Original Study

# INVESTIGATING THE EFFECT OF BLOOD CONTAMINATION ON THE SHEAR BOND STRENGTH OF GC G-PREMIO BOND TO ENAMEL

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**ABSTRACT**

**Aim:** In recent years, the use of composites has increased. Working with adhesive systems is sensitive and influenced by various factors. the present study was conducted with the aim of investigating the effect of blood contamination on enamel when using G-Premio bond (GC Corporation).

**Materials & Method:** The buccal surfaces of 40 teeth were cut and then they were matched with sandpaper. The samples were divided into 2 groups of 20. Group A as the control and group B as the intervention group. In group A, the enamel surface was only rinsed and dried, and in group B, a drop of blood was applied to each sample surface in a standard form, and then they were rinsed and dried. G-Premio BOND (GC Corporation, Japan) was applied and cured on the surfaces. Then the composite was placed on the prepared surfaces and was cured.

The samples were subjected to 500 cycles of thermocycle and then placed under the pressure of the universal machine, and finally to determine the type of fracture, they were examined by stereo microscope device with 20X magnification.

**Results:** The mean shear bond strength of GC-G-Premio Bond adhesive to enamel in group A was 33.7 MPa and in group B was 22.9 MPa, which this difference was statistically significant ($p$=0.005). In evaluating the frequency of fracture type, the results showed that there is no significant difference between the two groups ($p$=0.429).

**Conclusion:** Based on the results of this study and previous studies, self-etch adhesives are not able to completely eliminate the contamination of enamel with blood, and after blood contamination, it is necessary to eliminate the contamination and re-etch.

**Key words:** *Enamel, Shear bond strength, Blood contamination, Self-etching adhesive.*

## Introduction

In recent years, the use of resin composite materials, especially light-cured composites, in the anterior and posterior teeth has increased due to the meeting of the beauty requirements requested by patients.[1]

Adhesive systems are sensitive and influenced by various factors. There are various intra-oral factors that can reduce the adhesion quality of the materials; such as high humidity of mouth, temporary cement residues, dental caries and contamination of the adhesive region with saliva and blood. Contamination of the adhesive surface with blood is a factor that may occur repeatedly during the restoration works. One of the prerequisite factors for obtaining satisfactory results is the complete establishment of operating environment isolation and prevention from contaminations during various stages of the work.[2]

Adhesive systems are divided into two groups of etch-and-rinse and self-etch. The etch-and-rinse adhesives completely remove the smear layer and open the dentinal tubules, but sometimes deeper areas demineralized by etching acid are not completely blocked by the resin, which causes the occurrence of nanoleakage overtime and has undesirable effect on the bond strength. Self-etch systems do not require the removal of smear layer, and simultaneously decalcification and penetration of the resin occur among enamel prisms. This clinical process has less complexity and less susceptibility to work due to lack of need for rinsing and lack of dependence on the level of dentin moisture.[3] Self-etch systems also summarize etching and priming steps in one step, as the result these systems lead to simplification of clinical stages and reduced technical sensitivity and increased work speed.[4,5]

The bonding strength is a very important and significant mechanical property in the clinical durability of tooth-colored restorations.[6]

The blood has a high protein content (about 6.7%) and includes macromolecules such as fibrinogen.[7] On the other hand, as dentin has protein absorption properties,[8] in the case of contamination with blood, these proteins create a layer on the dentin surface that prevents the penetration of resin to the dentin.[7] This could reduce bond strength by 30% to 70% in all adhesives,[9] and lead to effective reduction of the bond strength.[7]

One of a variety of self-etch adhesives, G-Premio bond (GC Corporation), has been released in the market, which is a self-etch single-bottle light-cured adhesive. According to studies and lack of sufficient information on the effect of blood contamination on the shear bond strength of this adhesive on the enamel, the present study was conducted with the aim of investigating the effect of blood contamination when using this adhesive on the enamel.

## Materials & Method

According to the reference,[10] the shear bond strength and standard deviation in the groups of control and blood-contaminated were 21±3.7 and 16.2±5.6, respectively. The sample size was calculated according to Minitab software and as 20 in each groups with 95% confidence and 90% power.

The samples were selected from pre-extracted teeth due to gingival disease or orthodontic purposes. In order to conduct this experimental study, 40 human molar and premolars without caries and restoration that were extracted due to gingival disease or orthodontic purposes were

collected over a period of three months before the beginning of the study. The teeth were kept in the 0.2% thymol solution for 24 hours after debridement and then were kept in the distilled water until the time of the test. The buccal surfaces were cut to a depth of 1 mm by a cylinder burs with a length of 3mm and a diameter of 1mm, and then were rubbed by a 600 grit sandpaper (silicon carbide) for 30 seconds and this way they were matched. The surface was rinsed and dried. the fresh capillary blood was taken from an individual's finger. The samples were divided into 2 groups of 20. Group A as the control and group B as the case group. In group A, the enamel surface was just rinsed and dried after preparation and in group B, a drop of blood was applied directly on the surface of each sample and was abandoned for 15 seconds without disturbance. Then the surface was rinsed and slowly dried with air. We applied the adhesive (G- Permio BONDTM, GC Corporation, Japan), which is a self-etch single-bottle light-cured adhesive, on the prepared surface according to the manufacturer's instructions. We waited 5 to 10 seconds and then dried the surface with mild air pressure for 5 seconds. Then we cured the adhesive with light. In the next step, we placed the light-cured composite (Filtek Z-250, A3-3M ESPE, USA) on the prepared surface by using Tygon Tube pieces with a height of 2 mm and an inner diameter of 1.5 mm, and cured it for 40 seconds. The samples were subjected to 500 thermal cycles using thermocycle machine (Vafaee, tc-300, Iran) at a temperature of between 5 ° C and 55 ° C with a residence time of 30 seconds and a transfer time of 10 seconds, and then they were placed in the universal machine (Universal testing machine MTD-500, SD mechatronic, Germany) with the blade speed of 1 mm/min in the bond location, and finally, to determine the type of fracture (adhesive, cohesive, mixed), all the samples were examined by two dentists using a stereo microscope (Carl Zeiss Inc, oberkochen, Germany) with 20X magnification.

T-test was used due to the normal distribution of data in the two groups. Due to the fact that this study is a laboratory study and is performed on pre-extracted teeth, there was no need for specific ethical considerations.

**Results**

The purpose of this study was to investigate the effect of blood contamination on the shear bond strength of GC G-Premio BOND to the enamel. For this purpose, 40 human molar and premolars without caries and restoration were selected and were divided equally into two groups of 20; A: without blood contamination (control group), B: after contamination of enamel with blood (intervention group). The shear bond strength of GC-G-Premio Bond adhesive to the enamel without contamination of enamel with blood and after contamination of enamel with blood was determined in megapascal (MPa). After collecting the data, the research data were analyzed statistically and the findings are presented in the sections below.

The mean and standard deviation of the shear bond strength of GC-G-Premio Bond adhesive to enamel without blood contamination of enamel and after contamination of enamel with blood in MPa, and comparison of both groups are reported in the Table 1 and Chart 1.

| Variable | Groups | | | | | | p-Value |
|---|---|---|---|---|---|---|---|
| | Without Blood Contamination | | | After Contamination of Enamel with Blood | | | |
| Adhesive Shear Bond Strength | N | Mean | SD | N | Mean | SD | 0.005 |
| | 20 | 33.7 | 12.4 | 20 | 22.9 | 10.4 | |

*Table 1: Mean, standard deviation and results of independent t-test for comparison of shear bond strength of the adhesive in studied groups*

As shown in Table 1, the mean and standard deviation of shear bond strength of GC G-Premio Bond adhesive to enamel in the control group (without blood contamination) was 33.7 (12.4) MPa and in the intervention group (after contamination of enamel with blood) was 22.9 (10.4) MPa. In order to compare the shear bond strength of GC-G-Premio Bond adhesive to enamel in two groups of control and intervention, considering the observation of assumptions of the normal distribution of the variable of shear bond strength of GC-G-Premio Bond adhesive to enamel based on the Kolmogorov-Smirnov test in the control group ($p$=0.778) and intervention group ($p$=0.928), and also the same variances of the variable of adhesive shear bond strength in both groups based on Leven test ($p$=0.545), the independent t-test was used.

As shown in Table 1, the results of independent t-test showed that there is a significant difference between shear bond strength of GC G-premio Bond adhesive to enamel in the two groups of intervention and control ($p$=0.005). The mean shear bond strength of GC G-Premio Bond adhesive to enamel without blood contamination of enamel was significantly higher than its strength after contamination of enamel with blood (Chart 1).

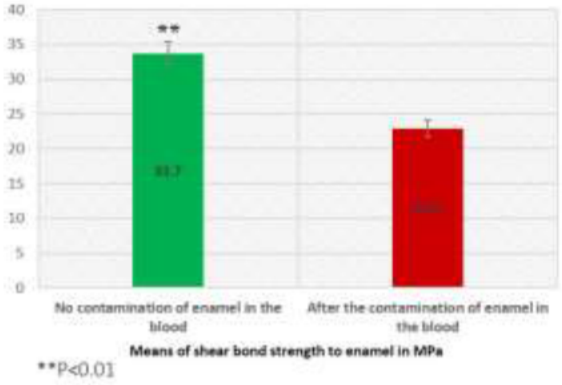


*Chart 1. The comparison of mean shear bond strengths to enamel in the studied groups in MPa*

As shown in Chart 1, the mean shear bond strength without blood contamination of enamel was significantly (10.8 MPa) higher than its strength after contamination of enamel with blood ($p$=0.005). The frequency distribution and

percentage of fracture types in the two groups of control (without blood contamination) and intervention (after contamination of enamel with blood), as well as the comparison of both groups based on the type of fracture are reported in Table 2 and Chart 2.

| Fracture Types | Groups | | | | p-Value |
|---|---|---|---|---|---|
| | Without Blood Contamination | | After Contamination of Enamel with Blood | | |
| | N | Mean | N | Mean | |
| Adhesive | 15 | 75 | 17 | 85 | 0.429 |
| Cohesive | 5 | 25 | 3 | 15 | |
| Total | 20 | 100 | 20 | 100 | |

*Table 2: Number, percentage and results of Chi-squared test (X2) for comparing fracture type in the studied groups*

As shown in Table 2, fracture type in the control group (without blood contamination) was 75% adhesive and 25% cohesive. In the intervention group (after contamination of enamel with blood), the fracture type was 85% adhesive and 15% cohesive. The results of Chi-squared test showed that there is no significant difference between the two groups in terms of fracture type ($p$=0.429).

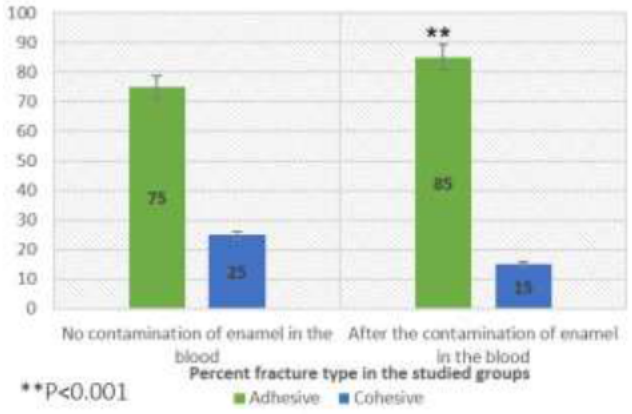


*Chart 2: The comparison of fracture type in the studied groups*

As shown in Chart 2, there is no significant difference between the two groups in terms of fracture type ($p$=0.429).

**Discussion**

Studies that have examined the effects of various contaminants such as water, saliva, plasma, eugenol or non-eugenol cements on the adhesion of adhesives to dental tissues, and they have shown that plasma reduces the bond strength to both enamel and dentin surfaces.[11-13] Researchers have stated that protein components in some contaminants such as blood can prevent monomers from penetrating into enamel rods and collagen network exposed by acid etch, and thereby reduce the bond strength.[10]

The type of adhesive system, the time of contamination, the surface studied (enamel or dentin) and blood type (fresh or with anticoagulants) are very diverse in various studies that examine the effect of blood contamination on adhesives, which complicates the analysis of the results.[14,15] In this study, fresh blood was used as the contaminant, as it has been shown in previous studies that adding anticoagulant reduces the bond strength.[15]

Self-etch adhesives have become very popular among dentists due to the lesser bonding components and work processes, the lack of need for rinsing, as well as less sensitivity after the work. During surface restoration of a tooth that is prepared without the use of rubber dam, there is a chance of bleeding from the gingival after phosphoric acid rinsing and contamination of restorative cavity. This problem does not exist in self-etch adhesives.[10]

One of a variety of self-etch adhesives, G-Premio bond (GC Corporation), has been introduced into the market, which is a self-etch single-bottle light-cured adhesive. According to studies and lack of sufficient information on the effect of blood contamination on the shear bond strength of this adhesive on enamel, this study was conducted with the aim of evaluating the effect of blood contamination when using this adhesive on the enamel.

In this study, the comparison of shear bond strength of GC-G-Premio Bond adhesive to enamel showed that there is a significant difference between shear bond strength of GC G-Premio Bond adhesive to enamel in two groups of intervention and control ($p$=0.005). The mean shear bond strength of GC G-Premio Bond adhesive to enamel without blood contamination was significantly higher than its strength after contamination of enamel with blood (Chart 1).

Also, the fracture type in the control group (without blood contamination) was 75% adhesive and 25% cohesive. In the intervention group (after contamination of enamel with blood), fracture type was 85% adhesive and 15% cohesive. The results of Chi-squared test showed that there is no significant difference between the two groups in terms of fracture type ($p$=0.429).

The results of this study are similar to the results of studies by Tachibana, Koppolu and Powers, and this indicates that blood contamination before applying adhesive reduces the shear bond strength to enamel. [16-18] On the other hand, the results of present study are in contradiction with the results of the study by Mendonça, which stated that blood contamination before applying primer do not reduce the bond strength.[10]

Koppolu *et al*. (2012), conducted a study with the aim of evaluating the effect of blood and saliva contamination on shear bond strength of a self-etch adhesive (Xeno III) to enamel and dentin. Fresh human blood and saliva were placed on the tooth surface before or after applying the self-etch adhesive (Xeno III). Then a resin composite (Filtek Z-250, A1-3M ESPE, USA) with a diameter of 3 mm was placed on the teeth for testing the shear bond strength. At the end, samples were subjected to the pressure of the universal machine to perform the test. The results showed that contamination of enamel with fresh blood before applying the adhesive reduces the shear bond strength of adhesive to enamel by 70%.[16]

Also, Tachibana *et al*. (2011), in the Brazil, examined the effect of blood contamination and elimination of this contamination with different detergents on bond strength of a self-etch adhesive to dental tissues. Heparinized blood and fresh blood, that were taken from a specific person, were placed on the surfaces and dried. A single-step adhesive (Clearfil S3 Bond, Kuraray, Osaka, Japan) was applied on the surface of the samples and then composite cylinders were placed at a height of 0.5 mm and a diameter of 0.8 mm on the tooth surface. After 24 hours, shear bond strength test and evaluation of fracture under the pressure of the universal machine (Mini Instron 4442, Instron; Norwood, MA, USA) were performed. The groups without blood contamination (22.05 ± 3.95), similar to the present study, showed more bond strength than the blood contaminated group (2.99 ± 1.92). So that fresh blood contamination reduced the adhesive bond strength by 90%.[17]

Powers *et al*. (1995), conducted a study in the US with the aim of evaluating the bond strength of adhesives and resin composites to enamel and dentin in different conditions (without contamination, saliva contamination, plasma contamination, zinc oxide eugenol cement contamination, non-eugenol cement contamination). The commercial bonding material (Gluma 2000; Bayer AG, Dormagen, Germany) and its composite (Pekafill; Bayer AG) were applied on tooth surfaces in two states (contaminated, re-etched). The samples were debonded after 24 hours under tension by the universal machine. Among the control group, the highest bond strength to enamel and dentin was recorded as 20 MPa and 12 MPa, respectively. Contamination reduced the bond strength by 20% to 100%. The re-etching without any other mechanical action increased the bond strength even to the same level as the control group.[18]

Mendonça *et al*. (2010), in the Brazil, investigated the effect of contamination of enamel and dentin with blood on the bond strength of a two-step self-etch adhesive (Clearfil SE Bond) in vitro. The samples were randomly divided into 10 groups (n=10) based on the time of contamination (before the primer or after resin bonding) and the way of eliminating it (drying or rinsing and drying). A drop of blood was applied directly on the surface of each sample and was abandoned for 15 seconds without any disturbance. At the end, the samples were placed under the pressure of the universal machine (Model 4440 Instron, Canton, Mass. USA) until being debonded. The overall results showed that blood contamination reduces the bond strength of this type of adhesive; while the results of study on enamel in this study showed that blood contamination before applying primer do not reduce the bond strength.[10] This difference with the present study is probably due to the difference in the type of adhesives used in the two studies. Clearfil se bond is a type of two-step self-etch adhesive that in the first step prepares the dental substrate and primes it (condition and prime), and bonding is applied after drying the surface; as a result, self-etch primer cleanses or hydrolyzes the blood, so that, in the next step, resin bonding could be bond to the enamel without any interfering factor. While GC G-Premio Bond adhesive was used in the present study, which is a type of self-etch single-bottle adhesive and all of the etching agents of priming and bonding are placed in a bottle.

It seems that all researchers have consensus on the fact that blood contamination of enamel before applying adhesive reduces the shear bond strength of self-etch single-bottle adhesives to enamel, and differences in the amount of this reduction is probably due to the difference in the commercial type of adhesives used, type of shear bond strength studied (micro or macro) and difference in the measuring devices.

**Conclusion**

The present study was conducted with the aim of investigating the effect of blood contamination on shear bond strength of a type of self-etch adhesive G-Premio bond (GC Corporation) to enamel. The mean and standard deviation of shear bond strength of GC-G-Premio Bond adhesive to the enamel in the control group (without blood contamination) was obtained as 33.7 (12.4) MPa and in the intervention group (after contamination of enamel with blood) obtained as 22.9 (10.4) MPa.

As shown in Table 1, the results of independent t-test showed that there is a significant difference in the shear bond strength of GC G-Premio Bond to enamel between both intervention and control groups ($p$=0.005). the mean shear bond strength of GC G-Premio Bond adhesive to the enamel without contamination of enamel with blood was significantly higher than its strength after contamination of enamel with blood.

Based on the results obtained in this study and previous studies mentioned, single-step self-etch adhesives are not able to completely eliminate the blood contamination of enamel, and after blood contamination, there is need for elimination of the contamination and possibly re-etching.

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