Original Article

The Effect of Different EDTA-Irrigation Time on the Microtensile Bond Strength of Resin Sealers and Root Canal Dentine

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Abstract

This study aimed to determine how the duration of EDTA irrigation affects bond strength. The 160 extracted human premolars were decoronated and embedded in resin block. Root canals were prepared by using the NiTi rotary files and distilled water irrigation, and irrigated with 5 % NaOCl. In group 1, this was followed by irrigation with distilled water, while in groups 2-5, this was followed by irrigation with 17 % EDTA for 1, 3, 5, and 10 min, followed by distilled water. Two specimens of each group were used for scanning electron microscopic observation. The remaining specimens were divided into 2 groups—AH Plus and MetaSEAL (n = 15 each). The specimens were prepared for microtensile tests. The failure mode was identified, and the bond strength value was analysed using one-way ANOVA and Tukey's HSD post-hoc test. The 10-min EDTA-treated specimens (group 5) showed greater microtensile bond strength than non-EDTA-treated specimens (group 1) ($p < 0.001$) in MetaSEAL group. The fractured surface showed mixed failure accounted for the majority of failures in all groups. In SEM observation, the NaOCl group showed a smear layer covering the dentine surface, but the EDTA groups showed an absence of smear layer and various depths of demineralized dentine and exposed collagen. In conclusion, the duration of EDTA irrigation affected on the microtensile bond strength of the methacrylate resin sealer and root dentine.

Keywords: AH Plus, EDTA, MetaSEAL, Microtensile bond strength, Root canal sealer

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Introduction

The elimination of infection within the root canal system, followed by three-dimensional hermetic filling of the root canal spaces is accepted to be the main key to success in endodontic treatment.[1-3] Microleakage of a root canal-treated tooth is the main cause of endodontic failure.[4-6] The traditional obturation technique employing gutta-percha and zinc oxide eugenol sealer is prone to leakage, because of the high solubility of these materials.[7,8] Therefore, to reduce leakage, a root canal sealer has an important role to seal well. This has led to the development of resin sealers.

It is necessary to condition the radicular dentine appropriately to obturate the root canal with the distinct sealer types. If a root canal sealer produces high bond strength, it might reduce the leakage.[9] Obturation using a resin sealer requires dentine surface treatment, such as removing the smear layer to improve bond strength[10,11]; this is commonly done by using a final flush with EDTA and sodium hypochlorite (NaOCl)[12-14] However, NaOCl, a strong oxidizing agent, leaves behind an oxygen-rich layer on dentine surfaces, which inhibits methacrylate resin polymerization[15] and decreases bond strength.[16]

An appropriate irrigation protocol for resin sealer-based root canal obturation has not been reported to date. The majority of studies tend to recommend a final flush with EDTA, followed by water[9,11,17], but the optimal EDTA irrigation duration is not clear. Thus, this study aimed to establish how the duration of EDTA irrigation affects bond strength.

Materials and Methods

Tooth selection

The study protocol was approved by the Ethics Review Committee for Research, Chulalongkorn University. One hundred and sixty single-root human premolars, which were extracted for orthodontic reasons, were stored in distilled water at 4°C until use. Based on 2 perpendicular radiographic views, teeth with roots that were shorter than 13 mm, had an opened apex or calcified root canal, had cracks, fractures, caries, or restorations, were excluded.

Tooth preparation and root canal dentine treatment

All teeth were decoronated at 2 mm above the cemento-enamel junction using a slow-speed diamond saw (Isomet™ 1000 Presicion Saw, Buehler, IL, USA). One millimeter was subtracted from the working length directly obtained using K-file no. 15 (Dentsply Maillefer, Ballaigues, Switzerland). The root canals were embedded in self-cure clear resin tubes, and were then prepared using the rotary files (ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland), starting with S1 to F5, and were irrigated with distilled water 1 ml in needle syringe gauge 25 with slightly vertical agitation; 1 mm shorter than working length between changing each file. Finally, all canals were flushed with 1 ml of distilled water before treatment of the root canal dentine surface.

The root canals were irrigated with 5 % NaOCl (10 ml) for 2 min, and were then divided into 5 groups (Fig. 1b) (n = 32 per group). Group 1 was irrigated with 10 ml of distilled water. Groups 2 to 5 were irrigated with 5 ml of 17 % EDTA in 1, 3, 5, and 10 min, respectively, and then they were irrigated with 10 ml of distilled water. All canals were dried with paper points.

Sample preparation

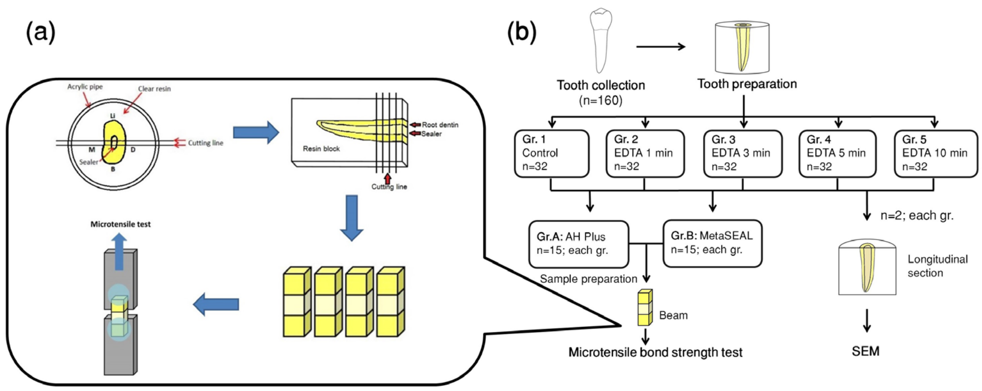
The root dentine surface of 2 specimens from all groups were observed by scanning electron microscopy (SEM, Quanta 250, FEI, Oregon, USA), while the remaining specimens were prepared for tests of bond strength between the root canal dentine surfaces.

Sample preparation for microtensile bond strength test

The root canals were randomly sealed with resin sealer using a needle syringe (gauge 23). Group A was sealed with an epoxy resin (n = 15) (AH Plus, Dentsply

DeTrey, Konstanz, Germany) and group B was sealed with a methacrylate resin sealer (n = 15) (MetaSEAL, Parkell Inc., New York, NJ, USA). Regarding the methacrylate resin sealer group, the sealer underwent light-cured activation for 20 seconds after application. The specimens were then kept in a 100 % humidity incubator at 37°C for a period 3 times longer than the standard setting time (AH Plus: 8 hours and MetaSEAL: 16 hours).

Microtensile test specimens were prepared by cutting beam-shaped samples from the coronal one-third of the root canal, using the slow-speed diamond saw. The 0.6 × 0.6-mm-thick beams were cut at the widest part of the canal that consisted of 2 interfaces (Fig. 1a). At least 4 samples were cut from each specimen. The median bond strength of these samples was recorded as the microtensile bond strength of that root canal.



Gr, group

Figure 1 *Schematic illustration of (a) sample preparation method for the microtensile bond strength test, and (b) research methodology.*

Sample preparation for SEM observation

Two specimens from all groups were cut perpendicularly to the root axis to observe the dentine surface in a controlled root region (13 mm from the root apex); then, they were cut longitudinally through the centre of the bucco-lingual width of the canal, to expose their internal portion.

The specimens were cleaned with distilled water in an ultrasonic bath, fixed in 2.5 % glutaraldehyde for 24 hours, and then washed with phosphate-buffered saline, before being serially dehydrated. The internal and lateral surfaces of the root canal, representing the cross-sectional and longitudinal views of dentinal tubules, were viewed by SEM after being sputter-coated with gold.

Microtensile bond-strength testing

The specimens were subjected to a tensile force at a crosshead speed of 1 mm min^{-1}. After fracture, the cross-sectional area (in mm^2) of each sample was measured under 45x magnification, using a stereomicroscope (SZ61, Olympus, Tokyo, Japan) and the failure mode determined. The maximum tensile force that fractured the specimen was recorded and used for bond-strength calculation (MPa). The failure mode was classified as adhesive failure, cohesive failure in the sealer, cohesive failure in the dentine, or mixed failure.

SEM observation

The root canal surfaces of prepared specimens were observed by SEM at 10000X and 25000X. Both cross-sectional and longitudinal views were photographed.

Statistical analysis

Bond-strength values of each type of sealer were analysed by one-way analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test (α = 0.05). All statistical analyses were performed using SPSS software version 22 (SPSS Inc., Chicago, IL, USA).

Results

Microtensile bond strength test

The means and standard deviations of microtensile bond strength are given in Table 1. Treatment of the root canal dentine surface with NaOCl, EDTA, followed by distilled water, groups 2-5, did not promote high bond strength in epoxy resin. For methacrylate resin sealer, 1-, 3-, and 5-min EDTA irrigation group (group 2-4) showed not significantly higher than treatments without EDTA (group 1) (p = 0.139, p = 0.179, and p = 0.099, respectively), whereas 10-min EDTA irrigation group (group 5) promoted significantly higher bond strength than treatments without EDTA ($p < 0.001$).

Table 1 Microtensile bond strength.

Group	NaOCl DW	NaOCl EDTA 1 min DW	NaOCl EDTA 3 min DW	NaOCl EDTA 5 min DW	NaOCl EDTA 10 min DW
AH Plus	10.45±2.97	12.62±3.17	11.38±2.98	12.23±4.71	12.62±5.05
MetaSEAL	14.90±5.41^{A}	20.53±8.10^{A,B}	20.24±7.37^{A,B}	20.91±5.45^{A,B}	26.15±5.93B

Bond strength is given in Mpa; measurements are given as mean standard deviation
The same superscript capital letters indicate the absence of significant differences in microtensile bond strength for each row (p > 0.05).
DW, distilled water

Failure mode

The failure mode is presented in Fig. 2. The predominant failure mode throughout groups was mixed failure, no cohesive failure within the dentine occurred. A markedly higher number of cohesive failures in the sealer were found in the 10-min EDTA groups of methacrylate resin sealer.

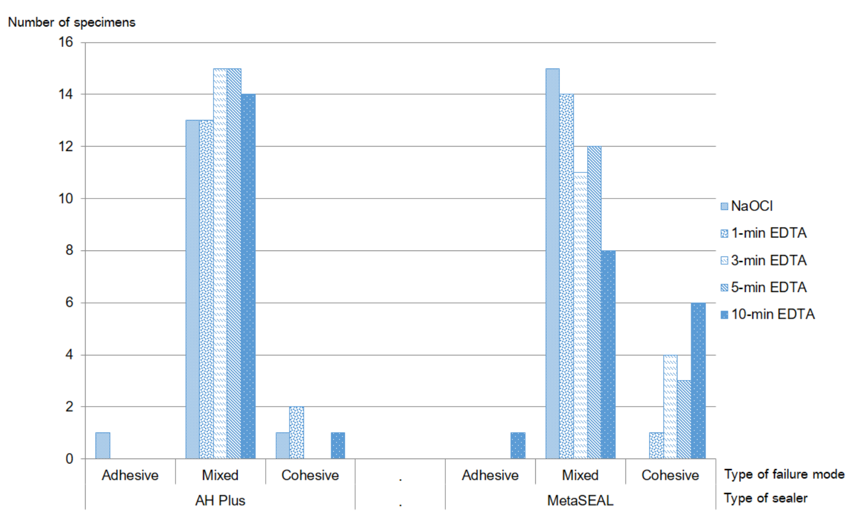


Figure 2 *The number of failure modes of AH Plus and MetaSEAL in different irrigation protocols.*

SEM observations

Group 1 (NaOCl; Fig. 3a and 3b) showed an amorphous smear layer covering the dentine surface, and no dentinal tubules were seen. Longitudinal sections of dentinal tubules (Fig. 3c) demonstrated short collagen fibrils in the intertubular dentine, but rarely in the peritubular dentine (Fig. 3c; arrows).

Group 2 (1-min EDTA, Fig. 3d and 3e) showed no smear layer, and generally patent dentinal tubules, demineralized dentine surface in some areas, and generally exposed integral collagen fibrils. In longitudinal sections (Fig. 3f), collagen fibrils on the intertubular dentine were more visible than in group 1, and collagen fibrils were exposed on most of the peritubular dentine.

Group 3 (3-min EDTA; Fig. 3g and 3h) showed the absence of a smear layer, entirely patent dentinal tubules, generalized demineralization of the dentine surface (which was deeper than that seen in group 2), and a vast integral collagen fibril network. In longitudinal sections (Fig. 3i), the collagen fibril appearance on the intertubular dentine and peritubular dentine were similar to that in group 2, but a collagen fibril network was present in the demineralized dentine on the wall of the root canal (left side).

Group 4 (5-min EDTA; Fig. 3j and 3k) showed a similar surface to that in group 3, but the demineralized dentine area and exposed integral collagen fibril network were larger than those in group 3. In longitudinal sections (Fig. 3l), more collagen fibrils were present on the intertubular dentine and peritubular dentine and along dentinal tubules than in group 3.

Group 5 (10-min EDTA; Fig. 3m and 3n) appeared similar to group 4; however, dentine demineralization was deeper and dense collagen bands were present. In longitudinal sections (Fig. 3o), dense collagen bands were seen, and other areas were similar to group 4.

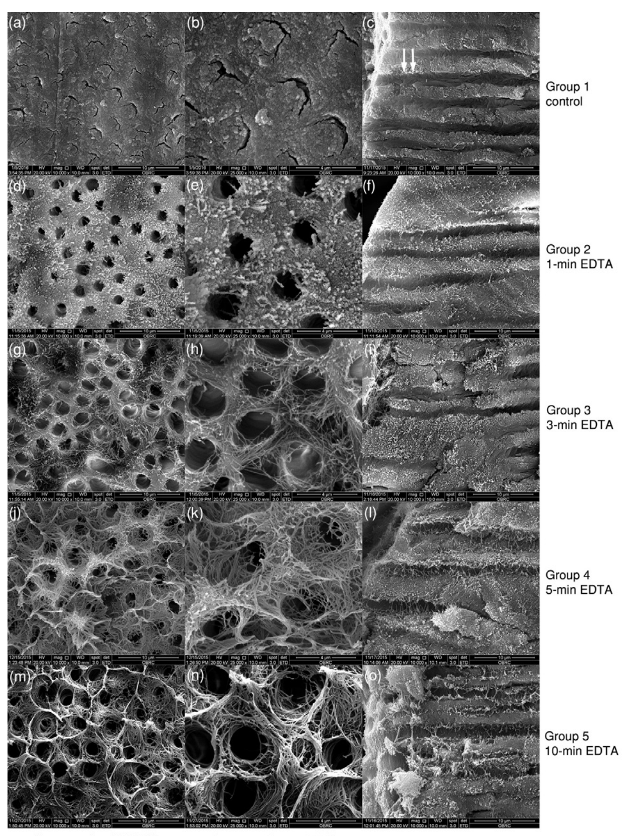


Figure 3 *Representative scanning electron microscope micrograph of radicular dentine specimens.*

Discussion

Root canal obturation is aimed at comprehensive three-dimensional filling of the root canal, to prevent reinfection into the root canal system.[2,18] The bondability of the root canal sealer to root dentine is thought to improve sealing ability and the stability of the root-filling materials. Generally, it was recommended 1 to 5 minutes EDTA irrigation time as smear layer removal protocol[19] and the previous study showed excessive erosion in root dentine by 10-min EDTA and followed by NaOCl irrigation,[20] therefore, this study carried out the duration of EDTA irrigation at 1, 3, 5, and 10 minutes that affected bond strength.

For the epoxy resin sealer (AH Plus), the treatment of the root canal dentine surface with NaOCl, EDTA (1- to 10-min), and followed by distilled water did not significantly increase the microtensile bond strength compared with the control group. This result did not well correlate with previous findings[9,11], which reported that a high bond strength of resin sealers was associated with final irrigation using a decalcifying agent, because of differences in their methodologies. The adhesion of epoxy resin sealer (AH Plus) to dentine was found that it adhered by mechanical lock from sealer penetration in dentinal tubules[10,21] and formed a covalent bond between the amino groups of the dentine collagen and epoxide rings.[22,23] From SEM observations, a 1-min EDTA irrigation resulted in demineralization of dentine in some areas and short exposed collagen fibrils, while longer irrigation (3-10 min) tended to result in deeper demineralization and longer exposed collagen fibrils in a duration-dependent manner. All EDTA irrigation groups showed absence of smear layer, the integrity of collagen fibrils and no denatured collagen fibrils. It seems that penetration of the sealer into dentinal tubules[24] and the quality and amount of collagen fibrils may less affect the bond strength of the epoxy resin sealer.

For the methacrylate resin sealer (MetaSEAL), the higher bond-strength value in the EDTA groups correlated with previous findings.[11,17,25] A longer duration of EDTA irrigation tended to promote a higher strength of resin sealer-dentine bonding. From SEM observation described above, irrigation with EDTA causes chelation of calcium from the exposed dentinal collagen, which is important for adhesion of the methacrylate resin sealer. The sealer was self-adhesion, which was incapable of etching through the smear layer[26] and adhered by hybridization to collagen fibers.[27,28] Base on this finding, it seems that removal of the smear layer, and the integrity and quantity of collagen fibrils affect the bond strength of the methacrylate resin sealer. Additionally, a longer duration of EDTA irrigation tended to result in more cohesive failure than no EDTA or a shorter EDTA irrigation duration. In clinical situation, however, cohesive failure within a root canal sealer will not occur if the root canal obturate with core materials.

Several bond-strength testing methods have been used previously, for example, push-out test[29-31], shear test[32,33], and microtensile test.[34,35] In our study, the microtensile test, which is commonly used to test adhesion effectiveness of bonding agents was selected because it reflects the interfacial bond strength in small area, and minimize friction force.[36] However, microtensile test also has limitation to test in low bond strength materials such as zinc oxide eugenol based sealer (data not shown).

The study is limited in that only the coronal third of the root was used as a representative of root dentine, because the middle and apical third of the root canal were too small for preparing specimens for microtensile testing. Apical third and middle third of root canal dentine has fewer dentinal tubules than coronal third of root canal dentine and consequently, fewer sealer penetration into dentinal tubules is occurred.[37,38] Therefore, we assumed that bond strength value of resin sealers to middle third and apical third of root canal dentine might become lower than coronal third of root canal dentine respectively.[35] To evaluate bond strength at only one interface (between root dentine and sealer, it was necessary to fill the root canal only with sealers.

Conclusion

The duration of EDTA irrigation affects the microtensile strength of the bond between the methacrylate resin sealer (MetaSEAL) and root canal dentine. Final irrigation with 5 % NaOCl, 17 % EDTA and distilled water increased the bond strength of resin sealers. Ten minutes of EDTA irrigation could enhance adhesion of the methacrylate resin sealer to the root canal dentine. However, as various factors enhance bond strength, further studies are warranted.

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