# Evaluation of a new orthodontic bonding system (Beauty Ortho Bond)

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## ABSTRACT

Background: The purpose of the current study was to evaluate the efficacy of a new orthodontic bonding system (Beauty Ortho Bond) involving the shear bond strength in dry and wet environments, and adhesion remnant index (ARI) scores evaluation in regard to other bonding systems (Heliosit and Resilience Orthodontic Adhesives).

Materials and methods: Sixty defect free extracted premolars were randomly divided into six groups of 10 teeth each, mounted in acrylic resin, three groups for a dry environment and three for a wet one. Shear bond strength test was performed with a cross head speed of 0.5 mm/min, while surfaces of enamel and bracket-adhesive-enamel surfaces were examined with stereomicroscope For ARI scores evaluation. Data were analyzed by one way analysis of variance, least significant difference, student's t-test, and Fisher exact test.

Results: The mean shear bond strength showed highest values for Resilience adhesive followed by Beauty Ortho Bond and Heliosit adhesives respectively both in dry and wet environments. Interestingly, there was a non-significant difference (P<0.05) between Resilience and Beauty Ortho Bond adhesives using least significant difference at dry environment. In wet environment the Beauty Ortho Bond showed an acceptable mean shear bond strength value (6.39 Mpa) which is considered as a clinically acceptable value. Adhesive remnant index scores demonstrated a tendency towards score 1 in dry environment, and towards score 3 in wet environment, the scores also showed a non-significant difference (P<0.05) between Resilience and Beauty Ortho Bond adhesives using Fischer exact test.

Conclusion: Beauty Ortho Bond is less sensitive to wet environment than Resilience and Heliosit adhesives, therefore it has an advantage during clean up, as it reduces the risk of enamel damage during debonding procedure.

Keywords: Beauty Ortho Bond, Shear bond strength, light cured composite. (J Bagh Coll Dentistry 2015; 27(1):175-181).

## **INTRODUCTION**

Since the introduction of the concept of the acid etching that was invented by Buonocore in  $1955^{(1)}$ , then the direct bonding of orthodontic appliances to enamel with composite resin was introduced by Newman in 1965 <sup>(2)</sup>.At current time, the bonding of different attachments, such as brackets and tubes, on the enamel surface is a routine clinical procedure, typically using a resin composite adhesive <sup>(3-6)</sup>.

Many researches described the use of phosphoric acid for creating micro-irregularities in the enamel surface to enhance mechanical interlocking, the effect of time factor <sup>(7,8)</sup>, and concentration of phosphoric acid <sup>(9)</sup> have been investigated to determine the most suitable technique of enamel preparation.

In spite the bond strength to the tooth structure is favorable in restorative dentistry, the bond strength in orthodontics must give two durable requirements, it must be so sufficient to retain the brackets but low enough to allow easy removal and cleanup of the adhesive remnants during brackets debonding procedure <sup>(10)</sup>.

It has been recommended that the bond strength values must fall in the rage of six to eight Mega Pascal, which are sufficient to get a clinically effective orthodontic bonding procedure (5,10,11)

The higher bond strength may increase the risk of enamel chipping <sup>(12)</sup>. Although acid etching of enamel may cause micro-roughness about 10-20 micrometer <sup>(4,9)</sup>. Most orthodontists accept enamel surface acid etching as a routine technique which has a risk of iatrogenic enamel deterioration, such as surface staining stemming from increased surface porosity, discoloration by resin tags retention in enamel, enamel fracture, and chipping <sup>(10,13)</sup>. Advances in an adhesive technique have let orthodontists to incorporate new adhesives, composite resins and bonding techniques into clinical practice, the invention of self-etching primer is to expedite the bonding procedure by combining etching and priming into a single step <sup>(14)</sup>. Furthermore, saving time and reducing procedural faults, their lower etching capability, due to higher pH relatively to the acid etch technique, which it might decrease the potential for iatrogenic enamel damage (14-16). The purpose of the current study was to evaluate the efficacy of a new orthodontic bonding system (Beauty Ortho Bond<sup>®</sup>)involving the shear bond strength in dry and wet environments, and adhesive remnant index scores evaluation in regard to other bonding systems.

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## MATERIALSAND METHODS

### **Tooth specimens**

Ninety four human healthy maxillary first premolars teeth were collected for orthodontic purposes from 15-25 years old Iraqi patients seeking the orthodontic treatment, all the teeth were examined for any visible decalcification, hypoplastic areas by using light curing unit, any tooth that has a defect was discarded, and only 60 teeth were included in the current study, then the teeth were washed with water and stored in normal saline in a sealed box for 1-3 months at room temperature  $(22^0 \pm 3)$  and the normal saline changed it periodically until bonding procedure (17,18), then the 60 teeth were randomly allocated into six groups of ten for shear bond strength measurement.

#### Mounting of the teeth:

The roots of the included teeth were serrated by a diamond disk, made a retentive wedge shaped to increase the teeth retention inside the self-cured acrylic blocks, then each tooth was fixed on a glass slide in a vertical position using soft sticky wax at the root apex, so that the middle third of the buccal surface was oriented to be parallel to the analyzing rod of the surveyor, so that the force could be applied at right angle to the enamel-bracket interface <sup>(19)</sup>.

Another tooth was fixed on the glass slide about 1 cm away from the first tooth and was oriented in the same manner, then three more teeth were placed and fixed on the glass slide in the same way of the second tooth in order to have five premolars fixed on the glass slide one cm apart, having the middle third of the buccal surface of each tooth parallel to the analyzing rod of the surveyor and the occlusal surface of each tooth oriented to the same height by using a stone disc bur <sup>(20)</sup>, then the two L-shaped metal plates were painted with a thin layer of a separating medium (Vaseline), and placed opposite to each other in such a way to form a box around the vertically positioned teeth with the crowns protruding. Then the powder and liquid of the cold cured acrylic were mixed and poured around the teeth to the level of the cement-enamel junction of each tooth. After cold cure acrylic resin setting, the two L-shaped metal plates were removed, the sticky wax used for fixation of teeth in the proper orientation removed too and the resulting holes filled with cold cure acrylic, slightly adjustment of the acrylic blocks was done using a portable engine. After mounting, the teeth were stored in normal saline solution to prevent dehydration until bonding procedure<sup>(21)</sup>.

## **Preparation of bracket-bonded specimens**

The 60 teeth were separated into six equal groups (10 teeth each), three groups for a dry environment testing were separated and the other three groups for a wet environment testing. 60 new stainless steel standard edgewise Ultratrim brackets (Dentaurum/ Germany) were used with coarse mesh base with surface area of 10.165 mm2, the buccal surface of all teeth were cleaned and polished using non-fluoridated pumice (DFL Minmet Refractories Corp; ShiJiaZhuang/ China) for 30 seconds (each tooth), then washing with water spray for 10 seconds, drying with air syringe for 10 seconds, oil and humidity free, then each one of the three bonding systems was applied on 20 teeth (10 teeth in dry environment and 10 in wet), the three bonding systems (Table 1) in the current study were classified into the following groups:

Group 1 (An adhesive system with a primer and needs an etchant): The enamel surfaces were treated with 37% phosphoric acid etching agent (Etching agent, Resilience; OrthoTechnology; Fl/USA) and allow the etchant to remain for 30 seconds, washed for 20 seconds with an air/water spray, and dried for 20 seconds with an oil and humidity free air stream, the etched enamel surface should be of a white chalky appearance, a primer (Sealant resin, Resilience light cure adhesive; OrthoTechnology; Fl/USA) was applied with a cotton applicator on etched enamel surface, then the metal bracket base was bonded with adhesive paste (Resilience light cure adhesive; OrthoTechnology; Fl/USA), seat the bracket on the tooth with light pressure and after positioning press firmly to place not express all the paste from under the bracket, remove any gross excess of paste that expresses from around the bracket, light cure the adhesive from two directions (incisal and gingival or mesial and distal) by a light cure unit (Radii Plus, high powered cordless led curing light, SDI; Austria). Excess bonding was removed with a small scaler. All samples were light cured for 20 seconds (10 seconds for each proximal side) following the manufacturer instructions, after performing the bonding procedure the specimens were immersed in normal saline solution and stored in incubator at  $37^{\circ}$ C for 24 hours before the bond strength testing (22,23)

Group 2(An adhesive system with no primer but needs an etchant): The enamel surface was treated with 37% phosphoric acid (Total Etch, IvoclarVivadent clinical; Schann/Liechtenstein), and allow the etchant to remain on enamel surface

for 30 seconds, washed for 20 seconds with an air/water spray and dried for 20 seconds with an oil and moisture free air stream, the enamel surface becomes chalky white, apply the translucent low viscosity adhesive agent (Heliosit orthodontic adhesive, Ivoclar Vivadent clinical; Schann/Liechtenstein) to the under surface of the metal bracket, then position the bracket on the tooth surface, cure with the light unit (Radii Plus, high powered cordless led curing light, SDI; Austria), and follow the previous instructions.

Group 3(An adhesive system with no etching gel but with a self-etching primer with fluoride releasing property) (Figure 1): Primer A and primer B were mixed, then the solution was rubbed on enamel surface for approximately three seconds. An air jet was briefly applied to the enamel surface, and the paste (Beauty Ortho Bond, shofu; Kyoto /Japan) was applied onto the back of bracket base, then position the bracket on the tooth surface, cure with the same light unit in groups one and two, and follow the same previous instructions.

Each primer-composite combination was tested under two different enamel surface conditions: dry and saliva application (wet), therefore:



Figure 1: Beauty Ortho Bond (Shofu; Kyoto /Japan)

Groups 4, 5, and 6: Apply the same bonding, systems in groups 1, 2, and 3 respectively but in wet environment, to achieve a saliva contaminated condition (wet environment), human saliva from one donor was applied with a brush on to the buccal surfaces without air jet "after priming in groups 1 and 3, and after etching in group 2". Then, the brackets were bonded with composite paste, for the group 6 (in wet environment testing of Beauty Ortho Bond), also apply Salivatect paste on tooth surface, not just the standard paste on base of bracket. Each bonding procedure was done by the same operator. Excess bonding was removed with a small scaler. All samples were light cured for 20 seconds (10 seconds for each proximal side) following the previous manufacturer instructions, after performing the bonding procedure the specimens were also immersed in normal saline solution and stored in incubator at  $37^{\circ}C$  for 24 hours before the bond strength testing <sup>(22,23)</sup>.

#### **Shear bond strength test** (Figure2)

The embedded specimens (teeth in the acrylic blocks) were secured in a jig attached to the base plate of an Instron universal testing machine (Tinius Olsen, Model 1150, England). A chiseledge plunger was mounted in the movable cross head of the testing machine and positioned so that leading edge was aimed the at the enamel/composite interface, the speed of the cross head was 0.5mm/minute, and the maximum load necessary to debond the bracket was recorded, the force required to remove the brackets was measured in Newtons (N), and the shear bond strength  $(1MPa = 1N/mm^2)$  was then calculated by dividing the force values by the bracket base area  $(10.165 \text{mm}^2)$ .



Figure 2: The embedded specimen (teeth in the acrylic block) was secured in the sliding jig

#### **Residual adhesive**

After debonding procedure, all teeth and brackets were examined under X20 magnification of a stereomicroscope (Olympus, Tokyo, Japan), the adhesive remnant index (ARI) scores were evaluated by the same operator, enamel surface was scored using the criteria proposed in ARI of Wang et al. <sup>(24)</sup>, as follows:

- **Score 1**= the failure occurred between bracket base and adhesive.
- Score 2= Cohesive failure occurred within the adhesive itself.
- Score 3= Adhesive failure occurred between adhesive and enamel.
- **Score 4**= Enamel detachment.

#### Statistical methods

All statistical analyses were performed with the Statistical Package for Social Science (SPSS for windows, 16.0, Chicago, Illinios/USA). Descriptive statistics, including the mean, standard deviation, minimum, and maximum shear bond strength values were calculated for the six groups, furthermore, number and percentage of observations for ARI scores were also evaluated. Inferential statistics, including one way analysis of variance (ANOVA) was used to determine any statistical difference between shear bond strength values of the three bonding systems in both dry and wet environments, least significant difference was used to compare between each two groups of bonding systems in both dry and wet environments.

A Student's *t* test was used to compare the shear bond strength data of Ortho Beauty Bond "BOB" with other bonding groups under different environments, Fisher exact test was used to test the exact relationship of ARI scores values between dry and wet environments.

The levels of probability in statistical evaluation were: significance at  $0.05 \ge P > 0.01$ ; highly significance at  $0.05 \ge P > 0.001$ ; and non-significance at P > 0.05.

## RESULTS

The descriptive statistics of shear bond strength for each group of the three adhesive systems are presented in table 2. In both dry and wet environments, highest mean shear bond strength values were found in groups 1 and 4 (Resilience Light Cure Adhesive), followed by groups 3 and 6 (BOB), and groups 2 and 5 (Heliosit Adhesive) respectively. When Orthodontic comparing the three adhesive systems, there were highly significant differences (P=0.000) in the mean shear bond strength values using ANOVA test in both dry and wet environments. The results of the Student's t-test for the independent samples (between dry and wet environments) revealed highly significant differences (P=0.000) in the mean shear bond strength values, with lower mean values in wet environment (groups 4, 5, and 6) than dry environment (groups 1, 2, and 3), as shown in table 2.

The least significant difference (LSD) test for the three adhesive systems in dry environment showed that there were highly significant differences (P=0.000) between groups 1 and 2, and groups 2 and 3, while there was a no significant difference (P>0.05) between groups 1 and 3. In wet environment there were highly significant differences (P=0.000) between the three adhesive systems (groups 4, 5, and 6), as shown in table 3.

Adhesive remnant index (ARI) scores (table 4) indicate the site and mode of bond failure for the three adhesive systems in dry and wet environments.

The ARI scores of the three adhesive systems had a slight tendency towards score 1 (failure between bracket base and adhesive) in dry environment, while revealed a slight tendency towards score 3 (adhesive failure between adhesive and enamel) in wet environment.

The scores showed a highly significant difference  $(0.05 \ge P > 0.001)$  between groups 1 and 4, and a significant difference  $(0.05 \ge P > 0.01)$  between groups 2 and 5, and a non-significant difference (P>0.05) between groups 3 and 6, respectively, when Fisher exact test was used.

## DISCUSSION

Ever since the procedure of bonding was introduced by Neuman into orthodontic practice <sup>(2)</sup>, there has been a constant endeavor to improve the qualities of materials in bonding procedures. The search still continues, ideally, the bond strength needs to be optimum rather than too much or too less. Excessive bond strength increases the risk of damage to enamel during debonding and too weak bond strength results in frequent bond failures during the course of treatment. According to Reynolds, the optimum bond strength should be in the range of 5.9-7.8 Mega Pascal <sup>(25)</sup>. Researchers have been attempting to gain the best quality and gentlest techniques for bonding orthodontics brackets, various new developments have been made to help improve the technique, these developments have concentrated on improving bond strength, decreasing bonding time, reducing the number of bonding steps, and decreasing the adhesive remnants. All these advances have worked towards creating the best bonding protocol while maintaining enamel health during treatment and after debonding. As newer and more efficient products are marketed worldwide, evaluation of their interactions with other orthodontic products available to practitioners, as well as their effect on enamel must be performed.

The current study compared shear bond strength of the new adhesive (BOB) with two adhesives (Resilience and Heliosit) available in Iraqi market. All of these adhesives were light cured type. It is important to mention that the mean shear bond strength values for all the adhesive (Resilience, Heliosit, and BOB) were greater than 6-8 MPa previously reported <sup>(25,26)</sup>, as

an adequate bond strength for routine bonding procedure in both dry and wet environments, with highest mean shear bond strength value for Resilience followed by BOB and Heliosit adhesives respectively in dry condition, except for the Heliosit adhesive in wet condition, showed lowest and unacceptable (to the suggested range) mean shear bond strength value, as shown in table 2, this may be due to variation in the amount of glass filler in Resilience and BOB, and the glass filler absence in Heliosit. Furthermore, contamination of enamel with saliva after priming as in groups 4, 5, and 6 decreases the mean shear bond strength values for the three adhesives, although it still practically adequate except for Heliosit, where it was unacceptable in wet environment (Condition 5). The decrease in shear bond strength in wet environment comes in agreement with another research <sup>(27)</sup>, which stated that humidity decreases the shear bond strength because the saliva consistency could have caused that the adhesive was not in contact with the enamel surface etched at the time of polymerization of the adhesive system, thus producing poor mechanical retention.

The least significant difference test showed that there was a non-significant difference between groups 1 and 3 (Resilience and BOB adhesives) in dry condition, because both of the adhesives exhibited high mean shear bond strength values, as shown in tables 2 and 3.

In general the shear bond strength tests involve numerous variables and are technique-sensitive, so the same bonding study can have varying results under different experimental conditions, or when performed by different orthodontists. In addition an in-vitro bonding enamel is very different from an in vivo bonding environment. Factors such as the patient's enamel composition and saliva contamination can cause the same bonding research to yield different results when performed in intraoral environment. Thus it is important to conduct more clinical studies.

In Orthodontics, the site of bonding failure is important because an effort is made to maintain an intact and sound enamel surface after debonding. Thus, in order to minimize the risk of enamel damage, it is more desirable for bond failure to occur within the adhesive or at the bracket-adhesive interface than the adhesiveenamel interface. However, a smaller adhesive remaining after debonding, the perfect bonding procedure would be, because it would leave a healthy enamel surface without large amount of adhesive to remove.

When evaluating the adhesive remnant index (ARI) scores for the three adhesives, in dry

conditions groups 1, 2, and 3 had showed a slight tendency towards score 1(failure between bracket base and adhesive),this implied that the bond strength would be strong at that locus, in wet conditions groups 4, 5, and 6 had showed a slight tendency towards score 3 (adhesive failure between adhesive and enamel), this implied that the bond strength at enamel bonding resin interface would be weak at that locus because of wet condition (Saliva).

When Fisher exact test was used to compare the scores between dry and wet conditions, the scores showed a non-significant difference (P>0.05) between groups 3 and 6, that means the BOB is less sensitive to wet condition than the other two adhesives (Resilience and Heliosit), and the more frequent bond failure occurs at the enamel-adhesive interface, this may be due to the hydrophilic nature of the salivatect paste of BOB that allows to function in the presence of saliva contamination, perhaps displacing or diffusing through it, to infiltrate and polymerize within the micromechanical retention of the etched enamel surface, so BOB anticariogenic property that comes from the fluoride releasing ability because of presence of pre-reacted glass ionomer filler, might help orthodontists to decrease the risk of enamel damage and to remove residual adhesive form the enamel surface easily at debonding.

However, a smaller adhesive remnant can mean less chair time for removal of the adhesive remaining after debonding. The ideal bonding system would leave a healthy enamel surface without large amounts of adhesive to remove, and thus prevent iatrogenic enamel loss. Furthermore, the locus of bond failure is determined by a complex combination of contributory factors including the direction of the force applied, enamel pretreatment, the adhesive itself, and the bracket type.

Within the limitation of this in vitro study, the following conclusions should be drawn:

- 1. All these adhesives demonstrated adequate bond strength to withstand orthodontic forces throughout the experiment, except for Heliosit, which exhibited unacceptable bond strength in wet condition.
- 2. In regard to BOB, saliva contamination of enamel after priming had little effect in bond strength, it has an advantage during clean-up of the enamel surface, as it reduces the risk of enamel fracture at the time of bracket debonding.

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#### الخلاصة

الخلفية : الغرض من هذه الدراسة هولتقيم فعالية مادة لاصقة تقويمية جديدة ( بيوتي اور ثو بوند ) من خلال حساب قوة اللصق القصية في البيئة الجافة والرطبة وكذلك تقييم معامل بقايا المادة اللاصقة بالمقارنة مع بقية انواع اللواصق .

المواد والطرق : ستون سنًّا ضاحكاً خالى من الاضرار تم تقسيمها الى سنة مجاميع ,تحتوي كل مجموعة على عشرة اسنان ، ثبتت هذه الاسنان بواسطة مادة الاكريليك؛ ثلاثة مجاميع لللبينة الجافة وثلاثة للبينة الرطبة. تم أحتساب قوة اللصق القصية بجهاز الانسترون ذي ألرأس الصليبي وبسرعة 0,5 ملم/دقيقة. بينمااسطح واجهاتوصلات المينا والمواد اللاصقة

لللبيئة الجافة ونلائة للبينة الرطبة بم احتسب فوة اسعن منصب بجهر ، مسرون في مرس مسيبي ر. في مريب من مريب من مع والحاصرات السنية تم فحصها بواسطة مجهر لتقييم معامل بقايا المادة اللاصقة. النتائج : معدل قوة اللصق القصية كانت اعلى قيمة في مادة الرزيلينس ذات النصوئي ثم في بيوتي اورثو بوند واخيرا في هيليوست في كلامن البيئة الجافة والرطبة , من المثير للاهتمام لم يتم تسجيل فرق معنوي بين مادة الرزيلينس وبيوتي أورثو بوند في البيئة الجافة باستعمال اختبار أل أس دي كما وحق ما ومن المؤسم المعامل بين للاهتمام لم يتم تسجيل فرق معنوي بين مادة الرزيلينس وبيوتي أورثو بوند في البيئة الجافة باستعمال اختبار أل أس دي كما وجد قوة لصق مقبولة أيضا في البيئة الراطبة , هن المثير كما اضهر معامل بقاء المادة اللاصقة ميل نحو الدرجة الاولى في البيئة الجافة ، بينما اضهر ميل نحو الدرجة الثالثة في البيئة الرطبة ولم يظهر أي فرق معنوي في هذا المعامل بين

مادتي الرزيلينس وبيوتي اورثو بوند باستخدام اختبار فيشر.

الاستنتاج : مادة بيوتي أورثو بوند اقل تحسسا للرطوبة من بقية مواد اللصق وكذلك هي سهلة التنظيف مما يقلل من خطر تكسر مادة المينا اثناء عملية از الة لصق الحاصرات السنية من الاسنان.

Table 1. The bonding systems employed in the present study								
Material	Manufacturer	Components	Compositions					
Resilience light cure adhesive (With an etchant agent and primer)	OrthoTechnology; Fl/USA),	Etching gel Sealant resin (Primer) Paste	Bis-GMA, glass filler, photosensitive catalyst TEGDMA, silane-treated quartz,amorphous silica, camphorquinone					
Heliosit orthodontic adhesive (With an etchant agent and no primer)	IvoclarVivadent clinical; Schann/Liechtenstein)	Etching gel Translucent low viscosity paste	Urethane dimethacrylate,Bis-GMA, decadioldimethacrylate,silicon dioxide, catalysts, stabilizers					
Beauty Ortho Bond (With self- etching primer)	Shofu, Kyoto, Japan	Primer A Primer B Paste Salivatect	Water, acetone, others Phosphoric acid monomer, ethanol TEGDMA, S-PRG filler, Bis-GMA					

 Table 1: The bonding systems employed in the present study

Table 2: Descriptive statistics, comparative statistics for the shear bond strength (Mpa) of the
three adhesive systems in both dry and wet environments

Bonding	Dry environment				Bonding Wet environment				T-test (d.f.=18)		
System	Min	Max	Mean	SD	System	Min	Max	Mean	SD	t-test	p-value
Group 1 (n=10)	10.32	12.81	11.48	0.744	Group 4 (n=10)	8.01	10.01	9.02	0.648	7.92	.000**
Group 2 (n=10)	5.80	7.32	6.57	0.490	Group 5 (n=10)	2.04	3.05	2.45	0.400	20.6	.000**
Group 3 (n=10)	10.06	11.73	10.97	0.563	Group 6 (n=10)	5.98	7.03	6.39	0.300	22.7	.000**
ANOVA	d.f.	Mean Square	F-test	Sig.	ANOVA	d.f.	Mean Square	F-test	Sig.		Highly cant 0.05
	29	72.84	197.3	.000**		29	109.2	495.9	.000**	$\geq P >$	0.001

 Table 3: Least significant difference test for shear bond strength between the adhesive systems in dry and wet environments

Dry environment (SE=0.271)				Wet environment (SE=0.209)				
Groups		Mean Difference	<b>P-Value</b>	Groups		Mean Difference	<b>P-Value</b>	
Group 1	Group 2	4.906	.000**	Group 4	Group 5	6.567	.000**	
Group 1	Group 3	0.503	.075	Group 4	Group 6	2.625	.000**	
Group 2	Group 3	4.403	.000**	Group 5	Group 6	3.942	.000**	
**-Highly significant 0.05 > $P < 0.001$								

\*\*=Highly significant  $0.05 \ge P > 0.001$ 

 Table 4: Scores of the adhesive remnant index for the three adhesive systems in dry and wet environments, and the difference between them using Fisher exact test

ARI	Dry	v Environm	ent	Wet Environment			
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	
Score	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	
1	8.00(80)	6.00(60)	1.00(10)	3.00(30)	2.00(20)	1.00(10)	
2	2.00(20)	2.00(20)	8.00(80)	0.00(0)	0.00(0)	7.00(70)	
3	0.00(0)	2.00(20)	1.00(10)	7.00(70)	8.00(80)	2.00(20)	
4	0.00(0)	0.00(0)	0.00(0)	0.00(0)	0.00(0)	0.00(0)	

Group 1 vs. 4: Fisher Exact Test= 0.0832, p-value= 0.003 \*\*, d.f.=1

Group 2 vs. 5: Fisher Exact Test= 0.519, p-value= 0.03 \*, d.f.=1

Group 3 vs. 5: Fisher Exact Test= 0.782, p-value= 1, d.f.=1

\*= Significant  $0.05 \ge P > 0.01$ ; \*\*=Highly significant  $0.05 \ge P > 0.001$