INTERNATIONAL
ENDODONTIC JOURNAL

doi:10.1111/iej.13005

The recovery effect of proanthocyanidin on microtensile bond strength to sodium hypochlorite-treated dentine

Y. Wang[1,2], C. Chen[1], H. L. Zang[1] & Y. H. Liang[1,2]
[1]Department of Cariology and Endodontology, Peking University School and Hospital of Stomatology, Beijing; and [2]Dental Clinic Peking University International Hospital, Beijing, China

Abstract

Wang Y, Chen C, Zang HL, Liang YH. The recovery effect of proanthocyanidin on microtensile bond strength to sodium hypochlorite-treated dentine. *International Endodontic Journal*, **52**, 371–376, 2019.

Aim To evaluate the recovery effect of proanthocyanidin (PA) on microtensile bond strength (μTBS) to sodium hypochlorite (NaOCl)-treated dentine.

Methodology Fifty-five freshly extracted third molars with intact dental crowns, no caries or restorations were sectioned to expose a sound middle layer of dentine and were randomly divided into 11 groups. In the blank control group, dentine surfaces were immersed in deionized water for 20 min. In the negative control group, dental surfaces were immersed in 5.25% NaOCl for 20 min. In the other nine experimental groups, after immersion for 20 min in 5.25% NaOCl, followed by PA (5%, 10%, or 15%) treatment for 1, 5 min or 10 min. The NaOCl solution was renewed every 5 min. Then dentine surfaces were bonded using SE bond. Bonded samples were sectioned into dentine-resin sticks ($n = 45$) for microtensile bond strength testing (MPa). Failure modes were observed and classified into three types with a stereomicroscope. Microtensile bond strength data were analysed using one-way ANOVA. The confidence interval test was performed to analyse the recovery effect of PA on bond strength to NaOCl-treated dentine. The chi-squared test was used to analyse failure mode distribution.

Results After use of 5.25% NaOCl for 20 min, microtensile bond strength in the negative control group decreased significantly compared with that of the untreated group ($P < 0.05$). After a recovery treatment of 10% PA for 10 min or 15% PA for more than 5 min, the bond strength was restored to at least 90% of baseline ($P < 0.05$). No recovery effect on bond strength was detected after the application of 5% PA for 1 min ($P > 0.05$). Adhesive fracture was found to be the most common failure mode in the NaOCl-treated group. After the recovery application of PA, the proportion of mixed failures increased significantly ($P < 0.05$).

Conclusions Microtensile bond strength to NaOCl-treated dentine recovered after the application of either 5% PA for more than 5 min or 10% or 15% PA for more than 1 min. The application of PA before an adhesive procedure may immediately restore the compromised bond strength of NaOCl-treated dentine.

Keywords: dentine, microtensile bond strength, proanthocyanidin, sodium hypochlorite.

Received 26 February 2018; accepted 22 August 2018

Correspondence: Yu-Hong Liang, Department of Cariology and Endodontology, Peking University School and Hospital of Stomatology, Beijing 10081, China; Dental Clinic, Peking University International Hospital, Beijing 102206, China (e-mail: leungyuhong@sina.com).

Introduction

Sodium hypochlorite (NaOCl) is the most commonly used irrigant for root canal treatment because of its antimicrobial activity and capacity to dissolve organic substances (Peters *et al.* 2017). With advances in materials and technology in clinical practice, the

© 2018 International Endodontic Journal. Published by John Wiley & Sons Ltd

immediate restoration of root filled teeth with composite resins is increasing. However, it has been reported that the bond strength between dentine and composite resins could decrease after the use of NaOCl irrigation (Weston *et al.* 2007, Barutcigil *et al.* 2014, Gönülol *et al.* 2014, Pimentel *et al.* 2016).

Research has suggested that in addition to delaying the time prior to bonding (Bulut *et al.* 2006, Dikmen *et al.* 2015), the application of antioxidant solutions before an adhesive procedure may immediately restore the compromised bond strength of NaOCl-treated dentine (Prasansuttiporn *et al.* 2010, Manimaran *et al.* 2011, Pimentel *et al.* 2016). Compared with sodium ascorbate, proanthocyanidin (PA) has 20 times the antioxidant capacity (Castrillejo *et al.* 2011). PA is a group of polyphenolic natural products composed of flavan-3-ol subunits, and has generated much interest due to its collagen cross-linking and matrix metalloproteinases inhibition (Epasinghe *et al.* 2013, Liu *et al.* 2013, Scheffel *et al.* 2014). Recently, it has been reported that the antioxidation of PA enhanced the bond strength of oxidizing solution-treated dentine (Feiz *et al.* 2017). However, few studies have addressed the recovery effect of PA on bond strength for NaOCl-treated dentine. Therefore, this study sought to evaluate the reversal of microtensile bond strength (μTBS) decreases in NaOCl-treated dentine via treatment with PA with different application times and concentrations. The null hypothesis was that neither different application times nor concentrations of PA would restore the compromised bond strength of NaOCl-treated dentine.

Materials and methods

Specimen preparation

Fifty-five freshly extracted human third molars with completely formed crowns and without caries and restorations were selected. The teeth were cleaned using scalers to remove soft-tissue remnants and calculus and were stored in deionized water changed weekly at 4 °C for 3 months before use.

A diamond saw (SYJ-150; Shenyang Kejing Auto-instrument Co., Ltd., Shenyang, China) was used under running water to remove occlusal enamel perpendicular to the long axis of the tooth and thereby expose flat surface of sound dentine in the middle of the tooth. These dentine surfaces were examined under a stereomicroscope (Zoom 630; Shanghai Changfang Optical Instrument Co., Ltd., Shanghai, China) at 20× magnification to ensure the enamel had been completely removed. Subsequently, the exposed dentine surfaces were polished with 600-grit silicon paper under running water for 1 min and rinsed with deionized water for 1 min.

The teeth were randomly divided into 11 groups (five teeth in each group). In the blank control group, dentine surfaces were immersed in deionized water for 20 min. In the negative control group, dentine surfaces were immersed in 5.25% NaOCl solution for 20 min. In the nine experimental groups, dentine surfaces were immersed in 5.25% NaOCl for 20 min, followed by treatment with PA (at a concentration of 5%, 10%, or 15%) for specific time periods (1, 5 min, or 10 min). The NaOCl solution was renewed every 5 min. Solutions of 5%, 10% and 15% w/v PA were prepared by adding PA-rich grape seed extract powder (purity > 90%; MegaNatural-BP; Swanson Health Products, Fargo, ND, USA) to deionized water.

Bonding procedure

All dentine surfaces were rinsed with deionized water for 1 min. A two-step self-etch adhesive (Clearfil SE Bond; Kuraray Medical Inc., Tokyo, Japan) was applied to the treated dentine surface with applicator brushes in the following steps. The dentine surface was treated with primer for 20 s and dried with mild air flow. A thin layer of bond was applied and light-cured for 10 s. A composite resin (Clearfil AP-X; Kuraray Medical Inc.) was added to the bonded dentine in increments with thicknesses of approximately 5 mm, and each 2 mm layer of the composite was light-cured for 20 s using a light-curing unit (LEDition; Ivoclar Vivadent Manufacturing, Inc., Schaan, Liechtenstein) operating at 800 mW cm^{-2}.

Microtensile bond strength

After being stored in deionized water at 37 °C for 24 h, the adhesive samples were sectioned perpendicular to the bonded interface into slabs with a thickness of 1 mm with a diamond saw. The block of slabs was then rotated 90° and again cut perpendicular to the bonded interface to obtain sticks with a cross-sectional area of 1.0 mm × 1.0 mm. The sticks were carefully evaluated under a stereomicroscope at 20× magnification to exclude defects such as cracks or defects in the adhesives caused by bubbles. A total of 8–12 sticks were selected from a central region of

 © 2018 International Endodontic Journal. Published by John Wiley & Sons Ltd

each tooth to test μTBS (MPa; for five teeth in each group, $n = 45$).

For each stick, the thickness (a) and width (b) of the bonded area ($S = a \times b$) were measured using a vernier caliper (MNT-150; Shanghai Yingte Trade Co., Ltd., Shanghai, China). The stick was attached to the middle of the specimen platform of a μTBS tester (MicroTensile Tester; BISCO, Inc., Schaumburg, IL, USA) and was fixed with cyanoacrylate adhesive (Guangzhou Aibida Adhesives Co., Ltd., Guangzhou, China). Each stick was stressed to failure under tension by using μTBS tester at a crosshead speed of 1 mm min^{-1}. The imposed force (F, in N) at the time of fracture was recorded. The μTBS value was defined as,

$$\mu\text{TBS}\,(\text{MPa}) = \frac{F}{S}.$$

Failure mode analysis

Failure modes were analysed under a stereomicroscope at 45× magnification. Three types of failure modes were classified as follows: adhesive failure, occurred between resin and dentine; cohesive failure in dentine or in resin; and mixed failure, a combination of cohesive and adhesive failure.

Statistical analysis

μTBS data were analysed using one-way ANOVA and Write Student–Newman–Keuls test. The confidence interval test was performed to analyse the recovery effect of PA on bond strength to NaOCl-treated dentine. The chi-squared test was used to analyse failure mode distribution. The statistical significance level was set at $\alpha = 0.05$. All of the computational work was performed using SPSS (version 20.0; IBM, Chicago, IL, USA).

Results

The recovery effects of PA on bond strength to NaOCl-treated dentine are presented in Fig. 1; the effects are divided into four levels based on the results of the confidence interval test (Table 1). The recovery effect of PA indicates that the lower confidence limit of the bond strength of each experimental group reached 60%, 70%, 80% or 90% of the bond strength of the blank control group. There were significant differences among the 11 groups ($P < 0.05$). The ranking of bond strength values was 15%/10 min > blank control group (deionized water), 15%/5 min, 10%/10 min > 10%/5 min, 5%/10 min > 15%/1 min, 5%/5 min > 10%/1 min > 5%/1 min, negative control group (5.25% NaOCl). In the negative control group, μTBS decreased after immersion in 5.25% NaOCl for 20 min ($P < 0.05$).

The distribution of failure modes for the groups is presented in Table 2. Adhesive fracture was the most common failure mode in the NaOCl-treated group; after the recovery application of PA, the proportion of the mixed failures increased significantly ($P < 0.05$).

Discussion

In the present study, μTBS testing was used; this approach has been established as a versatile and reliable method for investigating dentine bond strength (Sano *et al.* 1994, Pashley *et al.* 1995). However, the test results may be affected by several factors, such as specimen geometry and specimen bond surface area. The specimen geometry used in this study was a stick shape to avoid additional stress (Ghassemieh 2008). The specimen bond surface area in the study was 1 mm^2 to obtain a uniform stress distribution and protect against fractures before testing (Armstrong *et al.* 2010).

In the present study, dentine bond strength was 50% lower than that of the blank control group (treated with deionized water) after treatment with 5.25% NaOCl for 20 min ($P < 0.05$). This result was consistent with the findings of previous studies (Prasansuttiporn *et al.* 2010, Barutcigil *et al.* 2014, Gönülol *et al.* 2014, Dikmen *et al.* 2015, Pimentel *et al.* 2016). Pimentel *et al.* (2016) reported that after 5.25% NaOCl application for 30 min and 17% EDTA for 3 min, dentine microtensile bond strength decreased 48% compared with that of untreated. With this decrease in dentine bond strength, the distribution of failure modes also changed. In the group not treated with NaOCl, fractures occurred mostly in a combination of cohesive and adhesive (68.9%), reflecting ‘integration’ of the composite resin, adhesive and dentine. However, the observed increase in adhesive failure after treatment with 5.25% NaOCl indicated the presence of a ‘weakened area’ in the bonding interface that reflected a reduction in bond strength.

Several studies have suggested that the mechanism of the adverse effect of NaOCl on dentine bond strength might involve damage to dentine collagen that affects the formation of hybrid layers (Carrilho *et al.* 2009). Another explanation for this decrease in

© 2018 International Endodontic Journal. Published by John Wiley & Sons Ltd

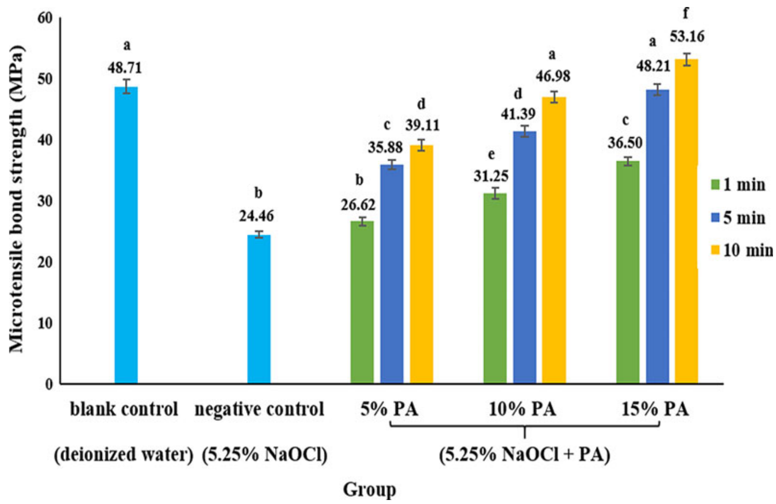

Figure 1 Microtensile bond strength in each group (MPa) Groups identified by same superscript letters are not significantly different ($P > 0.05$).

Table 1 Recovery effect of PA on microtensile bond strength to NaOCl-treated dentine

Recovery effect	Groups
60-70%	10%/1 min
70-80%	5%/5 min, 5%/10 min, 15%/1 min
80-90%	10%/5 min
>90%	10%/10 min, 15%/5 min, 15%/10 min

Table 2 Distribution of failure modes (%)

Group	Adhesive failure	Mixed failure	Cohesive failure
blank control[a]	24.4	68.9	6.7
Negative control[b]	93.3	6.7	–
5%/1 min[b]	88.9	11.1	–
5%/5 min[c]	62.2	35.5	2.2
5%/10 min[c]	60.0	37.8	2.2
10%/1 min[c]	68.9	31.1	–
10%/5 min[c]	51.1	44.4	4.4
10%/10 min[a,c]	35.6	60.0	4.4
15%/1 min[c]	60.0	40.0	–
15%/5 min[a,c]	44.4	48.9	6.7
15%/10 min[c]	51.1	44.4	4.4

Groups marked with the same superscript letter did not differ significantly with respect to failure mode distribution ($P > 0.05$).

bond strength is that oxygen free radicals generated by NaOCl via a chlorination reaction can inhibit polymerization in adhesive systems. Consequently, the bond strength between composite resin and dentine is reduced after NaOCl treatment (Lai *et al.* 2001, Manimaran *et al.* 2011, Dikmen *et al.* 2015).

In the present study, the recovery effect of PA on NaOCl-treated dentine was divided into four levels based on results of the confidence interval test (Table 1). No recovery of bond strength was detected after the application of 5% PA for 1 min ($P > 0.05$). However, the decreased bond strength of NaOCl-treated dentine could be reversed by treatment with 5% PA for more than 5 min or with 10% or 15% PA for more than 1 min ($P < 0.05$). The reverse to the NaOCl-treated dentine bond strength after PA treatment were both concentration and time dependent.

The reason of the observed recovery effect of PA may be partly explained by antioxidant activity of PA (Nuthong *et al.* 2009, Castellan *et al.* 2010). The molecular structure of PA includes many phenolic hydroxyl groups; these groups have strong antioxidative effects and can eliminate free radicals generated by NaOCl (Fine 2000). Furthermore, the phenolic hydroxyl group in PA can also contribute to stabilizing dentine collagen by reacting with proline in the collagen via a hydroxylation reaction (Bedran-Russo *et al.* 2007, Castellan *et al.* 2011, Zhou *et al.* 2016). This reaction may improve the quality of the hybrid layer, increasing dentine bond strength (Nuthong *et al.* 2009, Zhou *et al.* 2016). Interestingly, use of PA 15% for 10 min on NaOCl-treated dentine not only recovered but also increased the μTBS when compared with the blank control group in which NaOCl and PA were not applied. This finding might be partly explained by the collagen cross-linking effect of PA. There are phenolic hydroxyl groups in PA, which could interact with the collagen and introduce collagen cross-linking, then the bonded interface

© 2018 International Endodontic Journal. Published by John Wiley & Sons Ltd

could be stabilized. Venigalla *et al.* (2016) reported that the immediate dentine bond strength was enhanced by the application of 6.5 wt% PA for 2 min from 31.76 to 41.71 MPa ($P < 0.05$). The unpublished result from the present study also showed that the μTBS of dentine increased significantly after treatment with 15% PA for 10 min but without NaOCl, compared with the μTBS of dentine which was neither treated with PA nor NaOCl ($P < 0.05$).

The present study revealed that the application of 5% PA for a short time (1 min) was apparently unable to restore NaOCl-treated dentine bond strength. The increase in concentration and time of PA led to a greater degree of bond strength recovery. When the concentration of PA reached more than 10% and application time extended longer than 5 min, the bond strength of NaOCl-treated dentine recovered to at least 80% of baseline (the bond strength of dentine untreated with PA or NaOCl). From a practical perspective, 1 min of treatment with PA at a higher concentration (10% or 15%) could increase the bond strength significantly. Nevertheless, considering the adverse effects such as staining of dentine, use of PA with a higher concentration should be done with caution (Scheffel *et al.* 2014). One limitation of this study was that it only evaluated the immediate recovery effect of PA on bond strength to NaOCl-treated dentine. The durability of bonding improvement of PA to NaOCl-treated dentine, over time needs further work.

Conclusion

Treatment with 5% PA for more than 5 min or with 10% or 15% PA for more than 1 min restored the microtensile bond strength (μTBS) to NaOCl-treated dentine.

Conflict of interest

The authors have stated explicitly that there is no conflict of interest in connection with this article.

References

Armstrong S, Geraldeli S, Maia R, Raposo LHA, Soares CJ, Yamagawa J (2010) Adhesion to tooth structure: a critical review of "micro" bond strength test methods. *Dental Materials* **26**, 50–62.

Barutcigil C, Harorlı OT, Özcan E, Arslan H, Yıldız M (2014) Effects of ethylenediaminetetraacetic acid and sodium hypochlorite on the bond strength of bonding agents to pulp chamber lateral walls. *Journal of Dental Sciences* **9**, 229–34.

Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M (2007) Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. *Journal of Biomedical Materials Research Part B Applied Biomaterials* **80**, 268–72.

Bulut H, Turkun M, Kaya AD (2006) Effect of an antioxidizing agent on the shear bond strength of brackets bonded to bleached human enamel. *American Journal of Orthodontics and Dentofacial Orthopedics* **129**, 266–72.

Carrilho MR, Tay FR, Donnelly AM *et al.* (2009) Membrane permeability properties of dental adhesive films. *Journal of Biomedical Materials Research* **88**, 312–20.

Castellan CS, Pereira PNR, Viana G, Chen SN, Pauli GF, Bedranrusso AK (2010) Solubility study of phytochemical cross-linking agents on dentin stiffness. *Journal of Dentistry* **38**, 431–6.

Castellan CS, Bedranrusso AK, Karol S, Pereira PN (2011) Long-term stability of dentin matrix following treatment with various natural collagen cross-linkers. *Journal of the Mechanical Behavior of Biomedical materials* **4**, 1343–50.

Castrillejo VM, Romero MM, Esteve M *et al.* (2011) Antioxidant effects of a grapeseed procyanidin extract and oleoyl-estrone in obese Zucker rats. *Nutrition* **27**, 1172–6.

Dikmen B, Gurbuz O, Ozsoy A, Eren MM, Cilingir A, Yucel T (2015) Effect of different antioxidants on the microtensile bond strength of an adhesive system to sodium hypochlorite-treated dentin. *Journal of Adhesive Dentistry* **17**, 499–504.

Epasinghe DJ, Yiu CK, Burrow MF, Hiraishi N, Tay FR (2013) The inhibitory effect of proanthocyanidin on soluble and collagen-bound proteases. *Journal of Dentistry* **41**, 832–9.

Feiz A, Mosleh H, Nazeri R (2017) Evaluating the effect of antioxidant agents on shear bond strength of tooth-colored restorative materials after bleaching: a systematic review. *Journal of the Mechanical Behavior of Biomedical Materials* **71**, 156–64.

Fine AM (2000) Oligomeric proanthocyanidin complexes: history, structure, and phytopharmaceutical applications. *Alternative Medicine Review* **5**, 144–51.

Ghassemieh E (2008) Evaluation of sources of uncertainties in microtensile bond strength of dental adhesive system for different microtensile bond strength. *Dental Materials* **24**, 536–47.

Gönülol N, Kalyoncuoğlu E, Ertaş E (2014) Effect of sodium ascorbate on dentin bond strength after treatment with oxidizing root canal irrigants. *Journal of Dental Sciences* **10**, 139–44.

Lai SC, Mak YF, Cheung GS *et al.* (2001) Reversal of compromised bonding to oxidizedetched dentin. *Journal of Dental Research* **80**, 1919–24.

Liu Y, Chen M, Yao X, Xu C, Zhang Y, Wang Y (2013) Enhancement in dentin collagen's biological stability after

© 2018 International Endodontic Journal. Published by John Wiley & Sons Ltd

proanthocyanidins treatment in clinically relevant time periods. *Dental Materials* **29**, 485–92.

Manimaran VS, Srinivasulu S, Ebenezar AR, Mahalaxmi S, Srinivasan N (2011) Application of a proanthocyanidin agent to improve the bond strength of root dentin treated with sodium hypochlorite. *Journal of Conservative Dentistry* **14**, 306–8.

Nuthong P, Benjakul S, Prodpran T (2009) Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. *International Journal of Biological Macromolecules* **44**, 143–8.

Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM (1995) Adhesion testing of dentin bonding agents: a review. *Dental Materials* **11**, 117–25.

Peters OA, Peters CI, Basrani B (2017) Cleaning and shaping the root canal system. In: Hargreaves KM, Cohen S, Berman LH, eds. *Cohen's pathways of the pulp*, 10th edn. St Louis, MO: Mosby Elsevier, p 251.

Pimentel CA, Cecchin D, de Almeida JF, Gomes BP, Zaia AA, Ferraz CC (2016) Sodium thiosulfate for recovery of bond strength to dentin treated with sodium hypochlorite. *Journal of Endodontics* **42**, 284–8.

Prasansuttiporn T, Nakajima M, Kunawarote S, Foxton RM, Tagami J (2010) Effect of reducing agents on bond strength to NaOCl-treated dentin. *Dental Materials* **27**, 229–34.

Sano H, Shono T, Sonoda H *et al.* (1994) Relationship between surface area for adhesion and tensile bond strength-evaluation of a micro-tensile bond test. *Dental Materials* **10**, 236–40.

Scheffel D, Hebling J, Scheffel RH *et al.* (2014) Inactivation of matrix-bound mmps by cross-linking agents in acid etched dentin. *Operative Dentistry* **39**, 152–8.

Venigalla BS, Jyothi P, Kamishetty S, Reddy S, Cherukupalli RC, Reddy DA (2016) Resin bond strength to water versus ethanol-saturated human dentin pretreated with three different cross-linking agents. *Journal of Conservative Dentistry* **19**, 555–9.

Weston CH, Ito S, Wadgaonkar B, Pashley DH (2007) Effects of time and concentration of sodium ascorbate on reversal of NaOCl – induced reduction in bond strengths. *Journal of Endodontics* **33**, 879–81.

Zhou JF, Chiba A, Scheffel DL *et al.* (2016) Cross-linked dry bonding: a new etch-and-rinse technique. *Dental Materials* **32**, 1124–32.

 © 2018 International Endodontic Journal. Published by John Wiley & Sons Ltd