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# EDTA or $H_3PO_4$/NaOCl dentine treatments may increase hybrid layers' resistance to degradation: A microtensile bond strength and confocal-micropermeability study

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ABSTRACT

*Objectives:* The aim of this study was to reduce hybrid layer degradation created with simplified dentine adhesives by using two different methods to condition the dentine surface.
*Methods:* A smear-layer was created on flat dentine surfaces from extracted human third molars with a 180-grit/SiC-paper. Dentine specimens were conditioned before bonding with the following procedures: 37% $H_3PO_4$; $H_3PO_4$/0.5% NaOCl; 0.1 M EDTA; 0.1 M EDTA/0.5% NaOCl. Two etch-and-rinse adhesives: (Scotchbond 1XT or Optibond Solo Plus) were applied and light-cured. Composite build-ups were constructed. The bonded teeth were sectioned into beams, stored in distilled water (24 h) or 12% NaOCl solution (90 min) and finally tested for microtensile bond strengths ($\mu$TBS). Additional dentine surfaces were conditioned and bonded as previously described. They were prepared for a pulpal-micropermeability confocal microscopy study and finally observed using confocal microscopy.
*Results:* $\mu$TBS results revealed that both adhesives gave high bond strengths to acid-etched dentine before, but not after a 12% NaOCl challenge. Bonds made to acid-etched or EDTA-treated dentine plus dilute NaOCl, gave high $\mu$TBS that resisted 12% NaOCl treatment, as did EDTA-treated dentine alone. A confocal micropermeability investigation showed very high micropermeability within interfaces of the $H_3PO_4$, etched specimens. The lowest micropermeability was observed in $H_3PO_4$ + 0.5% NaOCl and 0.1 M EDTA groups.
*Conclusions:* The use of dilute NaOCl (0.5%) after acid-etching, or the conditioning of dentine smear layers with 0.1 M EDTA (pH 7.4) produced less porous resin–dentine interfaces. These dentine-conditioning procedures improve the resistance of the resin–dentine bond sites to chemical degradation (12% NaOCl) and may result in more durable resin–dentine bonds.


## 1. Introduction

To achieve optimal dentine bonding and sealing, the adhesive system should completely infiltrate the collagen network exposed during dentine acid-etching procedures.[1] Perfect resin infiltration can reduce the degradation of the collagen fibrils within the hybrid layer.[2–4] However, several studies have shown that the one bottle etch-and-rinse bonding systems do not completely infiltrate the demineralized dentine leaving unprotected collagen fibrils below and within the hybridized layer.[5–8]

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Long-term *in vitro*[9,10] and *in vivo*[7,11] studies have shown that the bond strength of resin-bonded dentine decreased over time due to collagen degradation within the hybrid layer.

Resin diffusion into and within the demineralized intertubular dentine matrices occurs via 20–30 nm wide interfibrillar spaces.[12,13] These narrow interconnecting channels for adhesive diffusion are not empty but contain proteoglycans that remain within the matrix after acid conditioning of the dentine.[14–16]

Acidic etchants remove the mineral phase of the smear layer, but the collagen component is not totally dissolved by phosphoric or citric acids.[17] Phosphoric acids may induce the gelatinization, the disorganization or denaturation of collagen that may trap residual mineral constituents within the hybrid layer. Removal of dentine proteoglycans by chondroitinase ABC increases the bond strength of etch-and-rinse adhesive systems to dentine and reduces the nanoleakage within the hybrid layer, due to a reduction of the amount of water content and enlarged interfibrillar spaces.[18] However, the use of such specific enzymes requires prolonged treatment times (i.e. 24 h) that are not suitable for clinical application. Therefore, alternative strategies must be developed to remove organic components from the demineralized layer prior to the bonding procedures.

Different conditioning procedures have been proposed: (1) sodium hypochlorite (NaOCl), a non-specific deproteinizing agent, has been suggested as an adjunctive procedure following the etch-and-rinse technique to improve the wettability of the bonding substrate,[19–21] facilitating intertubular and intratubular resin infiltration[22,23]; (2) milder dentine demineralization achieved using EDTA may avoid the denaturation of collagen[24] and improve the quality of the hybrid layer and its durability[25] due to the presence of more residual apatite crystallites left within the collagen matrix subsequent to the dentine conditioning.[26,27]

Confocal microscopy can be used to evaluate the micropermeability and the sealing ability of resin tags and hybrid layers after filling the pulp chamber of a bonded tooth with a water-soluble fluoroprobe such as Rhodamine B (Rh-B) for 3 h. The seepage of Rh-B into any water-filled spaces within the resin-bonded complex shows the different routes of fluid flow from the pulp chamber.[8] If the demineralized network is well infiltrated, there should be no water filled microporosities in continuity with (Rh-B) in the pulp chamber.

The aim of this study was to evaluate two different procedures for dentine conditioning in order to improve the resistance of resin–dentine bonds to degradation of hybrid layers. These aims were accomplished by evaluating the effects of EDTA or $H_3PO_4$/NaOCl dentine treatments on microtensile bond strength (μTBS) and micropermeability (μP) of hybrid layers created by two one-bottle etch-and-rinse adhesive systems.

## 2. Materials and methods

### 2.1. Specimen preparation

Human molars (age 20–40), extracted for surgical reasons under a protocol approved by an institutional review board of the King's College London, Dental Institute, London, UK were used in this study. The teeth were stored in 4 °C deionised water (pH 7.1) for no more than 1 month. The teeth were sectioned 1 mm beneath the cemento-enamel junction (CEJ) with a diamond wafering blade (high concentration XL 12205, Benetec Limited, London, UK) using a hard tissue microtome (Isomet 11/1180, Buehler, Coventry, UK) to remove the roots. The occlusal enamel was subsequently removed with a parallel cut to expose middle coronal dentine. The exposed dentine was polished with 180 grit silicon carbide paper to remove the diamond saw smear layer and replace it with a more clinically relevant smear layer.[28] The teeth were divided in to groups and subgroups (Table 1).

**Table 1 – Number of teeth used in each principal group and in each subgroups.**

| Principal groups and subgroups | Scotchbond 1 XT (μTBS/confocal μP/resin–dentine characterization) | | | Optibond Solo Plus (μTBS/confocal μP/resin–dentine characterization) | | |
|---|---|---|---|---|---|---|
| 37% $H_3PO_4$ | 5 | 3 | 2 | 5 | 3 | 2 |
| 37% $H_3PO_4$ + 0.5% NaOCl | 5 | 3 | 2 | 5 | 3 | 2 |
| 0.1 M EDTA | 5 | 3 | 2 | 5 | 3 | 2 |
| 0.1 M EDTA + 0.5% NaOCl | 5 | 3 | 2 | 5 | 3 | 2 |

### 2.2. Experimental design and bonding procedures

Two single-bottle, etch-and-rinse adhesives were used in this study: Scotchbond 1XT (SCH: 3M ESPE, St. Paul, MN) and Optibond Solo (OBS: Kerr Corp., Orange, CA, USA). There were four surface-treatments: (1) 15 s etch with 37% phosphoric acid (PA); (2) 15 s etch with 37% PA followed by 30 s treatment with 0.5% NaOCl; (3) 90 s etch with 0.1 M EDTA (pH 7.4); (4) 90 s etch with 0.1 M EDTA (pH 7.4) followed by 30 s treatment with 0.5% NaOCl.

The composition and application mode of the two commercially available one-bottle etch-and-rinse adhesive resin systems, Scotchbond 1XT and Optibond Solo Plus, used in this study are listed in Table 2.

The dentine surfaces were conditioned as per group assignment and rinsed for 30 s with deionised water. Excess water on the dentine surface was air dried (5 s) avoiding excessive desiccation of the surface. At least two coats of the bonding resin were then applied to the conditioned dentine and light-cured for 15 s with a light-curing unit with a blue light source (470 nm, ~600 mW $cm^{-2}$, Optilux VLC, Demetron Research Co., CT, USA). After applying the adhesive systems, the flowable resin composite Filtek Supreme XT™ (3M ESPE) was placed incrementally in five 1 mm layers to create 5 mm build-ups for both systems. Each layer was light-cured for 20 s with a final light-curing procedure of 40 s. The specimens were then stored in distilled water at 37 °C for 24 h.

### 2.3. Micro-tensile bond strength test

The resin–dentine specimens were serially sectioned after 24 h of water storage using a hard tissue microtome (Isomet