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ORIGINAL RESEARCH

Comparison of Chemical Aging and Water Immersion Time on Durability of Resin-Dentin Interface produced by an Etch-and-Rinse Adhesive

Bruna Uglik Garbui, Sergio Brossi Botta, Andre Figueiredo Reis, Adriana Bona Matos

ABSTRACT

Aim: The aim of this study was to analyze and compare the influence of short-term NaOCl-storage and long-term water storage on the microtensile bond strength (μTBS) of etch-and-rinse adhesive system to human dentin.

Materials and methods: Thirty-six third human molars were randomly divided into 6 groups (n = 6) according to the aging protocol: G1 (water, 24 hours); G2 (water, 6 months); G3 (water, 12 months); G4 (10% sodium hypochlorite – NaOCl, 1 hour); G5 (10% NaOCl, 3 hours) and G6 (10% NaOCl, 5 hours). A two-step etch-and-rinse adhesive (Adper Single Bond 2) was applied according to the manufacturers' instructions. A composite (Filtek Z250) was applied in four horizontal increments and was individually cured. Specimens were cut following the microtensile test technique, submitted to the different aging protocols, and tested in tension. The fracture pattern was observed in a stereomicroscope (40× magnification) and in a scanning electron microscope. The μTBS data were analyzed by ANOVA and Tukey's test ($\alpha = 0.05$).

Results: The effect of storage in 10% NaOCl for 1 or 3 hours was not significantly different from that of aging in distilled water (DW) for 6 or 12 months ($p > 0.05$). Beams immersed in DW for 24 hours and in 10% NaOCl for 5 hours showed the highest and lowest μTBS values respectively.

Conclusion: The aging protocols negatively influenced dentin bond strength. Aging specimens in 10% NaOCl for 1 or 3 hours can be an alternative method for long-term water storage (6 or 12 months) bond strength studies.

Clinical significance: This aging protocol allows a quick achievement of longitudinal bond strength data, so that results are available to the professionals in this area while the materials are yet present at the dental market.

Keywords: Laboratory research, Dental adhesive, Adhesion, Collagen, Dentin, Degradation, Durability, Microtensile bond test, Sodium hypochlorite.

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INTRODUCTION

The current approach of operative dentistry is focused on conservative restorative procedures. This is possible due to improvement of restorative techniques and polymeric materials for dental applications. However, hybrid layer degradation overtime is still a major issue.[1-6] Thus, replacement of adhesive restorations are normally necessary, leading to the so-called restorative cycle, where restorations are replaced by larger and more complex ones, compromising resistance of dental structure.[7]

Etch-and-rinse systems show excellent performance in dentin bond strength tests when short-term bond strength is evaluated (24 hours).[3,4,8-11] However, reduction in bond strength values is normally observed in long-term evaluations (6 months or more).[3,9-12] This reduction in restoration durability occurs due to hydrolysis of resin and/or collagen fibrils,[3,4] which causes degradation of the hybrid layer.[1,2,6,12,13]

Water storage is the most used procedure for specimen aging in durability tests of dentin-resin interfaces.[14-18] However, this method requires an idle period until results are obtained, and it is likely that the product under test becomes obsolete or out of the dental market. Thus, comparative studies of methods able to shorten this idle time are necessary and welcome.

Storage of specimens in 10% sodium hypochlorite (NaOCl) has been proposed with the intent to reduce experimental time.[14-18] Researchers[14-16] confirmed a higher reduction in bond strength (BS) and an increase in nanoleakage[14] for etch-and-rinse adhesive systems. However, the protocol for specimen aging with 10% NaOCl

is not yet standardized in the literature. Authors use different NaOCl immersion times, e.g. 2, 4, 5 or 6 hours.[18] Establishment of a standardized technique in order to compare results is essential. Tests of new formulations for dentin adhesives or new substrate treatments would be another important application, in which accelerated aging (within a few hours) could help to predict the long-term bond strength performance. Thus, storage in 10% NaOCl solution has shown to be a fast and reliable method for durability tests of the adhesive interface. However, the immersion time in this solution has not been defined so far.

Therefore, the aim of this study was to compare the influence of different specimen aging protocols on the BS of an etch-and-rinse adhesive system.

MATERIALS AND METHODS

Teeth Selection and Preparation

Thirty-six recently extracted human third molars (n = 6) stored for no more than 90 days in distilled water at 4°C were used in the present study. The occlusal surface was flattened with silicon carbide paper (240, 320 and 400) and a polishing device (100 rpm) with abundant water refrigeration. A standard smear layer was created # 600 SiC paper (60 seconds) before adhesive procedures.

Adhesive and Restorative Procedures

A two-step etch-and-rinse adhesive system (Adper Single Bond 2, 3M ESPE, St Paul, MN, EUA) was used according to the manufacturer's instructions (Table 1). After adhesive photo activation (Astralis 3 - Ivoclar Vivadent, Amherst, NY, EUA; 600 mW/cm^2), a composite resin (Filtek Z250, 3M ESPE) 'crown' was built in 4 horizontals increments (4 × 1 mm), which were cured (20 s) individually using the same light-curing unit.

Restored teeth were stored in distilled water at 37°C for 24 hours. Beams were obtained with a cross-sectional bonded area of approximately 0.9 mm^2 with a high-concentration diamond disk (Buehler Ltd. Lake Bluff, Illinois, EUA) under abundant water refrigeration, coupled with a precision cutting device (Isomet 1000, Buehler Ltd., Lake Bluff, IL, EUA—Proc FAPESP 05/04701-7). The beams were evaluated laterally under a light microscope (30× magnification) to check the adhesion area for failures. The specimens with visually detected defects on the adhesive interface were discarded.

Aging Protocol

Beams were randomly divided into 6 groups (n = 6), according to the aging protocol: G1 (water storage for 24 hours), G2 (water storage for 6 months), G3 (water storage for 12 months), G4 (10% NaOCl storage for 1 hour), G5 (10% NaOCl storage for 3 hours) and G6 (10% NaOCl storage for 5 hours).

After storage, specimens were individually fixed by their ends with a cyanoacrylate ester–based gel adhesive (Super Bonder—Loctite Brasil Ltd., Itapevi, SP, Brazil), to a jig attached to a Universal Testing Machine (EZ-Test 500N, Shimadzu Co, Kyoto, Japan) and tested in tension at a crosshead speed of 1 mm/min.

After the microtensile test, fractured specimens were mounted on metallic stubs and sputter-coated with gold (Denton Desk II, Denton Vacuum, LLC, Moorestown, NJ, EUA).

Failure Mode Evaluation

We examined fractured specimens using a stereomicroscope (Miview Digital Microscope Cosview Technologies Co. Ltd., Bantian, Longgang Dist, China) at 200× magnification to determine the mode of failure. Failure modes were classified as adhesive, cohesive failure of substrate, cohesive failure of restorative material or mixed (failures that involved adhesive interface and cohesive failure in substrate or adhesive interface and cohesive failure in restorative material).

For illustration of the fracture pattern, 12 representative specimens of each group were maintained for 48 hours in a desiccator (Sample Dry Keeper Simulate Corp, Tokyo, Japan) and then mounted on aluminum stubs with carbon cement. They were then sputter-coated with pure gold by means of a sputter-coating Denton Desk II (Denton Vacuum, LLC, Moorestown, NJ, USA) and observed with a scanning electron microscopy (SEM - JEOL JSM 6460, LV, Jeol Ltd., Tokyo, Japan, FAPESP # 00/08231-1) using acceleration voltage: 15 kV; working distance: 30 mm; spot size: 28 mm, so that microscopic fracture patterns and the morphology of the debonded interface could be studied.

Table 1: Adhesive system: Composition and application mode

Adhesive system	*Composition*	*Application mode*
Adper Single Bond 2 (3M ESPE, St Paul, MN, USA)	Bis-GMA, HEMA, dimethacrylates, methacrylate functional copolymer of polyacrylic and polyitaconic acids, ethanol, water, silica nanofillers, photoinitiators	1. Apply etchant for 15 seconds on dentin/enamel surface 2. Rinse with water for 30 seconds 3. Blot-dry dentin with a filter paper leaving it visibly moist 4. Apply adhesive on enamel/dentin surface 5. Gently air-dry for 10 seconds at a distance of 10 cm 6. Light-cure for 10 seconds

Photomicrographs were obtained for beam visualization (90× magnification) and fracture classification, and the most representative region of each surface was analyzed (1,000× magnification).

STATISTICAL ANALYSIS

Data were analyzed by one-way analysis of variance (ANOVA) and Tukey's test. The confidence level was set at $\alpha = 0.05$.

RESULTS

Microtensile Bond Strength

Mean values for the microtensile bond strength (μTBS), standard deviation (SD) and number of pretest failures for each group are presented in Table 2, when analyzed by the ANOVA and Tukey's test. Tukey test showed statistically significant differences among groups ($p < 0.05$). It was possible to observe that water storage for 24 hours (group 1) showed the highest μTBS values. Water storage for 6 or 12 months or in NaOCl storage for 1 or 3 hours showed no statistical difference between them. On the other hand, storage in NaOCl for 5 hours showed the lowest μTBS values.

Table 2: Mean (±SD) values for the μTBS test and number of pretest failures

Groups	*MPa*	*±SD*	*Valid beams*	*Beams lost*
G1–24 hours (H_2O)	54.9 a	8.3	100	1
G2–6 months (H_2O)	34.2 b	7.9	100	2
G3–12 months (H_2O)	32.0 b	3.4	99	10
G4–1 hours (NaOCl)	27.8 b	4.4	94	3
G5–3 hours (NaOCl)	32.4 b	3.0	99	4
G6–5 hours (NaOCl)	14.0 c	2.3	83	14

Stereomicroscopic Observation of Failure Modes

Figure 1 shows results for failure mode evaluation. All tested groups exhibited mainly adhesive/mixed fractures. The beams stored in water for 24 hours exhibited adhesive/mixed fractures (ca. 85%). Beams stored in water for 6 or 12 months exhibited predominantly adhesive/mixed fractures (ca. 80%). On the other hand, groups aged in NaOCl for 1 or 3 hours exhibited mostly adhesive/mixed fractures (ca. 70%) and about 15% dentin cohesive failure. Groups immersed in NaOCl for 5 hours exhibited about 50% adhesive/mixed fractures and 25% dentin cohesive fractures, and a significant number of pretest failures (14 beams) indicating weakening of specimens. Groups that were subjected to the NaOCl aging protocol exhibited higher number of dentin cohesive fractures than other groups.

In general, beams degraded from the periphery toward the center of the specimens regardless of the aging protocol.

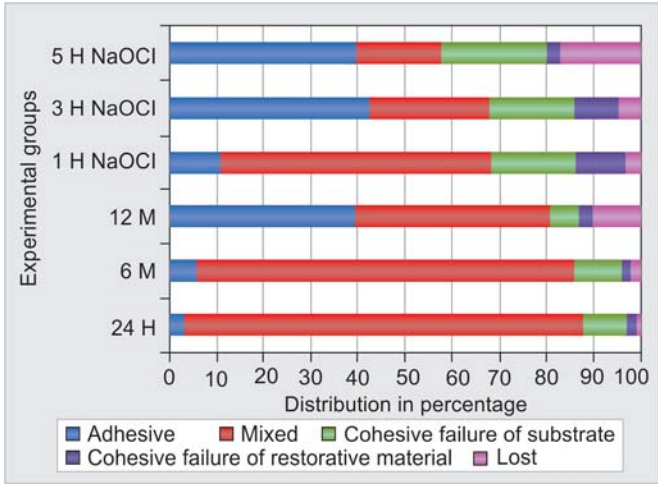


Fig. 1: Distribution of failure modes on test groups obtained with different protocols

Scanning Electron Microscope Observations

The effect of water storage for 24 hours can be observed in Figure 2A, where fracture occurred at the base of the hybrid layer. A flat surface, open dentin tubules, and a few tubules with resin tags inside them can be identified (Fig. 2B).

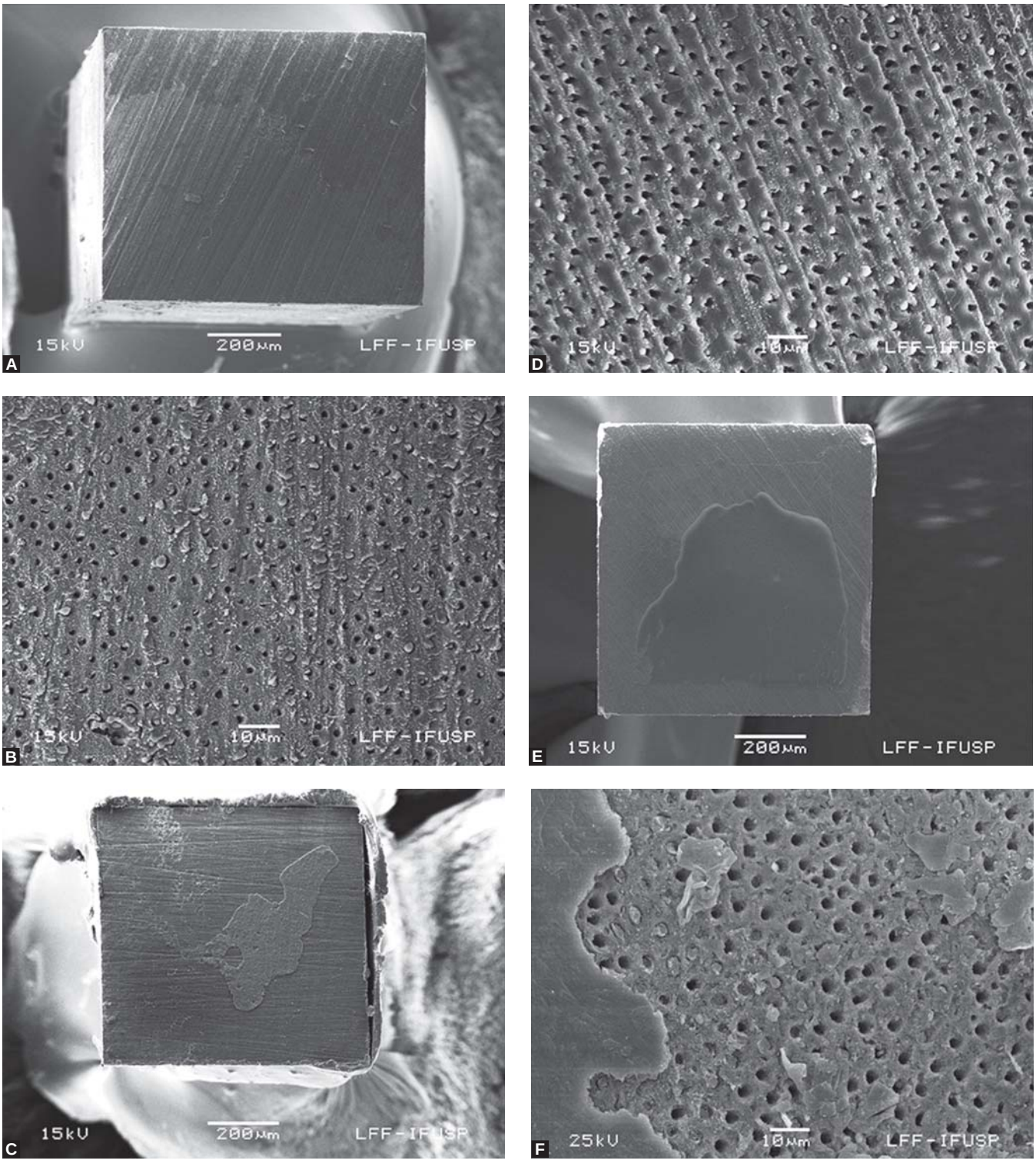
When specimens were submitted to water storage for 6 months the occurrence of mixed fracture with adhesive-covered areas in the center of the specimen (Fig. 2C) and areas with fracture at the base of the hybrid layer due to the presence of dentin tubules with resin tags inside them (Fig. 2D) can be observed.

After water storage for 12 months specimens showed mixed fracture where adhesive-covered areas can be observed at the center of the specimen (Fig. 2E). Open dentin tubules without presence of remaining resin tags can be observed at the periphery of the specimens (Fig. 2F).

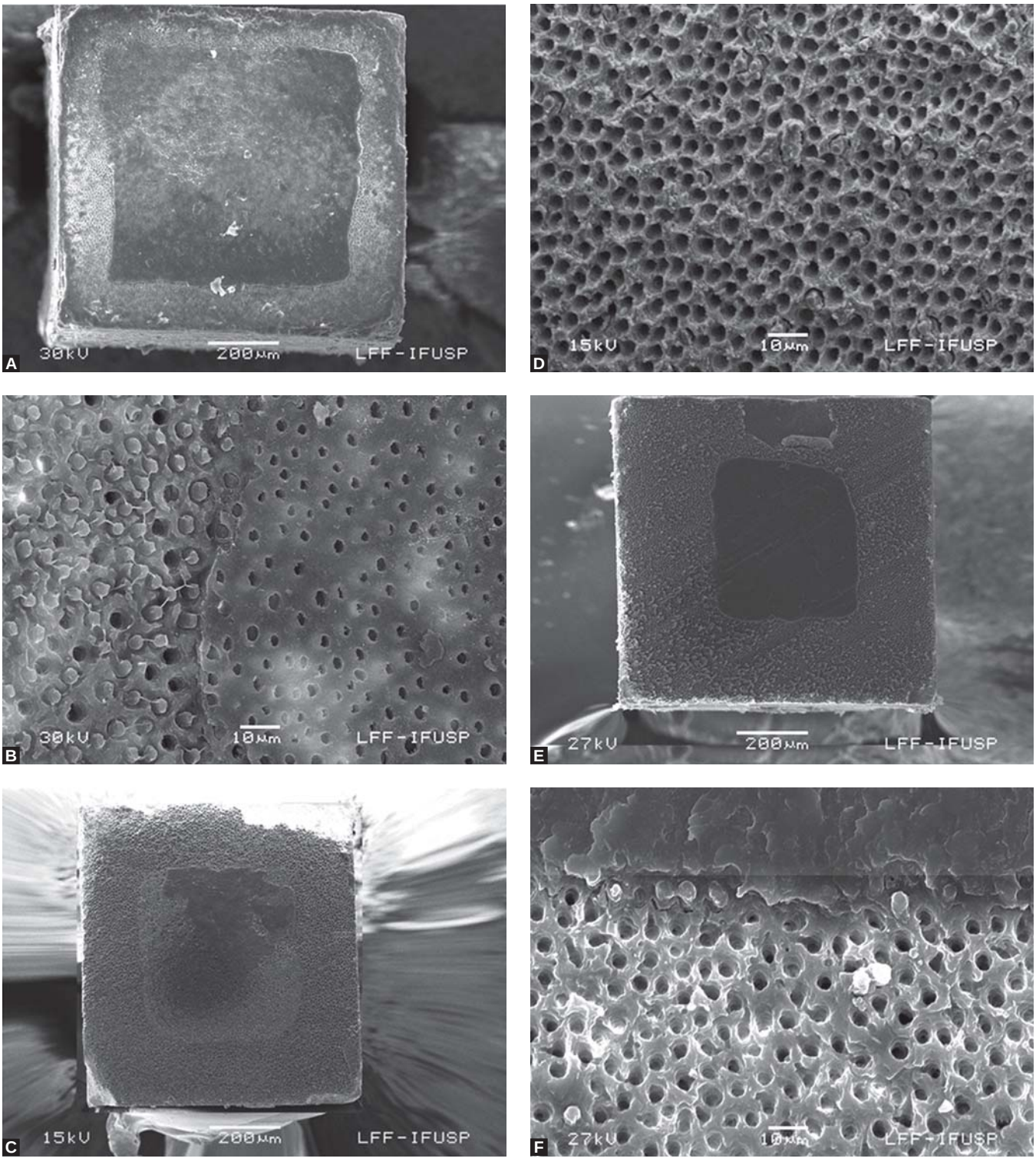
Storage under 10% NaOCl for 1 hour caused mixed fracture with adhesive-covered areas at the center of the specimens (Fig. 3A) and with dentin tubules filled with resin tags on the left side (Fig.3B). At the periphery of the specimens fracture of the hybrid layer base can be identified due to the presence of open dentin tubules.

The effect of storage in 10% NaOCl for 3 hours can be seen where a mixed fracture with adhesive-covered areas can be observed at the center of the specimens (Fig. 3C) with a lower extent compared to Fig. 3A. On the periphery of the specimens, fracture at the base of the hybrid layer with open dentin tubules (Fig. 3D) can be observed.

However, when the storage in 10% NaOCl was performed for 5 hours, mixed fracture can be observed with an adhesive-covered small region at the center of the specimen (Fig. 3E) and some regions clearly showed that the entire hybrid layer was dissolved. In addition, the dentin tubules were greatly enlarged (Fig. 3F).



Figs 2A to F: SEM photomicrographs of the failure pattern of μTBS beams submitted to water storage: (A) The effect of water storage for 24 hours can be observed (×90); (B) higher magnification of A (×1,000) showed that fracture occurred at the base of the hybrid layer with open dentinal tubules all over the dentinal surface; (C) when beams were stored for 6 months (×90), mixed fracture at the base of the hybrid layer can be seen with adhesive-covered areas in the center of the specimen and areas, it can also be identified a centripetal degradation of the adhesive interface; (D) due to the presence of dentin tubules with resin tags inside them, it can be assumed that fracture occurred at the base of the hybrid layer (×1,000); (E) after water storage for 12 months specimens (×90), the same centripetal pattern of degradation occurred with the occurrence of mixed fracture, where adhesive-covered areas can be observed at the center of the specimen; (F) open wide dentinal tubules without presence of remaining resin tags (×1,000)



Figs 3A to F: SEM photomicrographs of the failure pattern of μTBS beams submitted to sodium hypochlorite storage: (A) Storage in 10% NaOCl for 1 hours caused mixed fracture with adhesive-covered areas at the center of the specimens (×90); (B) in higher magnification of A (×1,000), a surface with dentin tubules filled with resin tags at the periphery of the specimens as well as regions with fracture of the hybrid layer base due to the presence of open dentin tubules; (C) after 3 hours of 10% NaOCl storage, a larger centripetal degradation could be observed when compared to the effect in A, a mixed fracture with adhesive-covered areas can be observed at the center of the specimens; (D) in higher magnification (×1,000) in the periphery of the specimens, fracture at the base of the hybrid layer with open dentin tubules can be observed; (E) however, when the storage in 10% NaOCl was performed for 5 hours, mixed fracture (×90) can be observed with an small region covered by adhesive at the center of the specimen and some regions clearly showed that the entire hybrid layer was dissolved; (F) in this higher magnification of E (×1,000), adhesive interface were intensively degraded with dentin tubules greatly enlarged by the deproteinizing effect of the NaOCl

DISCUSSION

Although water storage and thermocycling are established techniques for aging of the adhesive interface, both methods require long experimental periods.[14] Due to this limitation, aging of adhesive interfaces using 10% NaOCl solution has been proposed because it provides results more rapidly.[14-16,18-21]

NaOCl is a nonspecific deproteinizing agent that in aqueous solution forms superoxide radicals (O_2^-), which induce peptide chain oxidation in proteins, such as collagen.[22] This solution can also cause chlorination of the N-terminal end of proteins and formation of hypochlorous acid.[23] Some of these chloramines are associated with an increase in the susceptibility of collagen fibrils degradation.[24]

This degradation potential of NaOCl solution is responsible for the effective removal of organic compounds from the resin/dentin interface. This is due to its ability to dissolve the collagen fibrils that were not encapsulated by the adhesive resin,[18,19] thus, generating a higher degradation of the bonded interface and lower bond strength values.[14,19,20]

In the present study, bond strength values for specimens stored in distilled water for 24 hours were significantly higher than those of specimens stored in water for 6 months or 1 year, corroborating the results established in the literature.[9-12,25] The reduction in bond strength values can be explained by chemical hydrolysis of the resin and/or collagen fibrils, since the depth of dentin demineralization resulting from phosphoric acid application usually exceeds the capacity of resin monomer diffusion.[3] This difference in results is due to the formation of a demineralized resin zone partially infiltrated and without protection at the base of the hybrid layer.[26,27] As a consequence, partially infiltrated zones occur,[28,29] which are vulnerable to degradation,[30] thus, jeopardizing stability of adhesion between resin and dentin.

As occurred in the groups aged in distilled water, bond strength values observed after aging in 10% NaOCl solution for 1, 3 and 5 hours were lower than those obtained in the control group (specimens tested after 24 hours storage in water),[14-18,20] reinforcing that this is a method for accelerated aging of specimens.

No significant difference was found when bond strength values of groups stored in 10% NaOCl for 1 and 3 hours were compared, in accordance with previous investigations.[15] However, it was observed that μTBS values were significantly reduced when specimens were stored in 10% NaOCl for 5 hours. A possible explanation for this finding is that the longer the specimen remains in contact with NaOCl solution, it is able to break peptide chains of collagen fibrils by installing the process of degradation of the adhesive interface. Scanning electron microscope (SEM) observation demonstrated that the entire hybrid layer was clearly dissolved in some regions after aging in NaOCl for 5 hours. In addition, dentin tubules were greatly enlarged. The higher number pretesting failures in this group can be related to the weakening of the underlying dentin.

When bond strength values of groups stored in water and in NaOCl solution were compared, it was found that μTBS of specimens stored in water for 6 and 12 months were similar to those aged in 10% NaOCl solution for 1 hours or 3 hours. Studies comparing aging in 10% NaOCl for 3 hours and in distilled water for 6 or 12 months were not found in the literature. The most similar study available compared aging in 10% NaOCl for 3 hours and storage in artificial saliva for 6 months[14] and the results shown therein are similar to those found in our study.

In bond strength mechanical tests, fractured specimen surfaces generate information on the fracture mechanism. This type of analysis is important to verify, if the test was conducted adequately, inducing tension in order to break the bonded interface and not the adherent or adhered elements. Bond strength tests must induce uniform tension in order that the interface is tested, not the substrate.[31,32]

In general, a higher amount of mixed and adhesive fractures was detected in this study regardless of the aging protocol used. Beams stored in water for 6 or 12 months and in 10% NaOCl for 1, 3 and 5 hours degraded specimens from the periphery toward the center thus exhibiting centripetal bond interface degradation as observed in the literature.[15,20] Degradation was higher in the specimens stored in NaOCl for 5 hours.

When specimens were stored in 10% NaOCl for 5 hours, a higher frequency of dentin cohesive failures was verified because NaOCl can remove dentin organic components increasing its porosity,[33] causing substrate weakening and, consequently, a higher number of cohesive fractures. Therefore, a 5 hours exposure to NaOCl can be considered excessive, since, it caused reduction of μTBS results but also changes in the substrate. On the other hand, aging in NaOCl for 1 to 3 hours caused a degradation pattern very similar to that observed with water storage for 6 and 12 months and similar μTBS values.

CONCLUSION

It was concluded that storage of specimens in 10% NaOCl solution is valid as an aging protocol with the advantage of allowing us to get, after only 1 to 3 hours, BS data equivalent

to those of water storage for 6 to 12 months. Clearly, the immersion time in 10% NaOCl solution should be chosen by the researcher. Studies with the use of these protocols for accelerated aging of specimens and different adhesive systems are necessary.

CLINICAL SIGNIFICANCE

This aging protocol allows a quick achievement of longitudinal bond strength data, so that results are available to the professionals in this area while the materials are yet present at the dental market.

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ABOUT THE AUTHORS

Bruna Uglik Garbui

Department of Operative Dentistry, School of Dentistry, University of Sao Paulo, Brazil

Sergio Brossi Botta

Department of Operative Dentistry, School of Dentistry, University of Sao Paulo, Brazil

Andre Figueiredo Reis

Department of Restorative Dentistry, Guarulhos University Guarulhos, Sao Paulo, Brazil

Adriana Bona Matos (Corresponding Author)

Department of Operative Dentistry, School of Dentistry, University of Sao Paulo, Brazil, e-mail: bona@usp.br