

## Influence of chlorhexidine application on longitudinal adhesive bond strength in deciduous teeth

Vicente Castelo Branco Leitune<sup>(a)</sup>  
Fernando Freitas Portella<sup>(a)</sup>  
Priscila Veit Bohn<sup>(a)</sup>  
Fabrício Mezzomo Collares<sup>(a)</sup>  
Susana Maria Werner Samuel<sup>(a)</sup>

<sup>(a)</sup>Conservative Dentistry Department, Laboratory of Dental Materials, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

**Abstract:** The aim of this study was to evaluate the influence of applying 2% chlorhexidine for 30 seconds after phosphoric acid conditioning of dentin on the immediate and long-term bond strengths in deciduous teeth. The occlusal enamel was removed from 40 human sound deciduous molars, which were exfoliated by natural means, and the dentin was conditioned with 37% phosphoric acid for 15 seconds and washed with running water. The specimens were divided into two groups of 20 teeth. The test group received an application of 2% chlorhexidine for 30 seconds prior to a three-step etch-and-rinse adhesive system, whereas the control group received only the adhesive system. Three cylindrical restorations were made with a composite resin for each tooth. Ten teeth in each group were submitted to a microshear bond strength test after 24 hours, while the remaining teeth were stored in distilled water at 37 °C for 6 months before testing the microshear bond strength. The test group had a higher bond strength than did the control group after 6 months of storage. No statistical differences were found when groups with the same dentin treatment were compared at different times. Short applications of chlorhexidine at low concentrations prevent hybrid layer degradation and positively affect bond strength over time.

**Descriptors:** Chlorhexidine; Tooth, Deciduous; Matrix Metalloproteinases.

### Introduction

The longevity of restorations is directly affected by the integrity of the hybrid layer. Degradation of polymer networks and collagen fibrils over time could compromise the long-term bond strength of adhesive systems.<sup>1</sup> One mechanism for collagen fibrillar network degradation is the collagenolytic activity of endogenous matrix metalloproteinases (MMPs).<sup>1,2</sup>

MMPs are a group of zinc-calcium-dependent enzymes that regulate the physiological and pathological mechanisms of collagen-based tissues.<sup>3,4</sup> MMPs are present in the latent form in the dentin substrate.<sup>2</sup> However, when the inorganic scaffold surrounding the collagen fibrils of dentin is missing due to caries and/or acid etching,<sup>4</sup> collagen fibers become denuded and express MMP activity, which begins the degradation process. As in an enzymatic process, aberrant expression of MMPs could be inhibited with the use of specific inhibitors (i.e., cysteine and serine protease inhibitors), which preserve the structural integrity of col-

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**Corresponding Author:**  
Fabrício Mezzomo Collares  
Email: [fabricao.collares@ufrgs.br](mailto:fabricao.collares@ufrgs.br)

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lagen fibers and could reduce the degradation of the hybrid layer.<sup>2</sup>

Chlorhexidine showed inhibitory activities for MMP-2, MMP-8, and MMP-9,<sup>5</sup> which are present in human dentin matrix. *In vivo*<sup>6</sup> and *in vitro*<sup>7</sup> studies have shown that dentin collagen degradation activity can be reduced by applying chlorhexidine to the dentin surface after the application of phosphoric acid and prior to the application of the adhesive system. Studies evaluating the effect of chlorhexidine after acid etching used an application time of 60 seconds.<sup>7-11</sup> In a few studies, researchers attempted to reduce this time to save both the professional's and the patient's time.<sup>10,12-14</sup> However, none of these studies evaluated the reduced time of chlorhexidine application on the dentin of deciduous teeth.

The purpose of this study was to evaluate the influence of applying 2% chlorhexidine for 30 seconds after phosphoric acid etching of dentin on immediate and long-term bond strengths in deciduous teeth.

## Methodology

Forty human sound deciduous molars, which were exfoliated by natural means, were selected for this study. The teeth were randomly assigned to a study group. This study was revised and approved by the local Ethics Committee. Freshly exfoliated teeth were immediately stored fully immersed in distilled water at 4 °C for no more than 6 months.

## Bonding procedures

The teeth were embedded in acrylic resin, and their occlusal enamel was removed to expose the dentin surface near the enamel. The exposed dentin was polished with 600 SiC sandpaper for 30 seconds in running water to produce a standardized smear layer.<sup>15</sup>

The dentin was acid conditioned for 15 seconds with 37% phosphoric acid and washed in running water for the same period; the dentin samples were then divided into 2 groups ( $n = 20$ ), according to the treatment of dentin. In the control group ( $G_{\text{control}}$ ), a three-step etch-and-rinse adhesive system (Scotch Bond Multi Purpose, 3M ESPE, St. Paul, MN, USA) was applied according to the manufacturer's in-

structions (primer for 15 seconds, air dried for 10 seconds, adhesive resin, and photoactivation). In the test group ( $G_{\text{CHX}}$ ), 1.5  $\mu\text{L}$  of a 2% chlorhexidine digluconate solution was applied with a microbrush for 30 seconds. The dentin was then gently dried with an absorbent paper, and the adhesive system was applied as in the  $G_{\text{control}}$  group. Three cylindrical restorations were made with a composite resin (Z350, 3M ESPE, St. Paul, MN, USA). The mean bonding area was  $0.95 (\pm 0.1) \text{ mm}^2$ . Adhesive resin and composite resin were photoactivated with a quartz tungsten halogen light curing unit (XL2500, 3M ESPE, St. Paul, MN, USA) with a light intensity of  $600 \text{ mW/cm}^2$ . The power of the light curing unit was gauged with a radiometer (Model 100, Demetron Research Group, Danbury, USA).

## Microshear bond strength test

The teeth were stored at 37 °C in distilled water for 24 hours and randomly assigned to one of 4 subgroups, according to the time of storage ( $n = 10$ ). Ten teeth from each subgroup were submitted to a microshear bond strength test after 24 hours ( $G_{\text{control 24h}}$  and  $G_{\text{CHX 24h}}$ ). The remaining teeth were stored for 6 months at 37 °C in distilled water and then submitted to a microshear bond strength test ( $G_{\text{control 6m}}$  and  $G_{\text{CHX 6m}}$ ).

For the microshear bond strength test, the embedded teeth were positioned in a device coupled to a universal test machine (EMIC DL-2000, São José dos Pinhais, Brazil). A steel wire with a cross section of 0.2 mm was positioned in the bottom of the cylinder, and a crosshead speed of 0.5 mm/min was applied until bond failure occurred. To express the bond strength in megapascals (MPa), the load upon failure was recorded in Newtons (N) and divided by the bond area ( $\text{mm}^2$ ).

## Statistical analysis

The normality of the data was evaluated by the Kolmogorov-Smirnov test. Statistical analysis was performed using two-way ANOVA and Tukey's post hoc test at the 0.05 level of significance. All analyses were performed with SigmaPlot 11.0 software for Windows.

## Results

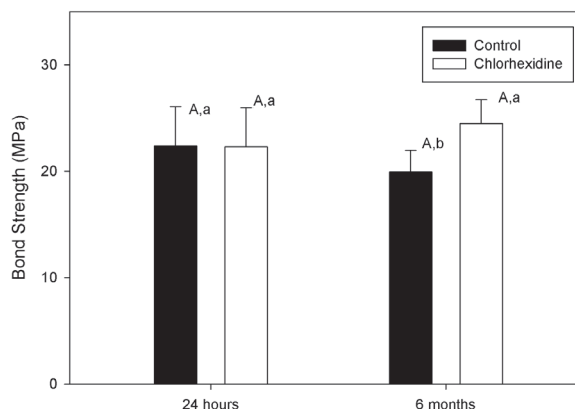
The results of the bond strength analysis are presented in Figure 1. The control group had a mean and standard deviation of 22.37 ( $\pm$  3.69) MPa at 24 hours and 19.93 ( $\pm$  2.05) MPa at 6 months. The test group (CHX) had a mean and standard deviation of 22.30 ( $\pm$  3.66) MPa at 24 hours and 24.48 ( $\pm$  2.24) MPa at 6 months. The results show a statistical difference between the control and test groups after 6 months of storage ( $p < 0.05$ ). No statistical differences were found when groups with the same dentin treatment were compared at different times ( $p > 0.05$ ).

## Discussion

In this study, the application of chlorhexidine for 30 seconds after phosphoric acid etching of deciduous dentin influenced the longitudinal adhesive bond strength. The group with chlorhexidine treatment had stronger bond strength than did the control group after 6 months ( $p < 0.05$ ). However, chlorhexidine showed no influence on the immediate bond strength in both groups ( $p > 0.05$ ).

The results of this study corroborate the findings of other studies that evaluated the re-hydration of dentin with 2% chlorhexidine after acid conditioning.<sup>7,8,12,13,16</sup> The chlorhexidine application showed no interference on immediate bond strength but resulted in higher bond strength values than the control group after 6 months, even when the chlorhexidine application time was reduced to 30 seconds.

During the bonding procedure with the etch-and-rinse adhesive systems, dentin is demineralized by the action of phosphoric acid. This process exposes a dense layer of fibrils of the organic matrix<sup>8,9</sup> composed primarily of type I collagen and proteoglycan,<sup>12,13</sup> which must be completely infiltrated with the adhesive resin to form the hybrid layer.<sup>12,16</sup> However, a decrease in the diffusion gradient of the resin monomers results in a layer of denuded collagen at the base of the hybrid layer.<sup>9,12,14,16,17</sup> These limitations of adhesive systems lead to the disarrangement of the collagen fiber network, which causes degradation by activating host-derived enzymes, such as MMPs, by acid contact and water uptake. The longevity of the adhesive/dentin bond is directly related



**Figure 1** - Bond strength of groups at 24 hours and 6 months. Different small letters indicate statistical differences between groups at the same time ( $p < 0.05$ ). Different capital letters indicate statistical difference in the same group at different times ( $p < 0.05$ ).

to the quality of the formed polymer<sup>18</sup> and the integrity of the uncovered collagen.<sup>6,19</sup> The uncovered collagen may be degraded by MMPs,<sup>2,6</sup> which leads to a degraded bond interface. The degradation of organic content occurs due to the gelatinolytic and collagenolytic activity present in latent forms within the dentinal matrix.<sup>3</sup>

Chlorhexidine, which is widely used as an anti-bacterial agent, has been investigated as an effective MMP inhibitor that does not have a negative effect on bond strength.<sup>20</sup> Inhibition of MMPs is related to the CHX cation chelating mechanism, in which metal ions such as calcium and zinc are sequestered,<sup>21,22</sup> and its interaction with sulfhydryl groups and cysteine residues present in the active site of MMPs.<sup>5</sup> The application of CHX as a method to rehydrate the conditioned dentin seems to be a recognized approach to minimize the effect of MMPs along the demineralized layer of collagen. Chlorhexidine interacts with the inactive matrix metalloproteinases present on collagen fibers.

Preservation of the hybrid layer is necessary to avoid bond strength loss over time. Chlorhexidine prevents only collagen degradation that does not interfere with polymer network stability, as demonstrated in a previous study<sup>23</sup> in which polymer degradation was so marked that the effect of chlorhexidine could not be verified. The polymer continues to be susceptible to water sorption and swelling; there-

fore, the leaching of the polymer from the hybrid layer creates spaces and exposes the collagen fibers, which can be degraded by MMPs. A storage period of 6 months was not sufficient for this phenomenon to occur, as seen in this study. Both the control and test groups presented no reduction in bond strength at 24 hours or 6 months.

Deciduous teeth were used in this study because they are less mineralized<sup>24</sup> than permanent teeth; therefore, they contain a higher content of organic material. Consequently, deciduous teeth are expected to experience more degradation of the hybrid layer over time. On the other hand, less collagen network disintegration is expected, which leads to a less noticeable reduction in bond strength when an MMP inhibitor is used. Previous studies have shown that a 60-second chlorhexidine application has an effect on deciduous teeth.<sup>25,26</sup>

Because the microtensile bond strength is a reliable *in vitro* metric to evaluate bond strength<sup>27</sup> and a positive correlation between microtensile and microshear bond strength tests was demonstrated,<sup>28</sup> the methodology utilized in this study was able to detect the differences observed between the tested groups. Furthermore, the common method used to evaluate simulated bond interface aging is to prepare specimens in beam forms with an adhesive 1 mm<sup>2</sup> in area and to soak the bonded specimens at 37 °C.<sup>29</sup> The restorations made for the microshear bond strength test are small (0.95 mm<sup>2</sup>), which allows the storage medium to infiltrate the bonding

area efficiently.

MMPs require calcium and zinc ions to maintain their proper tertiary structure and functional active sites; moreover, MMPs could hydrolyze specific peptides linkages in collagen peptides, thus disintegrating collagen fibrils.<sup>30</sup> The teeth were stored in distilled water to promote degradation, which could reduce the collagenolytic activity of the medium. However, differences in bond strength between the control and test groups were found after 6 months of storage, which indicates that aging occurred under the present study conditions without interfering with the results of the two groups' storage for 6-month comparison. *In vivo* studies of 30-second chlorhexidine application should be conducted to confirm the results of the present study.

Despite the advantages of using 2% chlorhexidine for 30 seconds after acid conditioning, the process requires one more step in the restorative procedure, which contrasts with the simplified clinical technique proposed. Numerous studies have been concerned with avoiding an increase in the clinical time of restoration and, thus, have included the use of chlorhexidine in a previous restoration step.

## Conclusion

Even at low concentrations and with a short application time (30 s) to dentin, chlorhexidine influenced the degradation of the hybrid layer and thus positively affected the *in vitro* bond strength over time.

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