# Influence of Blood Contamination on Bond Strength of a Self-etching Adhesive to Dental Tissues

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**Purpose:** The aim of this study was to detect the influence of (1) storage period of heparinized blood, (2) type of blood and presence of contaminant, (3) application mode of cleansing agents, and (4) efficacy of cleansing agents on contaminated enamel and dentin during the adhesion process of a one-step adhesive system.

**Materials and Methods:** One hundred four human molars were sectioned into halves along the long axis for enamel and dentin tests. Heparinized and fresh blood were obtained from the same donor, applied and dried to maintain a layer of dry blood on the top of samples. The cleansing agents used were hydrogen peroxide, anionic detergent, and antiseptic solution. A one-step adhesive system (Clearfil S3 Bond) was applied on the dental surface, and composite resin cylinders were built up using Tygon tubing molds. After 24 h, the μSBS test (1 mm/min) and fracture analysis were performed.

**Results:** There was no statistically significant difference in bond strength values regarding the storage period of heparinized blood and the types of blood. Groups without contamination presented higher bond strengths than contaminated groups. The application mode of the cleansing agents had no influence on bond strength results. There was no statistically significant difference among cleansing agents and they were as effective as a water stream in counteracting the effect of blood contamination.

**Conclusion:** Heparinized blood can be used as a contaminant for up to one week, and it is a reliable procedure to standardize the contaminant. The cleansing agents can be used without friction. A water stream is sufficient to remove blood contamination from dental tissues, before the application of a one-step adhesive system.

**Keywords:** blood, contamination, self-etching adhesive system, bond strength.

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Although self-etching adhesive systems provide a faster application due to a reduced number of components and application steps, the risk of contamination by oral fluids is not eliminated. Achieving good moisture control is a great challenge in daily clinical practice. Blood and saliva contamination can occur, especially when rubber-dam isolation is not feasible, such as when carious lesions are located near or below the gingival margin. Since adhesive systems are very vulnerable to contamination by blood, contamination control is an important factor to obtain a successful and durable bond of composite resin to the tooth structure.[1,5,18,19,20,25,34,36,38]

To simulate gingival bleeding in laboratory studies, some investigators have used fresh capillary blood,[5,7,19,38] while other researchers have used venous blood samples with an anticoagulant.[11-13,18] Although the latter method is less labor intensive than the former, Dietrich et al[6] reported that the addition of an anticoagulant interferes with the interaction of blood and dentin (measured by marginal adaptation) that would normally occur in a clinical environment, thus obscuring the effect of blood contamination in laboratory studies. Among studies that used venous blood with an anticoagulant, blood used immediately

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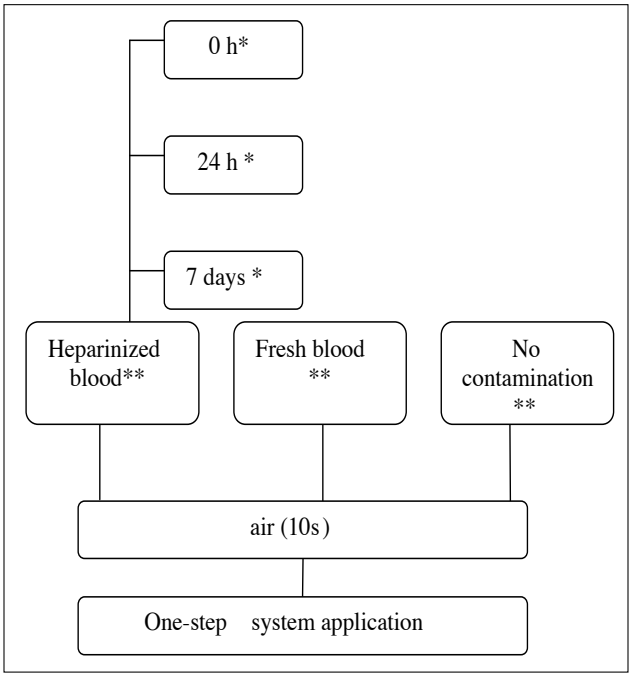


**Fig 1a** Design of Experiment 1(*): storage period of heparinized blood and 2(**): type of blood and presence of contaminants.

after preparation[11,12] yielded results which differed from those obtained when the collected and prepared blood sample was used within one week.[13]

Appropriate cleansing of blood-contaminated dental tissue is an important step in obtaining better adhesion. During endodontic treatment, antiseptic solutions are used to eliminate intracanal contaminants from dentinal walls by chemomechanical preparation. An antiseptic solution consisting of sodium hypochlorite (0.4% to 0.5%) and boric acid (4%) (Dakin's solution) is frequently used to disinfect canal walls, dissolving any remaining pulp tissue. It is a strong oxidizer and has a hemolytic and a hemoglobinolytic effect.[24] Other cleansing agents are used for various purposes in dentistry, such as anionic detergent solution or hydrogen peroxide.[2,17,26,27,35] Anionic detergent solution (0.125% sodium sulfate lauryldiethylene glycol ether) is used as a cavity cleanser.[22,29] It has a low surface tension and high penetration power, adsorption and emulsion properties, as well as a hemolytic effect[24] that assists in surface cleansing. Hydrogen peroxide has also been used in treating gingival disorders. The concentration and extent of exposure are the most important features, but the presence of organic and inorganic materials also influences the efficacy of this agent.[14]

The effectiveness is enhanced by the presence of trace metals, such as iron and copper, which accelerate decomposition of hydrogen peroxide into hydroxyl radicals according to the following reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH$ (hydroxyl radical). The overall effect of combining hydrogen peroxide with iron is a rapid decomposition of hydrogen peroxide with the intermediate formation of reactive oxygen as indicated above. Ultimately, oxygen and water are formed from the interaction of $H_2O_2$ and Fe: $2\ H_2O_2 + Fe\ (salt) \rightarrow 2\ H_2O + O_2$. This agent has a high surface tension and, when in contact with blood, the oxygen released has a hemolytic and a hemoglobinolytic effect.[21]

The aim of this in vitro study was to detect the influence of: (1) storage period of heparinized blood; (2) type of blood and presence of contaminants; (3) application mode of cleansing agents; and (4) efficacy of three different cleansing agents on contaminated enamel and dentin during the adhesion process of a one-step adhesive system.

## MATERIALS AND METHODS

### *Sample Preparation*

Samples of 104 freshly extracted, caries-free human molars, stored in distilled water, were used in this study, which was approved by the Research Ethics Committee of the University of São Paulo (USP) and had the informed consent of the donors (protocol 170/06). The teeth were sectioned along the long axis in order to obtain halves: one half was ground flat for enamel tests, while the other was ground to the point of dentin exposition. All experiments and factors were tested in both dental tissues.

This study was divided into four distinct experiments described in Figs 1a and 1b Twelve, 36, 24, and 32 teeth were used in experiments 1, 2, 3, and 4, respectively.

### *Experiment 1*

In this experiment, the influence of the storage period of heparinized blood on adhesion to enamel and dentin by means of a one-step adhesive system was tested using a microshear test.

### *Blood sampling and contamination protocol*

The blood sample used as the contaminant was obtained from one of the authors.

Fresh venous blood was collected with a disposable syringe from a Venae mediana antebrachii, immediately inserted in a Vacutainer tube containing 50 I.U. heparin per ml blood (Becton Dickinson; Curitiba, Paraná, Brazil).[6] This blood was used at 0 h, 24 h, or 7 days after collection. Except for the 0 h of the sample storage group, the collected blood samples were stored at 4°C.

The blood was applied to flat specimen surfaces and dried carefully with oil-free compressed air for 20 s from a distance of 10 cm. Care was taken to maintain a layer of dry blood on top of all samples[7] prior to adhesive system application.

### *Sample restoration*

A one-step adhesive system (Clearfil S3 Bond/ batch 00026A, Kuraray; Osaka, Japan) was applied to enamel and dentin, left for 20 s, dried with high-pressure air

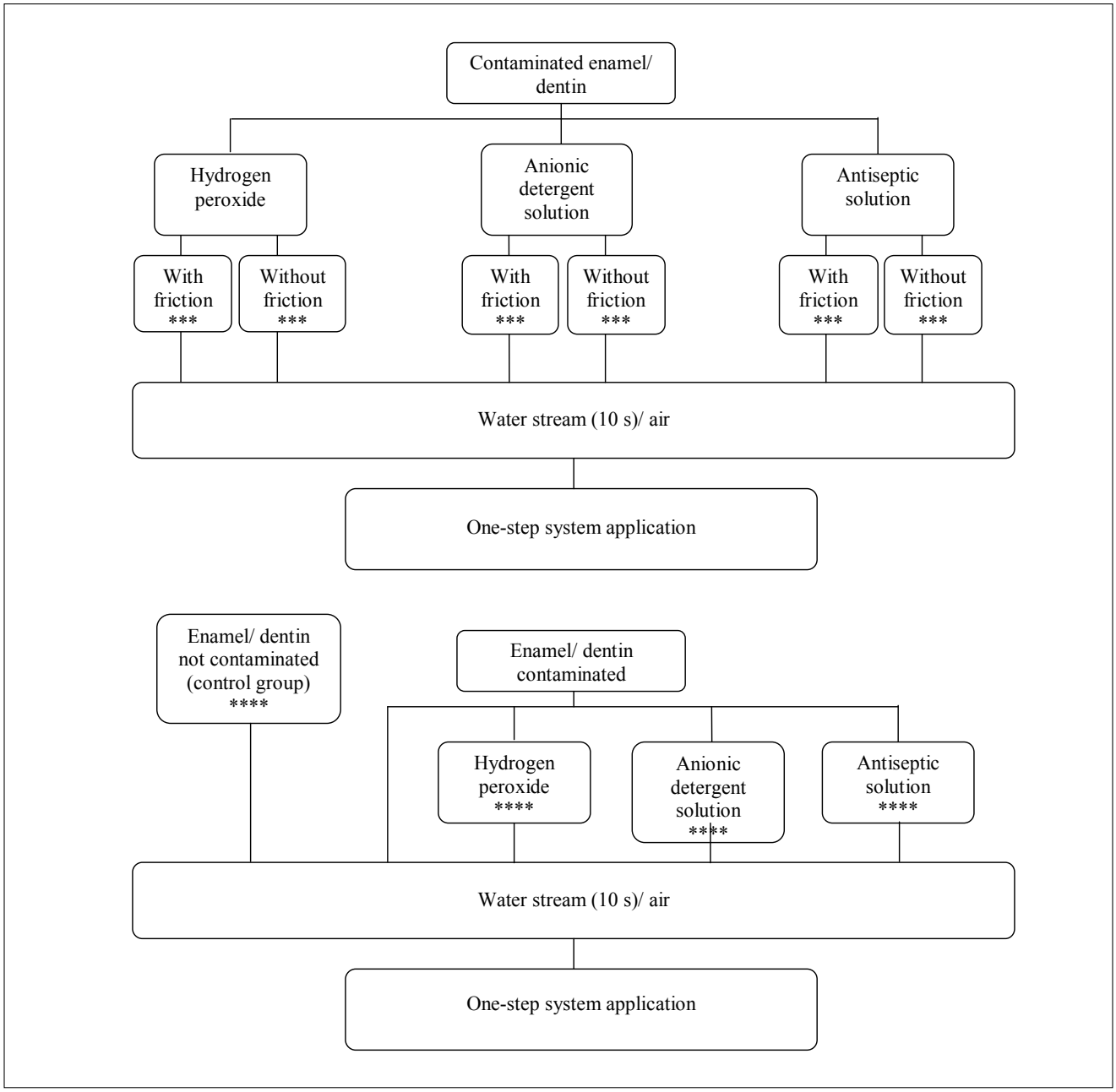


**Fig 1b** Design of Experiment 3(***): application mode of cleansing agents and 4(****): efficacy of three different cleansing agents.

flow (more than 5 s) and light cured for 10 s. Prior to light curing of the bonding resin, Tygon tubing molds (R-3603, Norton Performance Plastic; Cleveland, OH, USA) were mounted on the enamel and dentin surface to limit the bonding area. A microhybrid composite resin, shade A3 (Clearfil AP-X/ batch 01042A, Kuraray) was placed into the molds with a celluloid sheet matrix placed over the resin, gently pressed flat and photocured for 20 s. Because the Tygon molds were bonded tightly to the tooth surface by the simultaneous photocuring process of the bonding resin, no flash of composite resin extended onto the surface beyond the base of the mold. In this manner, two to four cylinders of resin, approximately 0.8 mm in diameter and 0.5 mm in height, were bonded to each dental surface. Specimens were stored at 23°C for 1 h prior to removing molds with a scalpel blade. The specimens were then stored in water at 37°C for 24 h.[28]

**Table 1 Mean microshear bond strengths and standard deviations (MPa) of enamel and dentin contaminated by heparinized blood (with different storage periods)**

| Substrate | Storage period | | |
|---|---|---|---|
| | 0 h | 24 h | 7 days |
| enamel | 1.13 ± 2.26 | 1.89 ± 3.78 | 1.70 ± 3.40 |
| dentin | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

***Microshear bond strength test (μSBS)***

Before the microshear test was conducted, all samples were checked under an optical microscope (40X magnification) for defects (Olympus SZ-PT; Tokyo, Japan). Samples that showed interfacial gap formation or bubble inclusion were excluded from the study and replaced by another sample.

Specimens were mounted in a jig so as to place the bonded resin/tooth interface parallel and as close as possible to a wire (diameter 0.20 mm) that was looped around the resin cylinder, in contact with half of the cylinder base for the microshear test, performed at a crosshead speed of 1 mm/min using a universal testing machine (Mini Instron 4442, Instron; Norwood, MA, USA). The microshear bond strength was calculated by dividing the maximum load at failure by the cross-sectional surface area of the bonded surface. If a spontaneous interfacial debonding occurred while the specimens were being mounted or sectioned, the bond strength was recorded as 0 MPa.[7,37]

***Fracture analysis***

All tested samples were examined under an optical microscope at 40X magnification to identify failure mode. The fractures were categorized as follows: type 1: adhesive failure between tooth substrate and adhesive resin; type 2: mixed failure with adhesive failure (type 1) and cohesive failure in tooth substrate; and type 3: cohesive failure in composite resin.

***Statistical analysis***

Data were analyzed using the Kruskal-WallistTest (Minitab 14 Software Minitab; State College, PA, USA) to perform group comparisons ($p < 0.05$).

***Experiment 2***

The influence of heparinized vs fresh blood used as a contaminant during the adhesion process by means of a one-step system in enamel and dentin was investigated by the same mechanical method.

***Contamination protocol***

The heparinized blood was obtained by the same method previously described in experiment 1. The fresh capillary blood was taken from the same donor (a needle-prick to alcohol-wiped forefinger) at the same time that specimens were mounted.[5,7,38] Both types of blood were applied over dental surfaces and dried carefully with oil-free compressed air for 20 s from a distance of 10 cm.

The sample restoration, microshear bond strength test, and fracture analysis followed the same steps used in experiment 1.

***Statistical analysis***

Data were analyzed using one-way ANOVA and Tukey's Simultaneous Test (Minitab 14 Software, Minitab) to perform group comparisons ($p < 0.05$). The analysis of fracture mode was also conducted.

***Experiment 3***

Experiment 3 investigated whether there is a difference in adhesion performance related to the application mode of the three substances used to cleanse contaminated dental surfaces. Enamel and dentin surfaces were contaminated with blood and dried carefully with oil-free compressed air for 20 s from a distance of 10 cm.

The cleansing agents used were 3% hydrogen peroxide, anionic detergent solution (0.125% sodium sulfate lauryldiethylene glycol ether), and antiseptic solution consisting of sodium hypochlorite (0.4% to 0.5%) and boric acid (4%) (Dakin's solution). They were applied on enamel and dentin surfaces in two different ways as described below:

- With friction: Cotton pellets soaked with each of the cleansing agents were applied for 10 s on the contaminated surface.
- Without friction: The cleansing substances were applied using a syringe to cover all surfaces, and were left undisturbed for 10 s.

After applying the cleansing agents, the surfaces were rinsed with water spray for 10 s and gently air dried. The same adhesive system used in experiments 1 and 2 was applied, and samples were prepared for the microshear bond strength test as previously described.

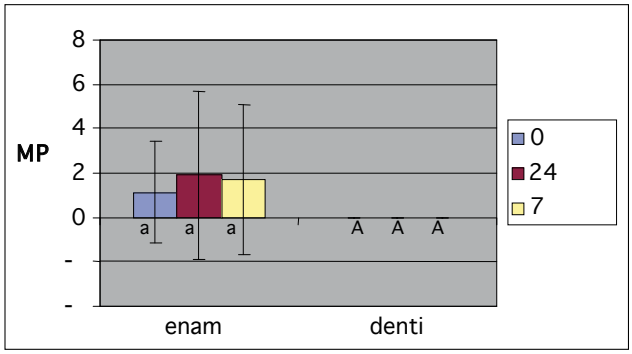


**Fig 2** Comparison of the microshear bond strength values of Clearfil S3 Bond to enamel and dentin after contamination with heparinized blood stored for 0 h, 24 h, and 7 days. The same letters are not significantly different within their group ($p > 0.05$).

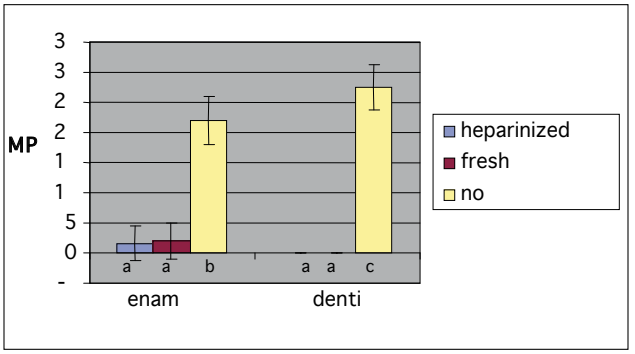


**Fig 3** Comparison of the microshear bond strength values of Clearfil S3 Bond to enamel and dentin contaminated (fresh blood and heparinized blood) or not. The same letters are not significantly different within their group ($p > 0.05$).

**Table 2 Mean microshear bond strengths and standard deviations (MPa) of enamel and dentin after different contaminations**

| | Substrate | Contaminant | |
|---|---|---|---|
| | Heparinized blood | Fresh blood | None |
| enamel | 1.58 ± 2.93 | 1.92 ± 2.99 | 22.05 ± 3.95 |
| dentin | 0.00 ± 0.00 | 0.00 ± 0.00 | 27.53 ± 3.69 |

***Statistical analysis***
The data were analyzed using the Mann-Whitney test (Minitab 14 Software, Minitab) to perform group comparisons ($p < 0.05$).

***Experiment 4***
In this experimental phase, the influence of three cleansing agents on the bonding performance of the one-step system applied over contaminated substrates was investigated.
The cleansing agents tested were 3% hydrogen peroxide, anionic detergent solution (0.125% sodium sulfate lauryldiethylene glycol ether), and antiseptic solution consisting of sodium hypochlorite (0.4% to 0.5%) and boric acid (4%) (Dakin's solution). All solutions were applied to contaminated enamel or dentin for 10 s, without friction, followed by water rinsing for 10 s. Another contaminated group of enamel and dentin was cleansed with a water stream only for 10 s. For the control group, non-contaminated enamel and dentin were used.

Blood contamination, sample restoration, microshear bond strength tests, and fracture analysis were conducted in the same manner used in experiment 3.

***Statistical analysis***
The data were analyzed using one-way ANOVA and Tukey's Simultaneous Test (Minitab 14 Software, Minitab) to perform group comparisons ($p < 0.05$).

## RESULTS

***Experiment 1***
No statistically significant difference was detected among experimental groups according to the storage period of the heparinized blood used ($p = 0.981$) for either enamel or dentin (Table 1, Fig 2). For this reason, experiments 2, 3, and 4 were performed using heparinized blood stored up to one week for contamination protocol standardization. One hundred percent adhesive failures (type 1) were observed in experiment 1 groups.

***Experiment 2***
For both enamel and dentin, the presence of blood contamination, irrespective of its type (fresh or heparinized), resulted in lower bond strength than in groups without blood contamination ($p < 0.05$) (Table 2, Fig 3), which

**Table 3 Mean microshear bond strengths and standard deviations of contaminated enamel cleansed with hydrogen peroxide, anionic detergent solution, or antiseptic solution (with or without friction), and failure analysis of the specimens**

| | | MPa | Type 1 | Type 2 | Type 3 |
|---|---|---|---|---|---|
| Hydrogen peroxide | with friction | 15.57 ± 3.83 | 100% | | |
| | without friction | 16.45 ± 0.86 | 90% | 10% | |
| Anionic detergent solution | with friction | 16.00 ± 3.25 | 100% | | |
| | without friction | 17.36 ± 2.83 | 100% | | |
| Antiseptic solution | with friction | 16.22 ± 3.56 | 100% | | |
| | without friction | 16.52 ± 1.86 | 100% | | |

Type 1: adhesive failure between tooth substrate or hybrid-like layer and adhesive resin; type 2: mixed failure of adhesive failure (type1) and cohesive failure in tooth substrate; type 3: cohesive failure in resin composite.

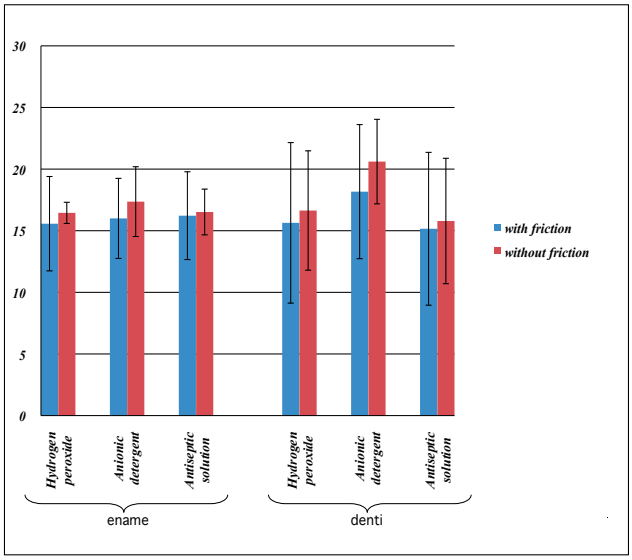


Fig 4 Comparison of the microshear bond strength values of Clearfil S3 Bond to contaminated enamel and dentin cleansed with hydrogen peroxide, anionic detergent solution and antiseptic solution in two different ways (with and without friction).

illustrates the strong influence that blood contamination has on bonding. Based on the results of experiment 1 and the fact that no significant difference was observed between heparinized and fresh blood, only heparinized blood stored up to one week was used in experiments 3 and 4. One hundred percent adhesive failures (Type 1) were observed in experiment 2 groups.

***Experiment 3***

The application method of the cleansing agents (3% hydrogen peroxide, anionic detergent solution, and antiseptic solution) had no influence on the microshear bond strength results of the adhesive system used in contaminated groups in enamel (Table 3, Fig 4) or dentin (Table 4, Fig 4) ($p > 0.05$). Adhesive failures (type 1) and mixed failures (type2) were observed in these experimental groups.

***Experiment 4***

All cleansing agents tested showed bond strengths similar to the group without contamination for both enamel ($p = 0.793$) and dentin (0.069). It is of prime importance to highlight that, although bond strengths for the cleansing agent groups are lower than that of the water group, they were not significantly different. All tested cleansing agents, as well as water, presented the same behavior, counteracting the negative effect of blood contamination in enamel (Table 5, Fig 5) and dentin (Table 6, Fig 6). Adhesive failures (type 1) and mixed failures (type2) were observed in these experimental groups.

**Table 4 Mean microshear bond strengths and standard deviations of contaminated dentin cleansed with hydrogen peroxide, anionic detergent solution or antiseptic solution (with or without friction), and failure analysis of the specimens**

| | | MPa | Type 1 | Type 2 | Type 3 |
|---|---|---|---|---|---|
| Hydrogen peroxide | with friction | 15.64 ± 6.51 | 70% | 30% | |
| | without friction | 16.64 ± 4.84 | 100% | | |
| Anionic detergent solution | with friction | 18.17 ± 5.44 | 78% | 22% | |
| | without friction | 20.61 ± 3.43 | 100% | | |
| Antiseptic solution | with friction | 15.16 ± 6.20 | 94% | 6% | |
| | without friction | 15.79 ± 5.09 | 71% | 29% | |

Type 1: adhesive failure between tooth substrate or hybrid-like layer and adhesive resin; type 2: mixed failure of adhesive failure (type1) and cohesive failure in tooth substrate; type 3: cohesive failure in resin composite.

**Table 5 Mean microshear bond strengths and standard deviations of contaminated enamel cleansed with different agents, failure analysis of the specimens**

| | MPa | Type 1 | Type 2 | Type 3 |
|---|---|---|---|---|
| Water | 18.27 ± 5.60 | 95% | 5% | |
| Hydrogen peroxide | 16.01 ± 2.53 | 95% | 5% | |
| Anionic detergent solution | 16.68 ± 2.83 | 100% | | |
| Antiseptic solution | 16.37 ± 2.54 | 100% | | |
| Control | 15.89 ± 3.74 | 100% | | |

Type 1: adhesive failure between tooth substrate or hybrid-like layer and adhesive resin; type 2: mixed failure of adhesive failure (type1) and cohesive failure in tooth substrate; type 3: cohesive failure in resin composite.

## DISCUSSION

Studies have shown that blood contamination does influence the bond strength to hard dental tissues,[5,6,7,11-13,34,36,38] but the best way to adequately deal with such contamination remains a question to be answered.

The literature on contamination during adhesive procedures contains many discrepancies, particularly in terms of the experimental design, such as in the type of blood used (fresh or heparinized) or the period of storage of the heparinized blood. Where some studies reported using the blood with an anticoagulant as a contaminant at the moment it was prepared11,12, other authors reported its use after one week.[13] The present study was conducted to verify the importance of this variable. The results showed that the heparinized blood samples did not change their characteristics as a contaminant for up to one week when compared with the periods of 0 h (immediately after the addition of heparin) and 24 h.

The second experiment of this project was conducted to test whether there were differences in bond strengths using fresh blood or heparinized blood. Opinions on this issue differ, with some authors using fresh blood,[5,7,19,38] while others[11,12,13,18] used blood with an anticoagulant. Some experimental procedures were standardized in or-

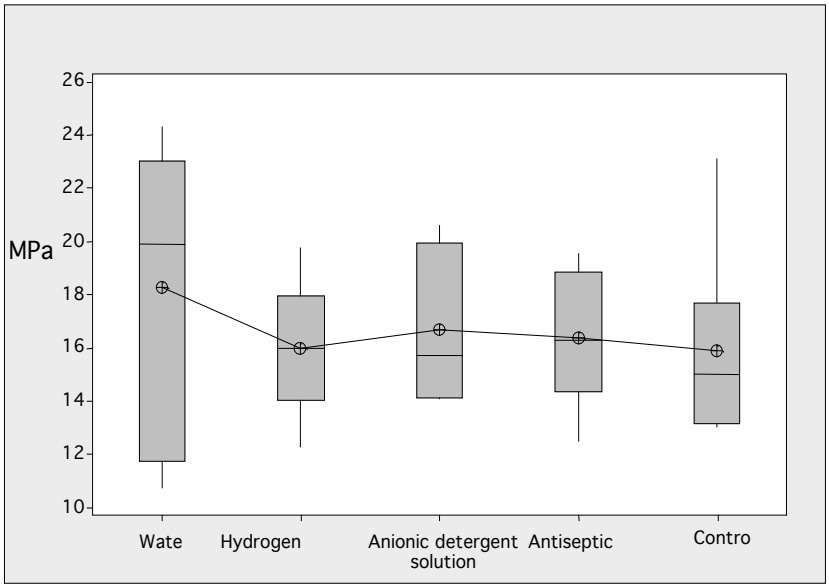


**Fig 5** Comparison of the microshear bond strength values of Clearfil S3 Bond to contaminated enamel cleansed with hydrogen peroxide, anionic detergent solution and antiseptic solution.

der to control external variables in the study, such as creating a layer of dry blood on top of the samples in order to simulate a worst-case scenario. For that test, the blood was air dried for 20 s from a distance of 10 cm,[7] and blood was taken from one female donor on the same day of the reproductive cycle that the experiment was performed to minimize hormonal variations that may occur in a female blood sample.[33]

The bonding ability of Clearfil S3 Bond to enamel and dentin was affected by the presence of contamination, in agreement with Oonsombat et al.[18] It is suggested that the presence of blood protein, together with macromolecules such as fibrinogen and platelets, can form a thin film on the surface,[3] which impairs adhesion to hard dental tissues.[20,38]

Dietrich et al[6] reported that the presence of an anticoagulant interferes with the interaction of blood and dentin when contamination occurs after acid etching, obscuring the effect of blood contamination in laboratory studies. However, in this study, the bonding ability was not statistically significantly different when contamination occurred with either heparinized blood or fresh blood.

The findings of the present study led us to use heparinized blood, stored up to a period of one week, to contaminate enamel and dentin in experiments 3 and 4.

In the third experiment, some cleansing agents[4,9,10,14,22,35] that are commonly used in dentistry were applied to remove the blood from dental surfaces in order to counteract the negative effects of blood contamination on adhesion to dental tissues. Some authors[22] suggest that the cleansing ability of some agents could be improved by friction with a cotton pellet, but the results of this study indicate that this procedure is unnecessary. Although the application mode (with/without friction or active/passive application) plays an important role in the action of some substances,[3] it did not effect the performance of the cleansing agents used in this study.

Based on this observation, in the fourth experiment, the cleansing agents were only applied to the surface of enamel and dentin without friction. In one group, only a water stream from an air-water syringe was used, and in all other groups, this water stream was used to remove the cleansing agent. The results of this experiment showed that bonding ability was not significantly different when contaminated enamel and dentin were cleansed with the agents proposed or only with a water stream. Additionally, results for these groups were not significantly different from those of the control group.

The literature shows that sodium hypochlorite oxidizes some components in the dentin matrix that are critical for the interfacial initiation of polymerization in some adhesive systems, which leads to lower bond strengths.[32] Other studies show that the application of sodium hypochlorite prior to the application of self-etching adhesives on dentin seems to positively influence the tensile bond strength of the self-etching adhesive.[8] In the present study, this agent neither increased nor decreased the bond strength, possibly due to the lower concentration present in the antiseptic solution applied on the dental substrate or because of the rinsing procedure used to remove residual cleansing agents.

During bonding procedures, hydrogen peroxide might break down to oxygen and water, generating bubbles or voids that interfere with resin infiltration into etched dentin.[31,32] This oxygen severely inhibits the interfacial polymerization of resin bonding materials.[16,23] Although reduction in bond strength of some adhesive systems applied to enamel and dentin may have been caused by the presence of hydrogen peroxide,[15,17,30,32] this product did not reduce the adhesion when applied for 10 s at 3%

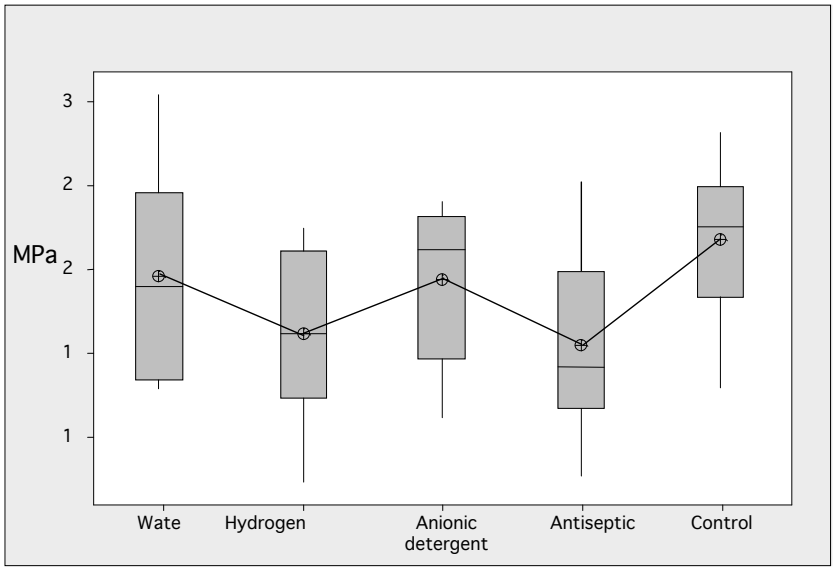


**Fig 6** Comparison of the microshear bond strength values of Clearfil S3 Bond to contaminated dentin cleansed with hydrogen peroxide, anionic detergent solution and antiseptic solution.

**Table 6 Mean microshear bond strengths and standard deviations of contaminated dentin cleansed with different agents, failure analysis of the specimens**

| | MPa | Type 1 | Type 2 | Type 3 |
|---|---|---|---|---|
| Water | 19.60 ± 6.34 | 85% | 15% | |
| Hydrogen peroxide | 16.14 ± 5.34 | 84% | 16% | |
| Anionic detergent solution | 19.39 ± 4.52 | 89% | 11% | |
| Antiseptic solution | 15.47 ± 5.42 | 84% | 16% | |
| Control | 21.80 ± 4.76 | 100% | | |

Type 1: adhesive failure between tooth substrate or hybrid-like layer and adhesive resin; type 2: mixed failure of adhesive failure (type1) and cohesive failure in tooth substrate; type 3: cohesive failure in resin composite.

concentration in this study. However, it is important to point out that the substance was washed for 10 s, a process that could have been responsible for the complete removal of remaining contaminant from the surface.

Large blood corpuscle elements can be completely rinsed away by a water stream, but a reaction between the exposed collagen meshwork and the blood protein components may inhibit primer infiltration into dentin.[7,13] However, in the present study, this did not occur, because no dentin collagen was exposed at the time contamination occurred – only before the application of the self-etching system.

This could explain the results obtained, since the groups in which the cleansing agents were used produced favorable results, just as did the groups in which a water stream alone was used. This outcome suggests that a 10-s water stream alone is sufficient to remove blood contamination from enamel and dentin, agreeing with Kaneshima et al.[13]

Finally, our study was effective in proving the negative interference of blood contamination on adhesion to enamel and dentin using a one-step adhesive system. In addition, some standardization of the contamination protocol was tested, and it is proposed that it should be used

in future studies related to blood contamination. However, it is important to remember that this is an in vitro study, and clinical research on this topic would provide relevant knowledge to professionals who deal with blood contamination in their clinical routine.

## CONCLUSION

The addition of heparin to blood samples did not change their characteristics as a contaminant for up to one week, when compared to the periods of 0 h (immediately after the addition of heparin) and 24 h. The addition of heparin to blood is a reliable procedure for standardizing the contaminant in experimental studies. The cleansing agents can be used without friction over contaminated surfaces of enamel and dentin. The use of a water stream for 10 s was enough to remove blood contamination on enamel and dentin, before the application of a one-step adhesive system.

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**Clinical relevance:** Heparinized blood can be used as a contaminant for up to one week, and it is a reliable procedure to standardize the contaminant. A water stream is sufficient to remove blood contamination from dental tissues before the application of a one-step adhesive system.