

## CORRELATION BETWEEN HYBRID LAYER THICKNESS, RESIN TAG LENGTH AND MICROTENSILE BOND STRENGTH OF A SELF-ETCHING ADHESIVE SYSTEM

Vanessa Rahal<sup>1</sup>, Fernanda G. de Oliveira<sup>1</sup>, André L.F. Briso<sup>1</sup>, Paulo H. dos Santos<sup>2</sup>, Maria L.M.M. Sundefeld<sup>3</sup>, Renato H. Sundfeld<sup>1</sup>

<sup>1</sup> Department of Restorative Dentistry, Araçatuba Dental School, São Paulo State University, Araçatuba, São Paulo, Brazil.

<sup>2</sup> Department of Dental Materials and Prosthodontics, Araçatuba Dental School, São Paulo State University, Araçatuba, São Paulo, Brazil.

<sup>3</sup> Department of Community and Preventive Dentistry, Araçatuba Dental School, São Paulo State University, Araçatuba, São Paulo, Brazil.

### ABSTRACT

The purpose of this study was to evaluate the correlation between the hybrid layer thickness, resin tag length and resin bond strength of a self-etching adhesive system to sound dentin tissue "in vivo". After performing restorative procedures and tooth extractions, ten specimens were sectioned in a mesiodistal direction. One dental section was used for light microscope analysis, in which both the resin tag length and hybrid layer thickness were measured, while the other section was analyzed using a microtensile test (0.5 mm/min). The fractured surface of the latter section was characterized using a stereoscopic magnifying glass (40× magnification). The results were subject to statistical

analysis using the Pearson Correlation Test ( $\alpha = 0.05$ ). The hybrid layer thickness, resin tag length and resin bond strength mean values were 2.19  $\mu\text{m}$  (0.34), 4.34  $\mu\text{m}$  (0.28) and 9.73 MPa (5.55), respectively. In addition, correlation tests between the resin tag length and the resin bond strength ( $r=0.014$ ) and also between the hybrid layer thickness and bond strength ( $r=0.43$ ), showed no statistically significant correlation. The microtensile bond strength of Adper Prompt L Pop self-etching adhesive system does not depend on hybrid layer thickness or resin tag length.

**Keywords:** Dentin bonding agents - Tensile Strength - Polarized Light Microscopy.

## CORRELAÇÃO ENTRE A ESPESSURA DA CAMADA HÍBRIDA, COMPRIMENTO DOS PROLONGAMENTOS RESINOSOS E RESISTÊNCIA ADESIVA À MICROTRAÇÃO DE UM ADESIVO AUTOCONDICIONANTE

### RESUMO

A proposta deste estudo foi avaliar a correlação entre a espessura da camada híbrida e comprimento dos tags com a resistência adesiva de um sistema adesivo auto-condicionante, quando aplicado em dentina hígida. Depois da realização dos procedimentos restauradores, dez espécimes foram seccionados ao meio na direção mesio-distal. Uma metade foi utilizada para análise em microscopia óptica, onde os comprimentos dos tags e espessura da camada híbrida foram mensuradas, a outra metade foi analisada por meio do teste de microtração (0,5 mm/min). As superfícies de fratura foram classificadas com o uso de lupa estereoscópica (aumento de 40×). Os resultados foram submetidos a análise estatística por meio do Teste de

Correlação de Pearson ( $\alpha = 0,05$ ). Os valores da espessura da camada híbrida, comprimento dos tags resinosos e resistência adesiva foram obtidas, sendo 2,19  $\mu\text{m}$  (0,34), 4,34  $\mu\text{m}$  (0,28) e 9,73 MPa (5,55), respectivamente. Além disso, o teste de correlação entre os comprimentos dos tags resinosos e a resistência adesiva ( $r=0,014$ ) e a espessura da camada híbrida e a resistência adesiva ( $r=0,43$ ), não mostraram correlação estatística. O teste de microtração do adesivo auto-condicionante Adper Prompt L Pop não apresentou correlação com a espessura da camada híbrida e o comprimento dos tags resinosos.

**Palavras-chave:** Adesivos dentinários- Resistência a tração- Microscopia de luz polarizada.

### INTRODUCTION

Progress in restorative techniques, particularly esthetics, has led manufacturers and researchers to seek improvement in the adhesive and resinous composite systems.

The advent of acid etching has provided excellent adhesive bonding of resinous materials to dental enamel<sup>1</sup>. Dentin, in contrast, is a more complex

substrate, and bonding to it continues to pose a challenge, despite recent progress in adhesive techniques<sup>2</sup>.

Nakabayashi<sup>3</sup> and other authors observed that the process of adhesive bonding to acid-etched dentin is mainly of micromechanical nature, and concluded that it is greatly improved by removing the smear layer from the dentin<sup>4,5</sup>.

More recently, new materials and techniques for acid etching dental tissues have been created, for example, the self-etching adhesive systems<sup>6,7</sup>, which can etch both enamel and dentin, and simultaneously infiltrate their adhesive monomers<sup>8</sup> into the micropores of the enamel and the demineralized collagen matrix.

Among the self-etching adhesives, there is the one-step type, considered more aggressive and capable of forming a hybrid layer similar in thickness to those formed with conventional adhesives<sup>9,10</sup>.

Laboratory studies with self-etching adhesive systems have shown the importance of the resin tags and hybrid layer for obtaining an adhesive bond to dentinal tissue<sup>7,11</sup>.

Among the ways of analyzing these resinous structures, some papers report that optical microscope analysis provides consistent information on large areas of the bond interface<sup>12,13</sup>. On the other hand, Sano et al., in 1994<sup>14</sup>, described the possibility of observing the behavior of adhesive systems by using the microtensile test, claiming that among other advantages, it allows the distribution of forces in a more homogeneous manner throughout the entire sample surface; thereby reducing the incidence of cohesive fractures and making it possible to obtain real bond strength values.

Studies on the bond strength of self-etching adhesive systems by application of the microtensile test have been performed<sup>15,16</sup>, but few have correlated the values obtained with the length of the resin tags and hybrid layer thickness. Therefore, the aim of this study was to analyze the correlation of the

length of resin tags and the thickness of the hybrid layer with the microtensile bond strength of a self-etching adhesive system, when applied to dentinal tissue “*in vivo*”. The null hypothesis tested was that the bond strength was not influenced by the thickness of the hybrid layer and length of resin tags.

## MATERIALS AND METHODS

### *Specimen Preparation and Restorative Procedures*

After the project had been submitted to and approved by the proper Research Ethics Committee, ten third molars indicated for extraction were selected from students of the dental undergraduate course. After dental prophylaxis, local anesthesia of the operating field was performed, in order to make wide Class I type cavity preparations involving practically the entire occlusal face, to a depth of 4 mm, standardized by the length of the active part of the 1094 diamond bur (KG Sorensen Indústria e Comércio Ltda., Barueri, São Paulo, Brazil) used. The diamond bur was mounted in a high-speed handpiece, and used under water and air cooling (MS 350 - Dabi-Atlante, Ribeirão Preto, São Paulo, Brazil).

A self-etching, one-step adhesive system Adper Prompt L Pop (3M/ESPE, St Paul, MN, USA) and resin composite Z-250 (3M/ESPE, St Paul, MN, USA) were used. Their compositions are provided in Table 1.

The ten teeth were divided into three groups according to the type of analysis to be performed on dentinal tissue, considering the following factors: resin tags, hybrid layer and microtensile bond strength (Table 2).

**Table 1: Materials used in the study.**

Material	Product Name	Lot	Composition	Manufacturer
Self-etching Adhesive System	Adper Prompt L Pop	216467	<b>Compartment/Liquid 1</b> Methacrylate Esters derived phosphoric acid, Bis-GMA, initiators based on camphorquinone and stabilizers	3M ESPE, St. Paul, MN, USA
			<b>Compartment/Liquid 2</b> Water, HEMA, polyalkenoic acid, stabilizers, methacrylate ester, fluoridated complexes	
Resin Composite	Z-250	6CR	UDMA, Bis-GMA, TEGDMA and inorganic load particles	3M ESPE, St. Paul, MN, USA

Source: 3M/ESPE, St Paul, MN, USA.

**Table 2: Study Groups.**

Adhesive System	Group I	Group II	Group III
Self-etching Adhesive Adper Prompt L Pop	Hybrid Layer Analysis N=10	Resin Tag Analysis N=10	Microtensile Bond Strength Analysis N=10

After the cavities had been washed with water and dried with smooth air jets, the adhesive system Adper Prompt L Pop (3M ESPE Dental Products, St Paul, MN, USA) was applied according to the manufacturer's instructions, i.e., under pressure on the dentinal surface for 15 seconds and dried with smooth air jets for 5 seconds. Then, after the applicator had been wet again, a second layer of adhesive was applied and following another application of air jets, it was light-activated for 10 seconds with an Ultralux halogen light source (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) at an intensity of 450 mW/cm<sup>2</sup>. At this time, the cavities received the application of resin composite Z-250 (shade A2) (3M ESPE Dental Products, St Paul, MN, USA) in layers, starting by inserting the resinous material against the vestibular and pulp walls, and then against the lingual and pulp walls until the entire cavity was filled. Each layer was light-activated for 40 seconds.

All the restorations were finished with diamond burs of the Gold Series No.1190F (K.G. Sorensen Indústria e Comércio Ltda., Barueri, São Paulo, Brazil), followed by the use of Enhance siliconized burs (Dentsply - Indústria e Comércio Ltda., Petrópolis, Rio de Janeiro, Brazil).

Immediately after this, the restored teeth were carefully extracted, taking every precaution necessary not to compromise the restored area.

Finally, the teeth were cleaned and sectioned in the mesio-distal direction, under constant irrigation, in a metallographic cutter ISOMET 1000 (Buehler, Lake Bluff, IL, EUA), to obtain two sections - vestibular and lingual.

### Optical Microscopy Analysis

The dental sections used for optical microscopy were decalcified in a solution containing equal proportions of 50% formic acid and 20% sodium citrate. Complete decalcification of the specimen was verified by taking radiographs<sup>13,17,18</sup>. After decalcification, the restorations were carefully removed, so that the remaining organic material could be embedded in paraffin.

The specimens were sectioned in the longitudinal direction of the clinical crown to a thickness of six micrometers, and sequentially mounted on glass slides. Fifteen slides of each specimen were selected, maintaining an interval proportional to the number of cuts obtained of each specimen. After staining the slides by the Brown & Brenn method, the best histological sections of each slide were analyzed by

Axiophot optical microscopy (ZEISS DSM-940 A, Oberkochen, Baden-Württemberg, Germany) at 1000× magnification, with a 100×/1.30 micrometric eyepiece (Fig.1). The measurements of the hybrid layer and resin tags were taken after meticulous analysis of the entire extent of the histological section with the aid of the software AxioVision Software Real (Version 4.3; Carl Zeiss MicroImaging Inc., Thornwood, NY, USA). For each factor under analysis, three measurements were obtained in each section, in which the resin tags analyzed and measured belonged to the same region of the hybrid layer analyzed and measured. Consequently, for each selected slide, the thickness of the hybrid layer and length of the resin tags would correspond to the mean of the three measurements taken. Thus, for each specimen, fifteen measurements were obtained for the factor hybrid layer, as well as for the factor resin tags.

### Microtensile Tests

The dental remainder was submitted to the microtensile bond strength tests, with its root end fixed to an acrylic block coupled to the metallographic cutter ISOMET 1000 (Buehler, Lake Bluff, IL, USA), which operated under water cooling at a speed of 800 rpm and static load of 80 grams. Sections were cut in planes across the plane of the dentin adhesive/resin composite interface, in the vestibular-lingual and mesio-distal directions of the tooth (Fig. 1).

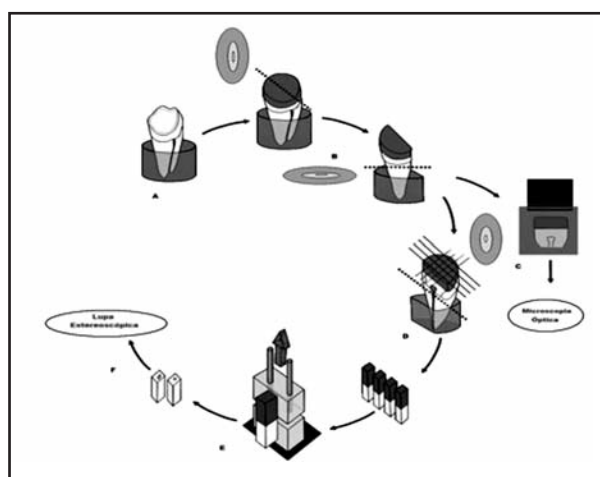


Fig. 1: Methodology used in the study. (A) Restorative Procedure. (B) Cutting the teeth into two sections. (C) Optic Microscopy Analysis (D) Transversal sections to obtain stick-shaped specimens for the microtensile test. (E) Microtensile test (F) Analysis of the fracture surface under stereoscopic microscope.

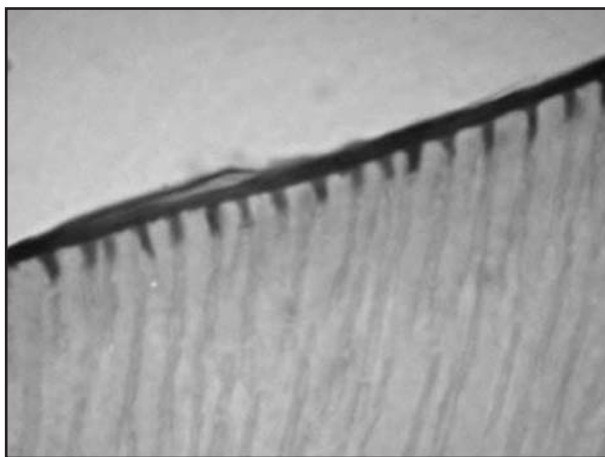


Fig. 2: Resin tags and hybrid layer observed by optical microscopy (Group II – E1) (1000× magnification).

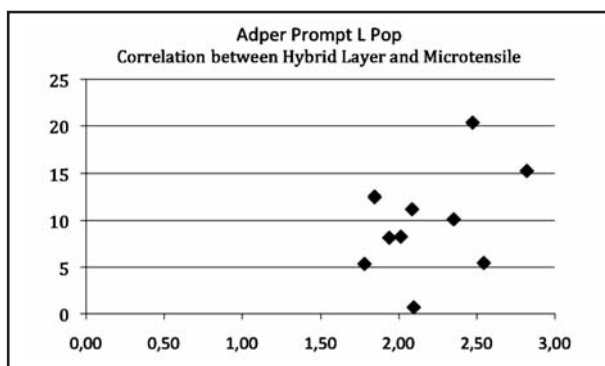


Fig. 3: Correlation test between hybrid layer and microtensile bond strength for the adhesive system Adper Prompt L Pop.

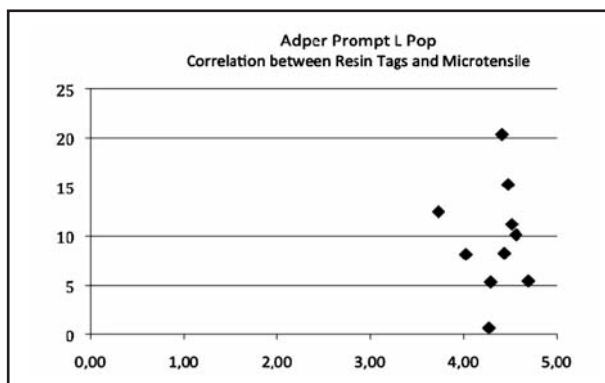


Fig. 4: Correlation test between resin tags and microtensile bond strength for the adhesive system Adper Prompt L Pop.

The sticks obtained were fixed to the Universal Test Machine Instron Model 4411 (Instron Inc., Canton, MA, USA), with cyanoacrylate adhesive (Super Bonder - Henkel Ltda., Itapevi, São Paulo, Brazil) and subject to traction with a 50N load cell at a speed of 0.5 mm/min until they ruptured.

The fractured area of each specimen was measured with a digital pachymeter (Digimes, Shinko Precision Gaging, Ind. Com. Ltda., China) to calculate the bond strength.

### Data Analysis

The results obtained were submitted to Pearson's Correlation test, at a level of significance of  $\alpha=0.05$ , to verify whether there was correlation between the resin tag lengths, hybrid layer thickness and the bond strength of the system studied.

### Fracture Pattern Analysis

After the microtensile test, the fracture patterns were analyzed with the aid of a stereoscopic microscope Stemi SV 11 (Carl Zeiss Company - DSM-940 A, Oberkochen, Baden-Württemberg, Germany) at 40× magnification. To reveal the fracture area of each specimen more clearly, the staining solution proposed by Ohkubo et al., in 1982<sup>19</sup> was applied to the region corresponding to the bond interface, which stained the resin-free dental structure. The criteria proposed by Figueiredo Reis et al.<sup>20</sup> were used to analyze the fracture patterns (Table 3).

## RESULTS

The microscopy analysis of the specimens showed the presence of resin tags with a mean length of 4.34 µm (0.28) and mean hybrid layer thickness of 2.19 µm (0.34), this being uniform and continuous, and present throughout the full extent of the cut (Fig. 2); the microtensile test showed a mean value of 9.73 MPa (5.55).

Application of Pearson's Correlation test, between the means of the factors microtensile bond strength and hybrid layer thickness ( $r=0.43$ ) and between those of microtensile bond strength and resin tag length ( $r=0.014$ ), showed absence of statistically significant correlation between the factors (Figs. 3 and 4).

Table 3: Criteria for Fracture Pattern Analysis (Figueiredo Reis et al., 2003 20 with modifications).

Type 1 (Mixed)	Type 2 (Adhesive)	Type 3 (Cohesive in Dentin)	Type 4 (Cohesive in Resin)
Partial fracture in dentin and adhesive or between resin and adhesive	Complete fracture in adhesive	Cohesive fracture in dentinal substrate	Cohesive fracture in the resinous material

As regards fracture pattern, 68% adhesive fracture, 26% mixed fractures and 6% cohesive fractures in resin were observed, no cohesive fracture in dentin being found (95% confidence) (Fig. 5).

## DISCUSSION

The excellent Brown & Brenn<sup>21</sup> staining method and common optical microscopy clearly showed the resinous structures in a large area of the bond interface of the decalcified dentinal tissue<sup>12,13,18,22-24</sup> in a single specimen.

The mean values of hybrid layer thickness, resin tag length and microtensile bond strength of 2.19  $\mu\text{m}$ , 4.34  $\mu\text{m}$  and 9.73 MPa, respectively, obtained in the present “in vivo” study (Table 2) were lower than those from other studies that used the same adhesive system “in vitro”<sup>13,23</sup>.

It should be pointed out that with the aim of making the results more compatible with real-life situations, in this study the adhesive system was applied “in vivo”, which may have contributed to these results, since the internal pressure in the dentinal tubules certainly diminishes the penetration of the adhesive system into the etched dentinal tissue<sup>25,26</sup>. Studies report that dentinal fluid pressure may cause lower bond strength values than those obtained in situations in which there is no pulp pressure present<sup>27-31</sup>. The low microtensile bond strength values obtained for the self-etching adhesive system used may also be related to the specimen storage conditions, in which the presence of moisture may have led to a degradation of the surface<sup>30,31</sup> due to the considerable permeability of the bond interface in this material<sup>30</sup>. In fact, Sauro et al., 2007<sup>25</sup> reported that the dentinal sealing desired after application of the adhesive system does not occur effectively in the self-etching systems due to the combination of the acid hydrophilic and hydrophobic monomers present in a single compartment, thus compromising the action of each of these components<sup>30,32</sup>.

In addition to these considerations, the application of the one-step self-etching adhesive system could lead to bond failures as a result of the excess water present in its composition, which results in incomplete polymerization of the adhesive system. Therefore, water sorption by hydrophilic resinous monomers, both in the hybrid layer and in the tags, contributes to low tensile bond strength values<sup>25,32</sup>. Indeed, the images obtained by common optical microscopy (1000 $\times$ ) showed a weak bond of this

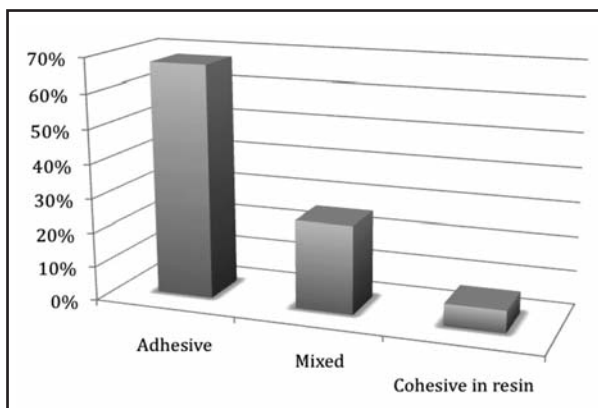


Fig. 5: Fracture patterns for the adhesive system Adper Prompt L Pop.

adhesive to dentin (Fig. 3), with the more frequent occurrence of adhesive fractures.

In addition to the abovementioned factors, it should be noted that the low pH found in the non-polymerized surface areas of this adhesive system, due to inhibition by oxygen, could result in a reduction in tertiary amines, thereby leading to poor polymerization of the adhesive material, and consequently, a reduction in bond strength between the materials could occur more easily<sup>25</sup>. Furthermore, the low concentration of hydrophobic components at the bond/dentin interface could be responsible for the low bond strength values, because they provide lower hydrolytic resistance<sup>16,23,31</sup>. Certainly, these factors contributed to failures at the interface between the resin composite and adhesive system<sup>32,33</sup>, thus limiting the clinical success of the restoration.

As in the “in vitro” research conducted by Oliveira et al., in 2009<sup>23</sup>, this study found no correlation between the values for the hybrid layer and resin tag length and the microtensile bond strength of the self-etching adhesive system Adper Prompt L Pop to dentinal tissue, when it was applied “in vivo”. This demonstrated that for this adhesive system, there may be no correlation between the variables under study.

The non-existence of correlation between the hybrid layer thickness and microtensile bond strength may also be related to the intrinsic mechanical properties of each material, such as the degree of conversion and concentration of hydrophilic monomers<sup>8,11,32</sup>.

The absence of correlation observed may be related to the findings of Spencer & Wang, in 2002<sup>32</sup>, who reported that the application of this same system promoted profound migration of low molecular weight molecules, such as hydrophilic monomers (HEMA).

Since the greater part of the resin tags and the base of the hybrid layer are formed by low molecular weight monomers, being weakly polymerized therefore reduces their contribution to bond strength<sup>4</sup>. Similarly, Van Landuyt et al., in 2007<sup>33</sup> reported that for this system, the thickness of the hybrid layer formed and length of tags did not appear to be the main factors responsible for an effective bond; but the quality of the dentin/bond interface is the main factor to consider in self-etching adhesive systems, as it is determined by the components that form the chemical composition of the adhesive.

#### CORRESPONDENCE

Dr. Vanessa Rahal  
Discipline of Restorative Dentistry  
São Paulo State University.  
Rua José Bonifácio 1193, São Paulo  
São Paulo, Brazil. 16015-050  
Email: vanessarahal@hotmail.com

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