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Scanning Electron Microscopic Examination of Enamel Surface after Fixed Orthodontic Treatment: In-Vivo Study

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SUMMARY
Introduction Therapy with fixed orthodontic appliances starts with bracket bonding and ends with debonding of brackets, leaving enamel surface varied.
Objective The aim of this pilot study was to examine enamel surface before and after debonding of orthodontic brackets by the use of scanning electron microscopy (SEM).
Methods Epoxy replicas of four patients' premolars indicated for therapy with fixed orthodontic appliances were made and brackets were bonded to their teeth with a different adhesives (Enlight, No-mix, Fuji Ortho LC and Heliosit Orthodontic) (n=4). Two months later, brackets on premolars were debonded and amounts of adhesive left on the tooth surfaces and the bracket bases were evaluated with the adhesive remnant index (ARI). After resin removal, epoxy replicas were made and the surface of premolars was evaluated with the enamel surface index (ESI). All replicas of premolars (n=32) were prepared for SEM examination and compared under different magnifications. Tooth damage was estimated based on correlation between ARI_{tooth} and ESI.
Results Pearson's χ^2 test showed no significant differences between ARI_{tooth} and $ARI_{bracket}$ of four materials used. Nonparametric correlations showed significant differences between ARI_{tooth} and $ARI_{bracket}$, ESI and ARI_{tooth}, and between ESI and $ARI_{bracket}$. Increasing of ARI_{tooth} is followed with the descent of $ARI_{bracket}$ and the ascent of ESI. Multivariate regression analysis showed a significant correlation between ESI and ARI_{tooth}.
Conclusion Most bond failures took place at enamel-adhesive interface. ARI_{tooth} was a predictor to enamel surface damage. The type of material did not affect enamel surface damage.
Keywords: adhesives; bracket debonding; materials testing; scanning electron microscopy (SEM)

INTRODUCTION

In 1955, Buonocore [1] demonstrated a new concept of acid etching in order to improve bonding between tooth surfaces and dental resins. Ten years later, Newman [2] developed a new technique of direct bonding of orthodontic brackets with composite resin. This technique led to many advantages: the treatment was more comfortable for patients, pretreatment separation was eliminated, gingival irritation was reduced as well as chairside time, oral hygiene was easier and aesthetics was improved [3]. However, there were some disadvantages as the loss of enamel during acid-etching [4] and decalcification of enamel around the bracket base [5]. Further studies were performed, and in 1972. Wilson and Kent [6] introduced new translucent cement, glass-ionomer cement. This new adhesive adhered to both enamel and metal, it was antimicrobial, as well as able to release and uptake fluoride and prevent decalcification [7, 8, 9]. Nevertheless, its bond strength was lower compared to composite resin [10]. A trend appeared to develop a new material which would have positive characteristics of both composite adhesives and glass-ionomer cements and would overcome disadvantages of both materials. This led to the development of modified composites and resin-modified glass-ionomer cements. The last ones are hybrids of composites and conventional glass-ionomer cements with possibilities for light curing, faster setting time and shear bond strength comparable to composite adhesives.

At the end of the orthodontic treatment the main concern is to turn the enamel surface back to its original state with minimal enamel loss and to return its original roughness. If this is not achieved, there is a great possibility of potential plaque traps and poor aesthetics. A variety of factors influences enamel loss. Composite adhesives and glass-ionomer cements differ in shear bond strength and remnant amount of adhesive left on the surface after debonding [11, 12, 13]. Removing remnant adhesive from these materials can be done with different types of instruments – pliers, scalers, sandpaper discs, diamond or tungsten carbide burs and ultrasonic instruments. The damage of enamel surface depends of both type of bur used and speed of rotating instruments [14, 15, 16].

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Many studies have examined enamel damage after bracket debonding using different parameters; the presence of perikymata after orthodontic treatment [17], enamel detachment index [18], composite remnant index and surface roughness index [15] and adhesive remnant index [19]. All these parameters give only the qualitative assessments of enamel surface, but lately there have been studies with three dimensional measurements of enamel surface that can also measure enamel loss [20, 21].

OBJECTIVE

The null hypothesis assumed in this study was that the enamel surface damage was dependent on the type of adhesive. The objective of this study was to compare composite resins (chemically cured, light-cured and light-cured with no adhesive required) and light-cured resin-modified glass-ionomer cement for the amount of remnant adhesive left on the enamel after bracket debonding.

METHODS

Four bonding materials were tested in this study, and their properties are shown in Table 1. The following composite resins and glass-ionomer cement were used: chemically cured composite No-mix (Dentaurum GmbH & Co. KG), light-cured composite Enlight (Ormco Co. SDS Inc.), light-cured composite with no adhesive required Heliosit Orthodontic (Ivoclar Vivadent AG) and light-cured resin-modified glass-ionomer cement Fuji Ortho LC (GC America Inc.).

Four patients (3 female, 1 male; mean age 19.75 years) indicated for comprehensive orthodontic treatment with fixed appliances, at the Clinic of Orthodontics, Faculty of Dental Medicine in Belgrade, were included in this study. The selection criteria included good oral hygiene, no decalcification on teeth and presence of all permanent premolars during the entire treatment. Informed consent was obtained according to the guidelines for human research subjects established by the Ethical Review Board at the Faculty of Dental Medicine in Belgrade. The patients were assigned randomly to one of the four bonding materials.

After cleaning of teeth from dental plaque, soft debris and dental calculus, impressions of the jaw (in which the fixed appliance would be bonded) were taken from each patient. A two phase impressions were taken with addition silicone Elite HD+, Zhermack SpA. Using these impressions replicas of all four premolars were made with i-pox plus, Audent AG. Afterwards, stainless steel brackets with 0.018 inch slots (Equilibrium®2, Dentaurum GmbH & Co. KG) were bonded to each patient. Each material that required light polymerisation was light-cured with the same visible light-curing unit (Optilux 501, Kerr Co., SDS Inc.).

Group 1: chemically cured composite No-mix. According to manufacturer's instructions, the buccal enamel surfaces were conditioned with 37% phosphoric acid for 15 seconds, rinsed for 5 seconds and dried thoroughly for 15 seconds. A thin film of No-mix activator was applied to the etched surface and bracket base. No-mix adhesive paste was applied over activator on the bracket base, and the bracket was positioned on the tooth and pressed firmly to expel the excess adhesive which was removed with a dental probe [22]. The brackets were left for 7 minutes to complete polymerisation.

Group 2: light-cured composite Enlight. According to manufacturer's instructions, the buccal enamel surfaces were conditioned with 37% phosphoric acid for 30 seconds, rinsed for 30 seconds and dried with oil-free and moisture-free air for 15 seconds until the appearance of chalky white surface. A thin layer of bond was applied to the etched surface and light-cured for 20 seconds. A thin film of Enlight adhesive was applied to the bracket base, and the bracket was positioned on the tooth and pressed firmly to expel the excess adhesive [22]. The excess adhesive was removed with a dental probe and adhesive was light-cured for 40 seconds.

Group 3: light-cured resin-modified glass-ionomer cement Fuji Ortho LC. The buccal enamel surface was conditioned with GC Ortho Conditioner (20% polyacrylic acid) for 20 seconds and rinsed for 20 seconds. The excess moisture was removed by blotting with a cotton pellet, leaving the surface moist. The encapsulated Fuji Ortho LC was triturated for 10 seconds, loaded into application gun and squeezed as a thin film onto the bracket base. The bracket was positioned and pressed firmly in order to expel the excess adhesive [22]. Adhesive was light-cured for 40 seconds (10 seconds from each side of the bracket base).

Group 4: light-cured composite, no adhesive required Heliosit Orthodontic. The buccal enamel surfaces were

Table 1. Properties of four bonding materials used in this study

Brand name	No mix	Enlight	Fuji Ortho LC	Heliosit Orthodontic
Manufacturer	Dentaurum GmbH & Co. KG	Ormco Co. SDS Inc.	GC America Inc.	Ivoclar Vivadent AG
Batch number	527053	740-0198	290391	560458
Description	Composite resin	Composite resin	Resin-modified glass-ionomer cement	Composite resin
Preparation	One paste and unfilled resin	One paste and unfilled resin	Powder mixed into liquid	One paste and unfilled resin
Adhesive required	Yes	Yes	No	No
Curing method	Chemically cured	Light-cured	Light-cured	Light-cured
Fluoride release	No	No	Yes	No
Enamel etching	Yes	Yes	Yes	Yes
Dry field	Yes	Yes	No	Yes

conditioned with 37% phosphoric acid for 30 seconds, rinsed for 30 seconds and dried with oil-free and moisture-free air for 15 seconds until the appearance of chalky white surface. A thin film of adhesive was applied to bracket base, and the bracket was positioned on the tooth and pressed firmly to expel the excess adhesive [22]. The excess adhesive was removed with a dental probe and adhesive was light-cured for 40 seconds.

After two months of wearing fixed appliance, each patient had their brackets removed from his four premolars with bracket removing pliers [23]. Debonding pliers were placed at outer wings of the bracket. After the bracket debonding, the amounts of residual adhesive were scored by microscopically evaluating of the adhesive remnant index (ARI) [19], both on tooth and debonded bracket bases. The $ARI_{bracket/tooth}$ scoring system consists of a 0-to-3 scale: 0 – no adhesive left on the bracket/tooth; 1 – less than half of the adhesive remained on the bracket/tooth; 2 – more than half of the adhesive remained on the bracket/tooth; 3 – all adhesive was left on the bracket/tooth, with a distinct impression of the bracket mesh. The remnant adhesive was removed with a tungsten carbide bur at low speed without water cooling [14] until the enamel surface appeared smooth and resin-free. The final polishing was achieved with a polishing cup [17]. After the removal of residual adhesive, two-phase impressions with addition silicone were taken and second replicas with i-pox plus were made.

First and second replicas of teeth (before and after debonding of brackets) (n=32) were cut with Buehler IsoMet, a low speed saw in order to gain samples suitable for positioning in SEM. Total of 48 samples were carefully cleaned with 25% alcohol solution, sputter-coated with gold in BAL-TEC SCD 005 and fixed to the specimen holder with conductive tape. The samples were observed by the use of JEOL scanning electron microscope JSM-6390LV and secondary mode images were made. The images of each sample were compared under magnifications of 10 times and 30 times. The magnifications of 10 times were used for evaluating ARI_{tooth}. The magnifications of 30 times were used for evaluating possible enamel damage according to the enamel surface index (ESI) described by Zachrisson and Årtun [17]. The ESI scoring system consists of 0 to 4 point scale: 0 – perfect surface with no scratches and distinct intact perikymata; 1 – satisfactory surface with fine scratches and some perikymata; 2 – acceptable surface with several marked and some deeper scratches with no perikymata; 3 – imperfect surface with several distinct deep and coarse scratches but no perikymata; 4 – unacceptable surface with coarse scratches and deeply marked appearance.

All bonding, debonding and clean-up procedures were carried out by the same operator (TS).

Statistical analysis

The statistical analysis was performed with the SPSS software package, ver. 12.0. Descriptive results for all indexes (ARI_{tooth}, $ARI_{bracket}$ and ESI) were calculated and expressed as frequencies, percentages, means and standard deviations and analysed with the Pearson's χ^2 test. The correlations between ARI_{tooth} and $ARI_{bracket}$, ARI_{tooth} and ESI, and between $ARI_{bracket}$ and ESI were examined with the non-parametric Spearman's test. Multivariate regression analysis was used to find predictor for enamel surface damage. In all tests statistical significance was defined as $p<0.05$.

RESULTS

Descriptive results for all indexes (ARI_{tooth}, $ARI_{bracket}$ and ESI) are shown in Tables 2, 3 and 4.

Table 5 shows distribution of frequencies of ARI_{tooth} and $ARI_{bracket}$ scores for four materials. Most of the materials had ARI_{tooth} scores in groups of 1 and 2, but Heliosit had also ARI_{tooth} score 0, while No-mix I showed also ARI_{tooth}

Table 2. Average ARI_{tooth} scores for four bonding materials

Material	Mean	Med	SD	Min	Max	95% CI
No mix	1.75	1.50	0.96	1	3	0.23–3.27
Enlight	1.75	2.00	0.50	1	2	0.95–2.55
Fuji Ortho LC	1.25	1.00	0.50	1	2	0.45–2.05
Heliosit	0.75	1.00	0.50	0	1	-0.05–1.55

Med – median; SD – standard deviation; Min – minimum; Max – maximum; 95% CI – 95% confidence interval

Table 3. Average $ARI_{bracket}$ scores for four bonding materials

Material	Mean	Med	SD	Min	Max	95% CI
No mix	1.75	2.00	1.26	0	3	-0.25–3.75
Enlight	1.50	1.50	0.58	1	2	0.58–2.42
Fuji Ortho LC	2.25	2.50	0.96	1	3	0.73–3.77
Heliosit	2.25	2.00	0.50	2	3	1.45–3.05

Table 4. Average ESI scores for four bonding materials

Material	Mean	Med	SD	Min	Max	95% CI
No mix	1.25	1.00	0.50	1	2	0.45–2.05
Enlight	2.50	2.50	0.58	2	3	1.58–3.42
Fuji Ortho LC	1.50	1.50	0.58	1	2	0.58–2.42
Heliosit	1.75	2.00	0.50	1	2	0.95–2.55

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Table 5. Frequencies (%) of ARI scores for tooth surfaces and their corresponding bracket surfaces ($ARI_{tooth/bracket}$) bonded with different materials

Material	$ARI_{tooth/bracket}$ (n=24/24)			
	Score 0 (n=2/3)	Score 1 (n=13/5)	Score 2 (n=7/11)	Score 3 (n=2/5)
No mix	0/25	50/0	25/50	25/25
Enlight	0/0	25/50	75/50	0/0
Fuji Ortho LC	0/0	75/25	25/25	0/50
Heliosit	25/0	75/0	0/75	0/25

Table 6. Frequencies (%) of ESI on teeth bonded with different materials

Material	ESI (n=24)				
	Score 0 (n=0)	Score 1 (n=11)	Score 2 (n=11)	Score 3 (n=2)	Score 4 (n=0)
No mix	0	75	25	0	0
Enlight	0	0	50	50	0
Fuji Ortho LC	0	50	50	0	0
Heliosit	0	25	75	0	0

score 3. The majority of specimens were in the groups of $ARI_{bracket}$ scores 1, 2 and 3, while No-mix had also $ARI_{bracket}$ score of 0. According to the χ^2 test, no significant differences were found between four materials in these two groups (ARI_{tooth} and $ARI_{bracket}$) (p>0.05). However, nonparametric correlations showed significant differences between ARI_{tooth} and $ARI_{bracket}$ with negative Spearman's correlation coefficient which means that if $ARI_{bracket}$ tends to increase, ARI_{tooth} tends to decrease.

Frequencies of ESI scores are shown in Table 6. No teeth bond with any of four materials showed neither perfect surface with no scratches and distinct intact perikymata (ESI score 0) nor unacceptable surface with coarse scratches and deeply marked appearance (ESI score 4). Most of materials were in the groups with ESI scores 1 and 2, while Enlight had ESI scores 2 and 3. The χ^2 test showed no significant differences among four materials (p>0.05). However, nonparametric correlations showed significant differences between ESI and ARI_{tooth} as well as between ESI and $ARI_{bracket}$. The Spearman's correlation coefficient between ESI and ARI_{tooth} was positive which means that if ESI increased ARI_{tooth} tended to increase, while the Spearman's correlation coefficient between ESI and $ARI_{bracket}$ was negative which indicates that $ARI_{bracket}$ tended to decrease, when ESI increased.

Also, multivariate regression analysis showed a significant correlation between ESI and ARI_{tooth} which means that ARI_{tooth} was predictor to enamel surface damage.

Figures 1–4 show representative SEM images of different values for ESI scores.

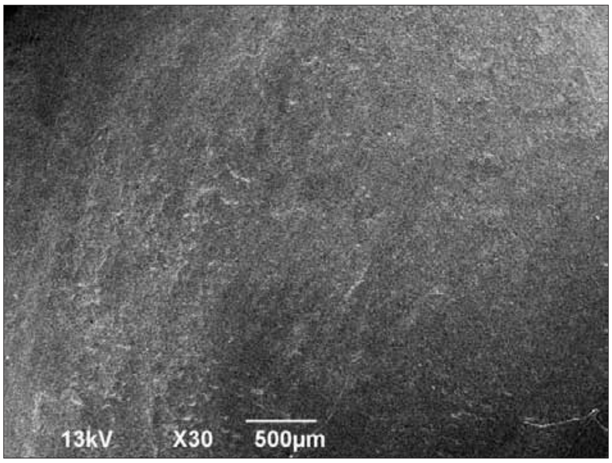

Figure 1. SEM image of enamel surface after removal of residual adhesive left after the use of No- mix (original magnification 30×), ESI score 1

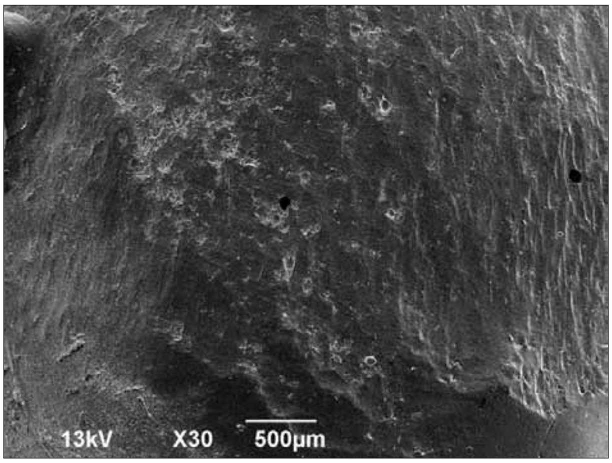

Figure 2. SEM image of enamel surface after removal of residual adhesive left after the use of Enlight (original magnification 30×), ESI score 3

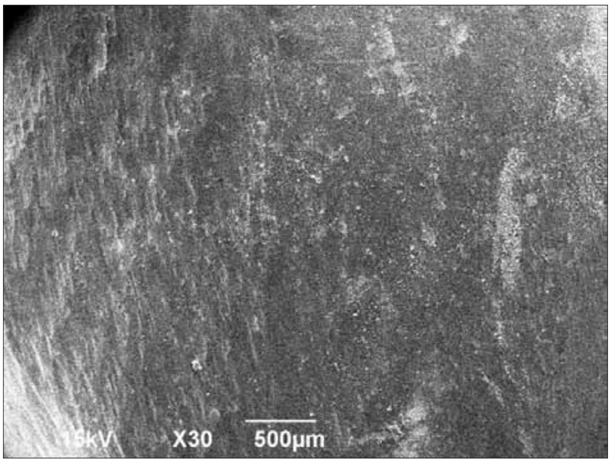

Figure 3. SEM image of enamel surface after removal of residual adhesive left after the use of Fuji Ortho LC (original magnification 30×), ESI score 2

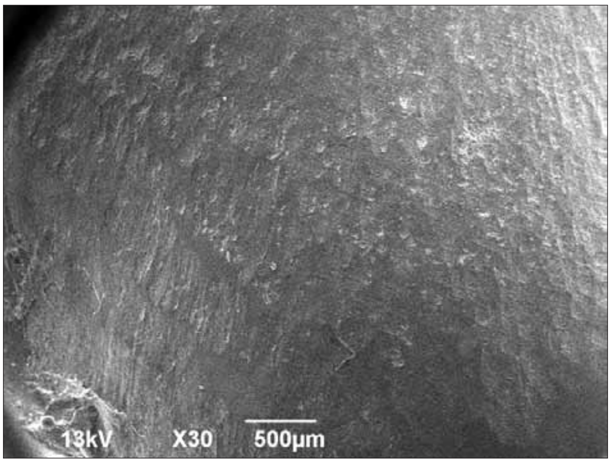

Figure 4. SEM image of enamel surface after removal of residual adhesive left after the use of Heliosit Orthodontic (original magnification 30×), ESI score 2

DISCUSSION

The null hypothesis that the enamel surface damage was dependent on the type of adhesive was rejected because multivariate regression analysis showed no significant correlation between the type of used material and enamel surface damage.

It was mentioned before that the main concern of each orthodontist at the end of the treatment is to revert original enamel surface roughness and appearance. If this is not achieved, the aesthetics may be unsatisfying. Furthermore, the loss of surface enamel and enamel prism endings exposal to organic acids in plaque as a consequence would lead to increased sensitivity to demineralisation and therefore development of dental caries or gingivitis.

The bond failure can be either adhesive (taking place at the enamel-adhesive interface or at the bracket-adhesive interface) or cohesive (in the enamel or in the adhesive). Cohesive failures in the enamel can easily be spotted macroscopically, while cohesive failures in the adhesive and adhesive failures can be determined with comparison of ARI_{tooth} and $ARI_{bracket}$ scores. In most cases (91.67%), ARI_{tooth} and $ARI_{bracket}$ scores of the same tooth had inverse proportions (ARI_{tooth} scores of 0, 1, 2 and 3 had comparable frequencies to $ARI_{bracket}$ scores of 3, 2, 1 and 0), meaning that most of the failures was at the enamel-adhesive or bracket-adhesive interface. However, in some cases (8.33%) the remnant adhesive on the tooth did not correspond to the type of failure on the bracket base, indicating that bond failure took place simply in adhesive. In this study, no cohesive failures in the enamel were noted. Overall, the most frequent ARI_{tooth} score was score 1 (54.2%), indicating bond failure close to the enamel-adhesive interface since less than half of the adhesive was left on the tooth. This was supported with results of $ARI_{bracket}$ scores with score 2 as the most frequent one (45.8%), suggesting that more than half of the adhesive remained on the bracket. All this implies weaker adhesion between the tooth and the adhesive than between the adhesive and the bracket which could facilitate clinicians to clean up the adhesive left on the tooth after debonding faster and with less enamel loss.

David et al. [24] used more distinguished qualitative method ranging the amount of residual adhesive left on the tooth after bracket debonding into 6 groups. They found that mean adhesive remnant weight and area was statistically equivalent for both composite resin and resin-modified glass-ionomer, with no advantage of either adhesive for these aspects. In keeping with the findings reported in our study, Shammaa et al. [25] also found the predominant mode of failure for the resin-modified glass-ionomer at the enamel-adhesive interface as well as majority of the brackets bonded with conventional light-cured composite adhesive. Our results partly agree with the results reported by Rix et al. [12], as well as by Summers et al. [13], that the predominant mode of bond failure for the resin-modified glass-ionomer was at the enamel-adhesive interface, but in the case of the conventional resin adhesive was at the adhesive bracket interface. Since the light-cured resin was used, former result was explained by the incomplete polymerization of the resin below the bracket base.

Lee and Lim [21] found that the resin-modified glass-ionomer group had lower ARI scores than the composite resin group. Still, in their study, a different type of conditioner was used, a conditioner that is indicated for dental pretreatment with a different composition compared to enamel referred Fuji Ortho conditioner used in our study. Consequently, in their study the bond strength between the enamel and resin-modified glass-ionomer would be lower and therefore result in fewer remnants of adhesive left on the tooth. Ireland et al. [26] also used the dental conditioner in their study which was milder than enamel one and resulted in less residual adhesive on the tooth after the use of resin-modified glass-ionomer cement compared to the light-cured resin adhesive.

ARI index that was used in this study is a very simple method of ranking remnant adhesive after debonding. Still, it is not objective method and therefore it can be very influenced by the operator. Also, ARI score can be affected by many factors; type of bonding technique (direct or indirect bonding) [23], type of bracket used or design of bracket base, type of acid used for etching (phosphoric or polyacrylic acid) [11, 26], type of material used (composite resin, glass-ionomer cement conventional or resin-modified) [12, 13], tooth position in the jaw (anterior or posterior) [22, 27] and side of tooth where brackets were bonded (buccal or lingual surface) [28].

In our study, a significant correlation between the remnant adhesive left on the tooth and surface appearance after clean up was found. The bond failure at the enamel-adhesive interface indicated a smaller amount of residual adhesive, reducing in that way the use of rotary instruments for clean up, and subsequent iatrogenic injuries. This contradicts with findings of Pont et al. [27], who found no significant differences between ESI and ARI_{tooth} scores. These findings may disagree due to different magnifications used during analysing samples. While we assessed enamel surface under magnification of 30 times, specimens of Pont et al. [27] were evaluated macroscopically. Our study showed that at the end of orthodontic treatment, in most cases (91.67%) the enamel surface was satisfactory or acceptable.

CONCLUSION

From this pilot study, the following can be concluded:

1. With four adhesive materials used after debonding of orthodontic brackets, most bond failures took place at the enamel-adhesive interface (ARI_{tooth} score of 1) which was correlated to the residual adhesive on the bracket base ($ARI_{bracket}$ score of 2).
2. ARI_{tooth} showed as a predictor of enamel surface damage. However, the type of adhesive made no influence on the remnant adhesive volume and enamel surface damage.

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3. There were significant correlations between ARI_{tooth}, $ARI_{bracket}$ and ESI. The more remnant adhesive there was on the tooth, the fewer remnants of adhesive would be on the bracket base and more satisfactory enamel surface would be obtained.

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Испитивање површине глеђи после терапије фиксним ортодонтским апаратом скенинг-електронском микроскопијом – студија *in vivo*

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КРАТАК САДРЖАЈ

Увод Терапија фиксним ортодонтским апаратом почиње поставком бравица, а завршава се њиховим уклањањем на крају терапије, након чега је површина глеђи измењена.

Циљ рада Циљ ове пилот-студије био је да се испита површина глеђи пре и после скидања ортодонтских бравица применом скенинг-електронске микроскопије (СЕМ).

Методе рада Израђене су реплике премолара шест пацијената код којих је индикована терапија фиксним ортодонтским апаратом и сваком од њих залепљене су бравице различитим адхезивом (*Enlight*, *No-mix*, *Fuji Ortho LC* и *Heliosit Orthodontic*). Два месеца касније бравице на премоларима су уклоњене, а количина преосталог адхезива на зубу и бравици одређена је индексом заосталог адхезива (енгл. *adhesive remnant index – ARI*). После уклањања адхезива начињене су реплике зуба и површина премолара је процењена помоћу индекса површине глеђи (енгл. *enamel surface index – ESI*). Све 32 реплике премолара припремљене су за испитивање применом СЕМ и упоређиване при различитом увеличању. Оштећења зуба су процењивана корелацијом вредности *ARI* зуба и *ESI*.

Резултати Пирсонов χ^2-тест није показао значајне разлике између вредности *ARI* зуба и *ARI* бравица у односу на четири коришћена материјала. Непараметарске корелације указале су на значајне разлике између вредности *ARI* зуба и *ARI* бравица, *ESI* и *ARI* зуба и *ESI* и *ARI* бравица. Повећање вредности *ARI* зуба било је праћено смањењем вредности *ARI* бравица и повећањем *ESI*. Мултиваријантна регресиона анализа показала је значајну повезаност *ESI* и *ARI* зуба.

Закључак Прекид везе најчешће се јављао на споју између глеђи и адхезива. *ARI* зуба се показао као предиктор оштећења глеђи. Врста материјала није утицала на оштећења површине глеђи.

Кључне речи: адхезиви; уклањање бравица; испитивање материјала; скенинг-електронска микроскопија (СЕМ)

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