

Full Length Research Paper

***In vitro* influence of 2% chlorhexidine on links established at the hybrid layer between collagen fibers and nano adhesive used in the adhesive system**

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In this study, 48 human teeth were chosen; they were extracted from natural sources. Sound dentin surfaces were exposed by operating low speed diamond disc at conventional speed with copious water spraying. The teeth were divided by randomizing technique, into two equal groups (n = 24): the control and the test group. Teeth in the control group followed the conventional method of etching, washing, applying adhesive, while teeth from the test group followed an intermediate stage of the application for 60 s of 2% chlorhexidine solution before applying adhesive system (Prime and Bond NT). After applying the adhesive, all the teeth received a standard cylindrical light curing composite restoration. Half of the teeth of each group were subjected to the bonding strength tests after 24 h and the other half, after 4 months period of keeping in distilled water at 37°C. The results show that there are no significant statistical differences at 24 h between the two groups while after 4 months, there was a positive statistical significance in favor of the group which followed a subsequent treatment with chlorhexidine. The application of 2% chlorhexidine might prevent hybrid layer degradation and this procedure has a beneficial effect on maintaining bond strength.

Key words: Teeth, chlorhexidine, matrix metalloproteinases, bond strength.

INTRODUCTION

One of the mechanisms that influence the adherence longevity of composite materials to tooth structure is determined by the links established in the hybrid layer between the network of collagen fibers in dentin and fluid resin used for infiltration (Van Meerbeek et al., 2003; De Munck et al., 2005; Breschi et al., 2008). Numerous recent studies put this in connection with activation of an enzyme group represented by endogenous matrix metalloproteinases (MMP-2, MMP-8 and MMP-9). These are normally present in latent form and inactive in the dentin. After demineralization caused by the acid attack from decay and/or application of phosphoric acid in ad-

hesive system, these enzymes can be activated (Gendron et al., 1999; Zhou et al., 2009; Tjaderhane et al., 1998). This begins a process of degradation of the network of collagen fibers which causes gaps in the connecting hybrid layer, with consequences of reducing capacity of bond strength of composite material.

Chlorhexidine (CHX) has been shown by numerous studies *in-vitro* and *in-vivo* to have the effect of influencing the activation of MMPs present in dentine (Chaussain-Miller et al., 2006; Hebling et al., 2005; Carrilho et al., 2007). In this way, it is possible in medium and long term to maintain the integrity of the hybrid layer and hence adhesion capacity of composite restorations to dental hard structures (Carrilho et al., 2007; Branco Leitune et al., 2011; Zhou et al., 2009).

The aim of this study was to evaluate *in vitro*, in short

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Figure 1. Dentin demineralization with 37% phosphoric acid.



Figure 2. Appearance of cylindrical restorations performed on a group of teeth.



Figure 3. Appearance of a tooth with cylindrical restoration from composite resin fixed for microshear bond strength test.



Figure 4. Appearance of some samples from the control group (G_{control}) prepared for the microshear bond strength tests.

and medium term, the impact of 2% chlorhexidine application on the bond strength of a restorative procedure that uses adhesive system as a fluid resin with mineral laden of nano hybrid type.

MATERIALS AND METHODS

The study began by requesting and obtaining the approval of the Ethics Committee of the Faculty of Medicine "V. Papiian", Sibiu to use for experiments, 48 human teeth extracted from natural sources (mobility grade 3 or extraction in orthodontic purposes). After extraction, the teeth were disinfected and then immediately submerged in distilled water and kept for 3 months at 4°C. All teeth followed the same treatment: removal of enamel and dentin discovery using a diamond disc with a 150 μm average grain for 30 s at conventional speed, under water refreshing. Dentin layer demineralization is shown in Figure 1.

So prepared dentine was conditioned for 20 s with 37% phosphoric acid and then washed in same time with water-air jet. These teeth were divided by simple random technique into two equal groups ($n = 24$): the control group teeth (G_{control}) and the tooth test group (G_{CHX}).

The teeth from control group were dried moderately by stippling with tissue paper and then the fluid adhesive resin was applied with a brush (Prime & Bond NT, Dentsply, De-Trey GmbH, Konstanz, Germany) according to the manufacturer's instructions (20 s brushing, moderate drying with air curing for 2 to 3 s followed by 10 s photopolymerization with a LED curing lamp (Starlight SX5, Mec-tron, Italy) with light intensity $>1200 \text{ mW/cm}^2$. The cylindrical restoration from composite resin used (Spectrum NT, Dentsply, De-Trey GmbH, Konstanz, Germany) had a standard diameter of 1.95 (± 0.2) mm. Appearance of some cylindrical restorations is shown in Figure 2.

Half of the control group teeth ($n = 12$) were rigidly fixed using a self-polymerizable resin in a cylinder mounting, and then submerged in distilled water and kept at a temperature of 37°C for 24 h. After this time, they were subjected to the restorations microshear bond strength tests, using the Instron 5587 machine. The other half of the control group teeth ($n = 12$) were stored in distilled water at 37°C for 4 months and after that they were mounted in self-polymerizable resin and the same microshear bond strength tests was done under the same conditions. Appearance of some teeth with cylindrical restorations mounted in resin is shown in Figure 3.

Teeth from the test group, after each-and-rains, were dried with tissue paper. After that, a solution of 2% chlorhexidine was applied by brushing for 60 s, followed by moderate drying, and then application of fluid resin; and finally, the cylindrical restoration using resin under the same conditions was performed as for the control group. The same procedure was followed where half of the teeth were tested after 24 h and the other half were tested after preserving for 4 months in distilled water at a 37°C. Appearance of some samples from the control group prepared for the bond strength tests is shown in Figure 4.

For testing the microshear bond strength, the teeth fixed in resin were positioned using a coupling device in the universal machine for testing tensile, compressive and buckling (Instron 5587, USA). The test machine was equipped with extensometer and provided with software for acquisition and processing the measured data

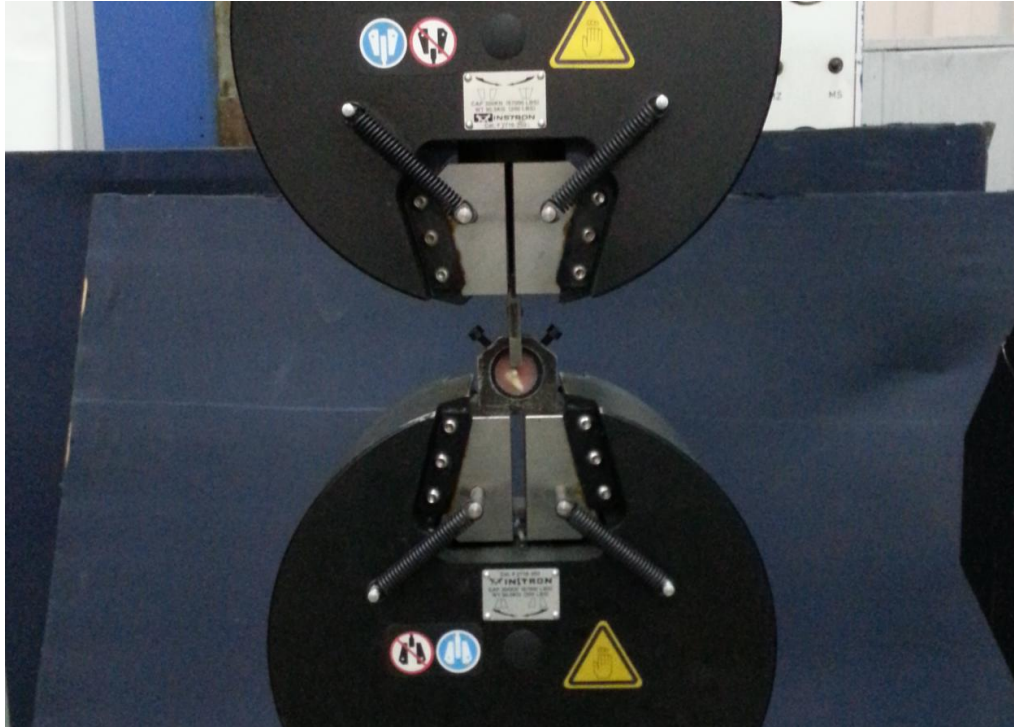


Figure 5. Detail of the test process for samples with INSTRON 5587 machine.

(Bluehill 2.0). The cursor of machine moved with a speed of 0.5 mm/min, during performance of the tests allowing data transfer and description of occurring phenomena using breaking graphs expressed in megapascals (MPa). A detailed look of a microshear bond strength attempt in INSTRON 5587 machine is shown in Figure 5.

Statistical analysis

For statistical analysis of data and results, we used two tests, the Kolmogorov-Smirnov test and the Paired samples t-test, using SPSS Statistics 17.0 for Windows.

RESULTS

After microshear bond strength tests on samples from the control and test groups at 24 h and after 4 months, and their processing with Bluehill 2.0 software, we obtained the following results shown in Table 1.

To assess normality of our data, we used the Kolmogorov-Smirnov test. In SPSS Statistics 17.0 for Windows, the P-values are listed as Asymp. sig. (2-tailed). Because for all tests we have P values between 0.599 and 0.994, we can accept the null hypothesis and affirm that our data do not differ from a normal distribution. In other words, for all tests, $p > 0.05$ and thus we concluded that our data are normal.

The control group mean was 21.99 MPa with a standard deviation of ± 0.71 MPa in samples examined at 24 h, while for the samples examined after 4 months, the

mean was 19.16 MPa and the standard deviation was ± 0.59 MPa. For the test group, we had a mean of 21.84 MPa after 24 h with a standard deviation of ± 0.89 MPa, respectively, a mean of 23.52 MPa with a standard deviation of 0.43 MPa after 4 months.

Graphical representation of the mean values of the bond strength after 24 h and after 4 months for samples from the control group and respectively from the test group is shown in Figure 6.

In order to compare the means between the control and the test groups at 24 h and respectively at 4 months, we used the Paired samples t-test, which provided as results, the t-statistic and the associated P value (if $p < 0.05$, then the means differ significantly). From the analysis of these data, we can say that no statistical differences were found when test and control groups were compared after 24 h ($P = 0.460 > 0.05$), while after 4 months, there were significant statistical differences between the control and test groups ($P = .000 < 0.05$).

DISCUSSION

Clinical protocol to achieve dental restorations with etch-and-rinse adhesive system causes loss of an important part of the mineral component of the dentin (Van Meerbeek et al., 2003; De Munck et al., 2005; Breschi et al., 2008). This action results at this level, an organic fiber network mainly represented by collagen fibers and proteoglycans of dentin structure (Van Meerbeek et al.,

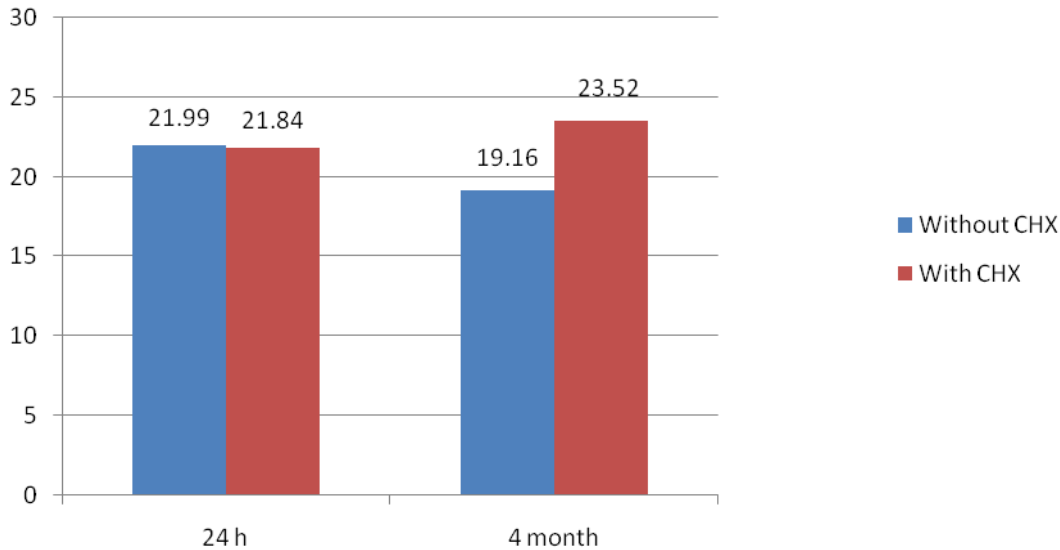


Figure 6. Graphical representation of the mean values of the bond strength (MPa) for the two groups.

Table 1. Values of bond strength tests expressed in MPa for the 12 samples of the experiment.

Sample	G _{control} -24 h	G _{CHX} -24 h	G _{control} -4 month	G _{CHX} -4 month
1	22.173	22.302	18.991	23.667
2	22.476	21.948	18.871	23.878
3	22.422	21.282	19.505	23.319
4	22.288	21.84	19.138	23.876
5	21.863	22.535	18.83	23.01
6	21.042	21.113	18.651	22.916
7	20.931	21.992	17.997	23.712
8	20.875	19.978	18.931	24.028
9	22.75	22.911	20.015	22.871
10	23.011	22.876	20.128	23.215
11	21.812	20.834	19.324	23.938
12	22.189	22.435	19.501	23.771

2003; Gendron et al., 1999; Zhou et al., 2009).

For good adhesion, the fluid acrylic resin of the adhesive system must complete infiltration, thus achieving a hybrid layer (De Munck et al., 2005; Breschi et al., 2008). Longevity of adhesion bonds from this layer depends on the quality of this resin and also on the collagen network integrity (Gendron et al., 1999; Tjaderhane et al., 1998; Campos et al., 2009). Collagen fibers may degrade over time under the influence of MMP enzymes if these are activated.

Many current clinical studies have proved that MMPs are present in dentin in inactive state. They are activated by the presence of acids in the case of dentin caries or in demineralizations with adhesive purpose (Tjaderhane et al., 1998; Chaussain-Miller et al., 2006). Chlorhexidine is a well-known general antibacterial agent for endodontic treatments which also prevent dentin MMPs activation

(Carrilho et al., 2007; Branco et al., 2011; Zhou et al., 2009).

Adhesion strength of a composite material can be expressed *in vitro* by determining the bond strength tension determined by applying a force attempt. Although the imagined model of cylinder restoration cannot represent accurately all clinical cases of restorations using composite materials, it can provide feasible information if the adhesion is based only on the system of micro retentions created by etching. By maintaining samples at 37°C for 4 months, we aimed at determining the bond strength of the used adhesive similar to those in the oral cavity.

The results obtained from this study confirm that applying a 2% solution of chlorhexidine may prevent MMPs activation. The effect of this action can be quantified by the statistically significant difference ($p < 0.05$) that is seen in time between samples that were treated with chlor-

hexidine and those that did not receive this treatment. Preserving the integrity of the hybrid layer is a general problem that occurs not only during composite restorations but with prosthetic reconstruction techniques based on adhesive collage, and can influence the longevity of restorations in this case.

Conclusion

In vitro application of 2% chlorhexidine for 60 s on demineralized dentin had a positive effect on the integrity of the hybrid layer responsible for adhesion of composite materials. Its influence occurs over time and can be explained by blocking the dentin MMPs activation, thus preserving the bond strength of such materials.

REFERENCES

- Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. (2008), Dental adhesion review: aging and stability of the bonded interface. *Dent Mater.* 24(1):90-101.
- Campos EA, Correr GM, Leonardi DP, Barato-Filho F, Gonzaga CC, Zielak JC (2009), Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing. *J Dent.* 37(2):108-14.
- Carrilho MR, Carvalho RM, de Goes MF, di Hipólito V, Geraldeli S, Tay FR, Pashley DH, Tjäderhane L (2007), Chlorhexidine preserves dentin bond in vitro. *J Dent Res.* 86(1):90-94
- Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. (2007), In vivo preservation of the hybrid layer by chlorhexidine. *J Dent Res.* 86(6):529-33
- Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. (2006), The role of matrix metalloproteinases (MMPs) in human caries. *J Dent Res.* 85(1):22-32
- De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. (2005), A critical review of the durability of adhesion to tooth tissue: methods and results. *J Dent Res.* 84(2):118-32, Feb
- Hebling J, Pashley DH, Tjäderhane L, Tay FR. (2005), Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. *J Dent Res.* 84(8):741-6.
- Tjäderhane L, T. Sorsa, V.-J. Uitto, M. Larmas, T. Salo (1998), The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. *J Dent Res.* 77(8):1622-9
- Leitune VC, Portella FF, Bohn PV, Collares FM, Samuel SM. (2011), Influence of chlorhexidine application on longitudinal adhesive bond strength in deciduous teeth, *Braz oral Res.* Vol 25, nr 5, 388-392
- Renée Gendron, Daniel Grenier, Timo Sorsa, Denis Mayrand (1999), Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. *Clin Diagn Lab Immunol.* 6(3):437-439
- Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. (2003), Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Oper Dent.* 28(3):215-35
- Zhou J, Tan J, Chen L, Li D, Tan Y. (2009), The incorporation of chlorhexidine in a two-step self-etching adhesive preserves dentin bond in vitro. *J Dent.* 37(10):807-812.