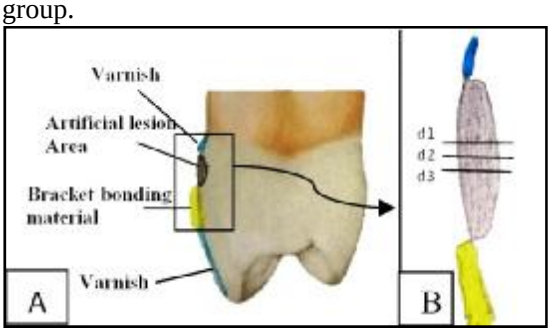

Figure 1: A; view of tooth profile illustrate areas of artificial lesion on buccal surface. B; enlarged artificial lesion demonstrating the three depth measurement at the centre

RESULTS

Caries penetration depth:

The results showed that the lowest caries penetration depth mean value of the groups of this study was for the group B while the highest caries depth mean value of the groups of this study was for the group D (table 1).

The result of ANOVA test showed that there was high statistical significant difference among these groups. LSD test also revealed statistically significant differences between each two groups (table 2) , all the treated groups have significant less caries penetration depth in comparison with the control group when P value ≤ 0.05.

Table 1: The Descriptive statistics of the experimental groups

Group	N	Mean	± SD	Min.	Max.
A	10	16	2.2	15	17
B	10	9.4	0.84	8	10
C	10	11.4	1.07	10	13
D	10	18.7	1.93	16	22

Table 2: LSD between each paired group

First group	Second group	Sig.
Group A	**Group B**	.000
Group A	**Group C**	.000
Group A	**Group D**	.010
Group B	**Group C**	.046
Group B	**Group D**	.000
Group C	**Group D**	.000

Histopathological study:

The histopathological finding of the examined groups demonstrated a carious lesion size graduating from large to small in correspondence with the fluoride defense measures abilities against demineralization utilized in this study being the largest in the control group followed by group A (APF) then by group C (Stannous and sodium phosphate fluoride) and finally the smallest lesion was found in the RMGIC group B. Group A exhibited an identified carious lesion between the enamel and dentine, for group B the area was represented by fine lines of demineralization diffused in remineralization layers that penetrated by the RMGIC whereas in group C a small isolated area of dark lesion overlaid by fluoridated mineralized area can be seen. Group D showed dark demineralization zone extends to dentine enamel junction, as can be seen in the figures 2,3,4,5.

Enamel surface pattern in the tested groups:

Variations in enamel surface pattern were shown in the results of this study. Group A has 40% of type IV of enamel surface , 40% of type V of enamel surface and 20% of type I of enamel surface while the group B has 60% of type IV of enamel surface , 40% of type V of enamel surface , while the group C has 80% of type V of enamel surface , 20% of type IV of enamel surface also the results showed that the group D has 60% of type IV of enamel surface , 20% of type III of enamel surface and 20% of type V of enamel surface.

DISCUSSION

Fluoride and fluoridated agents was the corner stone of defense mechanism against this decalcification surrounding the brackets that could be of irreversible type and threaten the esthetic success achieved with orthodontic treatment necessating additional operative work. The use of fluoride is one of the most studied, known and effective methods to prevent dental caries. The anti carious activity of topical fluoride has been attributed to its greater effect in the prevention of enamel decalcification rather than the remineralization of existing lesions due to its considerable capacity to reduce the minerals solubility in the enamel crystal lattice during acid challenge. [27] To the best of our knowledge, this study is the first comparison of these topical agents against enamel demineralization around orthodontic brackets in vitro condition by measuring lesion depth.

In the present study; by comparing to the control group all fluoride treated groups revealed statistically significant differences for artificial caries depth. The tested materials showed less mean lesion depth values that indicated less mineral loss than the control group. However, these significant differences vary among the

analyzed elements according to the preventive measure during the experimental phases, being the less subsurface lesion depth in group B followed by group C, then group A and finally the

Figure 2: Group A. A.. Photomicrograph view for enamel pretreated with APF shows lesion (arrow) in between enamel (E) and dentine (D). X4 B. Pitted, porous enamel surface (arrow). X4 C.. Other view shows glabular apposition of remineralizing matrix (arrows) with in between a demineralized dark area (arrows heads). X10

Figure 3: Group B.. A. A.. Rectangle demarked area of demineralized lesion of blue stains .X 4, B.. Magnifying view for previous figure A shows localized (blue) unmineralized lesion (arrow head), over layed with sparses thin hybrid-like ufine layer of infiltrated applied material (arrows).X10 C.. Longitudinal ground section for enamel tooth pretreatment with RMGIC shows dark demineralized lines interdigited with remineralized layers of translucent shiny lines (arrows) .X4

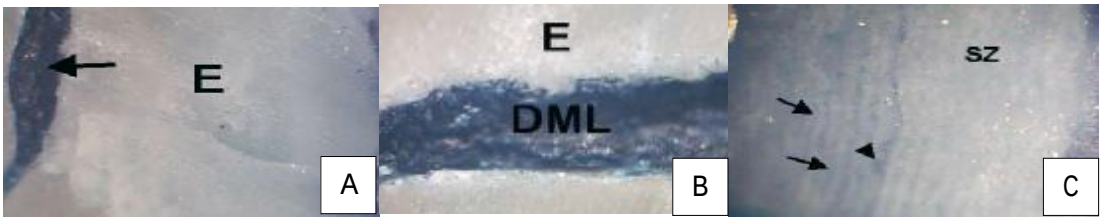

Figure 4: Group C. A..View for enamel surface (E) shows opaque area of minerlization and a lesion (arrow) shows blue stains Ground X4. B.. Magnifying view for figure B shows enamel (E) and demineralized lesion (DML).X10 C..Surface morphology of tooth enamel treated with stannous flouride shows smooth zone (SZ), fine porosities (arrow head, and occasional fine fissuring (arrows).X4

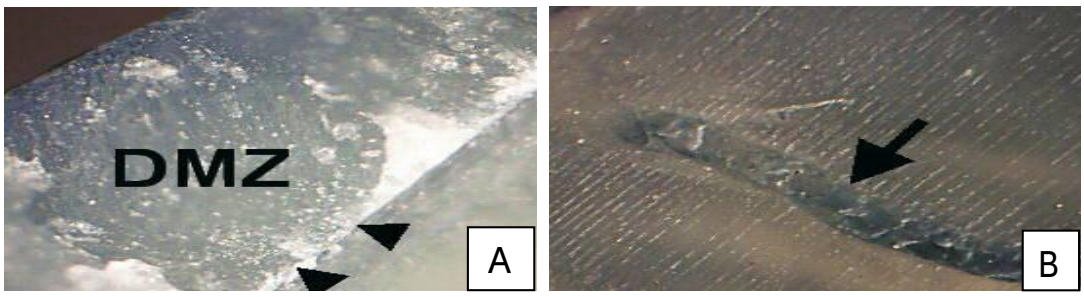

Figure 5: Group D. A.. Photomicrograph view for surface enamel of control group, showed dark demineralization zone extends to dentine enamel junction (arrow head). B.. longitudinal ground section shows extension of lesion (arrow) with rough pitted enamel. X4

control group, making the best lesion fighting measure is RMGIC 49% lesion depth reduction, as it well approved that this material can deliver regular amount of fluoride over months which provide excellent anti-caries activity during the prolong acidic exposure of examined enamel during this study. This superior anti-demineralizing activity of RMGIC to other tested groups was agreed with several studies. [23, 28, 29]

The RMGIC effect against decalcification was evaluated in researches with various methods, Schmit et al [28] found 50% smaller mean lesion depths at RMGI bonded groups when compared with the composite resin group in a comparable acid attack period to this study. Uysal et al [29] used a pen-type laser fluorescence device (DIAGNO-dent Pen) for demineralization evaluations, the RMGIC released fluoride changed the ratio between demineralization and remineralization reducing the lesions progression significantly more than the fluoride from the materials used in the other groups. Polychromatic cone-beam microtomographic system and depth measurement were utilized by Paschos et al [23] and also Fuji Ortho LC (RMGIC) was found to have significantly smaller lesion depth and less mineral loss in comparison with the other materials that comes in agreement with this study.

The result of group A showed 14% reduction in lesion depth in agreement with Esteves Oliveira et al [30] who assessed the APF effect on caries lesion progression inhibition and recorded 44% depth reduction, also a study by Mathew et al [31] reported 43% reduction in acid solubility in comparison to the control group by comparing the quantity of calcium dissolution into the demineralization solution using atomic emission spectrometry, the acidic challenge was for 24 h after 4 min of 1.23 also Villena et al[32] found APF gel application for one and four minute reduced mineral loss by 13% after 28 day of cariogenic challenge. Fluoride anticaries effect related to the reaction products formed on enamel during the professional treatment and their retention over time after the application, fluoride concentration released from 4 min APF application was reduced by 34% after 28 day of caries challenge, This reduction has been found in vitro [33,34] and in vivo [35] and is due to the release of loosely bound F formed on enamel by APF treatment to the oral environment [36] this would give good explanation to the lower anti caries capacity of APF compared to the sustained F release of RMGIC mentioned above, and frequent application of F in group C.

A 0.4% stannous fluoride gels scored 39% reduction in enamel demineralization. A study by Boyd [14] compared the use of a 1100 ppm fluoride tooth paste alone or together with either a daily 0.05% NaF rinse or 0.4% SnF_2 gel applied twice daily. He found 29% reduction in the demineralization of 0.4 % SnF_2 children brushed teeth compared to the 0.05 NaF, and both solutions provided additional protection against demineralization when compared to toothpaste. Recently, many investigations studied extensively solutions containing different concentrations of SnF_2 or combinations of different fluorides with $SnCl_2$. Hjortsjö et al [37] tested the effect of one minute exposure to a 0.78% w/v SnF_2 solution in a vivo model and found 67% enamel dissolution reduction after one min exposure to citric acid. However, the effect did not last for more than 1 day. [38] In an in vitro study using SnCl2 as the source of tin with amine fluoride and/or NaF as the source of fluoride., such solutions reduced tissue loss significantly, even when using a severe erosion cycle [15]. In an in situ model, used $SnCl_2$ mouth rinse yield 1,900 mg/kg Sn, NaF and amine fluoride yield 1,000 mg/kg F used once a day reduced erosive wear of enamel by 73%. [39]

Tin is thought to work through the incorporation into the surface enamel and/or the formation of a tin containing uppermost layers of the enamel [16, 40], the 39% artificial caries depth reduction presented by group C in this study would be supported by the above discussed studies investigated the SNF_2 effect on enamel acid resistance yet they can't mirror this study because either the experimental solution and/or the measuring procedures quite differ. Also the caries depth reduction significantly less than group B might be related to that F ion released from RMGIC is higher per day, whereas group C were significantly less caries depth penetration than APF group might be related to the frequent application and additional preventive effect yielded by tin precipitated layer provide by (0.4% Stannous fluoride, 1% sodium fluoride) gel in group C. An over view of the histopathological examination clearly revealed that the less caries depth groups the more mineralization deposit, on the other hand the histopathological scores showed that only group C has the majority of smooth flat uniform surface while the rest of the experimental groups have pitting surface score IV of a considerable percentage, indicating that there was a poor relation between the mean caries depth and amount of surface roughness, this study revealed that the three tried fluoridated measures were successfully able to reduce enamel dissolution.

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