Effect of Dentin Contamination with Hemostatic Agents and Cleaning Techniques on Bonding with Self-Adhesive Resin Cement

Background: Dentin contamination with hemostatic agents before bonding indirect restorations negatively affects the bond strength. However, the consensus on which materials could be used to clean contamination of hemostatic agents has not been explored. The aim of this study was to assess the effect of Katana Cleaner applied on the surface of dentin contaminated with hemostatic agents on the shear bond strength (SBS) of self-adhesive resin cement by comparing it with three other surface cleaners.

Material/Methods: Ninety dentin specimens were divided into a no contamination group (control) (n=10), 4 groups contaminated with 25% aluminum chloride (Viscostat Clear) (n=40), and 4 groups contaminated with 20% ferric sulfate (Viscostat) (n=40). Subsequently, 4 different cleaners were used for each contamination group (water rinse, phosphoric acid, chlorhexidine, and Katana Cleaner). Then, self-adhesive resin cement was directly bonded to the treated surfaces. All specimens were subjected to 5000 thermal cycles of artificial aging. The shear bond strength was measured using a universal testing machine.

Results: Two-way analysis of variance showed that the contaminant type as the main factor was statistically non-significant (p=0.655), cleaner type as the main factor was highly significant (p<0.001), and interaction between the contaminant and cleaner was non-significant (p=0.51). The cleaner type was the main factor influencing the bond strength. Phosphoric acid and chlorhexidine showed better performance than Katana Cleaner.

Conclusions: Cleaning dentin surface contamination with phosphoric acid and chlorhexidine had better performance than with Katana Cleaner.

Keywords: Adhesive cement • Composite Resins • Dentin-Bonding Agents • Polymers • Prosthesis Failure • Shear Strength

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Introduction

The adhesive interface between restorative materials and tooth structure plays a pivotal role in the long-term success and longevity of dental restorations [1]. Robust and durable bonding is crucial for both direct and indirect restorations (cementation), ensuring secure margins and preventing microleakage [2]. Despite significant advancements in adhesive dentistry, optimizing bond strength remains a key concern for clinicians and researchers [3].

Among the various luting agents, resin cements offer numerous advantages due to their exceptional bonding characteristics [5]. They exhibit strong bonding to enamel, dentin, and existing restorations, have superior mechanical properties and minimal solubility [6]. However, due to the increased demand for simplified clinical procedures, self-adhesive resin cements have become increasingly popular in dental practice [7-9].

Having a robust, impermeable, and well-defined connection of the restoration with the tooth structure offers numerous benefits [7]. A tightly sealed interface hinders microleakage of bacteria and food debris, effectively preventing secondary caries formation [10]. Several factors influence the efficacy of bonding, including surface cleanliness, wettability, roughness, viscosity, and proper adhesive activation [11]. Achieving optimal adhesion between dentin and resin cement presents a unique challenge due to the complex composition of dentin and the presence of contaminants and moisture [7-9].

Exposure to contaminants during clinical procedures is inevitable, impacting the tooth-restoration interface [12]. These contaminants, present in saliva, blood, gingival crevicular fluid, and hemostatic agents, can significantly compromise bond strength [12]. Blood macromolecules such as fibrinogen and platelets deposited on dentin surfaces have been shown to weaken the resin–cement bond, potentially leading to microleakage and secondary caries [13]. Hemostatic agents like ViscoStat and ViscoStat Clear have been found to negatively affect bond strength [14,15]. Their mechanism of action, involving platelet aggregation and clot formation, coupled with active ingredients such as ferric sulfate and aluminum chloride, can lead to dentin contamination, smear layer disruption, deminerализation, and degradation of composite and self-adhesive resin cements [16-20].

Achieving durable bond strength necessitates effective bleeding control and thorough cleaning protocols. Previous decontamination procedures have employed diverse techniques, with inconsistent outcomes [21,22]. Mechanical cleaning methods typically involve rotary instruments with pumice or ultrasonic scalers, while sandblasting with aluminum oxide is another approach [23]. Chlorhexidine, sodium hypochlorite, hydrogen peroxide, polyacrylic acid, phosphoric acid, and methacryloyloxydecyl dihydrogen phosphate (MDP) are widely used chemical cleaning agents [24].

Chlorhexidine remains the most prevalent oral antimicrobial agent and is the criterion standard for oral disinfectants. Studies have shown that 2% chlorhexidine is the preferred concentration in clinical and research settings [27-29]. While some studies suggest that 2% chlorhexidine can restore bond strength after hemostatic agent application, others report an undesirable decrease [26,28]. Similarly, phosphoric acid, which is commonly used for surface cleaning, has limited research-backed evidence. Notably, it can enhance chemical interactions between dentin and restorative materials, and restore self-adhesive resin cement bonding after contamination with aluminum chloride or ferric sulfate [29].

Recently, KatanaTM Cleaner (Kuraray Noritake Dental, Inc., Okayama, Japan), a novel MDP salt cleaner, has emerged as a potential cleaning agent. This mild (pH 4.5) cleaner, composed of MDP (a phosphate monomer) and an alkaline compound, demonstrates efficacy in cleaning intraoral and extraoral tooth structures and restorations [24,30-33]. Numerous studies have highlighted its effectiveness on zirconia surfaces [30-34]. The formation and strength of chemical bonds at the interface have been extensively studied in prior research. Contact angle measurements and time-of-flight secondary ion mass spectrometry (ToF-SIMS) have elucidated the nature of intermolecular interactions, providing insights into specific bonding mechanisms [35]. Additionally, Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) have been used to characterize the functional groups and chemical composition at the interface, further supporting the evidence for covalent or ionic bonding [33,36]. However, its impact on the shear bond strength (SBS) of resin cements to dentin remains unexplored.

Despite evidence suggesting Katana Cleaner’s effectiveness in cleaning zirconia surfaces contaminated with saliva and blood [24,30-33], its influence on self-adhesive resin cement SBS to dentin contaminated with hemostatic agents remains unknown. Therefore, this study aims to evaluate the SBS of self-adhesive resin cement applied after cleaning dentin contaminated with hemostatic agents using 4 different methods: water rinse, phosphoric acid, chlorhexidine, and Katana Cleaner. The null hypothesis was that there would be no significant difference in SBS after employing these cleaning agents. Conversely, the alternative hypothesis was that different cleaning methods would significantly impact SBS.

This study aimed to address a critical gap in knowledge regarding the efficacy of Katana Cleaner in optimizing resin cement bonding to dentin contaminated with hemostatic agents.
obtained results may provide valuable insights for clinicians seeking to improve the long-term success and clinical performance of indirect restorations.

**Material and Methods**

**Sample Size Calculation**

G*Power 3.1.9.7 statistical software was used to calculate the required sample size. Effect size $f$ was set to 0.4 at significance level of $\alpha=0.05$ and power of 0.8, with 9 test groups. The computed total sample size was 64 specimens, which requires 7 specimens/group. We used 10 specimens/group to overcome any specimen preparation errors or unexpected failures. Using the same parameters (effect size, significance level, and number of test groups) with a sample size of 90 specimens, the computed power of the study was 0.92.

**Ethics Approval**

Ninety sound maxillary and mandibular premolars were collected for 3 months from oral surgery clinics (Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia), and were extracted for orthodontic purposes, cleaned with ultrasonic scaler, and stored in distilled water. The study was approved by the Ethics Committee of the Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia (protocol number 125-10-22, approval date 11/10/2022).

**Specimens Preparation**

The root of each tooth was sectioned 1 mm below the cemento-enamel junction using a low-speed diamond saw (Allied Techcut Low-Speed Diamond Saw, Rancho Dominguez, CA USA). The crown parts were then embedded in chemically polymerized acrylic resin (Idofofast, Unidesa Odi, Madrid, Spain) for handling purposes during testing. The occlusal surface of each crown was cut at 1.5 mm from the cusp tip, then polished using silicon carbide paper (200, 400, and 600 grit) at 250 RPM) under water coolant to create enamel-free dentin surfaces (MetaServ 250 Grinder-Polisher, Buehler, IL, USA). Then, all the specimens were cleaned in distilled water using an ultrasonic cleaner for 10 min.

**Study Design**

The specimens were randomly divided into 9 groups using simple randomization, with 10 specimens per group. In the control group (group 1), the dentin was not contaminated with hemostatic agents and no cleaning techniques were implied. Groups 2, 3, 4, and 5 were contaminated with 25% aluminum chloride for 1 min (ViscoStat Clear, Ultradent, UT, USA) and cleaned by employing different cleaning techniques. Group 2 was cleaned by rinsing with water for 5 s and then dried. Group 3 was cleaned by rinsing with water, treated with 37% phosphoric acid etchant (FineEtch 37, Spident, Incheon, South Korea) for 15 s, then rinsed with water for 5 s, and air-dried to have a moist surface. Group 4 was cleaned by rinsing with water, treated with 2% chlorhexidine (Consepsis, Ultradent, UT, USA) using a micro-brush with rubbing motion for 1 min, rinsed with water, and then dried. Group 5 was cleaned by rinsing with water and treated with Katana Cleaner (Kuraray Noritake Dental Inc., Okayama, Japan), rubbed using a micro-brush for 10 s, then rinsed with water, and dried. Lastly, groups 6, 7, 8, and 9 were contaminated with 20% ferric sulfate for 1 min (ViscoStat, Ultradent, UT, USA) and then cleaned by rinsing with water for 5 s, 37% phosphoric acid etchant, 2% chlorhexidine, and Katana Cleaner, respectively, following the cleaning protocols of previous groups (Figure 1).

**Figure 1.** Flowchart summarizing the study.
Bonding Cement to Dentin

All specimens were dried and prepared for the application of a self-adhesive resin cement (RelyX™ U200 Automix, 3M ESPE, Seefeld, Germany). A standardized spilt putty mold was fabricated and seated on top of the occlusal part with a cylindrical opening in the middle to produce a cylindrical object with a 3 mm diameter and 4 mm height. The mold was firmly secured with a rubber band to avoid the flow of the resin cement out of the intended area. The self-adhesive resin cement was injected into the mold and light-cured for 40 s using an E-Morlite curing light (Apoza, New Taipei, Taiwan), with 1200 mW/cm² power. After that, the mold was disassembled, and each specimen was carefully inspected with 5× magnification loupes, and any excess cement was removed using a sharp scalpel.

Thermocycling and Shear Bond Strength Test

All specimens were subjected to 5000 thermal cycles at 5-55°C (Thermocycler THE-1100, Mechatronik, Pleidelsheim, Germany) to stress the bonding interface and simulate aging. Each thermocycling cycle required 1 min to be completed. This protocol corresponds to 6 months of intraoral simulation [37]. The SBS was measured using a universal testing machine (INSTRON, Norwood, MA, USA). The load was applied to the interface of the dentin-resin cement at a crosshead speed of 1 mm/min until failure (Figure 2).

Statistical Analysis

Statistical analyses were performed using SPSS, version 20 (IBM Corp, NY, USA). One-way analysis of variance (ANOVA) was used to compare the mean SBS of all cleaning groups, including the control group, for each of the 2 contaminant types. If there was a significant difference, the Bonferroni method was applied to perform pairwise comparison between each of the 2 cleaning groups. Each group was compared with the control group by conducting Dunnett’s t tests. Two-way ANOVA was used to study the effects of the 2 main factors (contaminant type and cleaner type) and their interactions on the SBS. Results were considered significant at P value <0.05. Two-tailed tests were performed for all statistical analyses.
Out of the 90 dentin specimens, 69 survived thermocycling, which was still more than the minimum sample size required (64 specimens) to achieve a statistical power of 0.8. A normality test (Shapiro-Wilk test) was performed to test the normality hypothesis of all quantitative variables, which were described as the mean, standard deviation, range, standard error of mean, and 95% confidence interval for the mean (Table 1, Figure 3). All variables were normally distributed, enabling the use of parametric tests. For 25% aluminum chloride, one-way ANOVA showed no statistically significant difference between the cleaning groups (P=0.09747). However, for 20% ferric sulfate, significant differences were observed among cleaning groups (P=0.002). Bonferroni’s method showed that chlorhexidine and phosphoric acid performed better than water (P=0.004 and P=0.043, respectively) and no significant difference was present between the other groups (P>0.05). Furthermore, each group was compared with the control by using Dunnett’s t test, and chlorhexidine was significantly different from the control group (P=0.013) (Table 2). No significant differences existed among the control group, phosphoric acid, Katana Cleaner, and water (P=0.119, P=0.961, and P=0.738, respectively).

The two-way ANOVA showed that the contaminant type as the main factor was statistically non-significant (P=0.655), cleaning agent type as the main factor was statistically highly significant (P<0.001), and interaction between contaminant and cleaner was statistically non-significant (P=0.51) (Table 3). Failure mode was evaluated using a stereomicroscope, and adhesive failure occurred in all the groups (Figure 4). Out of the 69 specimens, only 6 showed mixed failures (Table 1).

### Table 1. Descriptive statistics of the shear bond strength (MPa) of resin cement to dentin.

<table>
<thead>
<tr>
<th>Contaminants</th>
<th>ViscoStat Clear</th>
<th>ViscoStat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleaning agents</td>
<td>Control</td>
<td>Water Rinse</td>
</tr>
<tr>
<td>Number of specimen survived after thermocycling</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Mean (MPa)</td>
<td>11.46</td>
<td>10.90</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.62</td>
<td>2.94</td>
</tr>
<tr>
<td>Standard error of mean</td>
<td>1.63</td>
<td>0.98</td>
</tr>
<tr>
<td>95% CI lower bound</td>
<td>7.60</td>
<td>8.64</td>
</tr>
<tr>
<td>95% CI upper bound</td>
<td>15.32</td>
<td>13.16</td>
</tr>
<tr>
<td>Failure mode</td>
<td>Adhesive</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>2</td>
</tr>
</tbody>
</table>

### Figure 3. Comparison between the 2 contaminants’ mean values of shear bond strength.
Table 2. Multiple comparisons performed for 20% ferric sulfate groups.

<table>
<thead>
<tr>
<th>Bonferroni method (Compare between the groups)</th>
<th>Mean difference</th>
<th>Std. error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-7.58</td>
<td>2.47</td>
<td>0.043*</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>-10.12</td>
<td>2.55</td>
<td>0.004*</td>
</tr>
<tr>
<td>Chlorohexidine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>-3.68</td>
<td>2.55</td>
<td>1</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>-2.55</td>
<td>2.47</td>
<td>1</td>
</tr>
<tr>
<td>Chlorohexidine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>3.89</td>
<td>2.47</td>
<td>1</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorohexidine</td>
<td>6.44</td>
<td>2.55</td>
<td>0.16657</td>
</tr>
<tr>
<td>Katana Cleaner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>4.43</td>
<td>2.55</td>
<td>0.013*</td>
</tr>
<tr>
<td>Chlorohexidine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at P<0.05.

Table 3. Two-way ANOVA.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III sum of squares</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination</td>
<td>6.078</td>
<td>1</td>
<td>6.078</td>
<td>.202</td>
<td>0.635</td>
</tr>
<tr>
<td>Cleaning</td>
<td>624.254</td>
<td>3</td>
<td>208.085</td>
<td>6.904</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Contamination * cleaning</td>
<td>70.492</td>
<td>3</td>
<td>23.497</td>
<td>.780</td>
<td>0.51</td>
</tr>
<tr>
<td>Error</td>
<td>1597.467</td>
<td>53</td>
<td>30.141</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>14404.308</td>
<td>61</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Corrected total</td>
<td>2275.206</td>
<td>60</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Significant at P<0.05.

Figure 4. Failure mode evaluated under a light stereomicroscope at 20× magnification. (A) adhesive failure mode presentation. (B) Mixed failure mode presentation.
Discussion

The null hypothesis for the study was that there would be no significant difference in shear bond strength of self-adhesive resin cement to dentin contaminated with hemostatic agents after using different cleaning agents and it was rejected. We simulated clinical scenarios faced daily by clinicians (contamination of dentin with blood clotting agent) using the most widely available techniques to clean the contamination and tested their effectiveness in maintaining bond strength without contamination (control group). Hemostatic agents such as AlCl₃ and Fe₂(SO₄)₃ cause smear layer disruption, dentin demineralization, and diminution of the SBS of resin cements [15,20,38].

The variations in results can be attributed to the sectional characteristics and orientation of the dentinal tubules, which can alter the adequacy of the dentinal adhesives, as dentin is a biological, complex, and dynamic tissue made of crystals of apatite, water, and organic substances in the ratio of 47: 20: 33 by volume, respectively [41]. Dentinal tubules constitute 22% of the inner dentin (45,000-65,000 mm²) and 1% of the outer dentin (15,000-20,000 mm²), and have different diameters depending on their location: the bigger ones (3-4 μm) are near the pulp and the smaller ones (1.7 μm) are closer to the dentin-enamel junction [41,42].

The contact between dentin and resin cements relies on the adhesive penetrating the collagen after acid etching. The dissemination area between the resin cement and dentin representing the micromechanical bond of the restoration is known as the “hybrid layer” [43]. Collagen is a protein formed of glycine, lysine, and proline amino acids together with hydroxyproline and hydroxylysine. It is very stable and resistant to degradation because of its triple-helix structure. Hence, the extent of collagen crosslinking affects the mechanical and biochemical properties of the dentin [44].

In this study, the SBS of the dentin sample after cleaning with water only was significantly lower than that of the dentin samples in the other groups because the hemostatic residue could not be completely removed before the application of the adhesive system. These results confirmed the observations of different researchers that cleaning with only water leads to improper resumption of the SBS on the dentin [26,40].

Phosphoric acid removes the smear layer, thus dissolving the crystalline apatite in collagen, creating a leeway for the adhesive to glide between the collagen fibrils, and opening channels for resin infiltration [45]. Studies have shown that for both total-etch and self-etch adhesives, the SBS of the contaminated dentin surface is re-established by performing etching with phosphoric acid [40]. Other studies have reported lower SBS with phosphoric acid [12]. Because of its acidity (pH=−0.7), 37% phosphoric acid induces limited denaturation of the uncovered matrix metalloproteinases (MMPs) [46]. Activation of dentin MMPs can lead to collagenolytic activity within the hybridized dentin [45]. This could explain why phosphoric acid has lower bond strength than chlorhexidine. There are 23 known human strains of MMPs, and their activity is regulated by calcium and zinc [47].

MMPs are involved in several pathological dental conditions, such as periodontitis and caries. Recently, investigators have linked the degradation of the collagen matrix to the development of caries, in association with the SBS of the dentin [48]. The significantly higher SBS achieved by chlorhexidine in this study could be related to its ability to act as an MMP inhibitor. Chlorhexidine alters the three-dimensional structures of MMPs and depletes their metal ions (Ca²⁺ and Zn²⁺), which are necessary for their function [49].

In both mineralized and demineralized dentin, anionic molecules attach to cationic molecules in chlorhexidine. The amphiphilic chlorine molecule in chlorhexidine hinders the catalytic domain of MMP by binding to the zinc of the catalytic field. The reversible electrostatic bond between chlorhexidine and dentin depends on time and use of chlorhexidine in treated dentin. Several investigations have confirmed the prevention or deceleration of collagen fiber degradation after the use of chlorhexidine on the dentin surface, with no effect on SBS [50].

The MDP-containing Katana Cleaner showed a higher bond strength on contaminated zirconia [30,32]. In addition, the surface-active MDP salt in Katana Cleaner had a better decontamination effect on the computer-aided design and computer-aided manufacturing (CAD/CAM) resin blocks after contamination with saliva protein [51]. Therefore, in this study, we hypothesized that Katana Cleaner would have the same effect on contaminated dentin surfaces as on contaminated zirconia and CAD/CAM resin blocks. Because Katana Cleaner has free MDP salt and MDP salt of triethanolamine with a pH of 4.5, it is assumed to effectively eliminate saliva and/or blood contamination without removing hydroxyapatite from the tooth. Therefore, when Katana Cleaner is applied to the surfaces of the dentin and restorative material, the hydrophobic band of the MDP salt attaches to the contagion, decreasing its surface tension, and making it crumble [52].

Bonding strength to dentin after use of Katana Cleaner was lower than that in the chlorhexidine group. This result could be attributed to many factors and variations, mainly the activation of MMPs caused by acidity, which leads to degradation of collagen in the hybrid layer, which is a delicate coating of a resin-dentin mix embracing the resin and dentin molecules, protecting the bond from percolation and conferring strong acid protection. In addition, variations in the results as
a consequence of the degradation of the hybrid layer can occur owing to the presence of water in the adhesive interface, inadequate polymerization of the adhesive, incomplete infiltration of the adhesive, and failure to displace collagen-restricted or loose water by resin monomers.

To analyze the mode of failure, all specimens were collected after SBS testing was performed and were examined under a 100× magnification light microscope. The most common mode of failure in all groups was adhesive failure. Cohesive failure was not expected because of the low SBS results (Table 1).

This study has several limitations. One limitation is the use of a single type of resin cement (self-adhesive resin cement), as results may differ for different resin cement systems. Another limitation is that the dentin surface was the only bonding surface used, but contamination may also influence the enamel. In addition, we did not examine the surface of the dentin using a scanning electron microscope with high magnification to observe the influence of each surface treatment on the dentin surface, which would help understand the dentin’s reaction. Thus, further investigations are required to determine the best management after contaminating any bonding surface with hemostatic agents from different perspectives.

Conclusions

The following can be concluded from the present in vitro study:

- Dentin contamination with hemostatic agents is inevitable in different clinical scenarios; however, multiple effective methods for maintaining the bond strength are available.
- Phosphoric acid and chlorhexidine showed better performance than Katana Cleaner.

Declaration of figures’ Authenticity

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