

Effect of chlorhexidine, green tea and egcg as therapeutic primers to increase the durability of resin-dentin bond

Efeito da clorexidina, do chá verde e do EGCG como primers terapêuticos para aumentar a durabilidade da adesão resina-dentina

Daphne Camara BARCELLOS¹, Beatriz Maria FONSECA¹, Eduardo BRESCIANI¹, César Rogério PUCCI¹, Maria Amélia Máximo De ARAÚJO¹

1 – São Paulo State University (Unesp) – Institute of Science and Technology – São José dos Campos – SP – Brazil.

ABSTRACT

Objective: This study evaluated the effect of 0.2% chlorhexidine gluconate solution (CHX), green tea and active epigallocatechin-gallate (EGCG) used as therapeutic primers on the long-term bond strength of etch-and-rinse adhesive to dentin. **Material and Methods:** Eighty bovine incisors were worn to expose an area of dentin, that were acid-etched (37% phosphoric acid) and rinsed. The teeth were divided into 4 groups (n = 20): Group C (Control) - Single Bond; Group CHX - 0.2% CHX for 30s + Single Bond; Group EGCG - active EGCG gel at 10 μ M for 30 s + Single Bond; Group GT - aqueous green tea for 30s + Single Bond. Blocks of composite were fabricated and stored for 24 h or 6 months, sectioned into beams and submitted to microtensile tests. Results were analyzed by two-way ANOVA and Tukey's test (5%). **Results:** Mean (\pm SD) values (in MPa) were as follow: CHX (24 h) – 41.76 (\pm 2.62); C (24 h) - 40.81 (\pm 3.35); GT (24 h): 37.38(2.98); CHX (6 months) - 36.04 (\pm 3.52); EGCG (24h) - 35.91 (\pm 4.82); EGCG (6 months) - 35.75 (\pm 4.44); GT (6 months) - 31.95 (\pm 3.40); C (6 months): 30.05 (\pm 1.54). **Conclusion:** EGCG produced resin-dentin bonds that did not change after 6 months water storage but it decreased the immediate bond strength when compared to control and chlorhexidine groups.

KEYWORDS

Tensile bond strength; Dentin; Total-etch adhesives; Chlorhexidine gluconate; Green tea.

RESUMO

Objetivo: este estudo avaliou o efeito da solução de gluconato de clorexidina 0,2% (CHX), do chá verde e do componente ativo epigallocatequina 3-gallato (gel de EGCG) usados como primers terapêuticos sobre a resistência de união longitudinal de adesivo convencional a dentina. **Material e Métodos:** oitenta dentes bovinos foram desgastados para obter uma área de dentina plana, que foi condicionada (ácido fosfórico à 37%) seguida de lavagem. Os dentes foram divididos em 4 grupos (n-20): Grupo C (Controle) - Single Bond; Grupo CHX - 0,2% CHX por 30s + Single Bond; Grupo EGCG - gel de EGCG 10 μ M por 30s + Single Bond; Grupo GT - chá verde aquoso por 30s + Single Bond. Blocos de resina composta foram fabricados e armazenados por 24 h e 6 meses, seccionados e submetidos ao teste de resistência a microtração. Os resultados foram analisados por ANOVA dois-fatores seguido pelo teste de Tukey. **Resultados:** Valores de média (\pm Desvio-padrão) (MPa): CHX (24 h) – 41,76 (\pm 2,62)a; C (24 h) – 40,81 (\pm 3,35)ab; GT (24h): 37,38(2,98)abc; CHX (6 meses) – 36,04 (\pm 3,52) bcd; EGCG (24h) – 35,91 (\pm 4,82)cd; EGCG (6 meses) – 35,75 (\pm 4,44)cd; GT (6 meses) – 31,95 (\pm 3,40)de; C (6 meses): 30,05 (\pm 1,54)e. **Conclusão:** O gel de EGCG produziu uma interface adesiva dentina-resina que não alterou os valores de resistência de união após 6 meses de armazenagem em água, mas reduziu a resistência de união para o tempo de 24 h quando comparado com os grupos Controle e CHX.

PALAVRAS-CHAVE

Resistência de união; Dentina; Adesivos convencionais; Gluconato de clorexidina; Chá verde.

Clinical Significance

The EGCG negatively influenced the immediate bond strength, but produced resin-dentin bonds that did not change after 6 months water storage.

Significância Clínica

O gel de EGCG influenciou negativamente a resistência de união imediata, mas produziu uma interface de união dentina-resina que não alterou a resistência de união após 6 meses de armazenagem.

INTRODUCTION

Since Buonocore [1] introduced acid etching of enamel, advances have occurred in adhesive restorative procedures. With the development of adhesive systems, the desired retention of restorative material in the cavity changed dramatically the fundamentals of cavity preparation. Despite the immediate adhesion of adhesive systems to dentin seem effective [2], the longevity of the hybrid layer has been questioned as a consequence of hydrolysis of the adhesive interface over time [2,3].

Degradation of the hybrid layer may be triggered by various factors. The degradation of collagen fibers exposed due failures in hybrid layer formation has been accepted as a main factor in the degradation of the resin-dentin interface [3,4]. Dentin has endopeptidase enzymes, calcium and zinc-dependent, also called matrix metalloproteinases (MMPs) that can be activated by acid etching during adhesive procedure [4], and are responsible for rapidly degrade denatured collagen which were left unprotected after the process of demineralization by acid etching and not infiltrated by the adhesive monomers [5].

Among the MMPs identified in the organic matrix dentin are gelatinases (MMP-2 and -9) collagenase (MMP-8) and enamelysin (MMP-20) [6]. The collagenolytic activity of these enzymes tends to increase gradually, suggesting a self-activation or the progressive failure of its tissue inhibitors [6,7]. Several in vitro and in vivo studies have been accomplished with the aim of improving the adhesive interface using inhibitors of the MMPs in the adhesive protocol

[8 - 10].

Thus, the use of chlorhexidine digluconate (CHX) disinfectant solution prior to the use of a dentin bonding agent have been investigated in order reduce the degradation of the hybrid layer [8,10] due to their MMP inhibitory properties [11].

The green tea is rich in polyphenols, particularly the epigallocatechin-gallate (EGCG), which has hydrophobic interactions with collagenases and gelatinases enzymes and hydrogen bonds, capable of modifying the secondary structure of MMPs [12]. EGCG have been demonstrated its ability to inhibit the activity of these MMPs [12-15].

Alternative modification of adhesive protocols in dentin has been investigated to reduce the degradation of collagen fibers exposed in hybrid layer. CHX [8,10] and polyphenols in green tea [16,17] have been used as alternatives to improve the bond strength between the dentin and adhesive system at resin-dentin interfaces. Therefore, longitudinal evaluation of the dentin bond strength using these substances has become of primordial importance.

The aim of this study was to evaluate in vitro, the influence of CHX, Green tea and EGCG on the microtensile bond strength to dentin of the two-step total-etch adhesive at time intervals of 24 h and after 6 months period of storage in water at 37°C. This study tested two null hypotheses: 1) CHX green tea and EGCG applied prior to the adhesive system does not affect the immediate bond strength to dentin; 2) the storage period does not affect the bonding

effectiveness of two-step total-etch adhesive to dentin.

MATERIAL AND METHODS

Materials used in this study, chemical composition and manufacturers are presented in Table I.

Table I - Materials, composition and material's manufacturers

Materials	Manufacturers	Composition
Condac 37	FGM, SC, Brazil	Phosphoric acid 37%
Single Bond 2	3M ESPE St.Paul, MN, USA	Bonding agent: Bis-GMA, HEMA, dimethacrylate, methacrylate functional copolymer of polyacrylic and polytaconic acid, water, alcohol, photoinitiator
Amelogen Plus	Ultradent, Indaiatuba, SP, Brazil	Resin composite: Bis-GMA, TEGDMA, microparticles barium, amine, silicate, borate

* Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-Hydroxyethyl methacrylate; TEGDMA, triethylene glycol-dimethacrylate.

Eighty freshly extracted bovine incisor teeth were used in this study. The roots were sectioned with a steel diamond disc (KG Sorensen, Rio de Janeiro, Brazil) at the cemento-enamel junction. The labial surfaces were abraded using 400 grit abrasive papers coupled to a circular polishing machine (PA-10, Panambra, São Paulo, Brazil), under water cooling, to obtain a 5 X 5 mm area of flat dentin.

The dental surface was abraded to standardize the remaining dentin thickness at 2 mm, using a caliper thickness gauge (Golgran, São Caetano do Sul, Brazil). The smear layer was standardized using 600 grit abrasive papers coupled to a circular polishing machine, under

water cooling.

Bonding Procedures

The teeth were divided into 4 groups (n = 20), according to the adhesive protocol:

- Group C (Control): The surfaces were etched for 15 s with 37% phosphoric acid gel, rinsed and the excess moisture was removed with absorbent paper. Two layers of Single Bond total-etch adhesive (3M ESPE, St. Paul, MN, USA) were applied on the surface actively for 15 s and gently air dried for 10 s. The adhesive was light activated for 10 s with a LED light unit (Emitter A, Schuster, Santa Maria, RS, Brazil) with power density of 600 mW/cm².

- Group CHX: The surfaces were etched following the same protocol applied for Group C. An aliquot of 1.5 μ l of 0.2% chlorhexidine digluconate disinfectant solution (Byofórmula, São José dos Campos, SP, Brazil) was applied using a micropipette (International Labmate Limited, Hertfordshire, UK), prior to adhesive actively for 30s with mini brushes, and excess of CHX was removed with absorbent paper, leaving a moist substrate surface. The Single Bond total-etch adhesive (3M ESPE) was applied following the same protocol applied for Group C.

- Group GT: The surfaces were etched following the same protocol applied for Group C. An aliquot of 1.5 μ l of aqueous green tea was applied using a micropipette prior to adhesive actively for 30s with mini brushes. The aqueous green tea was prepared by infusing the herb (2 g Cammelia Sinensis exposed to steam for 180 ml of boiling water, with boiling time of 1 min to 100°C). Excess of green tea was removed with absorbent paper, leaving a moist substrate surface. The Single Bond total-etch adhesive (3M ESPE) was applied following the same protocol applied for Group C.

- Group EGCG: The surfaces were etched following the same protocol applied for Group

C. An aliquot of 1.5 μ l of 10 μ M EGCG gel (BR Patent 10 2014 014187 1 A2, University of São Paulo) was applied using a micropipette prior to adhesive actively for 30 s with mini brushes, rinsed for 30 s and the excess moisture was removed with absorbent paper. The Single Bond total-etch adhesive (3M ESPE) was applied following the same protocol applied for Group C.

Restoration placement

Composite resin blocks (Amelogen Plus, Ultradent, Indaiatuba, SP, Brasil), approximately 4 mm high were built on the treated surfaces. Each 2 mm portion was light activated for 40 s.

The bonded teeth were stored in distilled water at 37°C, for time intervals of 24 h or 6 months. The water was changed every week during the course of 6 months [16].

Microtensile Bond Strength Test

The test specimens were cut into parallel sections measuring approximately 1 mm, made from the mesial to the distal, and from the cervical to the incisal surface, using a diamond disc attached to a Labcut 1010 (Extec Technologies Inc., USA) cutting machine to obtain sticks, producing a minimum of 7 sticks per tooth. The sections were fabricated at low speed under water cooling to prevent stress induction at the bond interface.

The sticks were attached to a microtensile device in a universal testing machine (DL-1000, EMIC, São José dos Pinhais, PR, Brazil), with a 10 kg load cell, at a cross-head speed of 0.5 mm/min, in accordance with the ISO 11405 Standard. The bond strength data were expressed in Megapascals (MPa).

After the microtensile test, the specimens were analyzed with a 20x stereomicroscope (Stemi 2000 - Karl Zeiss, Germany). Fractures were classified as: cohesive in the substrate, cohesive in the composite, adhesive or mixed.

Statistical Analysis

Pre-test failures (PTFs) were not included in statistical analyses. The cohesive failure (dentin or composite) specimens were discarded from data analysis. The mean bond strength value for the beams originating from each tooth was calculated and used for the statistical analysis.

Data, expressed in MPa, were analyzed by two-way ANOVA (treatment X storage time) followed by Tukey test ($\alpha = 5\%$).

Scanning electron microscopy (SEM) examination

Two teeth from each group were prepared for SEM analysis. The specimens were sectioned perpendicularly to the bonding interface. The sections were polished with 2000 and 4000 mesh sheets. Phosphoric acid etchant was applied for 15 s, rinsed off with water for 10 s. Specimens were dehydrated, sputter-coated with gold-palladium and examined by SEM (high vacuum, 15KV, Everhart-Thornley Detector/ETD, 2000X magnification).

RESULTS

Two-way ANOVA revealed that EGCG group presented lower bond strength values compared with the other groups ($p = 0.00$) and storage in water for 24 h presented higher bond strength values compared with storage in water for 6 months ($p = 0.00$).

The cross-product treatment vs. storage time was statistically significant ($p = 0.00$) (Table 2). The Group CHX assessed after 24 h showed the highest bond strength values. The Group C with storage interval of 6 months presented the worst bond strength values. EGCG group presented lower immediate bond strength compared with the Groups CHX and C (Control). Significant reduction of the bond strength values were observed for all the groups after 6 months of water storage, except to EGCG group.

Table II - Overall microtensile bond strength values and the respective standard deviations (MPa) obtained in each experimental condition

GGroup	Treatment	Storage time	Mean (\pm S-D) Homogeneous sets
CCHX	Chlorhexidine	224 h	41.76 (\pm 2.62) a
CC	Control	224 h	40.81 (\pm 3.35) ab
GGT	Green tea	224 h	37.38 (\pm 2.98) abc
CCHX	Chlorhexidine	66 months	36.04 (\pm 3.52) bcd
EEGCG	EGCG	224 h	35.91 (\pm 4.82) cd
EEGCG	EGCG	66 months	35.75 (\pm 4.44) cd
GGT	Green tea	66 months	31.95 (\pm 3.40) de
CC	Control	66 months	30.05 (\pm 1.54) e

S-D = Standard Deviation.
Means followed by the same letters do not differ statistically ($p > 0.05$).

A low percentage of cohesive failure in dentin occurred for all groups after 6 months. The specimens presented PTFs only after 6 months of

water storage for all tested conditions. Figures 1 and 4 show SEM images obtained of the interfaces created in all groups.

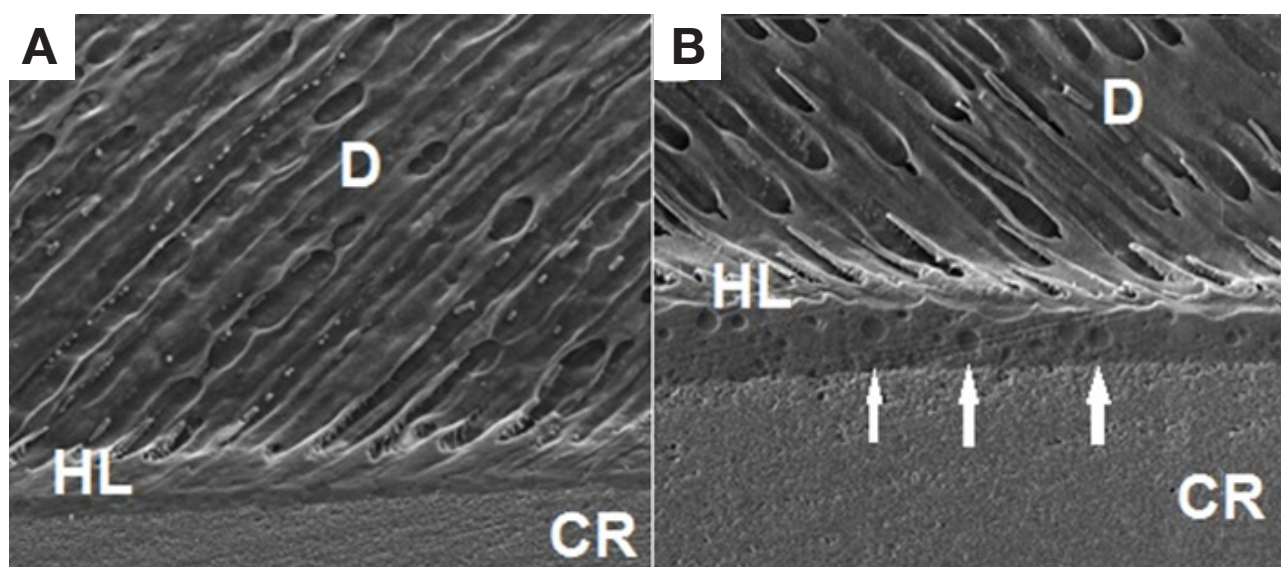


Figure 1 - A and B – Hybrid layer formed by Group C (Control), storage for 24 h and 6 months. A typical hybrid layer was created (Figure 1A). Adhesive resin with bubbles (arrows), which can indicate degradation due hydrolysis (Figure 1B). (Legends: CR – Composite resin; HL – Hybrid Layer; D – Dentin).

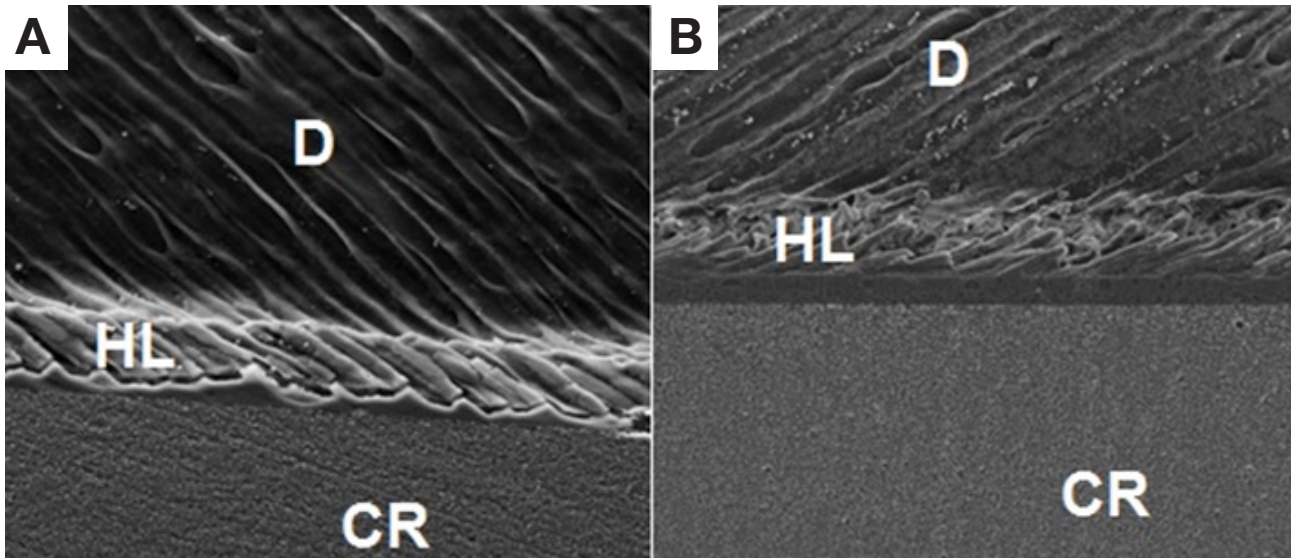


Figure 2 - A and B – Hybrid layer formed by Group CHX, storage for 24 h and 6 months. CHX used as a therapeutic primer did not interfere the formation of hybrid layer. (Legends: CR – Composite resin; HL – Hybrid Layer; D – Dentin).

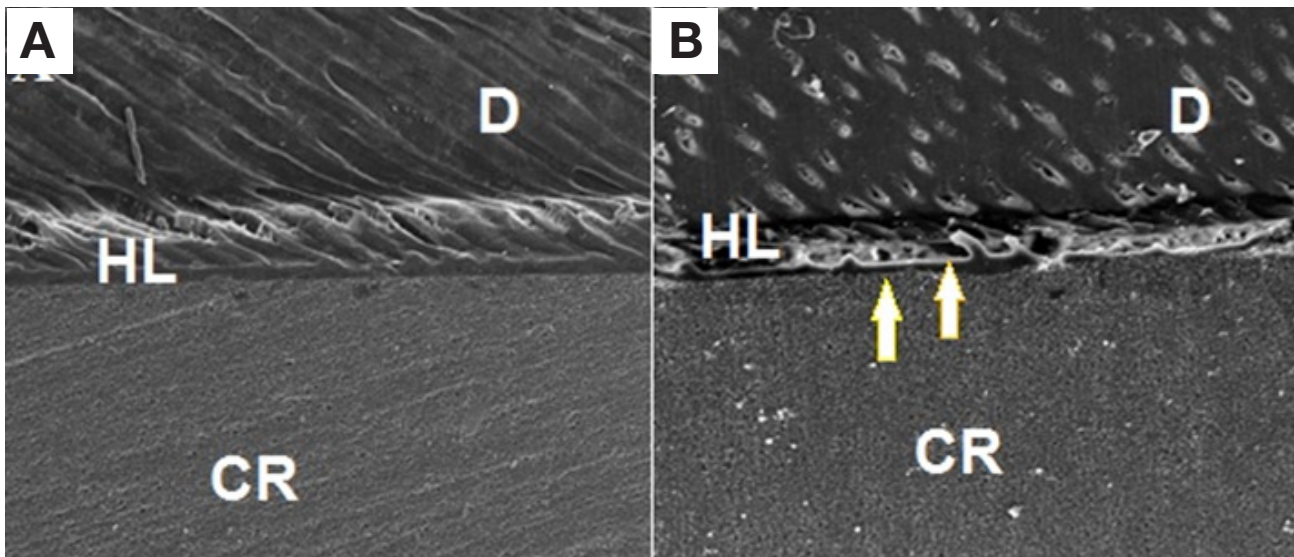


Figure 3 - A and B – Hybrid layer formed by Group GT, storage for 24 h and 6 months. A typical hybrid layer was created. GT used as a therapeutic primer created a typical hybrid layer. Interface with presence of gaps between resin and adhesive (arrows) (Figure 3B). (Legends: CR – Composite resin; HL – Hybrid Layer; D – Dentin).

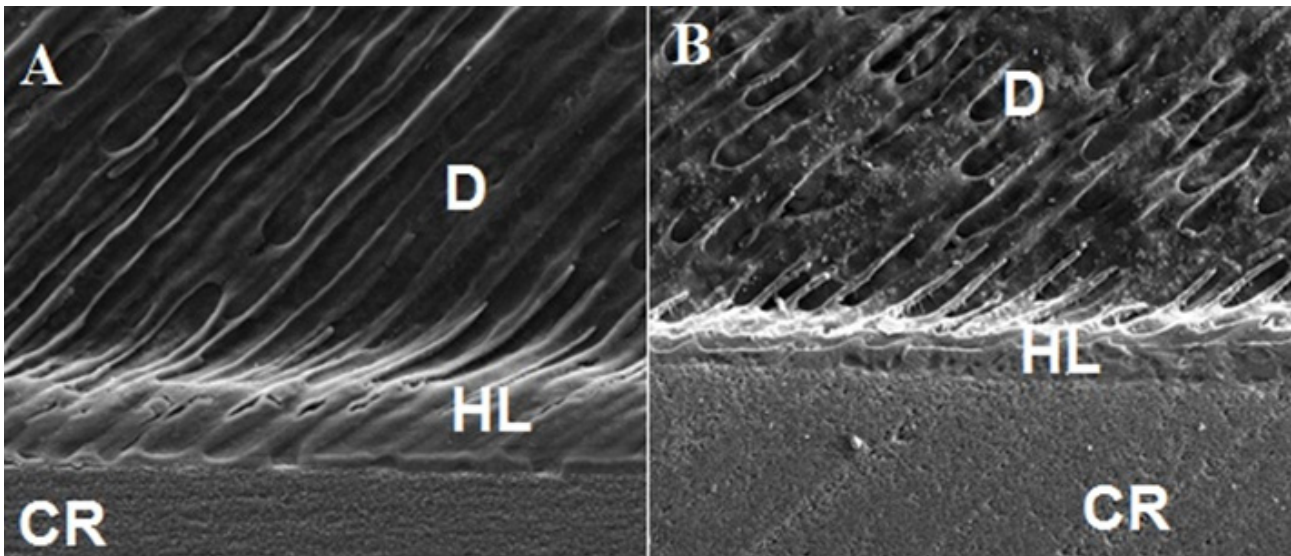


Figure 4 - A and B – Hybrid layer formed by Group EGCG, storage for 24 h and 6 months. EGCG used as a therapeutic primer created and interface micromorphology similar to Group C (Control). No areas of degradation of hybrid layer, over time, were observed (Figure 4B). (Legends: CR – Composite resin; HL – Hybrid Layer; D – Dentin.)

DISCUSSION

The first null hypothesis was rejected, because the EGCG showed lower bond strength values compared with the other groups (C and CHX). Some substances are being applied in adhesive protocol as potential MMP-inhibitors, such as CHX, green tea, and some polyphenols as EGCG [8,10,16,17].

CHX is an antiseptic and antimicrobial solution [18], which inhibits bacterial adhesion to dental surfaces by competing with calcium or retention sites. Therefore, this solution can prevent the formation of calcium-link between bacteria-dental surfaces or between bacteria-bacteria [19]. This mechanism may be effective in the inhibition of MMPs, since these are zinc and calcium-dependent proteases [8].

Green tea has been used for many years in the medical field because of its benefits to general health [20]. In dentistry, green tea has been used due to its antimicrobial action, low toxicity [21] and fluoride present in its composition [22]. Green tea has shown efficacy in decrease the progression

of erosion [23] in periodontal health maintenance [24], and the ability to inhibit MMPs [12].

Green tea has about 4.000 bioactive components, and a third consisting of polyphenols [25] that are related to their functional properties. Among the polyphenols, catechin EGCG is the most active and abundant [26]. Previous studies have shown that EGCG is effective in reducing acid production in biofilm by *S. mutans* [27] and halitosis [28]. Also, EGCG provides hydrophobic interactions with collagenases and gelatinases, and can modify the secondary structure of MMPs, inhibiting their activity [29].

Du et al. [16] observed that EGCG incorporation in Single Bond at 100, 200 and 300 $\mu\text{g/ml}$ can increase the bond strength to dentin immediate and can improve it durability after 6 months of in vitro water storage. However, our results demonstrate reduction in bond strength when EGCG was applied previously to adhesive. This result showed that the use of EGCG gel, as an adjuvant in dentin bonding, can chemically interfere the immediate adhesion of resin

materials. EGCG can be interacting negatively with dental adhesive and/or dentin, decreasing the immediate bond strength.

In this study, we used an aliquot of 1.5 μ l, which contained 0.00687 μ g of active EGCG, low concentration when compared to concentrations that Du et al.[16] incorporated in the Single Bond. Also, the gel solution was rinsed prior to the adhesive application, which can have reduced the final concentration of EGCG incorporated into the dentin. Maybe an aqueous solution with higher concentrations of EGCG, without the rinsing step, could improve the dentin bond strength and the bond durability of the adhesives. However, more research is needed for further evaluation, because it can be noted by SEM analysis, that EGCG used as a therapeutic primer created and interface micromorphology similar to Group C (Control) for 24 h of water storage and for 6 months, no areas of degradation of hybrid layer were observed (Figure 4A and 4B).

The second null hypothesis was rejected, because the storage time of 24 h presented higher bond strength values in comparison with the storage time of 6 months. These results are in agreement with various studies [30-32] that observed a reduction in dentin bond strength for the Single Bond when stored in water, due to degradation of the hybrid layer. Degradation of the hybrid layer can be due to: (1) the activation of MMPs by etching procedures, because MMPs are capable of degrading unprotected collagen fibers that are not infiltrated by the adhesive monomer [16]; (2) the incomplete polymerization of infiltrated monomers [33]; (3) the presence of residual solvents may contribute to the formation of a hypertonic zone at the bond interface, increasing its hydrophilicity [34] and (4) the water present in saliva and the intrinsic humidity of dentin (dentinal fluid) play a role in solubilizing the resin polymers [31,35].

For the interaction between "Treatment" and "Storage time", the Group CHX assessed

after 24 h showed the highest bond strength mean values. However, after storage interval of 6 months, significant reduction of bond strength was observed for Group CHX when compared to 24 h. Also, Group CHX assessed after 6 months storage showed higher bond strength when compared with Group C with storage interval of 6 months.

Ionized CHX presents cationic properties, being able to bind to negatively charged surfaces/target sites of diverse substrates. Thus, CHX remained retained in dentin substrates after formation of hybrid layer [35,37], promoting inhibition of the proteolytic activity in dentin and preserving the hybrid layer after longer periods of exposure to water [38].

According to Gendron et al. [11], two mechanisms of action are involved in the inhibition of MMP by CHX: chelation inhibiting MMP-2 and -9, and the interaction with cysteines and/or sulfhydryl groups present in the active site of MMP-8. Recent studies have shown that the use of CHX prior to adhesive system, without rinsing, significantly inhibited the MMPs activity in the hybrid layer [8,11,36,37] even at low concentrations [11].

It can be observed in this study that the use of CHX, as an adjuvant in dentin bonding, did not chemically interfere with the immediate adhesion of resin materials, because there is no difference in bond strength between Group CHX and Group C after 24h storage (Figure 1A and 2A). Also, one noted that the storage time did not interfere so strongly in the bonding of Group CHX when compared with Group C (Figure 2B), which can demonstrate a relative maintenance of bond strength with 0.2% CHX protocol (Group CHX) when compared to control protocol (Group C) (Figure 1B). Therefore, our results demonstrate that CHX can slow the degradation of the hybrid layer, extending the dentin bonding.

According to Carrilho et al. [37] and Brackett et al. [38], long-term action of CHX can

be explained by their confinement in the adhesive interface, since their removal through the dentinal fluid flow is reduced by the formation of resin tags that obliterate the dentinal tubules. Thus, CHX may remain trapped between the collagen fibers during the impregnation of interfibrillar spaces with fluid resin during application of the adhesive [39].

The Group GT assessed after 24 h showed similar bond strength mean values when compared to Group C (Control) (Figure 3A). Also, both groups showed decrease in bond strength after storage interval of 6 months (Figure 3B). The amount of polyphenols present in the aqueous solution of green tea prepared was 0.185 mg/ml, which corresponds to 400 μ M [23]. However, the total volume of aqueous solution applied was 1.5 μ l, which corresponds to 0.27 μ g of polyphenols applied on the dentin surface. Moreover, it is not known exactly for how long the polyphenols remain active after the preparation of green tea, because the polyphenols begin to oxidize after a certain time, which can lead to loss of their properties [23]. Therefore, the low concentration of polyphenols in the aqueous solution of green tea cannot be enough to prevent degradation on bond interface.

Although EGCG Group presented the lowest values of 24h bond strength when compared to the Group C, no significant reduction of bond strength was observed after 6 months of storage (Figure 3A and B).

These results corroborate previous studies that have shown that green tea polyphenols can inhibit the activity of collagenase on collagen degradation, especially EGCG [12,29]. Studies have shown that different concentrations of EGCG prior to Single Bond were able to maintain the bond strength after 6 months of storage in water [16,17].

Madhan et al. [29] speculate that a greater inhibition of collagenase by EGCG can be attributed

to hydrophobic interactions and adherence to the active sites of EGCG by collagenase through hydrogen bonds. In addition, EGCG has the ability to inhibit the activity and expression of MMP -2 and -9, being a non-competitive inhibitor, time- and dose-dependent, without chelation of calcium ion and zinc [13], which are essential for the activity of MMPs [40]. In addition, EGCG has low toxicity, even at high concentrations, and anti-inflammatory action, and may be safely used in any depth of cavity [41].

However, little is known about the actual benefits of the application of EGCG on dentin and its ability to inhibit MMPs. More studies are needed to better understand its mechanism of action and real benefits in maintaining the bond strength of resin-dentin.

CONCLUSION

Within the limitations of this study it can be concluded that:

- EGCG used as a therapeutic primer produced resin-dentin bonds that did not change after 6 months water storage. However, it decreased the immediate bond strength when compared to control group.

REFERENCES

1. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *J Dent Res.* 1955 Dec;34(6):849-53.
2. Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. In vitro degradation of resin-dentin bonds analysed by microtensile bond test, scanning and transmission electron microscopy. *Biomaterials.* 2003 Sep;24(21):3795-803.
3. Garcia-Godoy F, Tay FR, Pashley DH, Feilzer A, Tjaderhane L, Pashley EL. Degradation of resin-bonded human dentin after 3 years of storage. *Am J Dent.* 2007 Apr;20(2):109-13.
4. Mazzoni A, Pashley DH, Tay FR, Gobbi P, Orsini G, Ruggeri A Jr, et al. Immunohistochemical identification of MMP-2 and MMP-9 in human dentin: Correlative FEI-SEM/TEM analysis. *J Biomed Mater Res A.* 2009 Mar 1;88(3):697-703. doi: 10.1002/jbm.a.31920.
5. Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjaderhane L, et al. The requirement of zinc and calcium

- ions for functional MMP activity in demineralized dentin matrices. *Dent Mater*. 2010 Nov;26(11):1059-67. doi: 10.1016/j.dental.2010.07.006.
6. Sulkala M, Tervahartiala T, Sorsa T, Uitto VJ, Larmas M, Salo T, et al. Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin. *Arch Oral Biol*. 2007 Feb;52(2):121-7.
 7. Nishitani Y, Yoshiama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, et al. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. *Eur J Oral Sci*. 2006 Apr;114(2):160-6.
 8. Chang YE, Shin DH. Effect of Chlorhexidine Application Methods on Microtensile Bond Strength to Dentin in Class I Cavities. *Oper Dent*. 2010 Nov-Dec;35(6):618-23. doi: 10.2341/09-360-L.
 9. Lafuente D. SEM Analysis of hybrid layer and bonding interface after chlorhexidine use. *Oper Dent*. 2012 Mar-Apr;37(2):172-80. doi: 10.2341/10-251-L.
 10. Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjaderhane L, Mazzoni A, et al. Host-derived loss of dentin matrix stiffness associated with solubilization of collagen. *J Biomed Mater Res B Appl Biomater*. 2009 Jul;90(1):373-80. doi: 10.1002/jbm.b.31295.
 11. Gendron R, Greiner D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8 and 9 by chlorhexidine. *Clin Diagn Lab Immunol*. 1999 May;6(3):437-9.
 12. Demeule M, Brossard M, Page M, Gingras D, Beliveau R. Matrix metalloproteinase inhibition by green tea catechins. *Biochim Biophys Acta*. 2000 Mar 16;1478(1):51-60.
 13. Garbisa S, Sartor L, Biggin S, Salvato B, Benelli R, Albini A. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. *Cancer*. 2001 Feb 15;91(4):822-32.
 14. Sartor L, Pezzato E, Dell'Aica I, Caniato R, Biggin S, Garbisa S. Inhibition of matrix-proteases by polyphenols: chemical insights for anti-inflammatory and anti-invasion drug design. *Biochem Pharmacol*. 2002 Jul 15;64(2):229-37.
 15. Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. The role of matrix metalloproteinases (MMPs) in human caries. *J Dent Res*. 2006 Jan;85(1):22-32.
 16. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. *J Dent*. 2012 Jun;40(6):485-92. doi: 10.1016/j.jdent.2012.02.013.
 17. Santiago SL, Osorio R, Neri JR, Carvalho RM, Toledano M. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. *J Adhes Dent*. 2013 Dec;15(6):535-40. doi: 10.3290/j.jad.a29532.
 18. Ercan E, Özekinci T, Atakul F, Gül Kadri. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: In vivo study. *J Endod*. 2004 Feb;30(2):84-7.
 19. Gjermeo P. Chlorhexidine and related compounds. *J Dent Res*. 1989;68(Spec Issue):1602-8.
 20. Arab H, Maroofian A, Golestani S, Shafee H, Sohrabi K, Forouzanfar A. Review of the therapeutic effects of *Camellia sinensis* (green tea) on oral and periodontal health. *J Med Plan Res*. 2011;5(23):5465-9.
 21. Schmitz W, Saito AY, Estevão D, Saridakis HO. Green tea as a chemoprotector. *Semina: Ciências Biológicas e da Saúde*. 2005;26(2):119-30.
 22. Shu WS, Zhang ZQ, Lan CY, Wong MH. Fluoride and aluminium concentrations of tea products from Sichuan Province, PR China. *Chemosphere*. 2003 Sep;52(9):1475-82.
 23. Kato MT, Magalhães AC, Rios D, Hannas AR, Attin T, Buzalaf MAR. Protective effect of Green tea on dentin erosion and abrasion. *J Appl Oral Sci*. 2009 Nov-Dec;17(6):560-4.
 24. Kushiyama M, Shimazaki Y, Murakami M, Yamashita Y. Relationship between intake of green tea and periodontal disease. *J Periodontol*. 2009 Mar;80(3):372-7. doi: 10.1902/jop.2009.080510 .
 25. Tariq M, Naveed A, Barkat Ali K. The morphology, characteristics, and medicinal properties of *Camellia sinensis*' tea. *J Med Plants Res*. 2010;4(19):2028-33.
 26. Wang H, Provan GJ, Helliwell K. Tea flavonoids: their functions, utilization and analysis. *Trends Food Sci Technol*. 2000;11(4-5):152-60. doi: 10.1016/S0924-2244(00)00061-3
 27. Hirasawa M, Takada K, Otake S. Inhibition of acid production in dental plaque bacteria by green tea catechins. *Caries Res*. 2006;40(3):265-70.
 28. Xu X, Zhou XD, Wu CD. Tea Catechin EGCg suppresses the *mgI* gene associated with halitosis. *J Dent Res*. 2010 Nov;89(11):1304-8. doi: 10.1177/0022034510378682.
 29. Madhan B, Krishnamoorthy G, Rao RJ, Nair BU. Role of green tea polyphenols in the inhibition of collagenolytic activity by collagenase. *Int J Biol Macromol*. 2007 Jun 1;41(1):16-22.
 30. Osorio R, Pisani-Proença J, Erhardt MC, Osorio E, Aguilera FS, Tay FR, et al. Resistance of ten contemporary adhesives to resin-dentine bond degradation. *J Dent*. 2008 Feb;36(2):163-9. doi: 10.1016/j.jdent.2007.12.002.
 31. Toledano M, Osorio R, Osorio E, Aguilera FS, Yamauti M, Pashley DH, et al. Durability of resin-dentin bonds: effects of direct/indirect exposure and storage media. *Dent Mater*. 2007 Jul;23(7):885-92.
 32. Garcia RN, de Goes MF, Giannini M. Effect of water storage on bond strength of self-etching adhesives to dentin. *J Contemp Dent Pract*. 2007 Nov 1;8(7):46-53.
 33. Eliades G, Vougiouklakis G, Palaghias G. Heterogeneous distribution of single-bottle adhesive monomers in the resin-dentin interdiffusion zone. *Dent Mater*. 2001 Jul;17(4):277-83.
 34. Tay FR, Pashley DH, Garcia-Godoy F, Yiu CK. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part II. Silver tracer penetration evidence. *Am J Dent*. 2004 Oct;17(5):315-22.
 35. Park JG, Ye Q, Topp EM, Misra A, Spencer P. Water sorption and dynamic mechanical properties of dentin adhesives with a urethane-based multifunctional methacrylate monomer. *Dent Mater*. 2009 Dec;25(12):1569-75. doi: 10.1016/j.dental.2009.07.010.

36. Loguercio AD, Stanislawczuk R, Polli LG, Costa JA, Michel MD, Reis A. Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. *Eur J Oral Sci.* 2009 Oct;117(5):587-96. doi: 10.1111/j.1600-0722.2009.00663.x.
37. Carrilho MRO, Geraldini S, Tay F, de Goes MF, Tjäderhane L, Reis AF, et al. In vivo preservation of the hybrid layer by chlorhexidine. *J Dent Res.* 2007 Jun;86(6):529-33.
38. Brackett MG, Tay FR, Brackett WW, Dib A, Dipp FA, Mai S, et al. In vivo chlorhexidine stabilization of hybrid layers of an acetone-based dentin adhesive. *Oper Dent.* 2009 Jul-Aug;34(4):379-83. doi: 10.2341/08-103.
39. Breshi L, Cammelli F, Visintini E, Mazzoni A, Vita F, Carrilho M, et al. Influence of chlorhexidine concentration on the durability of eatch-and-rinse dentin bonds: a 12-month in vitro study. *J Adhes Dent.* 2009;11(3):191-8.
40. Yun JH, Pang EK, Kim CS, Yoo YJ, Cho KS, Chai JK, et al. Inhibition effects of green tea polyphenol (-)epigallocatechin gallate on the expression of matrix metalloproteinase-9 and on the formation of osteoclasts. *J Periodontal Res.* 2004 Oct;39(5):300-7.
41. Isbrucker RA, Bauschi J, Edwards JA, Wolz E. Safety studies on epigallocatechin gallate (EGCG) preparation. Part 1: genotoxicity. *Food Chem Toxicol.* 2006 May;44(5):626-35.

Daphne Camara Barcellos
(Corresponding address)

Av. Eng. Francisco José Longo, nº 777
Jardim São Dimas
12245-000 - São José dos Campos, SP - Brasil

Date submitted: 2016 Sep 17

Accept submission: 2016 Nov 25