# Effect of tetracycline on the bond performance of etch-and-rinse adhesives to dentin

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**Abstract:** This study evaluated the effect of modified tetracycline on the resin-dentin bond strength (μTBS), silver nitrate uptake (SNU) and solution homogeneity (SH) of two adhesives. Dentin surfaces were treated with phosphoric acid, rinsed off and either rewetted with water (control group - CO), 2% minocycline (MI), 2% doxycyline (DO) or 2% chlorhexidine (CH). Adhesive systems (Adper Single Bond 2 and Prime Bond NT) and composite were applied and light-polymerized. Specimens were sectioned to obtain bonded sticks (0.8 mm²) to test under tension at 0.5 mm/min. For SNU, specimens were immersed in silver nitrate and analyzed by EDX-SEM. SH was qualitatively analyzed after mixing the adhesives with different solvent-based solutions containing MI, DO and CH. Lower μTBS values were observed in the DO group compared with MI and CH ($p = 0.01$). Lower SNU was observed for MI and CH. The lowest μTBS for both adhesives was observed for the DO group ($p = 0.01$). Signs of phase separation were observed for DO with both adhesives. MI or CH used as rewetting solutions after acid etching did not affect the μTBS and hybrid layer quality.

**Descriptors:** Dentin-Bonding Agents; Chlorhexidine; Tetracycline; Tensile Strength.

## Introduction

There is a general consensus that resin-dentin bonds created by simplified adhesives deteriorate over time,[1] and this occurrence has been attributed mainly to degradation of the hybrid layer.[2] A decreasing gradient of resin monomer diffusion within the acid-etched dentin[3] results in incompletely infiltrated zones along the bottom of hybrid layers that contain denuded collagen fibrils.[4] These denuded collagen fibrils are vulnerable to degradation by endogenous metaloproteinases (MMPs) in a way that is similar to what occurs in caries and periodontal diseases.[5,6] Meanwhile it has been speculated that a subsequent resin elution from hydrolytically unstable polymeric hydrogels within the hybrid layers[7] leaves the collagen fibrils unprotected and also susceptible to the same degradation process. The literature has also described various pathological processes in which MMPs are implicated.[8]

MMPs are a group of 23 mammalian enzymes capable of degrading all extracellular matrix components. Human dentin contains collagenase (MMP-8), gelatinases MMP-2 and -9, and others.[9,10] These den-

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tin collagenolytic and gelatinolytic activities can be suppressed by protease inhibitors. There are several MMP inhibitors described in the literature and, among them, chlorhexidine (CH) and tetracyclines have been shown to be efficient adjuncts to periodontal therapy.[11]

So far, studies have evaluated the role of CH as MMP inhibitor to preserve resin-dentin bonds from degradation.[12-16] Tetracycline and their semi-synthetic forms (doxycycline [DO] and minocycline [MI]), in addition to acting as antibiotics, are very safe and potent MMP inhibitors.[17-20] The action mechanisms of chemically modified tetracyclines (CMTs) are thought to work through inhibiting the activity and secretion of MMPs and $Ca^{2+}$ chelation.[21] However, the effects of different tetracyclines used as rewetting agents after acid etching on the immediate performance of adhesive systems to dentin has not been previously investigated. Therefore the aim of this *in vitro* study was to evaluate the effects of DO, MI and CH on the immediate resin-dentin bond strengths and silver nitrate uptake of adhesive interfaces and homogeneity among adhesives and the solutions tested.

# Methodology

## Teeth selection and preparation

Forty extracted, caries-free human third molars were used. This study was approved by the local Ethics Committee under protocol # 6280/2009. A flat and superficial dentin surface was exposed on each tooth after wet grinding the occlusal enamel on # 180-grit SiC paper. The enamel-free dentin surfaces were further polished on wet # 600-grit silicon-carbide paper (Erios Prod. Odont. Ltda., São Paulo, Brazil) for 60 s. Teeth were divided in eight groups (n = 5) according to the combination of the main factors Adhesive (2 levels) and Rewetting solution (4 levels).

## Restorative procedure

An acetone solvent-based adhesive (Prime & Bond NT [PB], Dentsply De Trey, Konstanz, Germany) and a water/ethanol solvent-based adhesive (Adper Single Bond 2 [SB], 3MESPE, St. Paul, USA) were used. The surfaces were acid etched with phosphoric acid, rinsed off, air-dried and rewetted actively with water[22] (control group - CO), and aqueous solutions of 2% minocycline (MI), 2% doxycycline (DO) (Fleming drugstore, Ponta Grossa, Brazil) or 2% chlorhexidine digluconate (CH) (FGM, Joinville, Brazil) for 60 s. After that adhesive systems and resin composite (Opallis, FGM, Joinville, Brazil) were applied as described in Table 1. Five teeth were used for each experimental group.

The bonded teeth were longitudinally sectioned to obtain bonded sticks (0.8 $mm^2$ area)[13,15]. Each bonded stick was attached to a modified microtensile testing device, using cyanoacrylate resin, and subjected to a tensile force in a universal machine (Emic, São José dos Pinhais, Brazil) at 0.5 mm/min. Failure modes were evaluated at 400× (HMV-2, Shimadzu, Tokyo, Japan) and classified as described in Table 2.

## Silver nitrate uptake technique

Two bonded sticks from each tooth were not tested under tension, and were prepared for SNU evaluation according to an earlier protocol.[13,15] Briefly, the sticks were coated with two layers of nail varnish applied up to within 1 mm of the bonded in-

**Table 1 -** Adhesive systems (batch number): composition and application mode.

| Adhesive systems | Composition | Application Mode |
|---|---|---|
| PB (1223207A) | 1. Caulk Tooth Conditioner Gel 34% phosphoric acid<br>2. Adhesive – UDMA, PENTA, R 5-62-1 resin, T resin, D resin, silanated colloidal silica, cetylamine hydroxyfluoride, initiator, stabilizer and acetone | 1. acid-etch (15 s)<br>2. rinse (15 s)<br>3. air-dry (30 s)<br>4. active rewetting for 60 s<br>5. two coats of adhesive were applied slightly for 20 s<br>6. air-dry for 10 s at 20 cm<br>7. light polymerize (10 s – 600 mW/$cm^2$) |
| *SB* (# 9XJ) | 1. Scotchbond Etchant 35% phosphoric acid<br>2. Adhesive – Bis-GMA, HEMA, dimethacrylates, nanofilled colloidal silica (5 nm) polyalkenoic acid copolymer, initiators, water and ethanol | |

**Table 2 -** μTBS values and standard deviations (MPa) and fracture pattern (%) (*).

| | Prime & Bond NT | A/M | C | PT | Adper Single Bond 2 | A/M | C | PT |
|---|---|---|---|---|---|---|---|---|
| Control | 35.7 ± 4.5 A,B | 24 (75.0) | 0 (0) | 8 (25.0) | 42.1 ± 8.3 a | 34 (80.9) | 0 (0) | 8 (19.1) |
| 2% MI | 40.2 ± 12.1 A | 38 (77.6) | 0 (0) | 11 (22.4) | 36.9 ± 16.4 a | 33 (71.7) | 0 (0) | 13 (28.3) |
| 2% DO | 30.3 ± 8.2 B | 28 (50.0) | 0 (0) | 28 (50.0) | 40.2 ± 13.2 a | 36 (75.0) | 0 (0) | 11 (25.0) |
| 2% CH | 38.1 ± 8.3 A | 35 (87.5) | 0 (0) | 5 (12.5) | 42.8 ± 10.4 a | 32 (94.1) | 0 (0) | 2 (5.9) |

(*) Comparisons are valid within each adhesive system. Means identified with the same upper or lowercase letters are not significantly different (Tukey's test, $p > 0.05$). A/M – adhesive/mixed fracture mode; C – dentin or resin cohesive fracture mode; PT – pretest failures.

terfaces. The specimens were rehydrated in distilled water for 10 min before immersion in the tracer solution for 24 h. After that the sticks were placed in 50% ammoniacal silver nitrate and immersed in photo developing solution for 8 h under fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface. Specimens were then polished down (1,000-grit SiC paper and 1 and 0.25 lm diamond paste [Buehler, Lake Bluff, USA] using a polishing cloth), desiccated and sputter-coated with gold for analysis by a scanning electron microscope operated in backscattered electron mode and energy dispersive X-ray spectrometry ([EDX], SSX-550, Shimadzu, Tokyo, Japan). The working distance was 10 mm and the accelerating voltage was 15 Kv. The amount of SNU was measured following an earlier protocol.[22]

### Solution homogeneity

MI and DO (2%) were dissolved in water, acetone and ethanol. Sixteen μl of each adhesive was transferred to a small glass and mixed with 16 μl of each adhesive system. The mixture was stirred for 10-15 s. A digital image of each mixture was taken 30 s after mixing began, using a digital camera (D70 and AF-S VR Micro-Nikkor 105 mm, Nikon, Tokyo, Japan). Loss in clarity was interpreted as evidence of phase separation. Before mixing, all solutions were clear liquids.[2] This experiment was done in quadruplicate.

### Statistical analysis

The mean μTBS (MPa) and SNU (%) of all sticks from the same tooth were averaged for statistical purposes (n = 5 for experimental condition).

**Table 3 -** SNU and standard deviations (%) and statistical significance (*)

| | Prime & Bond NT | Adper Single Bond 2 |
|---|---|---|
| Control | 27.9 ± 4.9 B,C | 25.5 ± 4.9 b |
| 2% MI | 23.9 ± 3.6 A,B | 16.3 ± 4.2 a |
| 2% DO | 34.3 ± 3.8 C | 25.5 ± 4.3 b |
| 2% CH | 16.4 ± 5.4 A | 18.1 ± 3.9 a,b |

(*) Comparisons are only valid within each adhesive system. Means identified by the same upper or lowercase letters are not significantly different (Tukey's test, $p > 0.05$).

The μTBS and SNU were subjected to a one-way ANOVA for each adhesive system and a post hoc test (Tukey's test at $\alpha = 0.05$).

## Results

The μTBS and fracture pattern results are shown in Table 2. No significant difference was detected among groups for the SB adhesive ($p > 0.05$). For PB, lower μTBS values were observed when DO was applied compared with the other solutions ($p < 0.05$) (Table 1).

As regards SNU, significant differences were observed for both adhesives ($p < 0.05$). Lower % of SNU was observed for PB and SB when CH and MI was used in comparison with DO ($p < 0.05$) (Table 3 and Figure 1).

It was not possible to produce mixtures of MI and DO with acetone, as these were not soluble in acetone. MI and DO were only slightly soluble in ethanol (data not shown). Therefore SH was only tested with aqueous solutions of the aforementioned substances. Aqueous solutions of MI and DO were clear and completely transparent before mixing with

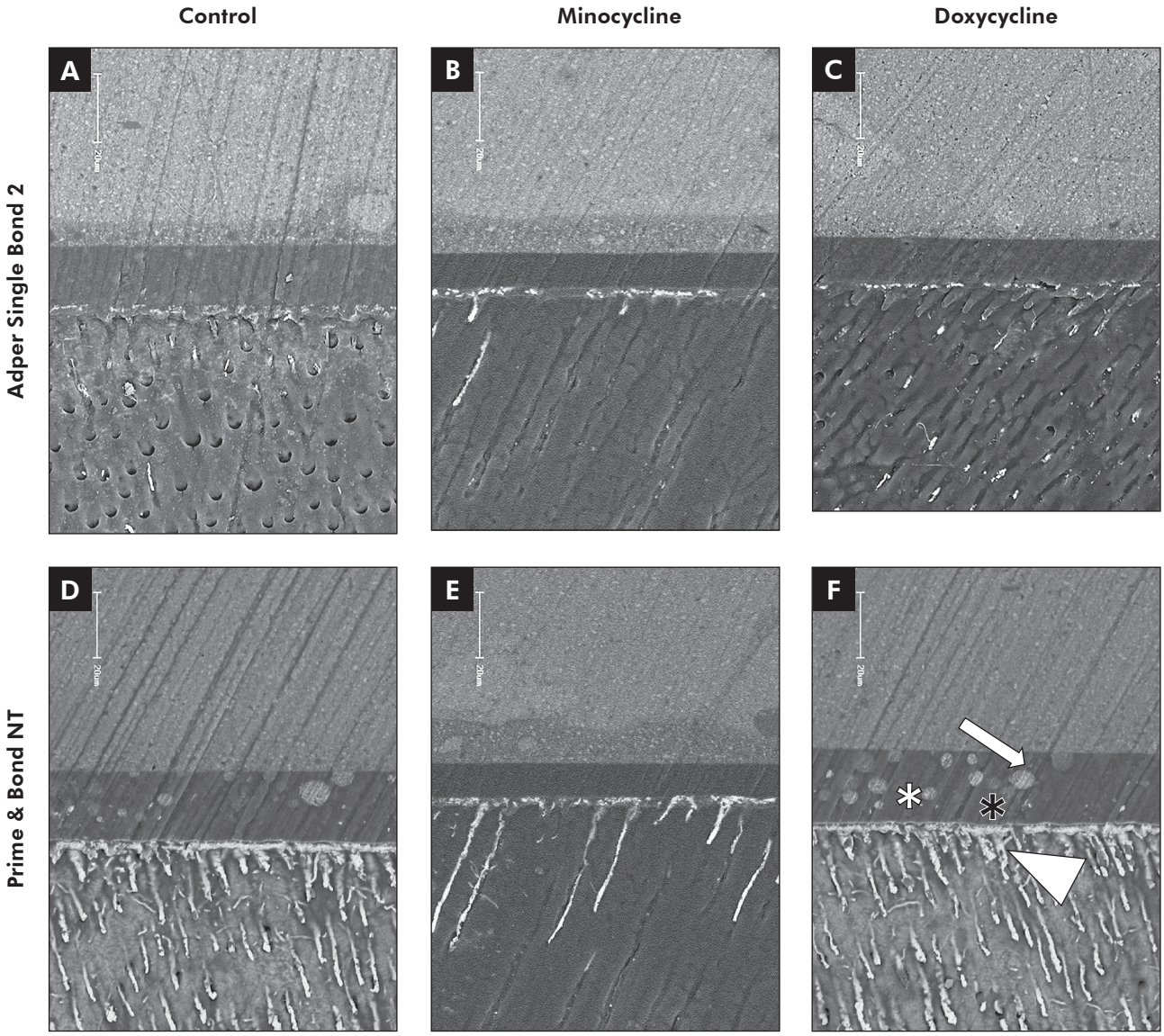


**Figure 1 -** Backscattered SEM images of the resin-dentin interfaces bonded with SB **(A to C)** and PB **(D to F)**. For all SB figures, the amount of silver penetration was lower and occurred practically only within the hybrid layer. Only few dentin tubules were infiltrated by silver nitrate. For PB, SNU deposition occurred almost throughout the entire thickness of the hybrid layer, mainly in the DO group. The presence of some globules in the adhesive layer **(F)** probably indicates phase separation (**arrow** = globules; **White asterisk** = adhesive layer; **black asterisk** = hybrid layer and **triangle** = dentin). Magnification: 1000×.

the adhesives (Figures 2A and 2D).

SB mixed with aqueous solution of DO showed a slight phase-separation (Figure 2B). No resin droplets, representative of phase-separation, were seen when SB was mixed with the aqueous solution of MI, and the adhesive was shown to be homogeneous and transparent (Figure 2D). PB mixed with MI and DO showed the presence of several resin droplets (Figures 2C and 2E). In contact with MI the solution turned opaque with few opaque resin droplets (Figure 2E).

## Discussion

Tetracyclines and their analogues (DO and MI) are a group of MMP inhibitors. These compounds are zinc-dependent endopeptidases that play an important role in the remodeling of connective tissue and are involved in embryogenesis, wound heal-

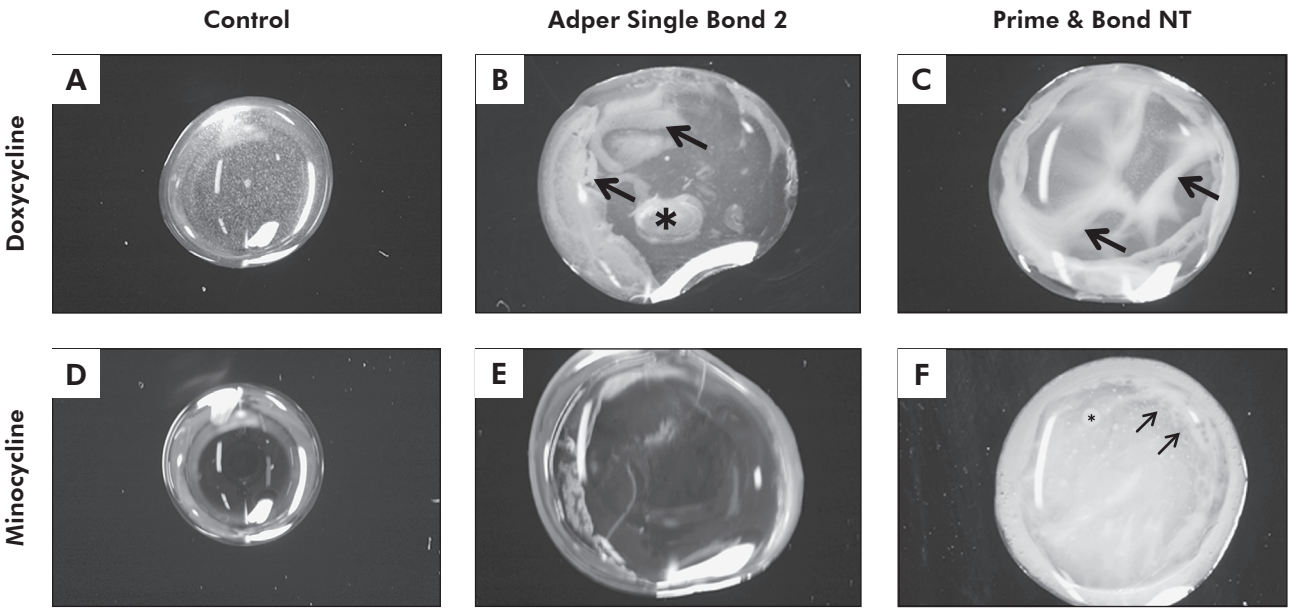


**Figure 2 -** Representative digital pictures of the MI and DO solutions **(A and D)** and the solution homogeneity after mixing these solutions with the adhesive systems. For SB, several resin droplets **(arrows)** and globules **(asterisk)**, representative of phase-separation were observed when mixing with DO **(B)**. No sign of phase-separation was observed when SB was mixed with MI **(E)**. For PB, signs of phase-separation, with many droplets, were observed in all parts of the specimens, when mixed with DO **(C, arrows)** and little **(F, asterisk)** and opaque droplets **(F, arrows)** when mixed with MI. Magnification: 100×.

ing, rheumatoid arthritis, and tumor invasion and metastasis.[17,21] There are MMPs that break down fibrillar collagen known as collagenases (MMP-1, MMP-8, MMP-13) and those that can affect basement membrane collagen (collagen IV) known as gelatinases (MMP-2, MMP-9).

DO and MI show a basic chemical structure consisting of a tetracyclic naphthacene carboxamide ring system, but they differ slightly. Doxycycline presents one hydroxyl group in carbon 5 of ring B and one methyl group in the carbon 6 of ring C, whereas minocycline has one amine group in carbon 7 of ring D.[17]

DO and MI can inhibit collagenases and gelatinases.[23] Inhibition of MMPs can theoretically occur at numerous levels attributable to the multiple steps involved in MMP transcription, protein synthesis, and enzyme activation through binding in the MMP active site. By binding to the active site zinc ion of the enzyme, modified tetracyclines can alter the conformation of the pro-enzyme molecule, thus blocking its catalytic activity in the extracellular matrix.[24] Whether or not this will bring the same benefits as those of chlorhexidine in preserving resin-dentin bonds over time has yet to be investigated.

Although MI and DO have quite similar chemical structures their results were very different in the present investigation. MI reached μTBS and SNU similar to that of CH irrespective of the adhesive system used. In Figure 2, one can observe that no phase separation was observed when MI was mixed with the SB adhesive (Figure 2C). The mixture looked like the aqueous solution of MI, with a translucent and reddish appearance. However, this was not the case when the aqueous solution of MI was mixed with the acetone-based system. Small resin droplets could be observed (Figure 2D), and although one may hypothesize that this is due to the insolubility of MI in acetone, one cannot rule out the possibility that other components of the adhesive may have played a role in the lack of solvent homogeneity. It is worth mentioning that the phase separation observed between MI and PB systems was not enough to jeopardize the resin-dentin bonds. The explanation for this, however, is still unclear to the author's understanding.

On the other hand, DO showed a very different pattern. DO cannot be used with acetone based

adhesive systems, because lower bond strength values as well as higher silver nitrate penetration were observed within the hybrid layer. This was also demonstrated by the high number of PT (Table 2), which denotes the fragility of the bonding interface. The lack of solubility of DO in acetone could be an explanation of why DO cannot be used as a rewetting agent for acetone-based systems. Figure 2B showed that when PB, the acetone-based system, was dropped onto the aqueous solution of DO, the material did not set properly, showing yellowish waves which probably represent phase separation domains.[25]

The formation of phase separation leads to entrapment of droplets within the adhesive layer.[25] These droplets themselves must reduce the μTBS of the adhesive system by acting as flaws during the μTBS test.[26] This is probably also responsible for the low fatigue resistance of materials with phase separation in comparison with those in which no phase separation is seen.

For the water/ethanol based system, μTBS values similar to those of the control group were obtained with DO, which would lead one to think that this MMP inhibitor is appropriate for this type of adhesive. This would be true if the adhesive interface rewetted with DO were not highly infiltrated by silver nitrate. Higher SNU within the adhesive interface indicated that the resin matrices contained several hydrophilic domains and/or hydrogel polymer formation.[27]

The amount of SNU at the SB interfaces was larger when the dentin was rewetted with DO rather than with CH and MI. This probably indicates the quality of the polymer formed within the hybrid layer when DO was applied beforehand. This may probably become more evident after some months of water storage, as this interface will be more prone to degradation through hydrolysis due to its higher initial hydrophilicity. Since hydrolytic degradation occurs only in presence of water, adhesive hydrophilicity and water sorption are directly correlated with hydrolytic degradation.[28]

Further studies also should be conducted to analyze the effects of different concentrations and application times of MI aqueous solutions. In addition, the association of MI with water and ethanol adhesive solutions should be investigated as well as the effects of MI application on the stability of the resin-dentin bonds over time.

## Conclusions

The use of a 2% aqueous solution of chlorhexidine and MI did not impair resin-dentin bond strength and the quality of the hybrid layer formation for both adhesives, although slight phase separation occurred for the acetone-based system. The use of 2% doxycycline should be avoided as it jeopardized the bond strengths and quality of the hybrid layer for both systems.

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