Effect of dentin contamination and cleaning on the bond strength of resin-modified glass ionomer cement

Efeito da contaminação e limpeza da dentina na resistência de união do cimento de ionômero de vidro modificado por resina

Efecto de la contaminación y la limpieza de la dentina sobre la fuerza de unión del cemento de ionómero de vidrio modificado con resina

Abstract
This study aimed to evaluate the effect of dentin contamination and cleaning techniques on the bond strength of resin-modified glass ionomer (RMGI)/dentin interfaces. 168 bovine teeth were selected and divided into twelve groups (n = 14), according to the contaminating agent (S - no contamination; Sa - saliva; Sg - blood; H - hemostatic) and cleaning technique (S - without cleaning; A - air and water spray; P - polyacrylic acid reconditioning 26%). All specimens were restored with Riva Light Cure (SDI) ionomeric material, sectioned (1.0 × 1.0 mm toothpicks), and subjected to the microtensile bond strength test and a thermocycling regimen (CT). In the immediate analysis, all contaminated groups without cleaning had the following adhesion values when compared with the control group. After thermocycling, in the groups without cleaning only the hemostatic was identified to the control. The Sa groups maintained the immediate analysis standard, while the Sg groups were similar to the control, regardless of the time analyzed. Groups H were similar to each other. In conclusion, the contaminants tested impaired the immediate adhesion of the ionomeric material to dentin. Substrate reconditioning was effective in specimens contaminated with saliva and hemostatic agent. However, bond strength values reduced after aging.

Keywords: Contamination; Dentistry; Glass ionomer cements; Tensile strength.
Safar et al. observed that salivary contamination of previously conditioned dentin compromised the bond strength of RMGI and dentin. They also observed that cleavage of dentin contaminated by eugenol by Kuphasuk et al., 2007 or recondition it with polyacrylic acid (Santana et al., 2008; Bertoz et al., 2013). Santana et al., 2008), blood (Brauchili et al., 2010; Pucci et al., 2016), and hemostatic agents (Francisconi et al., 2009; Gonçalves et al., 2019).

It is essential to keep the operating field clean and free of excess moisture to allow the carboxylic groups in the ionomer to react with the calcium ions in the tooth. However, anatomical and morphological characteristics of the cervical region interfere with the isolation of the operative field and result in surface contamination by saliva (Brauchili et al., 2010; Santana et al., 2008), blood (Brauchili et al., 2010; Pucci et al., 2016), and hemostatic agents (Kuphasuk et al., 2007).

Wangpermtam et al studied dentin contaminated by eugenol-based provisional cement residues and lubricating oil and demonstrated that the use of polyacrylic acid was ineffective in restoring the bond strength of RMGI and dentin. They also observed that cleaning agents such as chlorhexidine and pumice-based compounds left residues on the tooth surface, which interfered with the bond strength of the restorative material.

Safar et al. observed that salivary contamination of previously conditioned dentin compromised its bond strength with RMGI. They emphasized that the bond strength remained low, despite cleaning the dentin and reconditioning it with polyacrylic acid.
Shimazu et al. concluded that contamination with artificial saliva did not affect the adhesion of conventional GIC (Glass Ionomer Cement) and RMGI (Resin-Modified Glas-Ionomer) to bovine dentin and enamel (Tyas et al., 2004).

Despite this knowledge, the literature is still scarce on comparative studies investigating the effects of dentin contamination with saliva, blood, or hemostatic agents in resin modified ionomers and the cleaning protocols used in surface modification (Tay et al., 2001).

In this context, our study aimed to evaluate the effect of dentin contamination and the methods routinely used in substrate cleaning on the bond strength at the RMGI / dentin interface using the microtensile bond strength test after 7 days of storage and 10,000 thermal cycles. Three null hypotheses were tested: (1) The bond strength of the ionomeric material to dentin would not be affected by contamination with saliva, blood, or hemostatic agent; (2) Cleaning agents (air/water spray and 25% polyacrylic acid reconditioning) would not influence the bond strength of the ionomeric material to contaminated dentin; (3) The bond strength of the ionomeric material to dentin would not be affected by the analysis time.

2. Methodology

2.1 Teeth selection and adhesion area delimitation

The research project was submitted to the Animal Ethics Committee. A total of 168 newly extracted bovine incisors were selected and cross-sectioned, separating the crown from the root at the level of the cementoenamel junction. Subsequently, the pulp tissue was removed, and the teeth were stored in 0.1% thymol solution of neutral pH at 4 °C, up to one week until usage (Santana et al., 2008).

The palatine faces of the dental crowns were embedded in acrylic resin cylinders (JET, Classic Dental Articles Ltda, Campo Limpo Paulista, SP, Brazil), leaving the buccal faces exposed and parallel to the base of the cylinder to remove the enamel and create a flat dentin surface for the bonding test. A channel was prepared at the base of the resin cylinder for later use during dental preparation.

The resin blocks containing the dental crowns were placed on a self-polishing machine, Aropol E (Arotec Ind. And Com. Ltda, Cotia, Sao Paulo, Brazil), using 600-grit silicon carbide paper (Extec Corp., Enfield), CT, USA) under water-cooling to expose dentin. The distance between the center of the exposed dentin tissue and the base of the acrylic resin was measured with a digital caliper (Mitutoyo Corporation, Tokyo, Japan), positioned in the channel described above, to standardize cavity depth. Subsequently, the dentin was abraded to a depth of 1.0 mm beyond the dentin-enamel bond, measured with the caliper.

After obtaining a standardized flat dentin surface, a plastic matrix (8mm diameter, 3mm height) was positioned 2 mm from the cementoenamel junction to act as a mold for the ionomeric material.

2.2 Contaminating, cleaning agents, and restorative material

2.2.1 Contaminants

The teeth were divided into four groups, according to the contaminant used in the study: (1) no contamination, (2) human saliva, (3) human blood, and (4) hemostatic agent. Fresh saliva was collected from the experimenter. The same experimenter’s blood sample was collected, stored in a tube containing buffered trisodium citrate solution, and refrigerated at 4 °C (approximately) to avoid coagulation. The hemostatic agent of choice was Hemostop (Dentsply Ind. And Com. Ltda., Petrópolis, RJ, Brazil), arranged in a single vial for topical use.

The application of contaminants was preceded by oral prophylaxis using a Robinson brush and a mixture of pumice stone and water. The dentin surface was actively conditioned with 26% polyacrylic acid (Riva Conditioner - SDI Limited, Victoria, Australia) for 10 seconds, as recommended by the manufacturer. The dentinal surface was washed with air/water
spray for 10 seconds and dried with air jets for 10 seconds. A KG brush disposable applicator (KG Sorensen, Cotia, SP, Brazil) was soaked in each contaminant and actively applied to the dentin substrate for 2 minutes. The excess contaminant was removed with another disposable applicator.

2.2.2 Cleaning agents

The teeth were divided into three groups, according to cleaning method: (1) no cleaning, if the contaminant was not removed at all; (2) cleaning the contaminated surface with air/water spray for 20 seconds, followed by air drying for 20 seconds at a distance of 10 cm (7); (3) substrate reconditioning with 26% polyacrylic acid for 10 seconds, followed by rewashing and drying of the surface.

2.2.3 Restorative material

The restorative material selected for this study was the resin-modified glass ionomer cement, Riva Light Cure (SDI Limited, Victoria, Australia). The material is commercially available in single capsules activated by pushing the plunger until it is flush with the body.

The activated capsule was placed into an amalgamator (Ultramat II, SDI Limited, Victoria, Australia) and triturated for 10 seconds to mix the components of the ionomeric material. Subsequently, the capsule was placed into the applicator (Riva Applicator 2, SDI Limited, Victoria, Australia), and the material was inserted. Photoactivation was performed for 40 seconds using a 1100 mW/cm² light-curing unit (Poly Wireless, Kavo, Joinville, Santa Catarina, Brazil).

2.3 Group distribution

Thus, the distribution of specimens in the study groups, according to the surface treatments received that are summarized in the flowchart (Table 1).

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Cleaning</th>
<th>Acronym</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without contaminating (S)</td>
<td>No decontamination (S)</td>
<td>SS</td>
</tr>
<tr>
<td></td>
<td>Water and air spray (A)</td>
<td>SA</td>
</tr>
<tr>
<td></td>
<td>Polyacrylic Acid (P)</td>
<td>SP</td>
</tr>
<tr>
<td>Saliva (Sa)</td>
<td>No decontamination (S)</td>
<td>SaS</td>
</tr>
<tr>
<td></td>
<td>Water and air spray (A)</td>
<td>SaA</td>
</tr>
<tr>
<td></td>
<td>Polyacrylic Acid (P)</td>
<td>SaP</td>
</tr>
<tr>
<td>Blood (Sg)</td>
<td>No decontamination (S)</td>
<td>SgS</td>
</tr>
<tr>
<td></td>
<td>Water and air spray (A)</td>
<td>SgA</td>
</tr>
<tr>
<td></td>
<td>Polyacrylic Acid (P)</td>
<td>SgP</td>
</tr>
</tbody>
</table>

Source: Authors.
2.4 Storage, sampling, and thermal cycling

The restored teeth were stored in a humidifying chamber at 37 °C for 7 days (Tyas & Burrow, 2004). The crowns were cleaned with gauze soaked in distilled water, fixed on an acrylic plate, and sectioned perpendicular to the tooth/RMGI interface with a double-sided diamond disk (Buehler, Lake Bluff, IL, USA) mounted on Isomet 1000 metallographic cutter (Buehler, Lake Bluff, IL, USA) at a speed of 250 rpm and under water-cooling.

Initially, the specimens were cut into four sections incisocervically to obtain three “slices” per tooth. Next, they were mesiodistally resected to produce five more slices. Four slice from the central region of the restoration were analyzed, after eliminating the toothpicks from the extremities. The cross-sectional area was approximately 1.0 × 1.0 mm (+/- 0.2 mm), measured by a digital caliper (Mitutoyo Corporation, Tokyo, Japan) with an accuracy of 0.001 mm.

In each group, half of the sticks were subjected to the microtensile bond strength test, and the other half were subjected to 10,000 thermal cycles performed in a thermal cycling machine (Model 521-4D - New Ethics Ind., Com and Serv Ltda, Vargem Grande Paulista - SP, Brazil) and alternated between 5 °C and 55 °C water baths with a dwell time of 30 seconds.

2.5 Bond strength evaluation using the microtensile test

For microtensile bond strength test, the sticks were mounted on metallic stubs and fixed with cyanocrylate-based adhesive (Super Bonder Gel, Henkel Loctite Adhesives Ltda., Itapevi, SP, Brazil), positioning them in channels so that the adhesive interfaces were perpendicular to the direction of applied force. The Odeme Microtensile OM 100 test machine (Odeme Dental Research, Lucerne, SC, Brazil) was used at a speed of 0.7 mm/min until the sample fractured. The load required to break each toothpick was measured in Newtons and divided by the cross-sectional area of the toothpick in mm2 to calculate the bond strength in MPa.

Toothpicks that showed premature failures during any stage before the test were included in the means of the respective groups and given a value of zero.

The fractured surfaces of the specimens were examined using a stereoscopic magnifying glass at magnifications of 40x (Carl Zeiss, Oberkochen, Germany) to identify the fracture modes as follows: (A) cohesive dentin fracture, (B) adhesive interface fracture, (C) cohesive fracture in RMGI, (D) mixed fracture.

Representative specimens of fracture patterns were selected and observed under the EVO LS-15 scanning electron microscope (Carl Zeiss, Oberkochen, Germany) at magnifications of 1000×.

2.6 Statistical analysis

Kruskal-Wallis Test and Dunn’s post-test (p <0.05) were used to compare the differences in tensile bond strength values among the groups and the time comparison was performed using Wilcoxon test (p <0.05) as a non-parametric method.

3. Results

Table 2 shows that in the immediate analysis, all forms of contamination adversely influenced the bond strength of the ionomeric material to the dentin. Blood and saliva contamination resulted in significant drops in bond strength (both values were similar), while contamination with the hemostatic agent resulted in intermediate values. After thermocycling, the group contaminated with the hemostatic agent showed similar results to the control group, while the application of other contaminants produced the lowest bond strength values, which were similar. In a comparison of the analysis time between the groups, the SS and SaS groups only showed a reduction in bond strength after thermocycling.

When analyzing the comparison between the times, only the SS and SaS groups showed a reduction in bond strength
after thermocycling.

**Table 2.** Mean and standard deviation of the bond strength (MPa) results of the experimental groups that suffered contamination by saliva, blood and hemostatic agent before and after thermocycling.

<table>
<thead>
<tr>
<th>Acronyms</th>
<th>Contaminants</th>
<th>Bond Strength - Before Thermocycling</th>
<th>Bond Strength - After Thermocycling</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>Control</td>
<td>4.88 (3.17) A a</td>
<td>3.27 (1.44) A b</td>
</tr>
<tr>
<td>SaS</td>
<td>Saliva</td>
<td>1.87 (1.35) BC a</td>
<td>0.29 (0.43) B b</td>
</tr>
<tr>
<td>SgS</td>
<td>Blood</td>
<td>0.69 (1.08) C a</td>
<td>0.30 (0.57) B a</td>
</tr>
<tr>
<td>HS</td>
<td>Hemostatic</td>
<td>2.47 (1.79) B a</td>
<td>2.13 (1.41) A a</td>
</tr>
</tbody>
</table>

* Uppercase letters compare groups at a given time, lowercase letters compare different times in the same group. Source: Authors.

An analysis of the effect of cleaning treatments on saliva-contaminated dentin is shown in Table 3. The immediate analysis revealed that the group that received no cleaning (SaS) and the group that received the air/water spray treatment (SaA) showed the lowest bond strength values, which were similar. In contrast, the values obtained after substrate reconditioning (SaP) were similar to the control group (SS), confirming that this treatment was most suitable in salivary contamination of the field. After thermocycling, the SaS and SaA groups showed the lowest bond strength values, the statistical difference was significant between the groups while the SaP group values were similar to those of the control group.

Comparing the performance of the groups at different times of analysis, it was observed that the SS, SaS, and SaA groups showed significant reduction in bond strength values after thermocycling.

**Table 3.** Mean and standard deviation of the bond strength results (MPa) of the experimental groups that suffered saliva contamination and different cleaning methods before and after thermocycling.

<table>
<thead>
<tr>
<th>Acronyms</th>
<th>Contaminants</th>
<th>Bond Strength - Before Thermocycling</th>
<th>Bond Strength - After Thermocycling</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>Control</td>
<td>4.88 (3.17) A a</td>
<td>3.27 (1.44) A b</td>
</tr>
<tr>
<td>SaS</td>
<td>Saliva</td>
<td>1.87 (1.35) C a</td>
<td>0.29 (0.43) B b</td>
</tr>
<tr>
<td>SaA</td>
<td>Blood</td>
<td>2.89 (1.65) BC a</td>
<td>1.40 (1.11) B b</td>
</tr>
<tr>
<td>SaP</td>
<td>Hemostatic</td>
<td>4.62 (2.12) AB a</td>
<td>3.95 (1.03) A a</td>
</tr>
</tbody>
</table>

* Uppercase letters compare groups at a given time, lowercase letters compare different times in the same group. Source: Authors.

Table 4 shows that in the immediate analysis the proposed treatments were not effective in cleaning the substrate. It should be noted that the group that received no cleaning treatment (SgS) presented the lowest bond strength values before and after thermocycling. After thermocycling, the groups that received cleaning treatment (air / water spray and reconditioning) presented similar results to the control group and only the control group presented a significant reduction at this moment of analysis.
Table 4. Mean and standard deviation of the bond strength (MPa) results of the experimental groups that suffered blood contamination and different cleaning methods before and after thermocycling.

<table>
<thead>
<tr>
<th>Acronyms</th>
<th>Contaminants</th>
<th>Bond Strength - Before Thermocycling</th>
<th>Bond Strength - After Thermocycling</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>Control</td>
<td>4.88 (3.17)A a</td>
<td>3.27 (1.44) A b</td>
</tr>
<tr>
<td>SgS</td>
<td>Saliva</td>
<td>0.69 (1.08) C a</td>
<td>0.30 (0.57) B a</td>
</tr>
<tr>
<td>SgA</td>
<td>Blood</td>
<td>2.60 (1.86) B a</td>
<td>1.82 (0.86) A a</td>
</tr>
<tr>
<td>SgP</td>
<td>Hemostatic</td>
<td>2.87 (1.45) B a</td>
<td>2.79 (1.73) A a</td>
</tr>
</tbody>
</table>

* Uppercase letters compare groups at a given time, lowercase letters compare different times in the same group. Source: Authors.

When analyzing or performing the performance of different cleaning procedures against hemostatic-contaminated dentin, the control groups (SS) were compared with the groups identified with HS (uncleaned), HA (air / water spray) and HP (reconditioned).

Table 5 shows the acid-contaminated hemostatic agent (HP) group was detected in the control group (SS), showed no difference when compared to the non-decontaminated group (HS) and that caused by cleaning with air and water jet (HA). In the second moment of analysis, all groups were similar to each other. In the comparison between the observed analysis times, only the control group shows statistical difference, with reduction of their recovered values. Few premature failures were observed after thermocycling when used by air / water spray.

An analysis of mean the types of failures found before and after thermocycling revealed that all groups showed a higher number of adhesive and mixed failures (Graphic 1).
4. Discussion

The mechanism of adhesion of ionomeric materials to the dental structure is based on a dynamic process of ion exchange (From et al., 2004). His mechanism involves carboxylic groups that substitute substrate phosphate ions for the establishment of ionic bonds with calcium ions derived from partially dissolved apatite crystallites (Kiri et al., 2015). Thus, the greater the amount of mineral available, the greater the chemical bond tends to be (Cattani-Lorente, et al, 1999). Therefore, the need for close contact of the dental surface with the restorative material is clear.

For this purpose, it has been recommended that the surface to be restored should be conditioned with polyacrylic acid (Eiriksson et al, 2004), aiming at optimizing adhesion values (Tyas & Burrow, 2004). The presence of HEMA in the RMGI, which may favor the establishment of an ionomeric hybrid layer. In addition to chelation, the adhesion of the material is enhanced by the infiltration and polymerization of resin monomers in acid-etched dentin.

Chemical bonding to dentin, a low modulus of elasticity, and favorable clinical response in patients with dentin sensitivity (Francisconi et al., 2009; Pucci et al, 2016) make RMGI a viable treatment option for cervical lesions. However, cervical cavities are often contaminated by oral fluids. Several clinicians have reported the contamination of conditioned dentin with saliva, blood, or hemostatic agents. Our results corroborated with previous studies (Bertoz et al., 2013; Brauchili et al., 2010; Santana et al., 2008), indicating that surface contamination negatively influenced the adhesion of ionomers to dentin. Therefore, the first null hypothesis of the study was rejected.

Although saliva is 99% water, it also contains polysaccharides, proteins, and enzymes, which can create an organic layer on the dentin and prevent direct contact with the restorative material (Pucci et al., 2016; Safar et al 2016). Moreover, the presence of excess moisture can significantly affect RMGI Vickers Hardness and Flexural Strength and influence the bond strength test results (Cattani-Lorente et al, 1999). However, Shimazu et al. concluded that contamination with artificial saliva did not negatively influence surface adhesion, contrary to the results obtained in the present study.

Similarly, the presence of blood reduced the bond strength of the ionomeric material. Studies have (Brauchili et al., 2010; Eiriksson et al., 2004) suggested that the decrease in bond strength may be associated with the deposition of organic residue that prevents chemical bonding and micromechanical adhesion of the restorative material to dentin. According to
Eiriksson and colleagues (Eiriksson et al., 2004; Schwendicke, et al., 2016) blood formed a thin film of organic matter on the tooth that prevented contact with the restorative material.

Hemostatic agents control bleeding by coagulating the proteins present in the dentinal fluid. Pucci and colleagues observed that the application of hemostatic agents had a detrimental effect on hybrid layer formation and compromised the adhesion of the restorative material to the tooth.

The efficacy of the different cleaning methods was evaluated to reverse the effects of dentin contamination. When saliva and hemostatic agent-contaminated groups were subjected to cleaning methods, it was observed that only the group that underwent reconditioning showed results that were similar to the control group, indicating that this was the sole method to restore the bond strength of the material. This finding can be explained by the action of polyacrylic acid, which removes organic residues from the contaminated dentin, re-exposes intertubular dentin, and leaves hydroxyapatite to interact with the ionomer (From Munck et al, 2004; Perdigão et al, 2012).

When the blood-contaminated groups were analyzed, it was observed that none of the surface cleaning methods were able to reestablish the bond strength values. Therefore, the dental professional must exercise extreme caution in blood-contaminated dentin and use intensive cleaning methods such as blasting or prophylaxis, followed by reconditioning or repair (if needed), to promote adhesion at the interface.

In this context, Brauchli et al., 2010, rinsed dentin contaminated with saliva and blood with water and observed significantly lower bond strength values when compared with the control group. They proposed that the clinician must make an extra effort to avoid the contamination of dentin with blood as it has a detrimental effect on the material’s bond strength. Thus, the second null hypothesis was rejected.

Following the thermal cycling process, we observed a significant reduction in bond strength values, except in the saliva and hemostatic-contaminated groups with reconditioning, which showed statistical similarity. The findings were consistent with previous studies. Some studies have affirmed that thermally induced stresses produce volumetric changes in the restorative material and compromise the integrity of the tooth restoration interface. This behavior was possibly enhanced by the action of metalloproteinases present in saliva and dentin, which further weakened the bond in saliva-contaminated specimens over time (Tay et al., 2005). Thus, the third null hypothesis was rejected.

Emphasis should be given to the groups that received substrate reconditioning, which influenced initial bond strength. Polyanions such as polyacrylic acid, bind to dentin collagen matrices and endogenous proteases (Hashimoto et al., 2000), allow the calcium and phosphate ions in the dentin to react with acid monomers of the ionomer, and contribute to a durable bond at the restoration/dentin interface. This finding may also be associated with the ability of the hemostatic agent to remove smear layer due to its acidic nature, which may have positively influenced the chemical and micromechanical bond (Kuphasuk et al, 2007). Thus, a third hypothesis was also rejected.

5. Conclusion

Based on the results of the study, it is concluded that the contaminants tested impaired the immediate adhesion of the ionomeric material to the dentin. The reconditioning of the substrate was effective for contamination with saliva and hemostatic agent. However, cleaning agents did not restore the bond strength values of blood-contaminated samples. Thus, new studies are suggested that include new materials and contaminants for routine clinical use.
Acknowledgments

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References


