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Please cite this article: SHEAR BOND STRENGTH TO SOUND AND CARIES-AFFECTED DENTIN OF SIM-PLIFIED “ETCH-AND-RINSE” AND “SELF-ETCH” ADHESIVES AND THE HYBRID LAYER MICROMORPHOLOGY

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UDC:

DOI: https://doi.org/10.2298/VSP161220146D

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
SHEAR BOND STRENGTH TO SOUND AND CARIES-AFFECTED DENTIN OF SIMPLIFIED “ETCH-AND-RINSE” AND “SELF-ETCH” ADHESIVES AND THE HYBRID LAYER MICROMORPHOLOGY

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**Keywords:** Shear Bond Strength, Caries Affected Dentin, Hybrid Layer Thickness.

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ABSTRACT

**Purpose:** The purpose of this study was to compare shear bond strength (SBS) of currently available adhesive systems to sound dentin (SD) and caries-affected dentin (CAD) and elucidate the hybrid layer micromorphology.

**Materials and Methods:** Sixty extracted human molars with coronal carious lesions formed the experimental sample while additional sixty extracted intact human molars (impacted third molars) served as controls. Identification of a carious-affected dentin was carried out using visual identification (North Carolina Dentin Sclerosis Scale). Teeth from both the experimental and the control sample were allocated to one of the following three groups: Adper Single Bond Plus/Filtek Supreme XT (ASB/FS) (3M ESPE), AdheSE One/Tetric EvoCeram (AO/TEC) (IvoclarVivadent), and Prime&Bond NT/CeramX Mono (PB/CXM) (Dentsply). Bonding procedures utilized in this work were in line with the manufacturers’ instructions. The SBS was measured using a universal testing apparatus. Hybrid layer micromorphology was observed under scanning electron microscope (SEM). The SBS (in MPa), and hybrid layer thickness (in µm) were statistically analyzed using T-test, Mann-Whitney U-test, ANOVA, and Holm’s test.

**Results:** Mean SBS±Standard Deviation were: ASB/FS to SD=10.56±3.49; ASB/FS to CAD=10.06±2.55; AO/TEC to SD=7.01±2.05; AO/TEC to CAD=6.73±1.66; PB/CXM to SD=9.01±2.47; PB/CXM to CAD=7.83±1.42. A statistically significant difference was found between the bonding strength of ASB/FS and AO/TEC to both SD and CAD, and between ASB/FS and PB/CXM to CAD. Hybrid layer thickness was statistically significantly greater for ASB/FS than for PB/CXM. For the ASB/FS system, a statistically significantly thicker hybrid layer was formed on CAD than on SD. No hybrid layer could be observed for AO/TEC.

**Keywords:** Shear Bond Strength, Caries Affected Dentin, Hybrid Layer Thickness.
APSTRAKT

Cilj: Cilj ovog rada je poređenje otpornosti adhezivne veze na silu smicanja (SBS) aktuelnih adhezivnih sistema sa zdravim (SD) i karijesno izmenjenim dentinom (CAD) kao i analiza mikromorfologije hibridnog sloja.

Materijal i metode: Šezdeset ekstrahovanih humanih molara sa karijesom okluzalne površine izabrani su kao eksperimentalna grupa, a šezdeset ekstrahovanih intaktnih humanih molara (impaktirani treći molari) izabrani su kao kontrolna grupa. Karijesno izmenjen dentin prepoznat je upotrebom vizuelne identifikacije (North Carolina Dentin Sclerosis Scale). Zubi obe grupe podeljeni su u tri podgrupe zavisno od primenjenog kompozitnog sistema: Adper Single Bond Plus/Filtek Supreme XT (ASB/FS) (3M ESPE), AdheSE One/Tetric EvoCeram (AO/TEC) (IvoclarVivadent), Prime&Bond NT/CeramX Mono (PB/CXM) (Dentsply). Adhezivna procedura sprovedena je u skladu sa uputstvima proizvođača. SBS je merena na univerzalnoj kidalici. Mikromorfologija hibridnog sloja analizirana je upotrebom skening elektronskog mikroskopa (SEM). Otpornost adhezivne veze na silu smicanja u MPa i debljina hibridnog sloja u µm statistički je analizirana pomoću T-testa, Mann-Whitney U-testa, ANOVA i Holmovog testa.

Rezultati: Srednje vrednosti SBS±Standardna Devijacija iznosile su: ASB/FS na SD=10,56±3,49; ASB/FS na CAD=10,06±2,55; AO/TEC na SD=7,01±2,05; AO/TEC na CAD=6,73±1,66; PB/CXM na SD=9,01±2,47; PB/CXM na CAD=7,83±1,42. Statistički značajna razlika uočena je između ASB/FS i AO/TEC na obe forme dentina: na zdravom i na karijesno izmenjenom dentinu, a između ASB/FS i PB/CXM samo na karijesno izmenjenom dentinu. Debljina hibridnog sloja je statistički značajno veća za ASB/FS nego za PB/CXM sistem. Kod sistema ASB/FS debljina hibridnog sloja je statistički značajno veća na CAD nego na SD. Nije uočeno postojanje hibridnog sloja kod AO/TEC sistema.
INTRODUCTION

After removal of caries-infected dentin, a considerable area of the cavity floor comprising of caries-affected dentin with partially demineralized collagen remains\(^1\). Since caries-affected dentin has different mechanical, physical, and chemical properties from those characterizing sound dentin, achieving intimate adaptation of the resin composite and tooth tissue is significantly harder in the former case\(^2,3\).

For the purpose of implementing composite restoration adhesion to tooth tissue, it is important for both the primer and the resin to penetrate as deeply into demineralized dentin as possible, creating a structure known as a hybrid layer\(^4\). Hybrid layer quality depends on the adhesive chemical composition and the application technique used, as well as the tooth region and presence of caries-affected dentin\(^5\).

Contemporary adhesive systems may be classified as self-etch and etch-and-rinse (total-etch), according to the use of phosphoric acid as a surface etchant. The etch-and-rinse adhesives completely remove the dentin smear layer and smear plugs during acid conditioning. In the next step, resin penetrates into the demineralized zone and provides required adhesion\(^6\). However, this technique is very sensitive, potentially resulting in contamination due to inconsistencies in executing each step\(^7\).

The self-etch adhesives contain acidic monomers that provide simultaneous conditioning and priming of tooth tissue\(^8\). They enhance adhesive inter diffusion through the smear layer. Therefore, this method is deemed user friendly (as it requires fewer steps) and less sensitive (as it does not involve wet-bonding)\(^9\). In terms of their pH, self-etch adhesives can be classified as: (a) ‘ultra mild’(pH>2.5), (b) ‘mild’(pH≈2), (c) ‘intermediately strong’(1<pH<2), and (d) ‘strong’(pH≤1) self-etch approach\(^8\).

Adhesives can also be classified according to the number of steps required for their application, where by etch-and-rinse adhesives are divided into 3-step (separate application of acid, primer and adhesive resin) and 2-step (separate application of acid and mixture of
primer and adhesive resin). Similarly, self-etching adhesives can be 2-step (separate application of self-etching primer and adhesive resin) and all-in-one (application of self-etching primer and adhesive resin in one solution).

Bonding to caries-affected dentin is characterized by lower bond strength and inferior hybrid layer quality compared to bonding to sound dentin, irrespective of the type of adhesive system employed\textsuperscript{10}. For the etch-and-rinse adhesives, discrepancies between demineralization level and extent of resin monomer infiltration have been reported. As conditioning with phosphoric acid cannot completely remove mineral deposits inside dentinal tubules, resin infiltration depth can be compromised. The aforementioned issues contribute to lower bond strength\textsuperscript{1}. The etch-and-rinse adhesives form a thicker hybrid layer on caries-affected dentine (CAD) relative to that on sound dentin (SD)\textsuperscript{11}, with a greater prevalence of porous zones\textsuperscript{3}. Owing to the complete removal of the smear layer and smear plugs by etch-and-rinse adhesives, a large number of resin tags is produced\textsuperscript{6}. Self-etch adhesive systems have also demonstrated lower bond strengths to caries-affected dentin compared to sound dentin. During the application of self-etch adhesives, demineralization by acidic monomers and adhesive infiltration occur simultaneously. This approach results in fewer discrepancies in the level of demineralization and resin monomer infiltration. The hybrid layer formed by self-etch adhesives on CAD is usually thicker than that formed on sound dentin\textsuperscript{1}, with less pronounced resin tag formation. Only strong self-etch adhesives form typical resin tags in dentin\textsuperscript{8}.

The purpose of this study was to compare shear bond strength (SBS) of current adhesive systems to sound dentin (SD) and caries-affected dentin (CAD), as well as examine hybrid layer micromorphology. The null hypotheses were: (1) the tested composite systems bond equally well to sound and caries-affected dentin; (2) there is no difference between etch-and-rinse and self-etch adhesives in composite system in bonding to these respective substrates; (3) no important differences in the microstructure of the interfaces between the tested composite systems on respective substrates exist.
MATERIALS AND METHODS

Tooth Selection

Sixty caries-free and sixty molars with occlusal carious lesion were collected after obtaining informed consent from the patients, as approved by the Committee of Ethics of University of Novi Sad. Upon extraction, the teeth were cleaned with scalers and polished with pumice before being stored in 0.5% aqueous chloramine solution at 4°C. After seven-day storage period, the teeth were rinsed and transferred to distilled unionized water at 4°C and were used within one month from extraction\textsuperscript{12}.

Caries-affected Dentin Identification Procedure and Macroshear Bond Testing

Using self-curing polyester resin, the roots of thirty molars with occlusal carious lesion (Group A) and thirty caries-free molars (Group B) were individually embedded in polyvinyl chloride cylinders (PVC) of 22 mm diameter and 20 mm height. The tooth crown of every specimen protruded from the cylinder. Enamel of all occlusal surfaces was then removed using diamond bur (No:806 314110524014 NTI-Kahla, Gmbh, Germany) inserted in a high speed hand-piece under copious air-water spray. The exposed dentin surfaces of each specimen in Group A were ground with silicon carbide abrasive papers (SiC 600-grit paper, 3M) under running water using custom made grinding cylinder (see Fig.1) in order to obtain flat dentin surface perpendicular to the long axis of the tooth/PVC cylinder.

To obtain caries-affected dentin, we ground the samples forming Group B using the visual examination criteria set forth by the North Carolina Dentin Sclerosis Scale\textsuperscript{13}.

In each ground tooth, either SD or CAD was exposed, without revealing the pulp. Caries-affected dentin was identified according clinical (visual and tactile) examination guidelines, in accordance with the North Carolina Dentin Sclerosis Scale\textsuperscript{13}. Opaque, light yellow, or whitish dentin was classified as sound dentin. Glassy dentin, dark yellow, or slightly brownish was classified as caries-affected dentin (Fig 2).

Insert Figure 1

Insert Figure 2
Specimens from both groups were divided into three subgroups \((n=10)\), according to the adhesive/resin composite system used.

1. Subgroup 1: Adper Single Bond Plus/Filtek Supreme XT (3M-ESPE)
2. Subgroup 2: AdheSE One/Tetric EvoCeram (Ivoclar-Vivadent)
3. Subgroup 3: Prime&Bond NT/CeramX Mono (Dentsply)

Chemical composition and manufacturers’ instructions for tested adhesives are shown in Table 1.

Insert Table 1.

All implemented bonding procedures followed the manufacturers’ instructions and the pertinent test protocol guidelines, based on the ISO/TS 11405 specification of the bonding area limitation \(^{14}\). The specimens were placed in a custom made tool–specimen bracket, comprising of a metal cube into which a cylindrical hole corresponding to the specimen dimensions (22×20 mm) was bored, and a transparent PVC cylinder of 4×4 mm dimensions, for placing the resin composite build-up (Fig. 3). Assembling the aforementioned two components in the described manner allowed forming resin composite build-up perpendicular to the bonding dentin surfaces. These resin composite structures were layered gradually (in 2 mm increments), using a proprietary restorative resin composite of each adhesive. Each successive composite layer was light-cured using an LED curing device (SmarliteIQ2, Dentsply, Caulk, DE Milford, Serial No. B 21581).

Insert figure 3

The prepared specimens were stored in distilled water at 37°C for 24 h. Each specimen was used to determine shear bond strength using a universal testing machine (Instron Testing Machine Model 1122, Instron, Norwood, Massachusetts, US). Prior to testing, the specimens were secured in a specimen bracket fixed to the universal testing apparatus. A straight-edge guillotine was positioned as close as possible to the bonding interface and was aligned with the loading axis of the testing construction. A crosshead speed of 1 mm per minute was used. Shear stress was recorded in N (Newton), and the bond strength was calculated using the following equation:

\[
P\text{(MPa)} = \frac{F\text{(N)}}{S\text{(mm}^2\text{)}}\quad ^{15,16}
\]
The shear bond strengths were analyzed using T-test, Mann-Whitney U-test, two-way ANOVA, and Holm’s test, available in Primer of Biostatistics Statistical Software Program. In the two-way ANOVA analysis, adhesive/composite group and dentin substrate were the tested factors and the level of significance was set to \( p<0.05 \).

**Scanning electron microscopy (SEM) evaluation**

Scanning electron microscopy evaluation was conducted on 60 extracted human molars, divided into two groups according to the previously described criteria. Bonding substrates were prepared using the same procedure as that adopted shear bond strength testing. Once again, bonding procedures were in line with the manufacturers’ instructions (Table 1), and composite structures were constructed in bulk, in one 2 mm increments, applying the proprietary restorative resin composite of each adhesive. The teeth were then stored in distilled water at 37°C for 24 h. The bonded teeth with composite build-ups were sectioned parallel to the bonded surface to expose the dentin-adhesive interface. The specimens were polished under running water using SiC papers of increasingly finer grit (600, 1000 and 1200). For each polished specimen, CAD area was marked with a #11 scalpel under magnifying glass. The specimens were treated with 32% silica-free phosphoric acid gel (*Uni-Etch, Silica gel free, Bisco, Schaumburg, IL, LOT 0800012148*) for 60 s, followed by immersion in 2% sodium hypochlorite for 60 s to expose dentin-adhesive interface\(^\text{17}\). After rinsing with distilled water, the specimens were prepared under Environmental SEM (E-SEM) protocol, under a low vacuum and in wet conditions\(^\text{18}\). The specimens were examined by a SEM (*JEOL, JSM-6460 Low Vacuum, Tokyo, Japan*) at 1000x magnification\(^\text{17}\).

Insert Figures 4,5,6,7

**RESULTS**

**Shear Bond Strength Testing**
Descriptive statistics pertaining to shear bond strengths and the statistical significance of between-group differences are presented in Table 2.

Insert Table 2.

The results yielded by our investigation show that the adhesive system is a significant factor in determining shear bond strength. Greater bond strengths were achieved using the etch-and-rinse adhesives. The lowest bond strengths were noted for the AdheSE One-Tetric EvoCeram combination on caries-affected dentin. In addition, the obtained value was statistically significantly lower than that measured for the Adper Single Bond Plus-Filtek Supreme XT combination applied to both CAD ($p<0.01$) and SD ($p<0.02$) specimens. Prime&Bond NT-CeramX Mono exhibited statistically significantly lower bond strength on CAD than the Adper Single Bond Plus-Filtek Supreme XT ($p<0.03$). On the other hand, bond strengths of Prime&Bond NT CeramX Mono and AdheSE One Tetric EvoCeram were not statistically significantly different. Finally, none of the examined adhesives exhibited statistically significantly different shear bond strengths to sound dentin relative to caries-affected dentin ($p>0.05$).

**SEM analysis**

Hybrid layer thickness was measured using the SEM device (*NIH Image Analyser*) proprietary software (Fig 4,5,6,7). Insert figures 4,5,6,7,8A,8B

SEM images revealed that AdheSE One Tetric EvoCeram did not form the hybrid layer on the examined specimens.

The main statistical parameters pertaining to hybrid layer thickness are presented in Table 3, along with the statistical significance of between-group differences. As can be seen from the tabulated findings, greater hybrid layer thickness was formed on the CAD specimens. Insert Table 3.
Adper Single Bond Plus-Filtek Supreme XT system produced a statistically significantly thicker hybrid layer on both types of dentin than Prime&Bond NT-CeramX Mono system. On the SD specimens, hybrid layers were thinner, and the difference was statistically significant for Adper Single Bond Plus-Filtek Supreme XT system only. No hybrid layer could be observed on the specimens with AdheSE One-Tetric EvoCera.

**DISCUSSION**

Adhesion to dentin is an important step in placing composite restoration. Organic nature of dentin and its higher humidity relative to enamel makes bonding to this hard tissue very difficult\(^2\). Compared to SD, bonding to CAD is characterized by lower bond strength and inferior quality of the hybrid layer\(^10\). Owing to these discrepancies, bonding efficacy of different adhesive systems with SD and CAD has been extensively studied\(^18\).

Development of new adhesive systems requires valid laboratory methods for testing material properties, such as the shear bond strength test technique\(^19\). To standardize the test protocol, ISO/TS 11405 specification was established in 2003 year. The device recommended by this protocol is referred to as a “guillotine”\(^14\). In our study, the adhesives that were compared in terms of their bond strength included the corresponding composite provided by the same company. Consequently, conclusions can only be drawn at the level of the adhesive/composite combination\(^20\). According to Salz et al., “comparative bond strength tests are possible only at level of identical adhesive/composite combinations, and certainly not at the level of the adhesive alone”\(^21\). This assertion justifies the approach adopted in our investigation. Indeed, the goal of testing adhesives incorporating composites produced the same company is to inform their application in everyday practice.

In our study, comparison of the shear bond strengths of each adhesive to different substrates failed to reveal statistically significant differences. Consequently, the first null hypothesis was accepted. Shear bond strengths to SD and CAD were comparable, as the noted differences were not statistically significantly different. These results are in accordance with those reported by other authors\(^13,20\). For example, Pereira et al. posited that
the absence of statistically significant differences between the bond strengths of Adper Single Bond Plus adhesive with SD and with CAD can be attributed to large standard deviations, operator variability and/or technique sensitivity of this adhesive. Sonoda et al. also reported absence of statistically significant differences in the bond strength value of Prime&Bond NT to SD and CAD. These authors suggested that the caries retained after excavation, rather than the adhesive bond interface itself, is potentially the weakest part of the adhesive bond. Low bond strength values were reported for AdheSE One to SD and CAD in other investigations, in which statistically significant differences between the two could not be established. AdheSE One has a pH of 1.5 and is classified as an “intermediately strong” self-etch approach (pH in the 1–2 range). The functional monomer type in adhesive composition plays a crucial role in the self-etch adhesive performance. In AdheSE, bis-methacrylamide dihydrogen phosphate serves as a functional monomer. This molecule is characterized by a short spacer chain of phosphate functional monomer, which induces formation of unstable monomer calcium salts. Consequently, chemical interactions are less pronounced, resulting in a lower dentin bond strength. AdheSe is classified as a HEMA free adhesive. Presence of HEMA in the self-etch adhesive composition increases bond strength to dentin, as it provides good dentin wetting and hinders phase separation between the hydrophobic components and dentin. Low bond strength values of these adhesives can thus be attributed to the nature of their polymerization within dentin. Namely, photo initiator used in AdheSE One may contain acylphosphine oxides that do not react with many of the newer LED light curing units.

A comparison of the shear bond strengths of the tested composite systems revealed some statistically significant differences (Table 2). Consequently, the second null hypothesis was rejected. Specifically, the bond strength of composite system with etch & rinse adhesive (Adper Single Bond Plus+Filtek Supreme XT) was statistically significantly higher than that measured for the composite system with self-etch adhesive (AdheSE One+Tetric EvoCeram). These results correspond to the findings yielded by an extensive study in which more than 16000 shear bond strength tests were examined. However, as no statistically significant differences were noted between the bonding strength of the composite system with etch & rinse adhesive (Prime&Bond NT+ CeramX Mono) and that of the composite system with self-etch adhesive (AdheSE One+Tetric EvoCeram), our findings are not in line with those reported by Degrange et al. No differences between shear
bond strength of Prime&Bond NT and self-etch adhesives were found in the study conducted by Li et al.\textsuperscript{28}. Prime&Bond NT is a two-step acetone based etch & rinse adhesive characterized by high technique sensitivity. Acetone is unable to re-expand shrunken demineralized collagen\textsuperscript{29}. High technique sensitivity and the chosen bond testing method (macro shear) may result in low bond strength values\textsuperscript{30,31}.

SEM is typically used when investigating bonding mechanisms\textsuperscript{18}. However, the ultrastructural data pertaining to adhesive interface cannot be directly interpreted in terms of bond strength to tooth tissues. Micromorphological findings should always be carefully explained, as microscopic observations do not always correspond to the clinical findings\textsuperscript{17}.

Adhesion to dentin is a critical step in adhesive procedure. When using etch & rinse adhesives, the process commences with acid conditioning of dentin using phosphoric acid. This step results in a complete removal of the smear layer and smear plugs, leading to dentinal surface demineralization and exposure of the collagen fibrils\textsuperscript{32}. The second step comprises of a resin monomer penetration into this demineralized dentinal surface. As a result, a resin-matrix reinforced by collagen fibrils, called hybrid layer, is formed\textsuperscript{6}. Penetration of the resin monomer into opened dentinal tubules leads to resin tag formation. The hybrid layers formed with CAD are thicker than those of SD\textsuperscript{1-3,5,27,33}. As CAD is partially demineralized, it is more susceptible to acid-etching. This leads to the formation of a deeper demineralized zone. For both CAD and SD, discrepancies between demineralization depths and resin monomer penetration extent are common\textsuperscript{1}. The presence of highly acid resistant mineral deposits in dentinal tubules would interfere with resin monomer infiltration, as well as resin tag formation. Thus, hybrid layer formed with CAD is thicker and resin tags are less numerous, while lateral branches are less pronounced and shorter.

Self-etch adhesives form a thicker hybrid layer with CAD compared to SD, but are thinner than those obtained when etch & rinse adhesives are utilized\textsuperscript{3,33}. Typical resin tags in dentin are produced by strong self-etching adhesives (pH≤1) only, whereas they are rarely formed when mild and ultra-mild self-etching adhesives are employed\textsuperscript{8}. Since the AdheSE One is classified as an “intermediately strong” self-etch approach, typical resin tags are less numerous and are characterized by poor lateral branches (Fig.8 A,B).
Due to their pH, self-etching adhesives cannot dissolve acid-resistant mineral deposits in dentinal tubules of CAD. However, adhesive monomer penetration into CAD is hindered by a deeper mineralized zone, rather than adhesive pH. With the exception of those based on strong self-etching adhesives, these systems cannot dissolve the smear layer and smear plugs, and they remain as a part of hybridized complex. As the smear layer of CAD is enriched with disorganized collagen and mineral deposits, it may obstruct resin monomer infiltration. Thus, as we have found important differences in the microstructure of the interfaces between the tested composite systems on respective substrates, the third null hypothesis was rejected.

CONCLUSIONS

Noting the limitations of our study, the following conclusions can be drawn:

1. All tested composite systems bond equally well on sound and caries-affected dentin
2. The etch-and-rinse adhesives achieved stronger bond strengths
3. Adper Single Bond Plus-Filtek Supreme XT system formed a statistically significantly thicker hybrid layer on both types of dentin than Prime&Bond NT-CeramX Mono system

ACKNOWLEDGMENTS

This research is supported by Serbian Ministry of Education and Science projects 174005, III44003.
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Table

Table 1. Chemical composition and manufacturers’ instructions for tested adhesives

| Adhesive                | Chemical Composition                                                                 | Application                                                                                   |
|------------------------|---------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Adper Single Bond 2    | BisGMA, HEMA, dimethacrylates, ethanol, water, photoinitiator system, methacrylate    | 1. Apply Scotchbond Etchant (35%) to dentin. Wait 15 s and rinse for 10 s. Blot excess water using cotton pellet. |
| (3M-ESPE, St Paul, MN, USA) LOT N172405 | functional copolymer of polyacrylic and polyitaconic acids | 2. Immediately after blotting, apply 2–3 consecutive coats of adhesive for 15 s with gentle agitation using fully saturated applicator. Gently air thin for 5 s to evaporate solvent. Light-polymerize for 10 s. |
### Table 2. Mean SBS±Standard Deviation values, presence of statistical significance between groups

| Adhesive   | MeanSBS±St.dev (min,max) in MPa | MeanSBS±St.dev (min,max) in MPa | Significance CAD vs. SD |
|------------|---------------------------------|---------------------------------|-------------------------|
| CAD        |                                 |                                 |                         |
| SD         |                                 |                                 |                         |
| 1. ASB-FS  | 10.06±2.55 (5.09,12.89)         | 10.56±3.50 (6.37,16.51)         | non-significant         |
| Significance | p < 0.01 | p < 0.02 |
|--------------|---------|---------|
| 2. AO-TEC    | 6.73 ± 1.66 (4.93, 10.34) | 7.00 ± 2.05 (4.61, 11.3) | non-significant |

| Significance | non-significant | non-significant |
|--------------|----------------|----------------|
| 2 vs. 3      |                |                |

| Significance | p < 0.03 | non-significant |
|--------------|---------|----------------|
| 1 vs. 3      |         |                |

Table 3. Basic statistical parameters characterizing hybrid layer thickness and presence of statistical significance

| Adhesive | Mean HLT ± St. dev (min, max) in µm CAD | Mean HLT ± St. dev (min, max) in µm SD | Significance CAD vs. SD |
|----------|----------------------------------------|----------------------------------------|-------------------------|
| 1. ASB-FS| 5.32 ± 1.54 (2.89, 8.16)                | 3.39 ± 0.51 (2.23, 3.84)               | p < 0.01                |
|          | Significance 1 vs. 2 p < 0.05           | p < 0.05                               |
| 2. PB-CXM | 3.21 ± 2.68 (1.67, 4.54)                | 2.68 ± 0.91 (1.61, 4.44)               | non-significant         |

Received on December 20, 2016. Revised on September 13, 2017. Accepted on September 28, 2017. Online First October, 2017.