Universität Zürich
Zentrum für Zahnmedizin
Klinik für Präventivzahnmedizin, Parodontologie und Kariologie (PPK)
Direktor: Prof. Dr. med. dent. Thomas Attin

Arbeit unter Leitung von PD Dr. med. dent. Florian J. Wegehaupt
und PD Dr. med. dent. Tobias T. Tauböck

Influence of Different Pretreatments on the Microtensile Bond Strength to Eroded Dentin

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Shengjile Deari

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Influence of Different Pretreatments on the Microtensile Bond Strength to Eroded Dentin

Shengjile Deari[a] / Florian J. Wegehaupt[b] / Tobias T. Tauböck[c] / Thomas Attin[d]

Purpose: To evaluate the influence of different pretreatments on the microtensile bond strength (μTBS) of an etch-and-rinse adhesive to eroded dentin.

Materials and Methods: Thirty-six human teeth were ground down to their dentin layer and randomly divided into six groups (G1-G6; n = 6), G1 being the control group. Only in the test groups (G2-G6) were samples subjected to erosion using citric acid (pH 2.6) 10 x 2 min per day for five days. Between the erosive attacks, samples were stored in artificial saliva. After pretreatment – none (G1); none (G2); 2% chlorhexidine (30 s) (G3); prolonged primer application (1 min) (G4); roughening with a diamond bur (G5) and 10.5% NaOCl (1 min) (G6) – the adhesive Opti-Bond FL was applied. After the application of composite, samples were stored in water (7 d) and μTBS was determined. Data were evaluated using one-way ANOVA and Dunnett-T post-hoc test ($p < 0.05$).

Results: Eroded dentin without pretreatment (G2) resulted in significant reduction of μTBS compared with uneroded dentin (G1). μTBS after pretreatment with a diamond bur (G5) or NaOCl (G6) was not significantly different from that of the uneroded control group (G1). μTBS after pretreatment with chlorhexidine (G3) or with prolonged primer application (G4) was significantly lower than in the uneroded control group (G1), and not significantly different from the eroded control group (G2).

Conclusion: The present data suggests that μTBS to eroded dentin pretreated with bur abrasion or NaOCl is similar to the μTBS to sound, uneroded dentin.

Keywords: adhesion, dentin, erosion, microtensile bond strength.

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Paranormal nutritional habits, such as increased consumption of acid-rich fruit or beverages, as well as the long-term use of acidic medications, environmental exposure to acidic fumes, gastro-esophageal reflux disease, or eating disorders are some of the causative factors resulting in erosive tooth wear in patients.[7] Dental erosion is characterized by a pathological, irreversible loss of dental hard tissue due to chemical dissolution, caused by intrinsic or extrinsic acids without bacterial involvement.[40]

The prevalence of dental erosion in the younger population appears to have increased over the last few decades.[14] Erosive tooth wear may affect the whole dentition, but the occlusal surfaces (mandibular first molars) followed by the facial surfaces (anterior maxillary teeth) are predominantly affected.[14] The damage is often localized adjacent to the cementoenamel junction and manifests as a smooth, silky, glazed surface.[9,10] Progression of the erosive process results in a rounding and grooving of the cusps, or shallow cavities, and can lead to dental morphological changes and occlusal vertical dimension loss.[10] Furthermore, tooth hardness decreases significantly following demineralization, and the enamel layer becomes more susceptible to mechanical challenges, such as toothbrushing.[3,10] A clinical study investigating the erosive/abrasive tooth wear of enamel and

[a] *Resident Dentist, Clinic of Preventive Dentistry, Periodontology and Cariology, University of Zurich, Zurich, Switzerland. Performed the experiments, wrote the manuscript.*

[b] *Head, Division of Preventive Dentistry and Oral Epidemiology, Clinic of Preventive Dentistry, Periodontology and Cariology, University of Zurich, Zurich, Switzerland. Study idea and design, analyzed the data, critically revised and proofread the manuscript.*

[c] *Head, Division of Cariology, Clinic of Preventive Dentistry, Periodontology and Cariology, University of Zurich, Zurich, Switzerland. Participated in the study design and data analysis, critically revised and proofread the manuscript.*

[d] *Professor and Head, Clinic of Preventive Dentistry, Periodontology and Cariology, University of Zurich, Zurich, Switzerland. Realized the study idea and experimental design, proofread the manuscript.*

Correspondence: *Shengjile Deari, Clinic of Preventive Dentistry, Periodontology and Cariology, University of Zurich, Plattenstrasse 11, 8032 Zurich, Switzerland. Tel: +41-44-634-3284; e-mail: shengjile.deari@zzm.uzh.ch*

dentin in patients suffering from erosion reported a median wear of 36 µm in a six-month period.[5]

For the prevention of erosion, several treatment options have been postulated and investigated. Amaechi and Higham[1] considered possible approaches to prevent erosion at an early lesion stage. Their preventive strategies comprised treatment of the underlying medical disorders and diseases, use of remineralizing agents, fluoride mouthrinses, neutralizing agents and/or protective devices. Additionally, drinking habits should be changed and greater emphasis should be placed on health education.

The prevention potential of anti-erosive substances has been reported for different fluoride compounds, such as amine, sodium, or tetra fluorides.[33,36] Aside from these fluoride compounds, other anti-erosive compounds such as stannous chloride or cerium chloride have been investigated.[22,34] Another approach involves covering the lesion with sealant or adhesive, so further hard tissue loss can be prevented by minimizing the contact of the erosion-causing acids with the dental hard tissues.[27]

If the dentin becomes exposed and/or the loss of dental hard tissue results in a functionally and esthetically unacceptable condition, the teeth must often be restored. Since the amount of hard tissue that has to be removed prior to restoration should be kept to a minimum, the treatment of choice for the restoration of erosively worn dentitions may involve direct composite buildups applied using an adhesive. As a result of their improved physical and mechanical properties and due to improvements in wear resistance,[19,28,30,31] the indication of composites is no longer limited to small and medium-sized defects. Recent reports suggest that modern composites can also be successfully used for the rehabilitation of severely worn dentitions requiring vertical bite reconstruction.[2,12,29]

Concerning the use of adhesives on eroded dentin, a study by Zimmerli et al[39] showed lower bond strength to eroded dentin compared with sound dentin. As a result of this study, superficial preparation or minimal roughening of the eroded dentin with a diamond bur is recommended. A drawback of this method is that it results in additional removal of dental hard tissue. Other approaches that avoid further loss in preparing erosively altered surfaces should therefore be sought. One approach is to inhibit matrix metalloproteinases (MMPs) by application of 2% chlorhexidine (CHX) after etching.[11] MMPs may be activated by the low pH environment during erosion, leading to collagen and hybrid layer degradation. Pretreatment of dentin with CHX has been shown to result in an increased stability of the hybrid layer and therefore increased bond strength.[6] Thus, the physical durability of the adhesive interface can be improved. The adhesive interface may also be affected by a prolonged primer application time. During regular exposure of dentin to erosive acids, the dentin demineralizes, leaving a layer of collagen on the surface.[26] It is assumed that such a collagen layer can inhibit the interaction of dentin and the adhesive. The same effect can be observed if the dentin etching time is prolonged, therefore exposing more collagen. The primer then cannot penetrate the whole etched zone (collagen layer). To achieve a physical and chemical interaction, the adhesive has to fully penetrate the etched zone.[24] The hypothesis that a prolonged primer application time results in better penetration of the previously exposed collagen network must be evaluated, as no such in vitro studies exist to date. Sodium hypochlorite (NaOCl) is known to dissolve the collagen network. NaOCl is a non-specific proteolytic solution that removes organic components from teeth.[38] Therefore, NaOCl may offer the solution to improve bond strength to eroded dentin by removing the compact layer of collagen that has been exposed during erosive attacks. However, it must be borne in mind that dentin with depleted collagen is less suited to creating the hybrid layer.

Currently, there is no gold standard method for pretreating eroded teeth to yield reliable adhesive restorations. Hence, the aim of this study was to evaluate the influence of different pretreatments (CHX application, prolonged primer application time, bur abrasion, NaOCl application) on eroded dentin and to characterize the interfacial failure modes. The working hypothesis was that there is a difference in the µTBS achieved after the different pretreatments.

MATERIALS AND METHODS

Specimen Preparation

For this in vitro study, 36 extracted noncarious human molars were selected. The teeth were collected as by-products of regular dental treatments, and patients approved the use of their teeth for experimental purposes. After scaling and cleaning, the teeth were stored in tap water until use for a maximum of four weeks. To facilitate manipulation, the root tips were glued centrally on a specimen holder for scanning electron microscopes (Wenka, Karl Wenger; Courgenay, Switzerland) using superglue (Superglue no. 1733-2000, Renfert; Hilzingen, Germany). The teeth were then embedded in self-curing acrylic resin (Paladur, Heraeus Kulzer; Hanau, Germany) and ground parallel to the occlusal plane to remove the occlusal enamel and produce a smear layer. The teeth were ground with a polishing machine (Planopol-2, Struers; Ballerup, Denmark) at low speed (150 rpm) under constant water cooling with 180-grit silicon carbide paper (Buehler-Met II, Buehler; Lake Bluff, IL, USA) creating a roughening effect similar to that of an 80-µm diamond bur.[8] The surfaces were dried and checked under a stereomicroscope (Stemi 1000, Carl Zeiss; Feldbach, Switzerland) for the presence of enamel remnants or pulp tissue exposure in the central part. Afterwards, the teeth were randomly allocated to six groups (n = 6): uneroded control (G1), eroded control (G2), and four test groups (G3 to G6). Figure 1 illustrates the sample allocation and experimental procedures.

Demineralization

The specimens of groups G2 to G6 were cyclically demineralized and remineralized ten times daily for five days. A

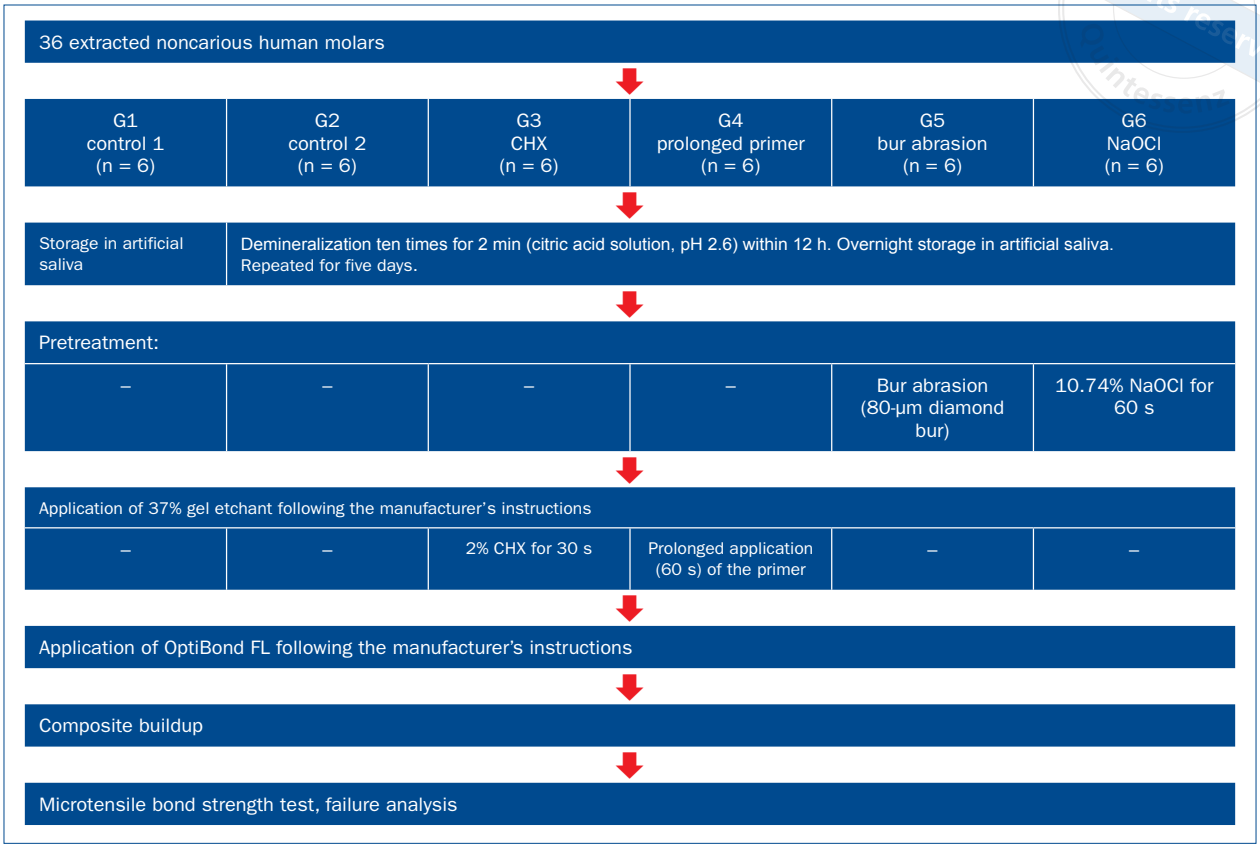


Fig 1 Sample allocation and experimental procedure.

cycle included the following processes: demineralization in 10 ml/specimen of citric acid for 2 min under agitation, rinsing with tap water for 5 s, and storage in 10 ml artificial saliva/specimen for remineralization under agitation. The solutions were renewed after each cycle. The samples were stored overnight (8 h) in artificial saliva without agitation. The samples of the uneroded control group (G1) (n = 6) were stored in artificial saliva, which was renewed after each cycle, but not exposed to acid.

The citric acid solution (Merck, VWR International; Zurich, Switzerland) with a concentration of 0.0094 M and a pH of 2.6, as well as the artificial saliva (pH 6.5), were freshly prepared daily. The artificial saliva contained 0.011 mmol/l ascorbic acid, 0.167 mmol/l glucose, 9.925 mmol/l NaCl, 1.530 mmol/l $CaCl_2 \cdot 2H_2O$, 2.991 mmol/l NH_4Cl, 17.036 mmol/l KCl, 1.974 mmol/l NaSCN, 2.425 mmol/l KH_2PO_4, 3.330 mmol/l urea and 2.395 mmol/l Na_2HPO_4 (Merck, VWR International GmbH, Zurich, Switzerland), according to Klimek et al.[16]

Restoration

After allocation to the six groups and demineralization of the five groups (G2 to G6), the teeth were treated as follows:

- G1 (uneroded/control 1): Application of the three-step etch-and-rinse adhesive OptiBond FL as per the manufacturer's instructions. For this purpose, the etchant (37.5% phosphoric acid, Lot 5062962, Syringe Kerr Gel Etchant, Kerr; Scafati, Italy) was applied for 15 s on the surface, rinsed for 15 s with water until the etchant was completely removed, and dried gently using an air syringe. Primer (OptiBond FL Prime, Lot 5055655, Kerr) was then applied with light brushing movements for 15 s and lightly air dried for 5 s until the dentin had a slight glassy appearance. The surface was then coated with a thin layer of adhesive (OptiBond FL Adhesive, Lot 5057827, Kerr) which was light cured for 20 s using an LED curing unit (Bluephase G2, Ivoclar Vivadent; Schaan, Liechtenstein). The curing unit had a light intensity in the range of 1150 to 1200 mW/cm^2, which was monitored each day by radiometer (Bluephase meter, Ivoclar Vivadent).
- G2 (eroded dentin/control 2): Application of OptiBond FL as per manufacturer's instructions.
- G3 (eroded dentin/CHX): After etching with Kerr Gel Etchant for 15 s, the dentin was treated with 2% chlorhexidie (CHX) (Merck, VWR International) for 30 s using flexible disposable applicators (Kerr Applicators, Kerr). The surface was then rinsed for 15 s with tap

water and gently dried. The primer (OptiBond FL Prime, Kerr) and the adhesive (OptiBond FL Adhesive, Kerr) were used in accordance with the manufacturer's instructions.

- G4 (eroded dentin/prolonged primer): Application of OptiBond FL following the manufacturer's instructions for Kerr Gel Etchant and the adhesive (OptiBond FL Adhesive, Kerr). However, the primer (OptiBond FL Prime, Kerr) was applied for a prolonged duration, 60 s instead of 15 s.
- G5 (eroded dentin/bur abrasion): Before application of OptiBond FL following the manufacturer's instructions, the surface was roughened using a water-cooled diamond bur (80-µm cylindrical diamond bur, FG 8305L/6, Lot 02623, Intensiv; Montagnola, Switzerland) to remove a thin layer of eroded dentin as suggested by Zimmerli et al.[39] To achieve consistent dentin removal (0.1 mm), the handpiece was attached to an appliance previously described by Wiegand et al,[37] which maintains a consistent load and height during preparation. The handpiece was applied with a load of 100 g at a rotation rate of 40,000 rpm.
- G6 (eroded dentin/NaOCl): Before application of the etchant and adhesive, the dentin surface was treated with 10.5% NaOCl (sodium hypochlorite, Lot V05053, Laboratorium Dr. G. Bichsel; Interlaken, Switzerland) for 60 s, rinsed for 15 s with tap water, and then gently dried.

Following the treatment described above, nanofilled composite (Filtek Supreme XTE, shade A1, Lot N549719, 3M ESPE; St Paul, MN, USA) buildups of 4 to 5 mm height were produced and applied in three increments to cover the whole exposed dentinal surface. Each composite increment was approximately 1.5 mm thick, which was checked using a periodontal probe.

Each increment was light cured for 20 s using the Bluephase G2 light-curing unit. After constructing the buildups, the specimens were stored for one week in tap water at 37°C.

µTBS Test

To determine µTBS, the specimens were cut longitudinally in two directions using a water-cooled diamond saw (Struers Accutom-50) with a diamond wheel (M1D10, Struers; size: 102 mm x 0.3 mm x 12.7 mm) to obtain nine rectangular sticks from the central portion of each tooth. The sticks were then cut parallel to the surface using a slow-speed saw (Isomet, Buehler) to obtain sticks with a length of 8 to 9 mm. The dimensions of each stick were measured with a digital caliper (Kisling; Zurich, Switzerland) and recorded to calculate the bonding area. The specimens had a cross-sectional bonding surface area between 0.855 mm^2 and 0.999 mm^2. All specimens were mounted at either end on a sandblasted (50-µm aluminum oxide) µTBS jig with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Ohtawara, Japan). The sticks were loaded under tension until failure in a universal testing machine (Zwick Roell Z010; Ulm, Germany) using a load cell of 200 N (KAF-TC, AST; Dresden, Germany) and a crosshead speed of 1 mm/min. The load at failure (N) divided by the bonding area (mm^2) yielded the tensile bond strength in MPa.

Failure Analysis

Failure modes were evaluated using a dual-head stereo zoom microscope (Wild; Heerbrugg, Switzerland) at 25X magnification and judged as cohesive (within dentin or the composite buildup), adhesive (between dentin and buildup), or mixed failure (both adhesive and cohesive). SEM images were taken (Zeiss Supra V50, Carl Zeiss; Oberkochen, Germany) after gold sputter coating (BAL-TEC SCD 030; Balzers, Liechtenstein) for 90 s.

SEM Analysis of Dentin Surfaces

Additionally, SEM images of all groups were prepared to visually evaluate the dentin surfaces after pretreatment. SEM images were taken after grinding (G1); erosion of the ground dentin (G2); erosion of the ground dentin, etching and treatment with CHX (G3); erosion of the ground dentin, etching and prolonged primer application (G4); erosion of the ground dentin and subsequent bur abrasion (G5); erosion of the ground dentin and treatment with NaOCl (G6).

Statistical Analysis

The µTBS of specimens that failed prior to testing (pre-test failures) was set at zero MPa. Data were entered in Microsoft Excel (Microsoft Office Professional Plus 2010, Microsoft; Redmond, WA, USA) and analyzed using SPSS (IBM SPSS Statistics for Windows, Version 21.0; Armonk, NY, USA). Descriptive statistics such as means and standard deviations were computed. The Kolmogorov-Smirnov test was used to investigate the normality assumption at the mean bond strength level. As data were normally distributed, differences between treatment groups with respect to the mean bond strength level were analyzed using one-way ANOVA followed by the Dunnett-T post-hoc tests. P-values < 0.05 were considered to be statistically significant.

RESULTS

Figure 2 illustrates the mean µTBS and standard deviations for all groups.

The µTBS in the groups where eroded dentin was prepared with a diamond bur (G5) (25.78 ± 9.41 MPa) or treated with NaOCl (G6) (27.13 ± 9.30 MPa) were not significantly different from the µTBS of the uneroded control group (G1) (24.88 ± 10.87 MPa) ($p = 0.839$ and 0.612, respectively). No significant difference was observed between the µTBS of groups G5 and G6 ($p = 0.760$).

The µTBS of the eroded dentin groups treated with CHX (G3) (9.33 ± 3.93 MPa) or with a prolonged application of primer (G4) (8.52 ± 3.92 MPa) were not significantly different from that of the erosion control G2 (10.06 ± 4.59 MPa) ($p = 0.869$ and 0.728 respectively).

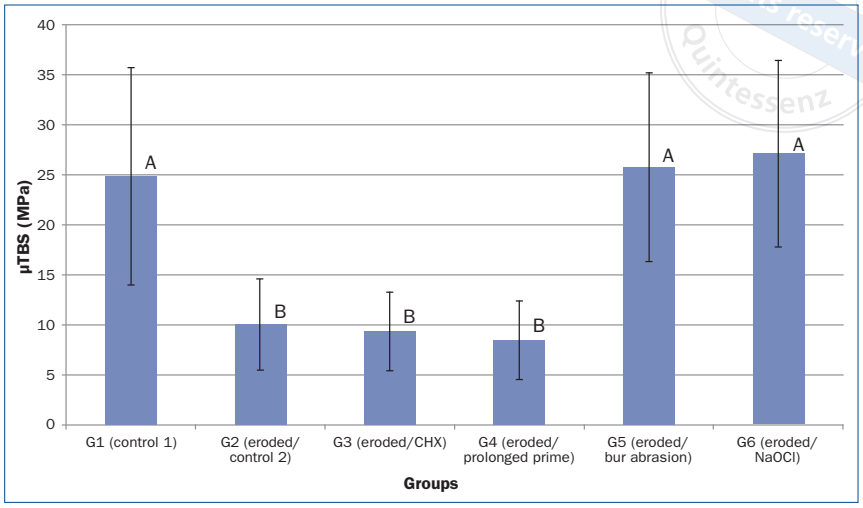


Fig 2 Mean (SD) µTBS in MPa for the six groups. Values that are not significantly different are marked with same capital letters.

The µTBS of G1, G5, and G6 were significantly higher than those of G2, G3, and G4.

Failure mode distributions are shown in Table 1. In all groups except G6, the most frequent failure mode was adhesive. Figure 3 presents representative SEM images of the dentin and composite side of the fractured surfaces. In adhesive failure, tags were disrupted and remnants of tags are visible in the dentinal tubules (Figs 3a and 3b). In cohesive failure, fractures within the respective substrates (dentin, composite) are visible (Figs 3c and 3d). The mixed failure mode shows both surface conditions (Fig 3e; dentin and composite).

SEM images of dentin surfaces are shown in Fig 4. In G1, a surface with smear layer and grinding marks is visible. Partially removed smear layer and exposed tubules are visible in G2. G3 and G4 clearly exhibit open dentinal tubules. The appearance of the G5 specimen is similar to that of G1, but with a smoother surface. In G6, the dentin surface displays occluded dentinal tubules.

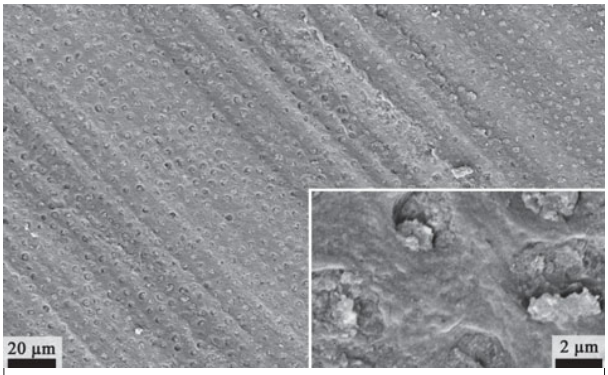
Table 1 Pre-test failures and distribution of failure modes for all groups

Group	Treatment (pre-test failures)	Failure mode of tested sticks		
		Adhesive	Cohesive	Mixed
G1	Noneroded / control 1 (9)	31	2	12
G2	Eroded /control 2 (13)	36	4	1
G3	Eroded / CHX (11)	38	5	0
G4	Eroded / prolonged primer application (8)	43	3	0
G5	Eroded / bur abrasion (3)	22	14	15
G6	Eroded / NaOCl (10)	17	3	24

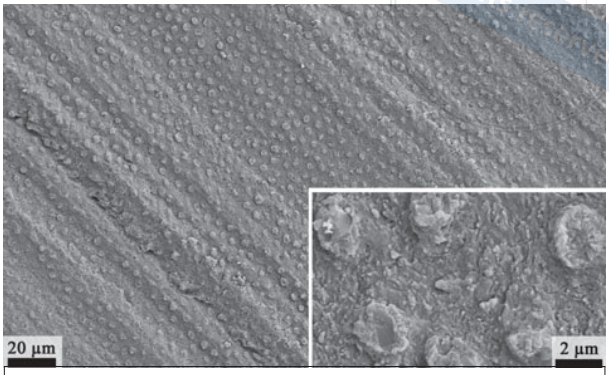
DISCUSSION

In this study, extrinsic erosive attacks were simulated by the use of citric acid with a pH of 2.6. The erosive attacks were performed at 2-min intervals, ten times daily for five days under agitation to simulate the clinical situation during the consumption of an acidic beverage. The pH was chosen according to Lussi et al,[18] where the pHs of acidic beverages were found to be between 2.2 and 3.7. The acidic challenge performed was modified from the pH-cycling model described by Ganss et al,[9] in which the attacks were performed 6 times per day for 10 min each for five days. Kirkham et al[15] showed that for the same total duration of erosive attacks (number of attacks per day multiplied by duration of attack), the total amount of mineral loss is higher if acid attacks are shorter and at an increased frequency. In this study, the duration of attacks was reduced and the frequency increased to compensate for the lower total duration of erosive attacks compared to the above-mentioned study.[9] To simulate the clinical situation with consumption of acidic beverages and saliva flow, the citric acid and artificial saliva were applied under agitation. Between the erosive attacks, the specimens were stored in artificial saliva in order to simulate remineralization, as performed in numerous previous studies.[4,13,35]

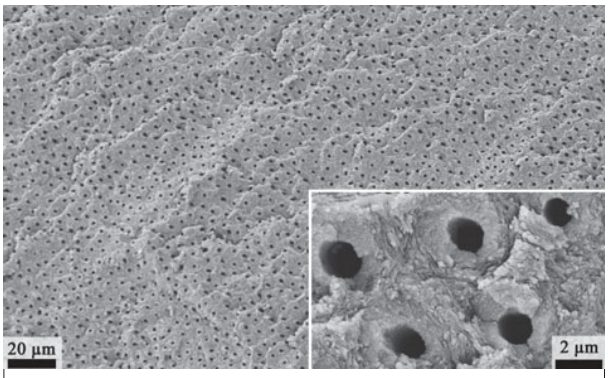
To minimize the number of possible influencing factors for all groups tested, the same adhesive was selected. OptiBond FL showed the best results in the evaluated properties, such as µTBS, nanoleakage, and in-situ degree of conversion on dentin.[17] Therefore, OptiBond FL can be considered a gold standard.[17] In support of this, Van Meerbeek et al[32] found that three-step etch-and-rinse adhesives such



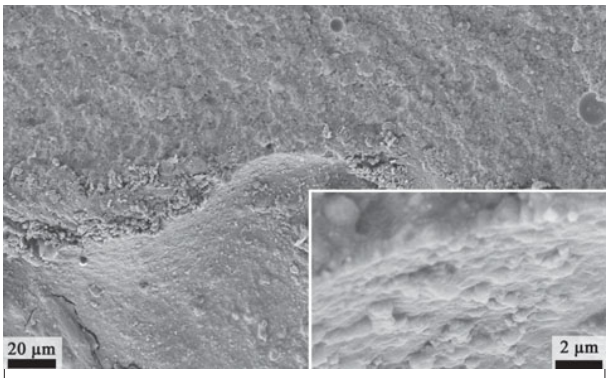
a Adhesive failure (dentin side)



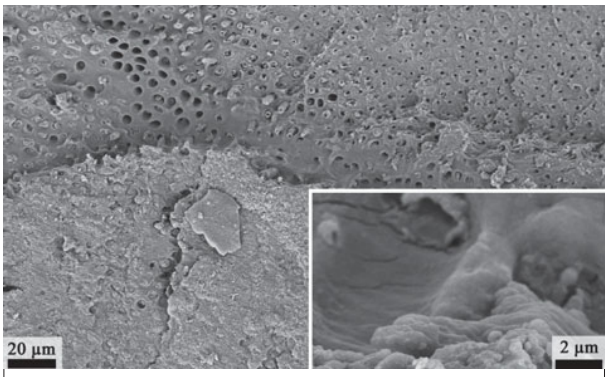
b Adhesive failure (composite side)



c Cohesive failure (dentin side)



d Cohesive failure (composite side)



e Mixed failure

Fig 3 Representative (SEM) images of the dentin and composite sides of the fractured surfaces.

as OptiBond FL, still provide superior performance in laboratory studies and clinical research. Thus, the adhesive OptiBond FL was used in the present study.

To assess the μTBS, a universal testing machine was used as described by Pashley et al.[20] With such methodology, it is possible to evaluate the adhesion of restorations to dental hard tissues. Compared to the shear bond strength test, the numerous advantages of this test method are accepted:[21] more adhesive and fewer cohesive failures are generated (as observed in the present study [Table 1]), and higher interfacial bond strength can be measured. However, there are also disadvantages.[21] For instance, measurements are labor intensive, technically demanding, and bond strengths < 5 MPa are challenging to measure. Nevertheless, due to the versatility and the many advantages over conventional shear bond strength and macrotensile bond strength testing, this procedure was chosen in the present study.

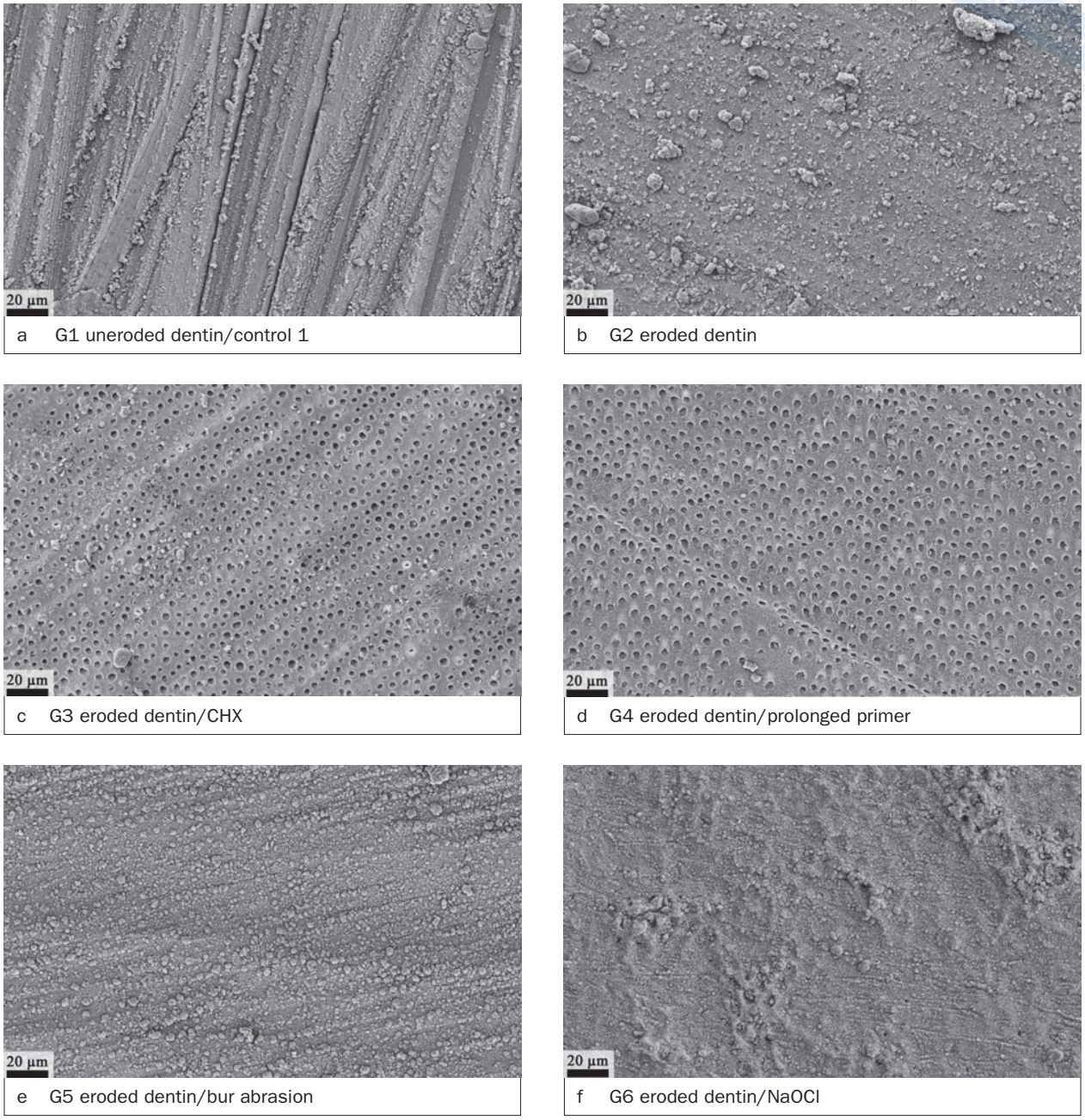


Fig 4 SEM images of dentin surfaces.

The working hypothesis was accepted, as significantly different μTBS were achieved after the different pretreatments. The chosen sequence of the pretreatments might have resulted in different thicknesses of the demineralization zone, which may have affected the outcome. However, the chosen sequence was based on the clinical situation.

After performing erosive attacks with citric acid solution, a significant decrease in the μTBS (uneroded G1 vs eroded G2 dentin) was observed, which is supported by the recent findings of Zimmerli et al.[39] Highly exposed dentin collagen may result in insufficient penetration during adhesive application, since adhesive components may not penetrate the whole depth of the demineralized layer produced by erosion. The resulting hybrid layer may contain porosities, resulting in inferior bond strength.[25]

The present study found that preparation with a diamond bur (G5) resulted in similar μTBS (Fig 2) and smoother dentin surfaces (Fig 4) compared with the uneroded control

group (G1). This approach was based on a recommendation by Zimmerli et al,[39] who found that preparation using a diamond bur increased bonding ability on eroded dentin. Nevertheless, such a procedure causes dental hard tissue loss. Consequently, in order to minimize hard tissue loss, other methods were also considered. Interestingly, NaOCl treatment also yielded μTBS similar to the uneroded dentin group (G1). As NaOCl is a nonspecific proteolytic agent, NaOCl is able to remove the exposed collagen from the demineralized dentin. It can be assumed that when exposed collagen is removed by NaOCl, the etchant and adhesive applied subsequently can react with the dentin as if no previous erosion had occurred. A study by Prati et al[23] showed that application of NaOCl after phosphoric acid etching resulted in higher bond strengths when used in conjunction with OptiBond FL.

Pretreatment with CHX was chosen, as an in vitro study by Carrilho et al[6] showed that the application of CHX after etching preserves the durability of the hybrid layer and the bond strength. Prolonged primer application duration was tested, because it has been suggested that this may result in better penetration of the exposed collagen. However, neither treatment with CHX nor prolonged primer application significantly increased the bond strength compared with the eroded control group (G2). It can therefore be assumed that the suggested effects were not achieved.

CONCLUSION

The pretreatment of eroded dentin by bur abrasion or concentrated NaOCl had a positive effect on μTBS, yielding values similar to those found under nonerosive conditions. As the pretreatment with NaOCl results in no additional dentin loss compared to bur abrasion, this approach seems favorable. However, further studies are needed to validate these findings by subjecting similar samples to thermomechanical loading, longer-term storage, and clinical application trials, in order to achieve a standard procedure for clinical use.

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Clinical relevance: Teeth with noncarious eroded surface lesions often require adhesive reconstructions using composites. Pretreatment with NaOCl improves the μTBS of an etch-and-rinse adhesive to eroded dentin without additional hard tissue loss, attaining values comparable to those observed on noneroded tooth surfaces.