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# EFFECT OF TOOTH AGE ON BOND STRENGTH TO DENTIN

## *EFEITO DA IDADE NA RESISTÊNCIA DE UNIÃO*

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This in vitro study evaluated the effect of tooth age on the tensile bond strength of Prime & Bond NT adhesive system to dentin. Human third molars from the five age groups were analyzed: A- 17 to 20yrs, B- 21 to 30yrs, C- 31 to 40yrs, D- 41 to 50yrs and E- 51 to 63yrs. The occlusal enamel was removed using a diamond saw under water cooling and the dentin surface was wet-ground with 600-grit SiC paper to obtain flat surfaces. The adhesive system was applied according to the manufacturer's instructions and a 6-mm high resin "crown" was built-up with resin composite. Teeth were stored for 24 hours in distilled water at 37°C and prepared for micro-tensile testing. Each specimen was mounted in a testing jig attached to a universal testing machine and stressed in tension at a crosshead speed of 0.5mm/min until failure. The means of tensile bond strength were (MPa): A- 21.42 ± 7.52[a]; B- 30.13 ± 10.19[a]; C- 31.69 ± 11.78[a]; D- 30.69 ± 8.47[a] and E- 35.66 ± 9.54[a]. No statistically significant difference was observed among the age groups ($p > 0.05$). The results suggested that the tensile bond strength of the adhesive system was not significantly affected by dentin aging.

**UNITERMS:** Dentin; Tooth age; Adhesive system; Bond strength.

## INTRODUCTION

Dentin is a hydrated biological composite structure composed of dentinal tubules, intertubular and peritubular matrices[17,30]. Each dentinal tubule is lined by peritubular dentin, which is characterized by a high mineral content. Intertubular dentin is found peripheral to the peritubular dentin separating the tubules, and its matrix contains 90 per cent type I collagen filled with apatite crystals[3,17]. Close to the pulp in newly erupted teeth, the dentin matrices are less mineralized and represent a small fraction of dentin area, whereas the tubules occupy a large area[6,14].

The main age-related change in older teeth includes a gradual enlargement of the peritubular dentin and intratubular mineral deposits, which often result in narrowed or completely occluded tubules[20,25]. Dentin sclerosis increases in teeth that have been subjected to attrition, to oral exposure of the dentin, to caries or dental restorative procedures[12,15,16,17]. Moreover, alterations in the organic fractions of dentin such as acid mucopolysaccharides, which are more predominant in mature teeth, may be found in certain instances with increased mineralization[17]. Therefore, a normal distribution of structural units depends on the developmental stage of the teeth[4,17,30].

Hybrid layer formation represents the main bonding mechanism of current dentin adhesives. This hybrid dentin-polymer structure is formed by impregnation of monomers into the demineralized intertubular dentin with subsequent polymerization. Resin infiltration into the dentinal tubules or resin tags also contributes to bonding. Studies show hybridization of the tubules' walls in the funnel-shaped portion, produced by acid etching[28,29].

The dentinal structure undergoes histological and physiological alterations with aging or maturation in vital teeth[4,12,17,20,25,26]. Thus, permanent teeth of elderly patients may have different morphology and composition, which may affect the results of acid etching or infiltration of resin monomer. The purpose of this study was to investigate the influence of age changes on the tensile bond strength of a one-bottle total etch adhesive system to dentin. In addition, SEM investigated regional variations of etched dentin as a function of the age of teeth.

## MATERIALS AND METHODS

Twenty-five extracted sound erupted third molars stored in distilled water containing thymol crystals were used within 4 months of extraction. The teeth were obtained according to protocols (46/2000) approved by the appropriate institutional review board of the Piracicaba Dental School – University of Campinas, and with the informed consent of the donors.

Teeth were grouped according to the patient's age and divided into five groups (n = 5). The age groups were: 17 to 20yrs, 21 to 30yrs, 31 to 40yrs, 41 to 50yrs and 51 to 63yrs. The occlusal enamel of the teeth was removed by a section perpendicular to the long axis of the tooth using a low-speed diamond saw under water cooling (Buehler Ltd., Lake Bluff, IL, USA) to expose areas of mid-coronal dentin. The flat dentin surfaces were wet-polished with 600-grit silicon carbide paper.

The dentin surfaces were bonded with Prime & Bond NT adhesive system (Dentsply De Trey, Konstanz, Germany) according to the manufacturer's instructions. The composition and application steps are described in Table 1. After bonding, TPH Spectrum resin composite (Dentsply De Trey, Konstanz, Germany) was incrementally applied to build up a 6-mm high crown. Three increments were applied and each one was light cured for 40s with a light-curing unit (Degulux Soft-Start, Degussa Hüls AG, Hanau, Germany). The bonded teeth were then stored in water at 37$^{o}$C.

After 24h, the teeth were serially sectioned into slabs with approximately 0.8 mm in thickness along the long axis of the tooth. Each slab was further vertically sectioned to produce some bonded sticks of approximately 0.7mm$^2$. Sections were made through the composite buildups and dentin using a low-speed diamond saw under water cooling (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA.), according to the "non-trimming" technique developed by Shono, et al.[24]. Five specimens were tested for each tooth.

The specimens were individually affixed with cyanoacrylate cement to the grips of the testing device, which was placed in a universal testing machine (Model 4411, Instron Co., Canton, MA, USA.). The sticks were pulled to failure in tension at a crosshead speed of a 0.5mm/min. The load at failure divided by the cross-sectional area at the site of fracture for each specimen was used to calculate the tensile bond strength in MPa. Data were statistically analyzed by one-way ANOVA and individual bond strength values were correlated with the tooth age by linear regression. Statistical significance was established at $\alpha = 0.05$.

The dentin side of failed specimens was lightly wet-abraded with 1000- and 2000-grit SiC paper to remove remnants of the adhesive agent, etched with 36% phosphoric acid for 15s and rinsed with water. For scanning electron microscopy (SEM), the samples were fixed in Karnovsky solution, post-fixed in osmium tetroxide solution, dehydrated in ascending alcohol concentrations, critical-point dried (CPD 030, Balzers, Balzer, Leichtenstein) and sputter-coated with gold (MED 010, Balzers, Balzer, Leichtenstein). Specimens were examined with a SEM (DSM 940A, Zeiss, Oberkochen, Germany), operated at 15 kV, and representative areas of the dentinal surfaces were photographed at 5,000 x and 10,000 x magnification.

## RESULTS

The one-way ANOVA (Table 2) revealed no statistically significant difference on the tensile bond strength to dentin among the age groups ($p = 0.248$). The mean tensile bond strength values and standard deviations for the age groups are presented in Table 3. The lack of influence of patient's age on bonding to dentin was confirmed by the absence of significant correlation between tensile bond strength and aging ($p = 0.162$ and $r = 0.335$).

Illustrative SEM photomicrographs of etched dentin are

**TABLE 1**- Components of Prime & Bond NT one bottle adhesive system used and procedures for bonding

| Components | Procedures | Manufacturer |
|---|---|---|
| 36% phosphoric acid | acid etching (15 s) and water rinse (15 s); | Dentsply De trey |
| PENTA, UDMA, Resin R5-62-1, T-resin, D-resin, nano-filler, initiators, stabilizer, cetyllamine hydroflouride and acetone. | remove of excess water by blotting with tissue paper; | Konstanz / Germany |
| | apply adhesive to wet surface and undisturbed for 20 s; | Lot number |
| | air dry gently (5 s); | 59712 |
| | light-cure (20 s). | |

Abreviattions: PENTA - Diphentaerythritol pente-acrylateomonophosphate, UDMA - urethane dimetacrylate.

presented in Figures 1 to 4. Acid etching opened the dentinal tubules and demineralized the intertubular and peritubular dentin for all age groups. In general, intertubular demineralization tended to decrease with aging. For younger teeth, the presence of intense exposed collagen fibrils after acid etching can be noted (Figures 1 and 2). Exposed outer peritubular collagen fibrils are shown in Figures 2 and 3. Figure 4 is a representative photomicrograph of old etched dentin. Some areas exhibit collagen fibrils embedded in mineral content due to a less aggressive or lower demineralizing effect of etching on the elderly groups.

## DISCUSSION

The bonding mechanism of resin to dentin is dependent on the microstructure of the substrate at the site of bonding[9,11,19,21,22]. Since dentin is a dynamic substrate[21], the quality of bonding has been analyzed as a function of aging. Studies have shown that the effectiveness in minimizing microleakage at the tooth-restoration interface, microgap formation and retention failures of dentin-bonded restorations can be influenced by dentin age[8,13,23].

Regarding the effect of dentin aging on the bond strength, Nakajima, et al.[18] showed much higher bond strength values to occlusal dentin of old molars than to young molars. The present findings confirm other reports on which similar tensile bond strengths to both young and old teeth were obtained for most adhesive systems[2,27]. In this study, although young teeth obtained lower bond strength means, no statistical differences were noted as well. The teeth used were free of caries and restorations; therefore, no natural alterations influenced the tensile bond strength of Prime & Bond NT.

Natural effects of physiological processes and microstructural changes in dentin as a function of tooth age include progressive deposition of peritubular dentin and tubule closure, which results in a significant reduction in the density of tubules and a decrease in dentin permeability[4,12,26]. Using earlier dentin bonding agents, studies have reported that the increased mineralization due to the aging phenomenon results in a less receptive or unfavorable substrate for bonding[5,8]. On the other hand, better dentin bond strength values were found in older teeth, due to the characteristics of collagen fibrils exposed on the dentin surface after acid etching[18].

Scanning electron microscopic studies of natural aged surfaces of cervical sclerotic dentin lesions submitted to acid pre-etching revealed that acid etchants did not adequately etch the aged sclerotic dentin surfaces for mechanical retention of the adhesive systems. Acid etchants were unable to effectively demineralize intertubular dentin and dissolve mineral salts or sclerotic casts within the tubules. This less effective conditioning may compromise resin infiltration in aged dentin and consequently the long-term durability of the restoration[5,10]. In the present study, the smear layer and smear plugs were completely removed by the acid, and collagen fibrils could be seen along the surface of the intertubular dentine, since the dentinal surfaces used for bonding were from intact internal mid-coronal occlusal dentin.

The application of Prime & Bond NT requires previous etching with 36% phosphoric acid. Scanning electron microscopy showed that the intertubular demineralization tended to decrease with age, and that the collagen fibrils after acid-etching, seemed to be more exposed in young teeth, probably because dentin has not become highly mineralized yet. However, such characteristics of exposed collagen fibrils did not affect the tensile bond strength. Tay, et al.[29] determined that a minimum pH value of 2.8 is required for the adhesive to effectively demineralize intact dentin within 30s. Thus, the acidity of this adhesive system (pH = 1.8) may be enough to promote a second demineralization of the underlying old dentin and allow a deep penetration of the resin monomer. This self-etching effect in highly

**TABLE 2**- One-way analysis of variance

| Source | Sum of squares | df | Mean square | F -Value |
|---|---|---|---|---|
| Age | 544.6937 | 4 | 136.1734 | 1.47* |
| Residual | 1848.7239 | 20 | 92.4362 | |
| Total | 2393.4177 | 24 | | |

* Statistically significant at the level of 5%

**TABLE 3**- Means of tensile bond strength (MPa) to dentin of five age groups

| Age Group | Mean ± SD | ANOVA |
|---|---|---|
| 17 - 20 | 21.42 ± 7.52 | a |
| 21 – 30 | 30.13 ± 10.19 | a |
| 31 – 40 | 31.69 ± 11.78 | a |
| 41 – 50 | 30.69 ± 8.47 | a |
| 51 – 63 | 35.66 ± 9.54 | a |

Mean values followed by the same letter were not significantly different mineralized zones may explain the fact that similar tensile bond strength values were obtained for the five age groups for Prime & Bond NT adhesive system, despite the effects of aging.

Among the multiple factors that can influence the effectiveness of dentin adhesives, age changes in teeth did not affect the resin-dentin bond strength. This becomes important because of the increased need for the restoration of elderly patients' teeth[1,7], due to a growing proportion of the dentate adult population.

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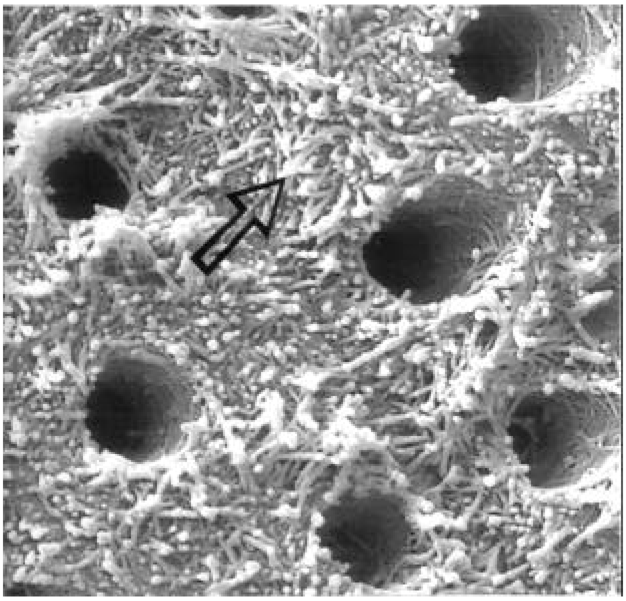

**FIGURE 1**- Aspect of the young dentin surface (18 years old) after removal of the inorganic components by acid etching. Note the collagen fibrils (arrow) in the intertubular demineralized dentin (Bar = 2 µm / 5,000 X)

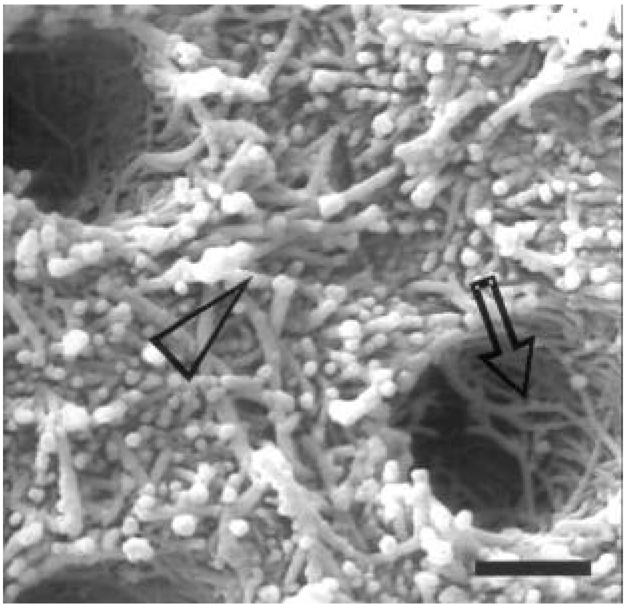

**FIGURE 2**- Cross-sectioned tubules showing intense exposed peripheral intertubular collagen fibers (arrow) and outer peritubular collagen fibrils (arrowhead) (20 years old) (Bar = 1 µm / 10,000 X)

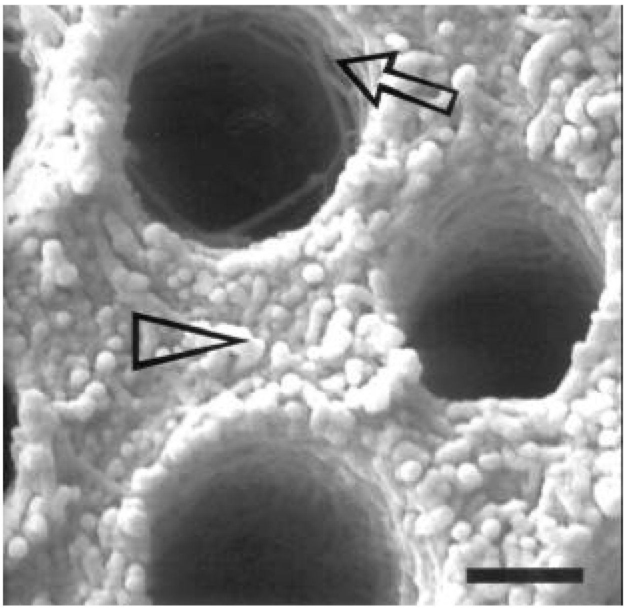

**FIGURE 3**- Cross-sectioned tubules showing exposed outer peritubular (arrow) and intertubular collagen fibrils (arrowhead) for a tooth from a 29 year-old patient (Bar = 1 µm / 10,000 X)

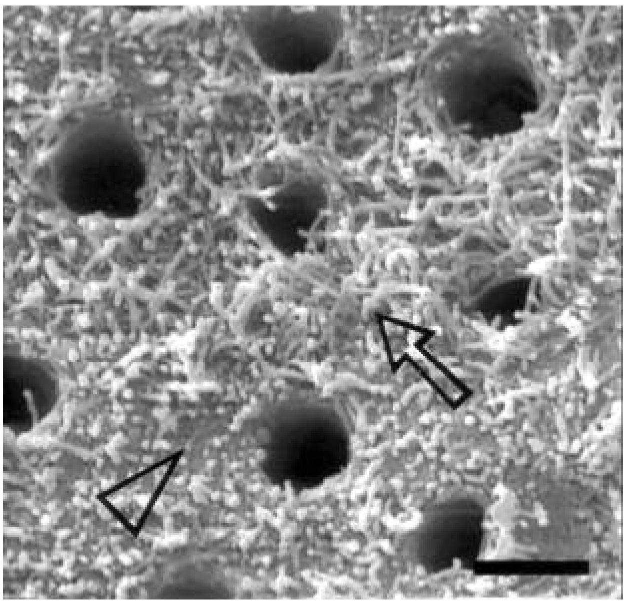

**FIGURE 4**- Surface view of old dentin (50 years old) etched with 36% phosphoric acid for 15 seconds. Note the collagen fibrils exposed (arrow) and collagen fibers embedded in mineralized areas (arrowhead) (Bar = 1 µm / 5,000 X)

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## RESUMO

Este estudo avaliou, *in vitro*, o efeito da idade na resistência à tração do sistema adesivo Prime & Bond NT no substrato dentinário. Foram utilizados terceiros molares humanos de cinco faixas etárias: A- 17 a 20, B- 21 a 30, C- 31 a 40, D- 41 a 50 e E- 51 a 63. O esmalte oclusal foi removido utilizando disco de diamante e a superfície dentinária abrasionada e planificada com lixa de SiC (600) sob refrigeração. O sistema adesivo foi aplicado de acordo com as recomendações do fabricante e um bloco de resina composta de 6 mm de altura foi confeccionado na superfície dentinária. Os dentes foram armazenados em água por 24 horas a 37$^{o}$C e preparados para o ensaio de micro-tração. Cada espécime foi fixado no dispositivo de micro-tração que estava acoplado a uma máquina universal de ensaio e testado numa velocidade de 0,5 mm/min. As médias de resistência à tração foram (MPa): A- 21,42 ± 7,52$^{a}$; B- 30,13 ± 10,19$^{a}$; C- 31,69 ± 11,78$^{a}$; D- 30,69 ± 8,47$^{a}$ e E- 35,66 ± 9,54$^{a}$. Nenhuma diferença significativa foi observada entre os grupos formados pelas faixas etárias ($p$ > 0,05). Os resultados sugerem que a idade não altera significativamente a resistência de união do sistema adesivo à dentina.

**UNITERMOS:** Dentina; Idade; Sistema adesivo; Resistência de união.

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