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# Effect of different application times of 1.3% green tea extract on the bond stability of etch-and-rinse adhesive system to dentin

# Efeitos de diferentes tempos de aplicação do extrato de chá verde a 1,3% na estabilidade de união de sistema adesivo convencional à dentina

Giovanna Caridade da Silva **Azevedo**[1] 0000-0002-6692-0868
Pedro Giorgetti **Montagner**[2] 0000-0002-7836-7131
Rafael Leonardo Xediek **Consani**[2] 0000-0003-4122-9234
Cecília Pedroso **Turssi**[1] 0000-0002-0078-9895
Roberta Tarkany Basting **Höfling**[1] 0000-0002-5345-5776
Flávia Lucisano Botelho do **Amaral**[1] 0000-0002-8934-6678

## ABSTRACT

**Objective**: The study aimed to assess the impact of different application times of 1.3% green tea extract on the bond stability of an etch-and-rinse adhesive system

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[1] Faculdade São Leopoldo Mandic Campinas, Departamento de Odontologia Restauradora. R. Dr. José Rocha Junqueira, 13, Ponte Preta, 13045-755, Campinas, SP, Brasil. Correspondence to: GCS Azevedo. E-mail: <gigicazevedo@gmail.com>.
[2] Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba, Departamento de Prótese e Periodontia. Piracicaba, SP, Brasil.

to dentin. **Methods**: Thirty third molars had their occlusal enamel removed to expose the dentin surface, which was then subjected to acid etching. The dentin fragments were divided into three groups based on green tea application time: 30 seconds, 60 seconds, and 0 seconds (control group, no treatment). The adhesive system (Adper Single Bond 2, 3M ESPE) was applied to the dentin, followed by the addition of four nanoparticulate composite resin cylinders (Filtek Z350 XT, 3M ESPE) to each sample. After 24 hours, two cylinders from each sample underwent microtensile bond strength testing, and the remaining two were tested after six months of storage in a solution simulating biological fluid. The fracture modes were analyzed under a stereomicroscope, and data were subjected to Fisher's exact test (α=0.05). **Results**: Results showed that after 24 hours, the 30-second Green Tea Group had significantly higher bond strength compared to the control group ($p$<0.05). However, after six months, the bond strength of the 60-second Green Tea Group was significantly lower compared to the other groups ($p$<0.05). The Green Tea pre-treatment and storage time did not significantly affect the fracture modes ($p$>0.05), with most failures classified as mixed. **Conclusion**: In conclusion, while pretreatment with 1.3% green tea for 30 seconds increased immediate bond strength, it did not show a difference compared to the control group after six months, indicating limited long-term effectiveness.

**Indexing terms**: Dentin. Dentistry, operative. Green tea.

## RESUMO

**Objetivo**: O estudo teve como objetivo avaliar o impacto de diferentes tempos de aplicação de 1,3% de extrato de chá verde na estabilidade de união de um sistema adesivo de condicionamento ácido à dentina. **Métodos**: Trinta e três terceiros molares tiveram seu esmalte oclusal removido para expor a superfície dentinária, que foi então submetida ao condicionamento ácido. Os fragmentos de dentina foram divididos em três grupos com base no tempo de aplicação do chá verde: 30 segundos, 60 segundos e 0 segundos (grupo-controle, sem tratamento). O sistema adesivo (Adper Single Bond 2, 3M ESPE) foi aplicado à dentina, seguido pela adição de quatro cilindros de resina composta nanoparticulada (Filtek Z350 XT, 3M ESPE) a cada amostra. Após 24 horas, dois cilindros de cada amostra foram submetidos a testes de resistência de união por microtração, e os dois restantes foram testados após seis meses de armazenamento em uma solução simulando fluido biológico. Os modos de fratura foram analisados sob um estereomicroscópio, e os dados foram submetidos ao teste exato de Fisher (α=0,05). **Resultados**: Os resultados mostraram que após 24 horas, o Grupo Green Tea de 30 segundos apresentou resistência de união significativamente maior em comparação ao grupo-controle ($p$<0,05). No entanto, após seis meses, a resistência de união do Grupo Green Tea de 60 segundos foi significativamente menor em comparação aos outros grupos ($p$<0,05). O pré-tratamento com chá verde e o tempo de armazenamento não afetaram significativamente os modos de fratura ($p$>0,05), com a maioria das falhas classificadas como mistas. **Conclusão**: Em conclusão, embora o pré-tratamento com 1,3% de chá verde por 30 segundos tenha aumentado a resistência de união imediata, não mostrou diferença em comparação ao grupo-controle após seis meses, indicando eficácia limitada a longo prazo.

**Termos de indexação**: Dentina. Dentística operatória. Chá verde.

## INTRODUCTION

The adhesive systems have been continuously improved over the years to achieve more effective and long-term bond strength. These systems can create a strong interface between restorative materials

and the dental substrate, able to support an amount of mechanical loads and the tensile stress imposed by composite resin and they can be classified as etch-and-rinse, self-etching [1] and universal [2]. During time, it is common to find degradation of collagen in the hybrid layer occurring due to the activity of collagenolytic enzymes, such as Matrix Metalloproteinases (MMPs), leading to a failure in the bond strength of the restoration [3]. These enzymes are activated by the drop of oral pH [4], which typically occurs during phosphoric acid etching of dentin. Furthermore, their activity is exacerbated using etch-and-rinse adhesive systems, as the collagen fibers exposed after acid etching may not be fully covered by the adhesive system [5].

One way to prevent this degradation is through the application of solutions containing MMP inhibitors or collagen cross-linkers, which increase mechanical strength and stabilize collagen, making it more resistant to degradation [6]. Green Tea extract (GT) has been described as a natural inhibitor of MMPs [7,8] as observed in the previous studies that the use of a 0.2% aqueous solution of GT for 60 seconds, applied after acid etching, increased the immediate bond strength of the etch-and-rinse system to dentin but was not able to prevent the decrease in bond strength over time [9]. Considering that the application time may have been insufficient for the GT extract to induce changes in the dentin, longer application times have been tested. When applying 0.2% GT for 1 hour, although there was an increase in collagen's mechanical strength and there was no significant improvement in bond stability [10], and this application time is clinically not usual. Therefore, considering the relevance of study adhesion applying higher concentrations of GT extract for shorter periods can be an attempt to establish an efficient protocol to bond stability. Applying 1.3% GT extract for 30 seconds promote an increase in collagen's mechanical strength, protecting it against enzymatic degradation. However, the effect of this concentration on dentin bond stability has not yet been verified [11].

These enzymes are activated by a drop in oral pH [4], which usually occurs during dentin etching with phosphoric acid. In addition, their action is more aggravated when the etch-and-rinse adhesive systems are used, due to the exposure of collagen fibers after acid etching, which may not be completely covered by the adhesive system [5]. One way of preventing this degradation is by applying solutions containing MMP inhibitors or collagen crosslinkers, which increase mechanical strength and stabilize collagen, making it more resistant to degradation [11]. Among the substances with such properties, natural extracts such as GT stand out [7, 8]. In a previous study, it was observed that the use of an aqueous solution of GT extract at 0.2% for 60 seconds, applied after acid etching, increased the immediate bond strength of the etch-and-rinse system to dentin, but was unable to prevent a drop in bond strength over time [9].

Thus, this study aimed to verify the effect of different application times of 1.3% GT, for 30 seconds or 60 seconds, on the bond stability of an etch-and-rinse adhesive system to dentin. The null hypothesis tested was that neither the application time of the GT nor the samples' storage time would affect the bond strength of the etch-and-rinse adhesive system to dentin.

## METHODS

### Ethical aspects

The research project was approved by the Research Ethics Committee of the São Leopoldo Mandic School of Dentistry, Campinas, SP, Brazil, under protocol number 5,988,320.

**Experimental design**

The factors under study were the application time of the 1.3% GT solution for dentin pre-treatment, in 3 stages: 0 seconds (control group, no treatment); 30 seconds, and 60 seconds; and the storage time in a solution that simulates biological fluid, before the microtensile test, carried out at 24 hours and 6 months. The experimental units were made up of 30 fragments (n=30) of dentin, randomly divided between the three stages of pre-treatment (n=10). Samples were obtained from the same unit to be tested at both times and the justification for the sample size was based on a previous study [10]. The quantitative response variable was obtained using a microtensile test and the fracture pattern was obtained qualitatively. The materials cited, as well as their composition and mode of use, are described in chart 1.

**Chart 1**. Materials, composition and application method.

| Material<br>Batch Number# | Composition | Application Method |
|---|---|---|
| Phosphoric Acid Ultra Etch IndiSpense 35% (Ultradent, Salt Lake City, UT, USA)<br><br>#BKPVF | 35% Phosphoric Acid | Applied for 15 seconds, rinsed with water for 10 seconds, and dried with absorbent paper. |
| Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA)<br><br>#2230100849 | BisGMA, HEMA, diurethane dimethacrylate, polyalkenoic acid copolymer, camphorquinone, water, ethanol, and glycerol 1.3 dimethacrylate, 10% by weight of silica nanoparticles | Application of two layers, light air blow for 5 seconds, and light curing for 10 seconds. |
| Green Tea Extract 1.3% Aqueous Solution (compounded at a compounding pharmacy - Medicamenta, Campinas, SP, Brazil) | Cammelia sinensis | Applied passively on the dentin for the time specified in each experimental group after acid conditioning. |
| Nanoparticulate Composite Resin (Z350, 3M ESPE, St. Paul, MN, USA)<br><br>#2211800331 | BISGMA, UDMA, TEGDMA, PEGDMA, BISEMA, Zirconia, Silica, Aggregated Zirconia/Silica | Inserted into silicone tubes with a diameter of 1.14 mm and a height of 20 mm, and light-cured for 20 seconds. |

Note: HEMA: Hydroxyethyl Methacrylate; BIS-GMA: Bisphenol A-Glycidyl Methacrylate; UDMA: Urethane Dimethacrylate; BIS-EMA: 2,2-bis-4-2-(hydroxi-3- methylacriloxietoxi)-phenylpropane).

**Specimen preparation**

Third human third molars were extracted for reasons unrelated to the research and stored in a liquid that simulates biological fluid (5 mM HEPES buffer solution; 2.5 mM $CaCl_2$-$H_2O$; 0.05 mM $ZnCl_2$ and 0.3 mM $NaN_3$; pH 7.4) after extraction. The teeth were cleaned with scalpel blades and periodontal curettes

and sectioned with a 7020 diamond blade (KG Sorensen, Barueri, SP, Brazil) on a handpiece at low speed (Dentflex, Ribeirão Preto, SP, Brazil), separating the crown from the root. The teeth had their occlusal enamel removed, thus exposing the superficial dentin. The fragments were embedded in polystyrene resin (Piraglass Com Ind de Art Ornamentais, Piracicaba, SP, Brazil) in 2.0 cm diameter polyvinyl chloride casts, leaving upper surface of dentin exposed. After 24 hours, the specimens were removed from the casts and sanded on a rotary polisher (Aropol 2V, Arotec, São Paulo, SP, Brazil) with sandpaper (#400 and #600 grit) under irrigation, until the clean separation view between dentin and enamel.

**Restorative procedures**

The sectioned fragments were treated with 35% phosphoric acid (Ultra Etch Indispense, Ultradent, Indaiatuba, São Paulo, SP, Brazil) for 15 seconds, rinsed for 10 seconds and softly dried with absorbent paper (figure 1A and 1B). At this stage, the dentin fragments were randomly divided into three groups, according to the predefined time for each treatment, which was then applied to the dentin surface: 0 (zero) seconds (control group, no treatment); 30 seconds and 60 seconds.

The aqueous solution containing 1.3% GT extract (Medicamenta Farmácia de manipulação, Campinas, SP, Brazil) had a volume of 20 µl per fragment and was gently applied to the conditioned dentin using a micropipette (figure 1C), following the pre-determined time in each experimental group. The excess was removed with absorbent paper, keeping the dentin moist. The etch-and-rinse adhesive system was then applied according to the manufacturer's recommendations, as described in chart 1.

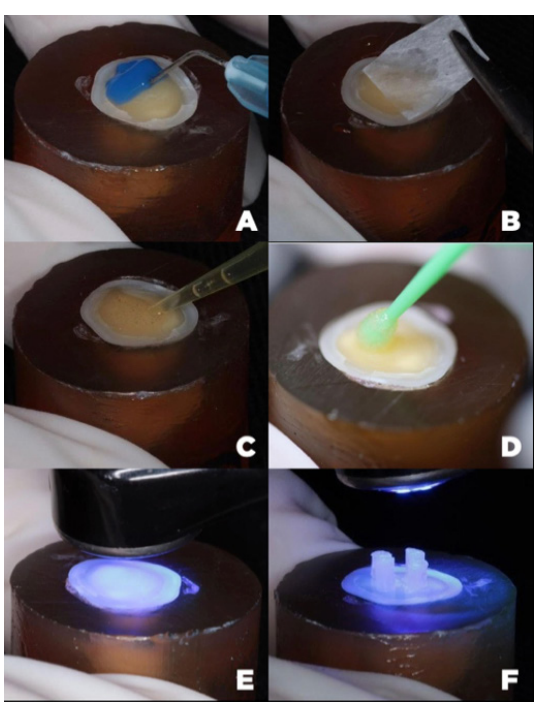


**Figure 1**. A: 35% phosphoric acid treatment for 15 seconds. B: Rinsed for 10 seconds and softly dried with absorbent paper. C: Aqueous solution containing 1.3% GT applied in dentin. D: Etch-and-rinse adhesive system. E: Photopolymerization for 10 seconds. F: Tubes construction with composite resin.

### Microtensile test

After treating each group with the GT, the etch-and-rinse adhesive system (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA) was applied according to the manufacturer's instructions using a disposable brush (Microbrush, KG Sorensen, Barueri, SP, Brazil) (figure 1D) and photoactivated for 10 seconds using an LED device (VALO, Ultradent, St. Lake City, UT, Brazil), operating at an irradiance of 1000 mW/cm$^2$, standard mode (figure 1E).

After applying the adhesive system, four Tygon tubes, 2 mm high and 1.14 mm in diameter, were adapted to the dentin surface and nanoparticulate composite resin (Z350 XT, 3M ESPE, St. Paul, MN, USA) was performed inside. The four cylinders were photoactivated together for 20 seconds using an LED light (VALO, Ultradent, Indaiatuba, SP, Brazil), operating at an irradiance of 1000 mW/cm$^2$ (figure 1F).

After performing the cylinders, the samples were stored in a bacteriological oven at 37°C. After 24 hours, two cylinders from each sample were subjected to the microtensile test in a Universal Testing Machine (EZ Test, Shimadsu, Japan) at a speed of 0.5 mm/min. The other two was stored for 6 months at 37°C in a solution simulating biological fluid composed of 5 mM HEPES buffer, 2.5 mM $CaCl_2$-$H_2O$, 0.05 mM $ZnCl_2$, 0.3 mM $NaN_3$ and pH 7.4 [12].

The force values obtained by the microtensile bond strength test were recorded in Newton (N) and then transformed into load/area values (MPa). The dentin fragment was an experimental unit, so the arithmetic mean of the two values obtained from the microtensile test at each time point was obtained from a single sample to refer to a single value for the experimental unit.

### Fracture mode analysis

After the microtensile test, the surfaces of the specimens were visually examined under a stereoscopic magnifying glass at 40x magnification (Eikonal Equip. Ópticos e Analíticos, model EK3ST, São Paulo, SP, Brazil) to classify the type of fracture that had occurred. The fractures could be classified into four types: adhesive (adhesion failure), cohesive in the tooth (fracture of a portion of the tooth), cohesive in the resin (resin failure), or mixed (adhesive and cohesive failure occurring together).

### Statistical analysis

All analyses were performed using the R statistical program (R Core Team – 2023 – R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Firstly, descriptive and exploratory analyses were performed on all the data. The bond strength data (MPa) was described with means, standard deviations, medians, minimum and maximum values and analyzed using a generalized linear model, considering a split-plot design, since samples tested at both times were obtained from the same experimental unit. Fisher's Exact Test was also used to analyze the fracture pattern variable and, as it is a categorical variable, it was described with absolute and relative frequencies.

## RESULTS

Table 1 and figure 2 show a large effect of the application of GT extract at 1.3% on the bond strength of the adhesive system ($f$=0.52). There was also a large effect of time on strength ($f$=0.41). The interaction

between the effects of group and time also showed a large effect, with $p$=0.0774, and was then unfolded following the recommendation [13]. After unfolding the interaction, it was observed that, at 24 hours, the group that received GT for 30 seconds showed greater bond strength than the group without tea ($p$<0.05). At 6 months, the group receiving GT for 60 seconds had lower bond strength than the other groups ($p$<0.05). It can also be seen that the group that received GT extract for 60 seconds did not follow the bond strength of the others and had a significant decrease in bond strength after 6 months ($p$<0.05).

The effect of GT on the fracture pattern was small and not significant ($p$>0.05), table 1 and figure 2. At both assessment times, most of the specimens had mixed fractures.

**Table 1**. Average values and standard deviation of the bond strength (in MPa), according to the concentration of the green tea extract solution applied to the dentin and the storage time.

| Group | Time | | | |
|---|---|---|---|---|
| | 24 hours | | 6 months | |
| | Mean (Standard Deviation) | Median (Maximum and Minimum) | Mean (Standard Deviation) | Median (Maximum and Minimum) |
| No treatment | 7.21 (2.20) Ab | 7.87 (4.40;11.0) | 8.12 (2.29) Aa | 7.93 (4.67;12.35) |
| GT 30 seconds | 10.88 (2.58) Aa | 10.51 (8.29;17.19) | 8.61 (3.08) Aa | 8.46 (5.37;15.35) |
| GT 60 seconds | 9.54 (4.05) Aab | 9.33 (2.51;17.54) | 6.58 (1.37) Bb | 6.32 (5.06;9.28) |

Note: Different letters (upper case horizontally and lower case vertically) indicate statistically significant differences, $p$≤0.05. $p$(group)=0.0988, $p$(time)=0.0539, $p$(interaction)=0.0774.

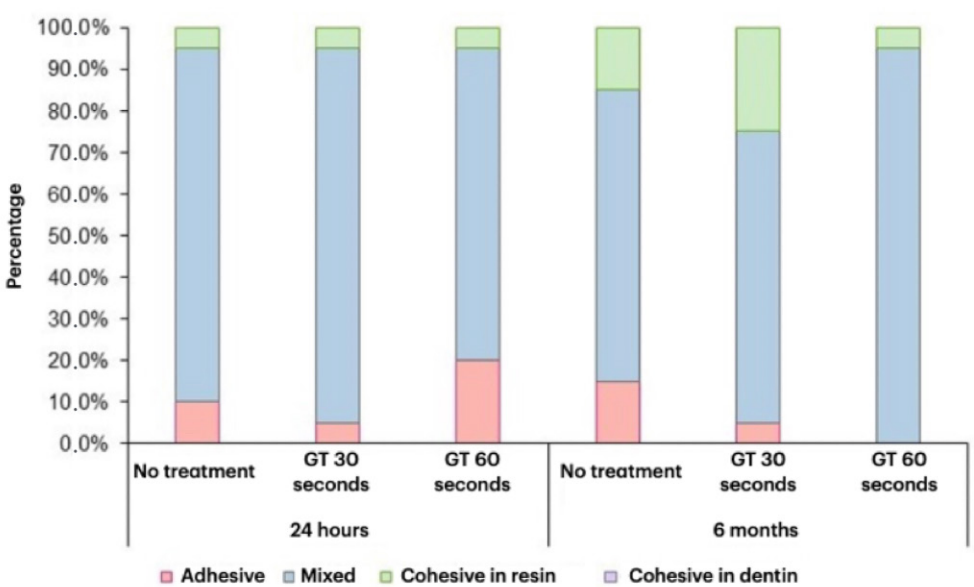


**Figure 2**. Distribution of failure patterns according to group and time.

## DISCUSSION

Considering looking up pre-treatments that promote biomodification of the dentin structure to improve the longevity of adhesive restorations, this study aimed to evaluate the effect of the application time of a 1.3% GT solution on the bond stability of the etch-and-rinse adhesive system to dentin. It was observed that both the time of dentin pre-treatment and the time the samples were stored statistically influenced bond strength, and the null hypothesis was therefore rejected.

The group exposed to GT for 30 seconds and whose bond strength was assessed after 24 hours, there was greater bond strength compared to the group that had no treatment (control group). This increase in bond strength can be attributed to the properties of GT, which is considered a natural inhibitor of MMPs [7,8], and a collagen cross-linking agent [6]. Consequently, dentin pre-treatment with GT can promote an increase in the mechanical strength of the collagen matrix, making it more resistant to degradation [6]. On the other hand, even in the immediate storage period (24 hours), it was observed that dentin pre-treatment for 60 seconds promoted intermediate bond strength, not differing from the control group. Possibly, the expected effect of the GT, described for the 30-second time, may have been hindered by possible deposition of particles from the GT, since the 1.3% solution was visually very dense and dark and there was sedimentation in the solution, which may have caused this type of result.

Six months after pre-treatment, the group that received GT for 30 seconds showed statistically similar bond strength to the control. In both groups, bond strength stability was observed over time. The present study opted to use the etch-and-rinse adhesive system, the application of which, in theory, can generate discrepancies between the depth of demineralization and that of infiltration of the adhesive monomers, leaving a strip of collagen exposed at the base of the hybrid layer, which would be subject to degradation by MMPs [3). Consequently, a drop in bond strength over time would be expected for the control group, as observed in other studies [14,15]. This degradation would be particularly stimulated by the storage used in the study, the solution simulating biological fluid, which contains calcium and zinc, which stimulate the activity of MMPs [12].

Thus, the bond stability seen even in the control group can be attributed to the storage time, since other studies that tested the etch-and-rinse adhesive systems also found bond strength stability after 6 months for this adhesive system [16]. There was no difference between the groups tested about failure mode, with a higher prevalence of mixed failures, which may indicate that the adhesive interface was preserved in some way, even after 6 months of storage. From the data in figure 2, it can also be seen that the samples that were subjected to the application of GT for 60 seconds performed less well than the other groups. This result can be attributed to the pre-treatment time, which may have promoted some deposition of the GT components on the dentin, as previously mentioned.

Clinically, the GT extract solution can be used at a concentration of 1.3% for 30 seconds, obtaining satisfactory bond strength after 24 hours, but its effect on bond stability must be proven in studies with longer storage times since the control group alone also showed bond strength stability.

## CONCLUSION

Although pre-treatment with GT at 1.3% for 30 seconds increased immediate bond strength, there was no difference compared to the control group after 6 months.

Conflict of interest: The authors declare that there are no conflicts of interest.

Data availability: The research data are available in the body of the document.

## Contributors

GCS Azevedo, data curation, investigation, methodology, project administration resources, writing – original draft, writing – review & editing. PG Montagner, investigation, methodology, writing – original draft, writing – review & editing. RLX Consani, Turssi CP, and RTB Höfling, writing – review & editing. FLB Amaral, data curation, investigation, methodology, project administration, resources, supervision, validation, visualization, writing – original draft, writing – review & editing.

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