Epigallocatechin-3-gallate increased the push out bond strength of an epoxy resin sealer to root dentin

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This study aimed to investigate the effect of epigallocatechin-3-gallate (EGCG) from green tea extract on the push out bond strength of an epoxy resin sealer to root dentin. Seventy single root canal premolars were decoronated and instrumented. The roots were randomly irrigated with different final irrigation protocols (n=16): 17%EDTA (EDTA), 17%EDTA followed by 2.5%NaOCl (EDTA+NaOCl), 17%EDTA followed by 1 mg/mL EGCG (EDTA+EGCG) and 1 mg/mL EGCG (EGCG). Other six root canals were received only 2.5%NaOCl as a control group. One root from each group (n=1) was excluded and prepared for SEM investigation. All root canals were then obturated and horizontally sectioned to perform the push out test. EDTA+EGCG group had the highest bond strength (p<0.05). EGCG group showed higher bond strength than EDTA group (p<0.05). The use of EGCG as a final irrigant significantly increased the push out bond strength of an epoxy resin sealer to root dentin.

Keywords: Bond strength, Collagen, Crosslinking agent, EGCG, Epoxy resin sealer

INTRODUCTION

Three dimensional filling of root canal system after chemomechanical preparation is recognized as the final objective of endodontic therapy1. Properly sealed root canal would prevent further reinfection and potential treatment failure providing long-term success in endodontic treatment6. The root canal sealer plays a major role in maintaining the integrity of the root canal seal. Sealing ability and bonding ability to root canal dentin are therefore the most desirable properties of an ideal endodontic sealer. The resin-typed sealer was introduced to endodontics with the goal of bonding with dentin as well as adhesive restorative materials. Among various types of sealers, an epoxy resin sealer is commonly used in endodontic treatment due to its superior physical properties8,9. An epoxy resin sealer produced the highest bond strength to root dentin compared with other types of sealers8, due to the covalent bonds forming between the epoxide rings and the exposed amino groups in the collagen network of root dentin9. Unfortunately, the final irrigants which have been recommended to use before root canal obturation caused a decrease in the bond strength of an epoxy resin sealer to root dentin7-9. Each endodontic irrigant has its own particular properties that could cause a change in dentin structure and subsequently has a negative effect on the adhesion of an epoxy resin sealer to root dentin10. Endodontic irrigants should be used with consideration of the condition of the dentin surface that is most suitable for bonding.

Various chemical irrigants have been used to enhance ability to clean the root canal system. Both ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) are well known and documented for their properties. Seventeen percent EDTA followed by NaOCl has been used as a final flush to create a clean, smear-free root canal dentin before root canal obturation11,12. Nevertheless, decreased bond strength of an epoxy resin sealer to root dentin after this final irrigation protocol was found13. Final irrigation with EDTA and NaOCl caused collagen degradation influencing the covalent bonds forming between an epoxy resin sealer and root dentin, thus decreasing the bond strength of an epoxy resin sealer to root dentin. It is advised to use only EDTA as a final flush when using an epoxy resin sealer to exposed the collagen fibrils in order to bind to the epoxide rings of an epoxy resin sealer10,13. However, collagen degradation in EDTA-treated dentin was observed over time interfering durability of resin-dentin bonds13.

Biodegradation of collagen in dentin in long term has become the greatest problem of resin-based material leading to interfacial bond failure15. One of the factors that causes collagen degradation is the host-derived matrix metalloproteinases (MMPs) presenting in dentin. The cross-linking agents have been used to improve the resistance to degradation and bond strength at resin-dentin interface. Epigallocatechin-3-gallate (EGCG), a collagen crosslinking agent, is the major polyphenol found in green tea. EGCG is considered as the most important active component in antimutagenic, antioxidative, anticarcinogenic, anti-inflammatory, antimicrobial, antiviral, antihypertensive and hypocholesterolemic properties of green tea, which received the great attention in field of nutrition, health and medicine16-19. In dental field, EGCG could be used as a storage medium for an avulsed tooth20-22. EGCG has been reported to enhance collagen stabilization and inhibit collagenase activity23. EGCG binds to collagen by hydrogen bonding and hydrophobic interaction24, crosslinking collagen and preventing from access of collagenase to the active site. The use of EGCG as a final irrigant may provide collagen stabilization, and
then improve the strength of resin-dentin bonds. This study aimed to investigate the effect of epigallocatechin-3-gallate (EGCG) from green tea extract as an endodontic irrigant on the push-out bond strength of an epoxy resin sealer to root dentin. The null hypothesis was that EGCG had no effect on the push-out bond strength of an epoxy resin sealer to root dentin.

MATERIALS AND METHODS

Sample preparation
The protocol for the present study was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Thailand (Number 40/2015, Study code: HREC-DCU 2015-044). Seventy-human premolars were selected from human extracted teeth for orthodontic purpose and stored in 0.1% thymol solution. The teeth presenting any resorption, calcification, crack, open apex or apical curvature were excluded.

The teeth were decoronated to a standardized length of 15 mm. A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to determine patency and 1 mm was subtracted to establish working length. The root canals were shaped with the ProTaper Universal NiTi rotary system (Dentsply Maillefer) to a standardized master apical file #30, 0.09 taper, while irrigating with 1 mL of 2.5% NaOCl (Faculty of Dentistry Chulalongkorn University, Bangkok, Thailand) between files. Irrigation was performed using a 5 mL disposable plastic syringe with 30-gauge needle passively placed up to 2 mL from the working length without binding.

EGCG was prepared by mixing the powder in the EGCG capsule (Giffarine, Thailand) with 35 mL distilled water to 1 mg/mL concentration. The quantitative analysis of EGCG in the EGCG capsule was determined by high-performance liquid chromatography (HPLC).

The roots were randomly divided into four groups (n=16) according to the final irrigation protocols: EDTA group, 5 mL of 17% EDTA 5 min; EDTA+NaOCl group, 5 mL of 17% EDTA 5 min followed by 5 mL of 2.5% NaOCl 1 min; EDTA+EGCG group, 5 mL of 17% EDTA 5 min followed by 5 mL of 1 mg/mL EGCG 5 min; EGCG group, 5 mL of 1 mg/mL EGCG 10 min. Other six root canals were received 5 mL of 2.5% NaOCl 1 min as a control group.

The samples were prepared by post-graduated endodontic student. They were coded in number and blinded to the examiner.

Scanning electron microscope (SEM) investigation of root canal dentin surface
One root canal from each group was taken for SEM investigation. The groove was cut on the buccal and lingual surface. The roots were then split with hammer and chisel. The root halves from the middle thirds were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 and prepared with an ethanol dehydration protocol. Each specimen was mounted on aluminium stubs, coated with gold/palladium and examined under SEM (JSM-5410LV, JEOL, Tokyo, Japan).

Push-out test
The root canals were dried with paper points and obturated with gutta-percha cone and epoxy resin sealer (AH plus, Dentsply Maillefer) using lateral condensation technique. Specimens were stored at 37°C and 100% humidity for 48 h and then embedded in clear self-cured resin in a plastic PVC mould. After leaving resin to polymerize overnight, each root was horizontally sectioned into 1 mm slice using a slow-speed, water-cooled diamond saw (Isomet 1000, Buehler, USA). Two slices were collected from the middle thirds of each root. The root slice was mounted to a universal testing machine (ez-s, Shimadzu, Japan). Metallic load applicator with rounded cross-sectional area of 0.5 mm in diameter was contact with only the root filling. The smaller diameter of the slice was positioned upward. The force was perpendicularly applied to the root canal filling material with 1 mm/min crosshead speed. The force at failure was recorded in Newton when interfacial failure or dislodgement was indicated by the sudden drop in the load-displacement curve. The push out bond strength was measured in MPa by dividing the force by the bonding area according to following formula:

\[
\text{Push out bond strength (MPa)} = \frac{\text{maximum load (N)}}{\text{adhesion area of root filling (mm²)}}
\]

The adhesion (bonding) surface area of each section was calculated as: \((\pi r_1^2 + \pi r_2^2) \times L\), where \(L = \sqrt{(r_1 - r_2)^2 + h^2}\); \(r_1\) and \(r_2\) are the larger and smaller radii, respectively, and \(h\) is the thickness of the section in mm.

Failure mode
After the push out test, the tooth slice was examined under stereomicroscope to determine the mode of failure. Each sample was evaluated at 20x magnification and classified into one of the categories.

1. adhesive failure at the dentin/sealer interface (no sealer visible on dentin walls)
2. cohesive failure within the sealer (dentin walls totally covered with sealer)
3. mixed failure (both adhesive and cohesive failures could be observed)

The effect of final irrigation protocols on push-out bond strength were analyzed by one-way analysis of variance. Post hoc comparisons were performed using Tukey multiple comparisons. The significance level was set at \(p<0.05\).

RESULTS
Table 1 shows the mean and standard deviation values of the bond strength and failure pattern. EDTA+EGCG group significantly showed the highest push out bond strength \((p<0.05)\). EGCG group showed higher bond strength compared to other irrigation regimens.
Table 1  Bond strength values and failure patterns of the experimental groups

| Groups            | Bond strength (MPa) Mean±SD | Failure pattern(n) |   |
|-------------------|----------------------------|-------------------|---|
|                   | Adhesive Cohesive Mixed    |                   |   |
| NaOCl (control)   | 0.37±0.11\textsuperscript{d} | 5                 | 0 | 5 |
| EDTA              | 1.54±0.31\textsuperscript{c} | 0                 | 12| 18|
| EDTA+NaOCl        | 1.28±0.28\textsuperscript{c} | 0                 | 9 | 21|
| EDTA+EGCG         | 3.59±0.39\textsuperscript{b} | 0                 | 18| 12|
| EGCG              | 2.16±0.42\textsuperscript{a} | 0                 | 11| 19|

Different letters represent statistically significant difference between groups (\(p<0.05\)).

**DISCUSSION**

Inability to establish and maintain the fluid-tight seal of the root canal system after chemomechanical preparation may lead to leakage, reinfection and treatment failure. Root canal sealer is essential for creating an impervious seal in disinfected root canal system. Although methacrylate resin-typed sealers have been developed to improve the bond with the root canal dentin, a conventional epoxy resin root canal sealer showed greater properties\textsuperscript{3,4}, and higher bond strength than those\textsuperscript{5}. Due to its desirable properties, an epoxy resin sealer is still a commonly used sealer and group) resulted in the lowest bond strength.

Regarding the failure mode, the mixed and cohesive failures predominated in all experimental groups. Adhesive failure was indicated only in the control group.

Figure 1 shows the SEM image of the root canal dentin surface following different final irrigation protocols. An exposed intact porous collagen network could be seen in all experimental groups. The control group showed a thick smear layer covering root canal dentinal wall compared to a smear-free, opened dentinal tubule in EDTA group (Fig. 2).

Fig. 2  (a) The dense smear layer covering the dentinal surface was presented in the control group which received only 2.5% NaOCl (magnification ×5,000). (b) A smear-free, opened dentinal tubule was clearly seen in EDTA group (magnification ×5,000).

Fig. 1  Demineralized uncollapsed collagen network was indicated in all experimental groups (a) EDTA+NaOCl group (magnification ×20,000) (b) EDTA group (magnification ×20,000) (c) EDTA+EGCG group (magnification ×20,000) and (e) EGCG group (magnification ×20,000). (d,f) A high magnification of c and e, respectively (magnification ×40,000).

strength than EDTA group with statistical significance (\(p<0.05\)). There was no significant difference in the mean bond strength value between EDTA and EDTA+NaOCl group (\(p>0.05\)). Final irrigation with NaOCl (control
may be considered a gold standard for new root canal sealer testing. The covalent bonds forming between the epoxide ring of an epoxy resin sealer and the exposed collagen network in root dentin has first been theorized to be involved in its high bond strength. The theory has not been widely accepted because of the lack of evidence. However, recent study confirmed the bond between the epoxide ring and the collagen network using a fourier transform infrared spectroscopy analysis. Thus, chemical irrigants should be used with consideration as it caused a change to the collagen network in dentin and affected the bond strength of an epoxy resin sealer. In this present study, the push out bond strength of an epoxy resin sealer was shown to be affected by the final irrigation protocols. The result of the bond strength to root canal dentin can be ordered: NaOCl(control)<EDTA<EGCG<EDTA+EGCG.

The null hypothesis was rejected. Group which irrigated with 17% EDTA followed by EGCG showed the highest bond strength. EGCG, a group of polyphenol, is well known for its powerful antioxidative activity. According to previous study, EGCG is capable of intermolecular crosslinking between collagen. Seven hydroxyl groups of EGCG react with carboxyl group in collagen molecule, forming esteric bond. Moreover, Madhan et al. demonstrated the involvement of hydrogen bonding and hydrophobic interactions as the major forces involving in the stabilization of collagen. EGCG has been treated to collagen-based biomaterials in order to improve their mechanical strength and biodegradation. We suggested that collagen stabilization by EGCG resulted in the highest push out bond strength in EDTA+EGCG group. In addition, EGCG group showed greater bond strength over EDTA group because EGCG also owns chelating ability. The exposed collagen after using EDTA, in fact, undergoes collapse according to the formation of interpeptide hydrogen bonding. EGCG not only binds to collagen causing collagen stabilization and preventing from access of collagenase to the active site, EGCG itself also potentially inhibits the collagenolytic activity by collagenase.

This study found no statistical difference between the final flush with EDTA and EDTA followed by NaOCl in contrast to the findings of Neelakantan et al. NaOCl is known to cause collagen degradation resulting in the decrease in the bond strength of an epoxy resin sealer as the epoxide ring of an epoxy resin sealer is unable to form the covalent bonds with the exposed amino group in the root canal dentin collagen network. However, in this present study, the SEM image revealed that the exposed porous collagen network remained intact even after final irrigation with 2.5% NaOCl for 1 min following 17% EDTA. This may explain those contradictory research results whether the final flush with NaOCl after 17% EDTA affects the bond strength of an epoxy resin sealer. The use of NaOCl final flush in higher concentration or longer time period may result in collagen degradation decreasing the bond strength of an epoxy resin sealer to root dentin.

Time used for irrigation in this study (5–10 min) seems irrelevant to clinical endodontic practice. Previous study demonstrated the improved mechanical properties of demineralized dentin according to potential collagen cross-link of EGCG after 12 h treated with EGCG. However, appropriated time for irrigation in clinic should not be too long. In our pilot study, 5 min EDTA irrigation is suitable to see clearly intact porous collagen in the middle third of root canal. In addition, another study reported the use of 10 min PA (Proanthocyanidins, a group of polyphenol found in grape seed) irrigation to enhance the bond strength and durability between resin-based sealer and root dentin. To best of our knowledge, this is the first study to use EGCG as an endodontic final irrigant in order to stabilize the collagen and improve the bond strength of an epoxy resin sealer. Further study on the proper concentration and time of EGCG used as a root canal irrigant is recommended.

Bond failure analysis after the push out test revealed that the most common types of failure mode were mixed and cohesive failure in accordance with Prado et al. This indicates high bond strength of an epoxy resin sealer to root dentin. The adhesive failure was found only in the control group because the dense smear layer along the whole canal inhibited the bond of an epoxy resin sealer and the collagen. The coronal third slices of the specimen were excluded in this study because they were unable to obtain circular shape canal. In addition, coronal third slices of all groups presented only cohesive failure in our pilot study since the oval-shaped canal was irrelevant to circular-shape load applicator.

Although studies found that some final irrigants decreased the bond strength of an epoxy resin sealer, there was no conclusive instruction for clinicians about the proper irrigants used before root canal obturation. The chemical irrigants could cause a change in chemical composition, dentin wettability and collagen degradation which potentially affected the sealing ability of the resin-typed sealer. The exposed collagen network should be preserved even when using an epoxy resin sealer. In this present study, the final flush with EGCG resulted in collagen stabilization which increased the strength of the covalent bonds formed with the epoxide ring of an epoxy resin sealer. In addition, EGCG final irrigation also prevents collagen degradation in long-term. Moreover, EGCG possesses additional antibacterial, antiinflammatory, and antioxidative properties, which could be benefit as a root canal irrigant to endodontic treatment included revascularization. Further study is needed to determine proper concentration and time used of EGCG and evaluate the biodegradation resistance of root dentin over time.

CONCLUSION

Within the limitations of this study, final flush with 1 mg/mL EGCG 5 min after 17% EDTA 5 min significantly increased the push-out bond strength of an epoxy resin sealer.
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