The effect of nano-hydroxy apatite on re-mineralize white spot lesions prior to orthodontic adhesive removal by different techniques (An *In vitro* comparative study)

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ABSTRACT

Background: White-spot lesion is one of the problems associated with the fixed orthodontic treatment. The aims of this in-vitro study were to investigate enamel damage depth on adhesive removal when the adhesive were surrounded by sound, demineralized or demineralized enamel that had been re-mineralized prior to adhesive removal using 10% Nano-Hydroxy apatite and to determine the effect of three different adhesive removal techniques.

Materials and methods: Composite resin adhesive (3M Unitek) was bonded to 60 human upper premolars teeth which were randomly divided in to three groups each containing ten sound teeth and ten teeth with demineralized and re-mineralized lesions adjacent to the adhesive. A window of 2 mm was prepared on the buccal surface of the tooth and painted with an acid resistant nail varnish except for the window. The demineralized enamel produced by immersion of teeth in demineralization buffer for 12 days.half of the demineralized window, was covered with acid – resistant red nail varnish, and the samples were then subjected to re-mineralization with 10% of nano hydroxyapatite. The adhesive was removed with either:(1) fiber reinforced composite bur in slow speed handpiece (SS); (2)12 fluted long flame carbide bur in high speed handpiece (HS); (3) ultrasonic scaler (US).damage to the enamel was assessed using stereomicroscope with grid eye piece.

Results: the greatest to least mean depth of damage with three different adhesive removal techniques to sound enamel was HS> US >SS and to demineralized and re-mineralized enamel were SS >US> HS. Sound enamel had the least amount of damage. Re mineralization before the adhesive removal highly significant reduced the amount of damage produced by all techniques compared with demineralized enamel.

Conclusions: When the demineralized enamel was present 12 fluted long flame carbide bur were found to be the least damage in adhesive removal technique and re-mineralization further reduced the amount of enamel damage **Key word:** Nano-hydroxy apatite, Re-mineralization, adhesive removal. **(J Bagh Coll Dentistry 2017; 29(2):90-96)**

INTRODUCTION

The remarkable risk associated with orthodontic treatment is the enamel demineralization when oral hygiene is poor. Inhibition of demineralization during orthodontic treatment is the largest challenges faced by orthodontist in spite of the recent development in caries prevention protocols. The progression of white spot lesions (WSLs) is related to elongated plaque collection around the brackets ^(1, 2).

Fixed orthodontic appliances did not only cause traditional oral hygiene procedures more complicated, but also increase the number of plaque retention regions on the surfaces of the teeth that are normally less liable to caries progression ⁽³⁾.

Following the introduction of fixed orthodontic appliance into the oral cavity, a fast decrease in the bacterial flora of plaque happens. More numbers of acidogenic bacteria are found in the plaque, most notably Streptococcus mutans, and Lactobacilli ⁽⁴⁾. Large numbers of bacteria are able to lower the pH of plaque in orthodontic patients to a greater level than in non-orthodontic

patients ⁽⁵⁾. Therefore, the advancement of caries process is quicker in patients with fixed orthodontic appliances. WSLs can become remarkable around the brackets within 1 month of bracket positioning, while the formation of regular caries usually takes at least six months ⁽⁶⁾. The most common location of these lesions are found on the buccal surfaces of teeth around the brackets, especially in the gingival region ⁽⁷⁾.

Earlier studies have demonstrated that the damage to enamel was caused by adhesive removal rather than removal of the bracket, preetch pumicing or during etching ⁽⁸⁾. The depth of damage to sound enamel, come from adhesive removal, has been showed to be as high as 150μ m ⁽⁹⁾, but varies based on removal technique used ^(8, 10).

An ideal adhesive removal technique would decrease iatrogenic damage while restoring the enamel to its pretreatment appearance, be clinically efficient for residual adhesive removal and have less discomfort or hazard to dental tissues. No one technique has been universally accepted as ideal or even superior to others in term of depth of damage to enamel and surface finish ^(11,12). A tungsten carbide bur in a high speed hand piece has been demonstrated to create better surface finish compared to the other

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techniques ⁽¹³⁻¹⁵⁾. A multiple step finishing approach has also been advocated with final polishing with rubber cups ⁽¹³⁾. Aluminium oxide discs ⁽¹⁶⁾, or silicon carbide coated polisher to produce the smoothest final surface ⁽¹⁷⁾.

However, all of these studies have examined adhesive removal from sound teeth without any surrounding demineralization. In addition to minimizing iatrogenic damage to WSLs using an appropriate adhesive removal technique, an option exists for the practitioner to re-mineralize WSLs prior to adhesive removal to further reduce enamel damage, and because there is no previous Iraqi studies have examined enamel damage produced by different adhesive removal techniques on sound, de-mineralized and subsequently re-mineralized enamel by using Nano hydroxy apatite therefore this study was conducted.

MATERIALS AND METHODS Sample selection:

From one hundred extracted human premolars 60 upper first premolars were selected, the extracted teeth were Collected from the Oral surgery department at the College of Dentistry (Baghdad University) and some private clinics in Baghdad city and stored in 0.1% of thymol solution (de-ionized water with thymol crystals) ⁽¹⁸⁾. The teeth were selected after examination with 10X magnifying lens. Any tooth with cracks, piting, WSL, or other enamel surface defects were excluded.

Teeth mounting:

Each tooth was fixed on a glass slide in a vertical position using soft sticky wax at the end of the root, so that the middle third of the buccal surface was oriented to be parallel to analyzing rod of the surveyor. After that a custom made cylindrical mold, made from plastic of 2cm in diameter and 2cm in depth, were painted with a thin layer of separating medium (Vaseline) and placed around the vertically positioned teeth with crowns protruding. Then the powder and liquid of the cold cured acrylic (Duracryl® Plus, Spofa Dental A kerr company 500gm powder and 250 ml liquid) were mixed and poured around the teeth to the level of the cemento-enamel junction of each tooth (19, 20). After mounting, the specimens were coded (G,Green; R,Red; B,Blue) and stored in deionized water solution with thymol until bonding to prevent dehydration and bacterial growth (21,22).

Polishing

The buccal surface of each tooth was polished using non fluoridated pumice in a rubber cup (for the standardization of this study one rubber cup was used for each tooth) attach to a slow speed hand piece then each tooth was washed with water spray, and dried with oil –free air.

Bonding procedure:

This was done using self-etching primer \ bond (3M, St Paul, MN, USA). A disposable brush was used for the application of the material on the enamel surface in gingivo-occlusal direction, then it was applied for a minimum 3-5 seconds per tooth with a light force. Then an air source (oil free air/water syringe) was used to deliver a gentle air for 1-2 seconds for each tooth to dry the primer in to thin film then light cured (woodpecker, china) for 20 seconds (according to manufacture instruction). For each sample a maxillary first premolar bracket (stainless steel brackets \Ortho Technology-USA) was allocated and the mesh bonding surface was covered with a separating film (Vaseline) prior to coating with composite resin adhesive (3M Unitek, Monrovia, CA, USA). They were then bonded to each tooth and the bracket was positioned in the middle third of the buccal surface parallel to the long axis of the teeth using a clamping tweezers. The adhesive was light cured for 40 second (20 seconds from mesial and 20 seconds from distal sides of brackets according to the manufacture instruction), at a distance of 5 mm (23). The bracket was then removed by tweezer leaving a relatively standardized bonded composite resin rectangles corresponding to the shape of maxillary first premolar bracket. Acid resistant red nail varnish was used to outline a 2 mm wide window around the complete area of composite because this is the most common area for WSL to form ⁽²⁴⁾. (Figure 1)



Figure 1: The steps of preparation the window on buccal surface of maxillary first permanent premolar around the adhesive

These teeth were then classified into six subgroups according to adhesive removal techniques:

✓ Group G 1 (SS): in this group the adhesive removal was done using fiber reinforced composite bur (American dental accessories, USA) in slow speed hand piece (5000-10000 rpm) with water spray; (sound enamel not exposed to demineralization and remineralization).

- ✓ Group R 1 (HS): in this group the adhesive removal was done using 12 fluted long flame carbide bur (Ortho technology) in a high speed hand piece with water coolant; (sound enamel not exposed to demineralization and remineralization).
- ✓ Group B 1 (US): in this group the adhesive removal was done using Ultra sonic scaler (Cavitron, Taiwan) with water coolant; (sound enamel not exposed to demineralization and re-mineralization).
- ✓ Group G 2 (SS): in this group the adhesive removal was done using fiber reinforced composite bur (American dental accessories) in slow speed hand piece (5000-10000rpm) with water spray; (the teeth exposed to demineralization & then re-mineralization)
- ✓ Group R 2 (HS): in this group the adhesive removal was done using 12 fluted long flame carbide bur (ortho technology/USA) in a high speed hand piece with water coolant; (the teeth exposed to demineralization & then remineralization).
- ✓ Group B 2 (US): in this group the adhesive removal was done using Ultra sonic scaler (cavitron Taiwan) with water coolant; (the teeth exposed to demineralization & then remineralization).

The control teeth (sound enamel) of each group were immersed in the deionized water and thymol crystl while the tested samples of each group were exposed to demineralization and then re-mineralization prior to adhesive removal. In order to produce WSLs in the exposed windows around the adhesive, all experimental samples were kept on the specimen jar lid, with 40 ml of the demineralization solution at pH 4.8 and 37°C. This solution was changed every 48 hours for 12 days according to the method proposed by White ⁽²⁵⁾. After the demineralization, each sample had acid resistant nail varnish painted over a randomly allocated half (right or left) of the demineralized window to create a demineralized half. the other half was exposed to a re- mineralizing solution containing (10% Nano hydroxyl apatite) at pH 7 then all the samples except the controls were suspended in to the re-mineralization solution for 30 days with 4 minute twice daily (once at morning and once at night) at 37°C with the solution changed every four days ⁽²⁴⁾. Following re-mineralization of sample, the nail varnish was carefully removed with acetone.

The adhesive was removed by using the technique appropriate for each group. In the SS and HS groups, new bur was used per sample. In the US group the same scaler tip was used for all samples. In order to achieve standardization for

adhesive removal, a modified dental surveyor with a suspending arm was used to control the hand piece orientation during adhesive removal. The samples of all groups were immersed in methylene blue at concentration of 2% for 24 hour in order to enhance the contrast in microscopic image then a ground section of 200 µm in thickness, perpendicular to the window surface were produced using an internal annulus saw minitom (Struers, Copenhagen, Denmark). The samples study were examined under stereomicroscope (Hamilton, Italy) to evaluate enamel damage depth after the removal of adhesive. Lesion depth was measured (in µm) for the demineralized and re-mineralized lesions and were compared with the sound enamel profile of the same section at magnification 4X using grid eye piece of steremicroscope. All of this procedures and measurments were done by specialist in oral histology department, Collage of dentistry, University of baghadad.

Statistical analysis

Descriptive statistics including means and standard deviations were measured for each group using Staitistical Package of Social Science (SPSS) software (version 19 chiccago, USA) for Windows xp. All data were examined for normality using shapiro-wilk test of normality. The depth of enamel damage were analysed using independent sample t test to compare the effect of demineralization and remineralization with control teeth, Paired sample t-test to compare the depth of enamel damage in the demineralization and re-mineralization enamel for all groups and also using analysis of variance (ANOVA) to compare the effect of adhesive removal techniques in each subgroup, finally least significant differences was used to show the significance between each two group after ANOVA test.

RESULTS

Table 1 showed the descriptive statistics that represent the mean and standard deviation of enamel damage depth in all techniques HS, SS and US for demineralized and sound enamel. Independents sample t-test showed a highly significant increase in the enamel damage depth in demineralized enamel group when compared with sound enamel group p-value=0.000.

Table 2 consisted of the descriptive statistics that define the mean and standard deviation of enamel damage depth in all techniques HS, SS and US for re-mineralized and sound enamel Independents sample t-test showed a significant increase in the enamel damage depth for HS technique p-value=0.029 and a highly significant increase in enamel damage depth for SS and US techniques when compared the re-mineralized enamel group with sound enamel.

Table 3 showed the descriptive statistics that represent the mean and standard deviation of enamel damage depth in all techniques HS, SS and US for re-mineralized and demineralized enamel. Paired t-test showed a highly significant increase in the enamel damage depth in demineralized enamel when compared with remineralized enamel with p-value=0.000. (The greatest increase in depth of enamel damage was seen in SS group followed by US and then the HS lastly, Table 4 summarized the mean and standard deviation (S.D.) of enamel damage depth for all studied techniques in control, demineralized and re-mineralized group. ANOVA test showed a highly significant differences between the three studied techniques in all groups (control, demineralized and re-mineralized) p-value=0.000. The control group showed that the least enamel damage depth found in SS technique followed by US and then the HS technique, while the demineralized and re-mineralized groups showed that the lowest enamel damage depth found in HS techniques followed by the US and then the SS technique. Table 5 using LSD test, showed a highly significant difference among sound, demineralized and re-mineralized groups between the (HS with SS), (HS with US) and (SS with US) in enamel damage depth.

Table 1: Effect of demineralization on Enamel damage depth (µm.) and comparing the effect of demineralization with the control group.

demineralization with the control group.							
Tashnismas	Change	Descriptive statistics		Groups' difference (d.f.=18)			
rechniques	Groups	Mean	S.D.	Mean Difference	t-test	p-value	
HS	Control	19.10	1.37	-20.40	-16.510	0.000	
	Demineralization	39.50	3.66			(HS)	
66	Control	7.60	2.17	110.90	-60.406	0.000	
33	Demineralization	118.40	5.38	-110.80		(HS)	
US	Control	15.90	2.77	-61.50	-32.448	0.000	
	Demineralization	77.40	5.32			(HS)	

Table 2: Effect of re-mineralization on Enamel damage depth (µm.) and comparing the effect of
re-mineralization with the control group

Techniques	Groups	Descriptive statistics		Groups' difference (d.f.=18)		
		Mean	S.D.	Mean Difference	t-test	p-value
пе	Control	19.10	1.37	1 70	-2.365	0.029
пъ	Remineralization	20.80	1.81	-1.70		(S)
88	Control	7.60	2.17	50.00	-30.568	0.000
55	Remineralization	57.60	4.70	-30.00		(HS)
US	Control	15.90	2.77	19.10	-14.167	0.000
	Remineralization	34	2.94	-18.10		(HS)

Table 3: Comparison between demineralization and re-mineralization groups of the enamel	l
damage denth (um)	

Tashnisuas	Chong	Descriptive statistics		Groups' difference (d.f.=9)		
rechniques	Groups	Mean	S.D.	Mean Difference	t-test	p-value
HS	Demineralization	39.50	3.66	18 70	17.732	0.000
	Remineralization	20.80	1.81	16.70		(HS)
SS	Demineralization	118.40	5.38	(0.90	46.240	0.000
33	Remineralization	57.60	4.70	00.80		(HS)
US	Demineralization	77.40	5.32	43.40	34.028	0.000
	Remineralization	34	2.94			(HS)

Groups	Techniques	Descri	ptive tics	(d.f.=29)		
oroups		Mean	S.D.	F-test	p-value	
	HS	19.10	1.37		0.000 (HS)	
control	SS	7.60	2.17	74.197		
	US	15.90	2.77			
	HS	39.50	3.66		0.000 (HS)	
Demineralization	SS	118.40	5.38	661.763		
	US	77.40	5.32			
	HS	20.80	1.81		0.000	
Remineralization	SS	57.60	4.70	306.682	0.000 (US)	
	US	34.00	2.94		(115)	

Table <u>4: Enamel damage depth (μm) using different adhesive removal tech</u>niques

Groups	Techniques		Mean	p-value
			Difference	
control	HS	SS	11.50	0.000 (HS)
		US	3.20	0.003 (HS)
	SS	US	-8.30	0.000 (HS)
Demineralization	HS	SS	-78.90	0.000 (HS)
		US	-37.90	0.000 (HS)
	SS	US	41.00	0.000 (HS)
Re-mineralization	HS	SS	-36.80	0.000 (HS)
		US	-13.20	0.000 (HS)
	SS	US	23.60	0.000 (HS)

Table 5: LSD test for the total sample

DISCUSSION

Until now there is no evidence-based clinical protocol for the removal of orthodontic adhesive in patients who exhibit white spot lesions around their orthodontic appliance in order to minimize iatrogenic damage to tooth enamel. Within the limitations of a laboratory based study, this study aimed to address this issue.

There are significant differences in the studies regarding the effects of different adhesive removal techniques on sound enamel and this is attributed to the differences in operator techniques, materials and the methods used to assess damage. The result of the present study for sound enamel found that the use of SS in group G1 for adhesive removal resulted in a significantly less damaging than the use of either US in group B1 or HS in group R1. This is the first study which measures the depth of enamel damage after adhesive removal with fiber reinforced composite bur in slow speed handpiece.

However, Karan et al $2010^{(26)}$. Sogra et al $2015^{(27)}$ found that the fiber reinforced composite bur created the smoothest enamel surface when compared with other methods. The US damage in group B1 found in the current study fell within this latter range in terms of the use of HS in group R1 for adhesive removal the results of the study

are equivocal. Hossien et al ⁽⁸⁾. Ireland et al. ⁽¹⁰⁾ found that US resulted in enamel damage in range of 1.3 μ m to 31.4 μ m and showed the greater damage to sound enamel in comparison with other removal techniques. On the other hand Krell et al ⁽⁹⁾ showed that a combined method utilizing pliers and an ultrasonic scaler produced damage of 38.5 ± 0.47 μ m which was significantly less than HS removal technique used in that study. In the present study, a relatively comparable degree of damage was created by US in group B1 and HS in group R1removal techniques; 15.90 μ m and 19.10 μ m respectively, and both were significantly higher than SS in group G1.

For demineralized and re-mineralized enamel the results of the present study, HS in group R2 was the least damaging technique. This is true for the depth of damage. HS were significantly lower than those of other removal techniques.US in group B2 resulted in a significantly less depth of damage to both demineralized and re-mineralized enamel in comparison with SS in group G2. It was noticed that the SS created a significantly greater area of damage to demineralized and remineralized enamel than other techniques.

These dissimilarities in enamel damage and surface finishing between techniques could be attributed to varieties in their mechanisms of adhesive removal. HS includes high blade torque which demands less pressure from operator hand piece. This makes it less susceptible to differences in density of enamel, and consequently it reveled to create damage to both demineralized and sound enamel. Unlike HS, SS removal has less torque demanding and greater pressure from operator hand piece, producing the least damage to sound enamel but fiercely cut and damaged remineralized and demineralized enamel. Demineralized enamel damage seems also to be affected by variations in the time required for the adhesive removal using different methods. It seems that there is significant variability exists between operators with considerations to this, attributed to variations in technique and experience.

Re-mineralization of enamel with 10% NHA led to a reduction in depth of damage regardless of the type of adhesive removal technique used. A postulated rationale for this is that the NHA treatment was able to increase the mineral content of demineralized enamel and became able to withstand the damaging forces applied during adhesive removal. This finding was in agreement with Cochrane et al (24) and Mayne et al (28) who found that the re-mineralization of a WSL surrounding an orthodontic bracket before bracket and adhesive removal might reduce the depth of enamel damage While the re-mineralized enamel was still damaged to a greater degree than sound enamel, it showed significantly less than that of demineralized enamel. The results of this in vitro study demonstrated that the re-mineralization would decrease the enamel damage prior to adhesive removal when WSL were found.

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المستخلص

الخلفية: البقعه البيضاء المتكونة على سطح السن قد تكون عرضه لا ضرار ميكانيكية اثناء ازالة لاصق تقويم الاسنان. هذه الدراسه المختبريه اجريت للتحقيق في عمق ضرر مينا السن عند ازالة اللاصق في حل كون االلاصقه محاطه بمينا سليمه او مينا فاقده للمعادن او في حال اعادة المعادن التي قد فقدت المعادن قبل ازالة اللاصق بأستخدام 10% nano hydroxyl apatite وتحديد اثر ثلاثة تقنيات مختلفه من تقنيات ازالة لاصق تقويم الاسنان.

مواد وطرق البحث: تم أختيار ستون سنا بشريا من الضواحك الطويه السليمه المقلوعة لاغراض تقويمية. قسمت الى ثلاث مجموعات كل مجموعه تتكون من عشرة أسنان سليمه و عشرة اسنان ذات مينا فاقده للمعادن في المنطقه المجاوره للاصقه تقويم الاسنان تم اعداد اطار يشمل2ملم على سطح المينا المحاذي للحاصرة باستخدام طلاء مقاوم للحامض باستثناء النافذه الميان الفاقده للتمعدن والتي انتجت بعد التعرض الى القواعد المخزن لمدة 12 يوم نصف النافذه الفاقده للمعادن كانت مغطاة بطلاء مقاوم للحامض باستثناء النافذه الميات لاعادة التمعدن والتي انتجت بعد التعرض الى القواعد المخزن لمدة 12 يوم نصف النافذه الفاقده للمعادن كانت مغطاة بطلاء اظافر مقاوم للحامض ثم تعرضت العينات لاعادة التمعدن بواسطة 10% mano hydroxyl apatite في منافه (1) بير ليفي معزز بمركب بهاندبيس بطيئة السرعه (2) 21مخد كاربايد بيرطولي اللهب بهاندبيس علية السرعه (3) قشارة با لموجلت فوق الصوتية وتم قياس عمق الضرر للمينا بواسطة المايكروسكوب المجسم وباستخدام عدسه مرقمة.

النتائج: كشفت نتيجة الدراسة بأن عمق الضرر للمينا السليمه من الاعلى الى الاقل كالاتي SS < US < HS. وعمق الضرر للمينا الفاقدة للمعادن والمينا بعد اعادة التمعدن كالاتي HS < US < SS. اعادة التمعدن قبل ازالة اللاصق يقلل كمية الضرر للمينا مع كل تقنيات ازالة الاصق عند مقارنته مع المينا الفاقدة للمعادن.

الاستنتاج: ضمَّن حدود هذه الدراسة المختبريه يمكن ان نستنتج ان استخدام 12 فلوتد لونك فليم كاربيد بير بواسطة هاندبيس عالية السرعة يقلل الضرر للمينا عندما تكون المينا المجاورة للاصقة فاقده للتمعدن,وان اعادة التمعدن قبل ازالة اللاصق يقلل من مقدار عمق الضرر للمينا.