Original Article

Reversal of Reduced Bond Strength to NaOCl-Treated Dentine with Sodium Ascorbate at Different Irrigation Times

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Abstract

The objective of this study was to compare the effect of 10% sodium ascorbate at different irrigation times on the microtensile bond strength of a two-step, total-etching adhesive to sodium hypochlorite-treated dentine. The roof of the pulp chamber and pulpal tissue of 49 extracted human mandibular third molars were removed. The teeth were randomly divided into 7 groups of 7 teeth each. The pulp chamber dentine was irrigated with distilled water (control), 5.25% sodium hypochlorite (NaOCl), or 5.25% NaOCl followed by 10% sodium ascorbate for 30 s, 1, 3, 5 and 10 min. The irrigated dentine was blot dried, bonded with a two-step, total-etching adhesive (Adper Single Bond II) and restored with a hybrid resin composite (Filtek Z250). After storage for 24 h in 37°C water, specimens were prepared and tested for microtensile bond strength using a universal testing machine at the crosshead speed of 1 mm/min. The data were analyzed using one- way ANOVA and LSD test at $p < .05$. Failure modes and the morphology of the treated surfaces were observed under scanning electron microscope. The bond strength of NaOCl-treated group was significantly lower than the other groups. The 10-min irrigation of sodium ascorbate produced a higher bond strength than the other irrigation times. However, irrigation with 10% sodium ascorbate on NaOCl-treated dentine for a duration less than 10 min prior to the application of a two-step, total-etching adhesive reversed the bond strength to a normal level. No morphological differences were found among the dentine surfaces treated with NaOCl followed by the different irrigation times of sodium ascorbate.

Key words: dentine adhesion; microtensile bond strength; sodium ascorbate; sodium hypochlorite; total-etching adhesive

Introduction

Endodontic therapy is considered to irreversibly alter the physical characteristics of tooth structure.[1] The tooth is weakened from the loss of tooth structure due to caries and subsequent endodontic preparation. Hence, the endodontically treated tooth requires reinforcement to maintain the optimal function and esthetics. Ray and Trope[2] indicated that the quality of permanent coronal restoration is of critical importance for endodontic success. Good adhesion between the restorative material and tooth structure results in good marginal sealing which provides defense against bacterial leakage. Restoration of endodontically treated teeth with bonded resin composite has been widely accepted to strengthen the weakened tooth structure.[3,4] Reportedly, the bonding capacity increased the fracture resistance of the remaining tooth structure.[5,6] This relies on using an dental adhesive via the mechanism of micromechanical interlocking with a hybrid layer and resin tags.[7]

Sodium hypochlorite (NaOCl) is generally used in endodontic treatment as a chemical irrigant to provide debridement, lubrication and dissolution of tissues.[8] NaOCl application on dentine surface adversely affects the bond strength of resin composite to dentine[9-11] especially with a total-etching adhesive.[9] The adverse effect of NaOCl may be related to its potential to damage the organic component of dentine. NaOCl also breaks down into sodium chloride and oxygen, which may interfere with the polymerization of resin-based materials.[12] A number of studies have revealed that application of 10% sodium ascorbate, an anti-oxidant[13], for 10 min on NaOCl-treated dentine reversed the bond strength to normal value.[11,14]

Even though the application of 10% sodium ascorbate for 10 min can reverse the bond strength, the duration of application time may be too long and not practical for clinicians. The purpose of this study was to evaluate the minimum time for application of this solution on NaOCl-treated dentine, which effectively reverses the bond strength. The null hypothesis was that the bond strength of a two-step, total-etching adhesive to NaOCl-treated dentine after irrigation with 10% sodium ascorbate for 30 s, 1, 3, 5, or 10 min are not different from the bond strength of that to untreated dentine**.**

Materials and Methods

Forty nine extracted human mandibular third molars without caries or defects were stored in 0.1% thymol solution and used within 3 months after extraction. Each tooth was cut horizontally with a slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) at the level of pulpal horn and the roof of pulp chamber was removed. Pulpal tissue was carefully removed with a spoon excavator and the pulp chamber dentine was rinsed with 10 ml of distilled water to remove debris then dried with a cotton pellet and paper point. Teeth were randomly divided into 7 groups of 5 teeth each. In group 1, the pulp chamber was irrigated with 10 ml of distilled water for 10 min (control group). In group 2, the pulp chamber was irrigated with 10 ml of 5.25% NaOCl (locally made, Department of Pharmacology, Faculty of Dentistry, Mahidol University, Thailand) for 10 min. In groups 3, 4, 5, 6 and 7, after irrigating with 10 ml of 5.25% NaOCl and drying, the pulp chamber was rinsed with 0.5 ml, 1 ml, 3 ml, 5 ml and 10 ml of 10% freshly prepared sodium ascorbate (Fluka, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) for 30 s,1 min, 3 min, 5 min and 10 min, respectively.

After irrigation, the pulp chamber of each tooth was dried, etched with 35% phosphoric acid (3M-ESPE, St Paul, MN, USA) for 15 s, rinsed for 15 s and then blot dried with a cotton pellet. The dentine at the wall of the pulp chamber was treated with a two-step, total-etching adhesive (Adper Single Bond II (Batch No. 200507260), 3M ESPE, St Paul, MN, USA). Two layers of bonding resin were applied, gently air blown and light-cured for 10 s using a light-curing unit (XL 3000, 3M Dental Products, St Paul, MN, USA) with a light intensity of 800 mW/cm^2. The bonded surface was incrementally filled with a hybrid resin composite (Filtek Z250 shade A2 (Batch No.20050210), 3M-ESPE, St Paul, MN, USA), and each 2-mm thick layer was cured for 20 s (Fig. 1A). All restorations were performed by a single operator. The restored teeth were kept in distilled water at 37°C for 24 h before the microtensile bond strength test.

Microtensile bond strength test

After completing the storage time, each restored tooth was sectioned longitudinally through the pulp chamber in the

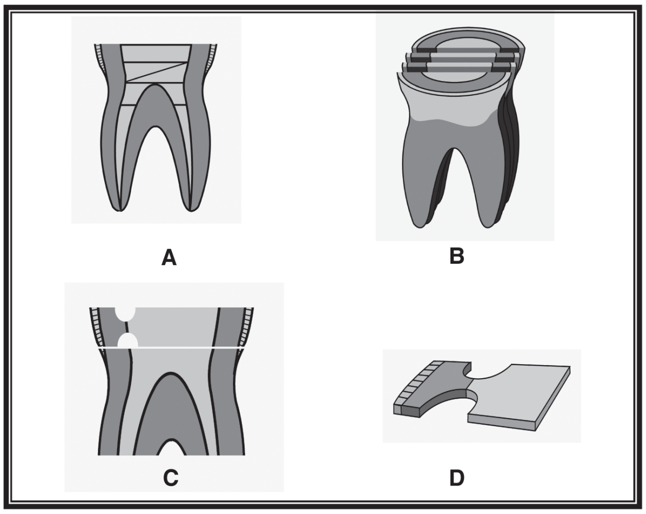

Fig. 1 *Schematic illustration of specimen preparation for microtensile bond test*
A: filling the pulp chamber with an incremental technique B: sectioning the restored tooth to obtain 2 slabs C: cutting off the root 2 mm from occlusal surface and trimming the slab to form a dumbbell- shaped specimen D: the dumbbell-shaped specimen

mesio-distal direction to obtain two slabs of 0.8 mm. in thickness (Fig. 1B). The root of each slab was removed from the crown approximately 2 mm. below the sectioned surface with a slow-speed diamond saw under copious water spray. The specimen was trimmed to form a dumbbell shape with the narrowest portion at the resin/dentine bonded interface (Fig. 1C and D) to produce a bonded surface area of 0.8 ± 0.2 mm^2 using a fine grit flame-shaped diamond bur (Intensive SA, Swiss Dental Products, Zurich, Switzerland) with a high-speed handpiece under copious water spray. The specimen was subjected to the microtensile bond strength test using a universal testing machine (Instron 5566, Instron Corp., London, UK) at a crosshead speed of 1 mm. /min until failure occurred. The microtensile bond strength was calculated and expressed in MPa. One-way analysis of variance was used to determine the effect of irrigating times on the microtensile bond strengths at a confidence level of 95%. Due to a significant factor, the least significant difference (LSD) analysis was used for multiple comparisons ($\alpha = 0.05$).

The fractured specimens in each group were examined under a scanning electron microscope (SEM) (JSM 5410 LV, JEOL, Tokyo, Japan) at 75x magnification. The failure modes were categorized as adhesive failure, cohesive failure in dentine and cohesive failure in bonding or resin composite. The area of each type of failure was converted into percentage of failure relative to the total bonding area.

SEM observation of surface morphology

Two teeth per group were employed for surface morphological study. The teeth in each group were prepared and the pulp chambers were treated with the irrigants in the same manner as described above. The specimens were fixed in buffered formalin for 24 h, critical-point dried, gold sputter-coated and examined under the SEM.

Results

Microtensile bond strengths of the two-step, total-etching adhesive to NaOCl-treated dentine after irrigation with 10% sodium ascorbate at different irrigation times are shown in Table 1. NaOCl significantly reduced the bond strength of the total-etching adhesive to dentine (7.93±3.50 MPa) when compared with control (18.65±2.45 MPa). No statistically significant differences were found among the bond strengths of the groups treated with 10% sodium ascorbate for 30 s, 1, 3 and 5 min and that of the control group. However, application with 10% sodium ascorbate for 10 min showed the highest bond strength (21.70±4.83 MPa) when compared with the other chemical irrigation-treated groups ($p < .05$).

Percentages of failure mode are shown in Table 2. The premature failure was not found in any specimen. Adhesive failures were predominantly observed in all groups. For the 5.25% NaOCl-treated dentine (group 2), no cohesive failure in resin and minimal cohesive failures in dentine were observed. Groups 3 to 7, which were treated with 10% sodium ascorbate for different periods, exhibited similar patterns of failure, mostly adhesive failures with a few of cohesive failures in bonding /resin composite and increased failure in dentine.

Table 1 *Means and standard deviations of the microtensile bond strengths (MPa) of each experimental group*

Group (n=10)	Treatment	Bond strength mean±s.d.
1	Distilled water	18.65±2.45 [a,c]
2	5.25% NaOCl	7.93±3.50 [b]
3	5.25% NaOCl + sodium ascorbate 30 s	17.19±3.31 [a]
4	5.25% NaOCl + sodium ascorbate 1 min	16.13±3.87 [a]
5	5.25% NaOCl + sodium ascorbate 3 min	16.49±4.26 [a]
6	5.25% NaOCl + sodium ascorbate 5 min	15.67±4.70 [a]
7	5.25% NaOCl + sodium ascorbate 10 min	21.70±4.83 [c]

Groups identified by different superscript letters are significantly different ($p < .05$)

Groups identified by the same superscript letters are not significantly different ($p > .05$)

Table 2 *Percentages of failure mode in each group (n=10)*

Group	Treatment	Percentage of failure		
		Adhesive	Cohesive in bonding resin composite	Cohesive in dentine
1	Distilled water	94.7	2.0	3.3
2	5.25% NaOCl	96.8	0	3.2
3	5.25% NaOCl + sodium ascorbate 30 s	79.7	5.5	14.8
4	5.25% NaOCl + sodium ascorbate 1 min	76.5	4.5	19.0
5	5.25% NaOCl + sodium ascorbate 3 min	82.4	2.8	14.8
6	5.25% NaOCl + sodium ascorbate 5 min	82.8	2.5	14.7
7	5.25% NaOCl + sodium ascorbate 10 min	77.3	13.2	9.5

Surface morphology of treated pulp chamber dentine was observed under the SEM at 3500 x magnification. In the control group (Fig. 2), scanning electron micrograph revealed predentine with largely opened dentinal tubules and unmineralized dentine matrix consisting of collagen fibrils. When the pulp chamber dentine was treated with 5.25% NaOCl, the organic matrix of predentine was dissolved, and the dome-shaped calcospherite structures with opened dentinal tubules were shown (Fig. 3A). Irrigation with 10% sodium ascorbate at different durations on the NaOCl-treated dentine surface demonstrated similar surface morphological appearances which were not different from the NaOCl-treated dentine in group 2 (Fig. 3).

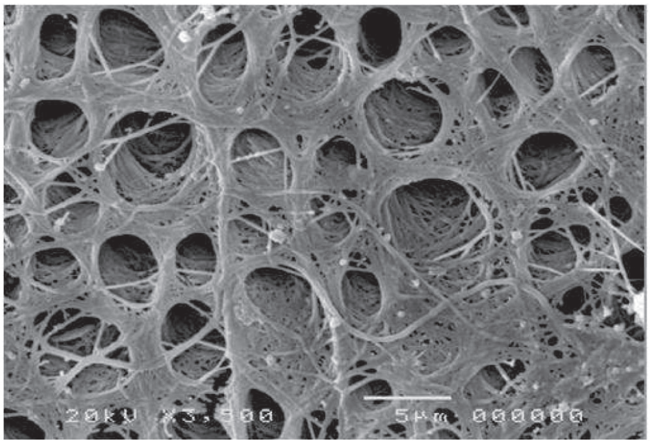

Fig. 2 *A SEM micrograph (x3500) of the pulp chamber dentine after rinsing with distilled water (control group)*

Discussion

Results of this study demonstrated that NaOCl, a common irrigation solution used in endodontic treatment, adversely affected the bond strength of Adper Single Bond II to dentine. This was in agreement with the results of previous studies.[9-11,13] Oxygen, which is broken down from NaOCl, might inhibit the polymerization of resin-based materials resulting in lower bond strength.[12,15] In addition, Nikaido et al[9] suggested that NaOCl damages the organic components of dentine. This might interfere with the penetration of monomer into the demineralized dentine and prevent the creation of a healthy hybrid layer resulting in low bond strength.[10]

Application of 10% sodium ascorbate on NaOCl-treated dentine (groups 3-7) significantly increased the bond strength of the adhesive to normal (control group) when compared with the NaOCl-treated group, this was in agreement with a previous study.[11] In another study[16] using flat grounded dentine surfaces, similar results were demonstrated that 10% sodium ascorbate reversed the bond strength of NaOCl-treated dentine. The results of the present study also showed that even though different bond strengths were found among the NaOCl-treated group and the sodium ascorbate groups, the treated dentine substrates revealed similar surface morphology (Fig. 3). NaOCl removed organic substances of predentin, found in the control group, and exposed the typically irregular structure of calcospherite. Application of 10% sodium ascorbate with different time durations revealed no effect on the morphology of NaOCl-treated dentine surface. This may indicate that the reversal of bond strengths was not due to the morphological change. The anti-oxidant property of sodium

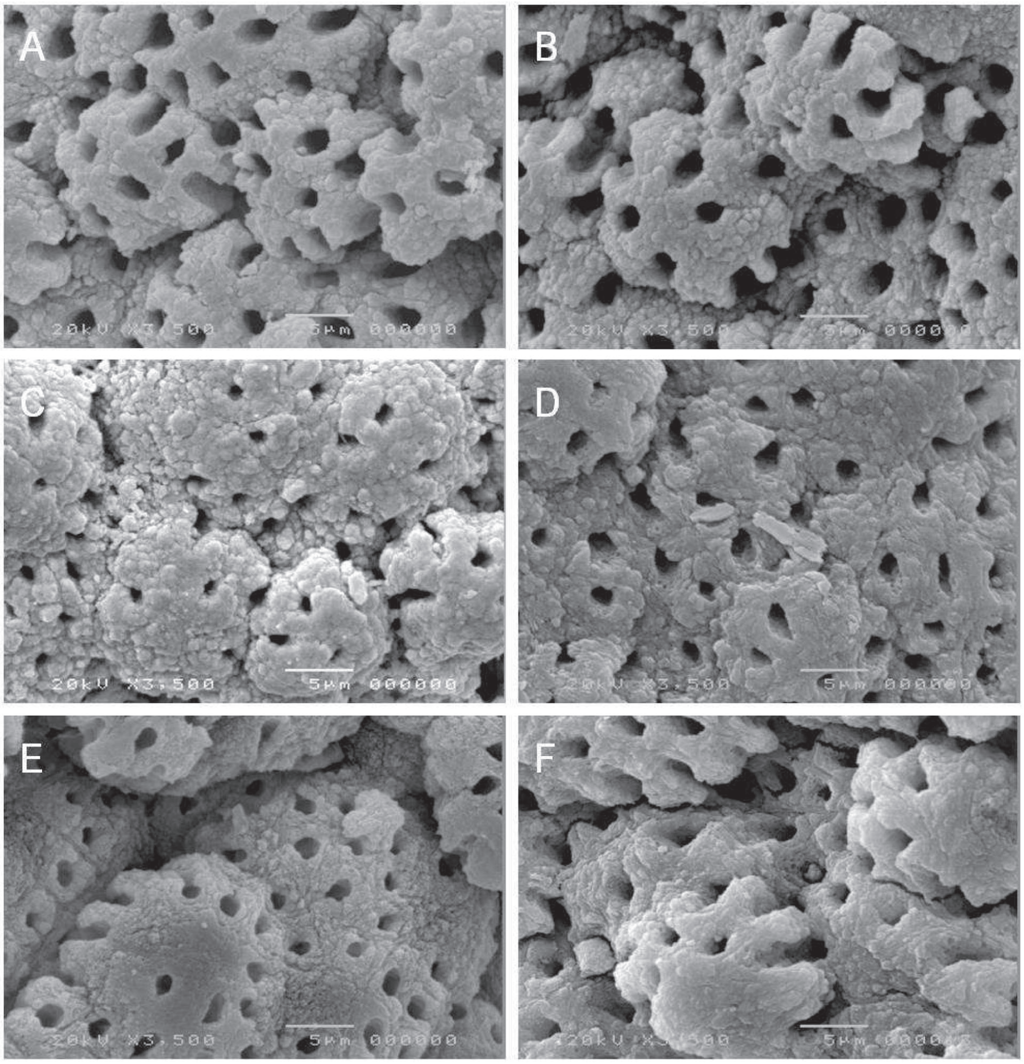

Fig. 3 *SEM micrographs of the pulp chamber dentine (x3500). A: after treated with 5.25% NaOCl for 10 min; B, C, D, E, and F: after treated with NaOCl followed by 10% sodium ascorbate for 30 s, 1 min, 3 min, 5 min, and 10 min, respectively*

ascorbate may neutralize and reverse the oxidizing effect of sodium hypochlorite by allowing free-radical polymerization of the adhesive to continue without premature termination, and thus the compromised bond strength is reversed.[16]

In the NaOCl-treated group, approximately 97% of the failures occurred at the resin/dentine interface and this adhesive failure tended to be reduced when sodium ascorbate was applied. This result seems to be correlated with the results obtained from the bond strength test. In this study, the data clearly demonstrated that the application of 10% sodium ascorbate either for short time durations (30 s, 1, 3, 5 min) or longer time (10 min) could reverse the bond strength of NaOCl-treated dentine similar to that of the control group (untreated), thus the null hypothesis is accepted. It is interesting that even the irrigation time of sodium ascorbate as short as 30 s could reverse the bond strength. This may suggest that the effect of sodium ascorbate is instantaneous. The sodium ascorbate used in this study; however, was a freshly prepared solution form. The instantaneous effect on dentine may be questionable for the commercial gel form, which the penetration and wetting property may be less than in the solution form. This requires further investigation. Some studies[17,18] in bleached enamel, converted sodium ascorbate into gel form and compared the effect of the gel form to its solution form. These studies revealed that both forms of sodium ascorbate increased the bond strengths to bleached enamel. However, the effective time of the gel form to reverse the bond strength has been reported to be longer than that of the solution form.[19] The 10-min irrigation time of sodium ascorbate significantly increased the bond strength when compared with the other sodium ascorbate-treated groups. This may be due to the longer time period and the greater volume of fresh sodium ascorbate solution to be in contact with the NaOCl-treated dentine surface. Thus, the more oxidized substrate might be neutralized.

De Munck et al[20] reported that the bond strength of two-step, total-etching adhesives decreased over time. Thus, it would be interesting to compare the long term durability of the bond strength of the two-step, total-etching adhesive in the 30 s, 1, 3, 5 min sodium ascorbate-treated groups to that of the 10-min group. Further study should be performed to clarify the mechanism of the reversal process as well as the long term durability of the bond strength. However, from a clinical point of view, application of sodium ascorbate for a shorter period was sufficient to reverse the bond strength to normal level and more practical than the 10-min application. The clinical implication of this study is that, with the use of sodium ascorbate, a dental practitioner can restore endodontically treated teeth immediately after finishing endodontic treatment with the two-step, total-etching adhesive (Adper Single Bond II).

Conclusion

Within the limitations of this study, irrigation of 10% sodium ascorbate solution for 30 s, 1, 3, 5 and 10 min on NaOCl-treated dentine significantly reversed the bond strength of the two-step, total-etching adhesive (Adper Single Bond II).

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บ ท วิ ท ย า ก า ร
Original Article

การคืนกลับของค่าแรงยึดบนเนื้อฟันที่ผ่านการล้างด้วยโซเดียมไฮโปคลอไรด์โดยการใช้โซเดียมแอสคอเบทที่ช่วงเวลาต่างกัน

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบผลของการใช้โซเดียมแอสคอเบทในระยะเวลาที่ต่างกันต่อค่ากำลังแรงยึดไมโครเทนไซล์ของสารยึดติดชนิดกรดกัดรวมกับเนื้อฟันของผนังโพรงเนื้อเยื่อในฟันที่ผ่านการล้างด้วยโซเดียมไฮโปคลอไรด์ โดยใช้ฟันกรามล่างมนุษย์ซี่ที่สามจำนวน 49 ซี่ นำมากรอตัดตัวฟันจากด้านบดเคี้ยวเพื่อกำจัดส่วนเพดานโพรงเนื้อเยื่อในฟันและกำจัดเนื้อเยื่อในออก แบ่งฟันเป็น 7 กลุ่ม กลุ่มละ 7 ซี่นำไปล้างโพรงเนื้อเยื่อในฟันด้วยน้ำกลั่น (กลุ่มควบคุม) โซเดียมไฮโปคลอไรด์ความเข้มข้นร้อยละ 5.25 หรือ โซเดียมไฮโปคลอไรด์ตามด้วยโซเดียมแอสคอเบทความเข้มข้นร้อยละ 10.0 เป็นเวลา 30 วินาที 1, 3, 5 และ 10 นาทีจากนั้นนำมาบูรณะโพรงเนื้อเยื่อในฟันด้วยเรซินคอมโพสิตร่วมกับสารยึดชนิดกรดกัดรวม (ซิงเกิลบอนด์ทู) เก็บฟันในน้ำที่อุณหภูมิ 37 องศาเซลเซียสเป็นเวลา 24 ชั่วโมง นำฟันที่บูรณะแล้วมาเตรียมชิ้นตัวอย่างเพื่อทดสอบค่าแรงยึดไมโครเทนไซล์ ศึกษาลักษณะการแตกหักที่เกิดขึ้นและลักษณะผิวฟันที่ผ่านการล้างด้วยน้ำยาต่าง ๆ ด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด วิเคราะห์ผลทางสถิติโดยใช้การวิเคราะห์ความแปรปรวนทางเดียวที่ระดับนัยสำคัญ .05 ผลการศึกษาพบว่าค่ากำลังแรงยึดไมโครเทนไซล์ของกลุ่มที่ล้างด้วยโซเดียมไฮโปคลอไรด์มีค่าน้อยกว่ากลุ่มทดลองอื่น ๆ อย่างมีนัยสำคัญ กลุ่มที่ใช้โซเดียมแอสคอเบทล้างเป็นเวลา 10 นาทีมีค่ากำลังแรงยึดสูงกว่ากลุ่มที่ใช้เวลาน้อยกว่า อย่างไรก็ตาม ค่ากำลังแรงยึดของกลุ่มที่ใช้โซเดียมแอสคอเบท น้อยกว่า 10 นาที ไม่ต่างจากกลุ่มควบคุม และไม่พบความแตกต่างในลักษณะพื้นผิวของฟันที่ล้างด้วยโซเดียมแอสคอเบทที่ทุกระยะเวลา