

## Comparison of the effect of two MMP inhibitors on microtensile bond strength to dentin in class I cavities

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### Abstract

**Aim:** To compare the effects of pretreatment with doxycycline and chlorhexidine on microtensile bond strength to dentin in class I cavities restored with composite resin.

**Materials and Methods:** Class I cavities were prepared on the occlusal surface of 30 freshly-extracted caries-free molars. In group 1, 2% chlorhexidine was applied to the acid etched surface, placed in contact for 20 seconds, and gently air dried without rinsing. In group 2, 2% doxycycline was applied to the acid etched surface, placed in contact for 20 seconds, and gently air dried without rinsing. In group 3, the control group, just the conventional bonding procedure was used without any pre-treatment. The cavities were restored with resin composite using a bulk method. After thermocycling, the specimens were serially sectioned to obtain resin dentin sticks with a rectangular cross-sectional area of approximately 1mm<sup>2</sup> using microtome. The microtensile bond strength values were tested using Universal testing machine at crosshead speed of 0.5 mm/min until failure. The results obtained were analyzed by One-way ANOVA and Tukey's HSD tests for statistical significance.

**Results:** The pre-treatment with chlorhexidine and doxycycline showed a significant effect in preserving the *in vitro* bond strength, when compared to the control group (p=0.000). Among the test groups, the chlorhexidine treatment resulted in a higher bond strength value than doxycycline group (p=0.000).

**Conclusion:** Pre-treatment with MMP inhibitors resulted in higher bond strength values. Chlorhexidine group showed a significantly higher bond strength value compared to the Doxycycline group.

**Keywords:** Chlorhexidine, Dentin, Doxycycline, MMP inhibitors, Microtensile bond strength.

### Introduction

Evolution and advances in adhesive dentistry have enormously contributed to increase in resin-dentin bond strength. But, the durability of bonding between adhesives and the dentin matrix is still a concern.<sup>1</sup> Premature loss of bond strength is one of the problems that affect adhesive restorations.<sup>2</sup> The loss of bond strength has mainly been attributed to degradation of the hybrid layer at the dentin-adhesive interface and deterioration of the dentin collagen fibrils.<sup>3</sup> The diffusion of resin monomer into the demineralized dentin after acid etching shows a decreasing concentration gradient.<sup>4</sup> This results in unprotected and vulnerable collagen fibrils at the bottom of the hybrid layer,<sup>5</sup> which may become sites for collagen hydrolysis by host derived matrix metalloproteinase (MMP) enzymes.<sup>6</sup>

MMPs are a group of calcium and zinc dependent host-derived enzymes<sup>7</sup> which are trapped within the mineralized dentin matrix during tooth development.<sup>8</sup> Human dentin contains at least collagenase (MMP-8), gelatinases (MMP-2 and -9), and enamelysin (MMP-20). L Dentin collagenolytic and gelatinolytic activities can be inhibited by protease inhibitors,<sup>13</sup> indicating that MMP inhibition could be useful in the preservation of hybrid layers.<sup>14</sup>

Chlorhexidine functions as a potent MMP inhibitor,<sup>15</sup> and prevents or minimizes the auto-

degradation of exposed collagen fibrils within incompletely-formed hybrid layers, thereby contributing to the long term stability of the hybrid layer and bond strength.<sup>16</sup> A study by Carrilho and others<sup>14</sup> showed that 2% chlorhexidine application after acid etching, preserves both the durability of the hybrid layer and bond strength *in vitro* of aged specimens. Another *in vitro* study by Chang and others<sup>3</sup> showed that thermos cycling for 10,000 cycles resulted in a significant bond strength reduction in the control group, but it showed less reduction in groups treated with chlorhexidine. In addition in a study Brackett and others<sup>29</sup> showed that in caries-free permanent teeth, degradation of the dentin hybrid layers beneath Class I resin composites *in vivo* is not extensive at six months; however it appeared to be slowed by the use of a chlorhexidine solution after acid etching. Although sensitivity to chlorhexidine is rare, contact dermatitis is a common adverse reaction to chlorhexidine.<sup>27</sup> Case-reports with Ig-E mediated allergic reactions to chlorhexidine have been published. So an alternative to chlorhexidine is indicated in persons with known sensitivity to chlorhexidine. Doxycycline, with significant action on preserving the bond strength is a viable option. Doxycycline, a member of the tetracycline family has the ability to down-regulate the MMPs.<sup>17</sup> Doxycycline inhibits the catalytic activity of human collagenases and gelatinases.

The current in vitro study compared the effects of pretreatment with Doxycycline and Chlorhexidine on microtensile bond strength to dentin in Class I cavities restored with composite resin, after thermocycling.

## Materials and Methods

### Cavity Preparation

Thirty freshly-extracted caries-free molars, stored in saline, were employed in this study. The occlusal enamel was ground flat using a model trimmer under running water, then abraded with wet 600-grit silicon carbide abrasive paper to expose a flat dentin surface that permitted placing the occlusal cavity margins in dentin. Class I cavities (4mm long, 4mm wide, 2mm deep) were prepared in dentin using a diamond bur in a high-speed handpiece with copious air-water spray. At this time, the specimens that showed visible pulp exposure were excluded from the study.

### Restorative Procedure

All the cavities were submitted to the bonding protocols using a two-step etch and rinse (Adper Single Bond 2, 3M ESPE, St Paul, MN, USA) adhesive system and a light-curing composite (Z-350, 3M ESPE). All the materials were handled according to the manufacturer's instructions. The materials used in this study are listed in Table 1. The specimens were randomly divided into three groups of 10 teeth each. All preparations were etched with 37% phosphoric acid for 15 seconds, rinsed with water spray for 10 seconds and gently dried for 10 seconds. In group 1, 2% chlorhexidine was applied to the acid etched surface, placed in contact for 20 seconds, and gently air dried without rinsing. In group 2, 2% doxycycline was applied to the acid etched surface, placed in contact for 20 seconds, and gently air dried without rinsing. In group 3, the control group, just the conventional bonding procedure was used without any pre-treatment. Following the pre-treatment sequences of the individual groups, the cavities were filled with resin composite using a bulk method. An additional 2-mm thick resin composite was built-up and each increment was polymerized for 40 seconds. The resin composite was then built up for microtensile bond strength. All the specimens were submitted to 10,000 cycles of thermocycling, with the temperature changing from 5°C to 55°C, with a dwell time of 15 seconds and an interval time 10 seconds each.

### Measurement of microtensile bond strength

After thermocycling, the specimens were serially sectioned into 1-mm thick resin slabs, and they were then rotated 90° and sectioned again to obtain resin dentin sticks from the cavity floor with a rectangular cross-sectional area of approximately 1mm<sup>2</sup> using microtome. Three sticks were obtained from each restoration; therefore thirty specimens were used in each group. The bonded surface area was calculated

before each test by measuring the narrowest portion to the nearest 0.01 mm using a digital caliper (Mitutoyo, Tokyo, Japan). Each specimen was fixed to a custom-made testing jig (Geraldelli's device) with a cyanoacrylate glue (Model Repair II Blue, Dentsply-Sakin, Japan), and subjected to a tensile load at a crosshead speed of 0.5 mm/min until failure (Instron 4411, Instron Corporation, Canton, MA, USA).

### Statistical Analysis

The results obtained were analyzed by One-way ANOVA and Tukey's HSD tests for statistical significance. The statistical significance was set at the 0.05 probability level.

### Results

The results of the microtensile values are summarized in Table 2. The pre-treatment with chlorhexidine and doxycycline showed a significant effect in preserving the in vitro bond strength, when compared to the control group (p=0.000). Among the test groups, the chlorhexidine treatment resulted in a higher bond strength value than doxycycline group (p=0.000).

### Discussion

Long term dentin bonding of resin adhesives is threatened by disaggregation of the hybrid layer owing to the activation of dentin MMPs. The activities of endogenous collagenase and gelatinase derived from demineralised dentin are thought to induce the degradation of bonded dentin matrix in the hybrid layer. Therefore application of specific MMP inhibitors which can suppress dentin collagenolytic and gelatinolytic activities<sup>13</sup> are recommended to restrict deterioration of hybrid layers. The reason for doing microtensile bond strength testing in this study was that this method permits measurement of bond strength without cohesive failure of dentin. It also permits multiple measurements to be made within a single tooth.<sup>28</sup>

Chlorhexidine has been found to have desirable MMP-inhibition properties (MMP-2, -8 and -9) even at low concentrations, possibly resulting from its Zn<sup>2+</sup> cation-chelating property.<sup>15</sup> The beneficial effect of chlorhexidine in preserving the bond strength has earlier been proved in vitro<sup>14,19</sup> and in vivo studies.<sup>6</sup> Recent studies with chlorhexidine have shown that the use of chlorhexidine after acid etching resulted in initial bond strengths comparable with those of the controls.<sup>20-22</sup> But after 10,000 cycles of thermocycling, which represent a service year,<sup>23</sup> the reduction in bond strength seen in chlorhexidine group was significantly less than that seen in control group.<sup>3</sup> So in the present study, the comparison between the actions of MMP inhibitors on the microtensile bond strength values was done after thermocycling of all the specimens.

Doxycycline is a broad spectrum antibiotic of the tetracycline family. It also has the additional action of

differential inhibition of the activity of members of the MMP family and inhibition of MMP synthesis.<sup>24,25</sup> Doxycycline can inhibit the catalytic activity of human collagenases and gelatinases.<sup>18</sup> Doxycycline inhibits the expression of MMP-8 and MMP-9.<sup>26</sup>

In this study, pre-treatment with MMP inhibitors, Chlorhexidine and Doxycycline resulted in significantly higher microtensile bond strength values compared to

the control group, and also the chlorhexidine group showed significantly higher values when compared to groups with doxycycline pre-treatment. Analysis of the results of this study indicates beneficial effects of both chlorhexidine and doxycycline on the preservation of dentin bond strength, in which chlorhexidine has a more promising effect.

**Table 1: Materials used in the study**

Material brand name	Composition	Manufacturer
Scotchbond Etchant	37% phosphoric acid	3M ESPE Dental Products, St Paul, MN, USA
Adper Single Bond 2	Ethyl alcohol, Bis-GMA, HEMA, Glycerol, 3-dimethacrylate, Acrylic acid copolymer, Itaconic acid, Diurethane dimethacrylate, Water, Colloidal filler	3M ESPE Dental Products, St Paul, MN, USA
Filtek Z-350	Bis-GMA, UDMA, TEGDMA, Ethyl methacrylate, inorganic fillers	3M ESPE Dental Products, St Paul, MN, USA

**Table 2: Overall means & standard deviations of Microtensile Bond Strength values (MPa)**

Group	Mean microtensile bond strength values (MPa) (Mean ± SD)
Chlorhexidine group	23.77 ± 0.47
Doxycycline group	20.57 ± 0.86
Control	15.56 ± 0.86

## Conclusion

1. Pre-treatment with MMP inhibitors (Chlorhexidine and Doxycycline) resulted in higher bond strength values, in aged specimens.
2. Chlorhexidine group showed a significantly higher bond strength value compared to the Doxycycline group.
3. So among the two MMP inhibitors, Chlorhexidine appears to be promising in enhancing the durability of bond between composite resin and dentin.
4. Doxycycline can perform as an alternative to CHX in specific cases where CHX is contraindicated.

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