



Research Article

**EFFECT OF CONTAMINATION BY TWO DIFFERENT HAEMOSTATIC AGENTS AND USE OF CHLORHEXIDINE CLEANING AGENT ON THE SHEAR BOND STRENGTH OF COMPOSITE TO DENTIN USING A SEVENTH GENERATION BONDING AGENT – AN IN VITRO STUDY**

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Haemostatic agent, aluminium chloride, ferric sulphate, Chlorhexidine, dentin bonding agent, shear bond strength.

**ABSTRACT**

**Aim:** To investigate the effect of 20% aluminium chloride and 20% ferric sulphate as haemostatic agents on shear bond strength of seventh generation bonding agent after the use of 0.2% Chlorhexidine solution as a cleansing agent.

**Materials and Method:** The occlusal enamel of fifty mandibular molars was removed to expose midcoronal dentin and mounted in autopolymerizing resin 1 mm apical to CEJ. Then the specimens were randomly divided into 5 groups (n=10):

**Group 1,** Optibond All in One (Kerr) bonding agent application

**Group 2,** Application of Hemostal+Optibond

**Group 3,** Application of Hemostal+Chlorhexidine rinse+Optibond

**Group 4,** Application of Viscostat+Optibond

**Group 5,** Application of Viscostat+Chlorhexidine rinse+Optibond

For all the groups, a composite resin rod was built up on the occlusal dentin surface and light cured and incubated followed by testing in universal testing machine.

**Results:** All groups showed statistically significant difference compared to Group 1. Significant difference was seen between Group 2 and Group 3 and also between Group 4 and Group 5.

**Conclusion:** Contamination with haemostatic agent decreases the shear bond strength of self etching adhesive to dentin. Chlorhexidine as rinsing agent partially recovers the shear bond of contaminated dentin.

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**INTRODUCTION**

Operative dentistry demands meticulous isolation of the field for adequate visibility, ease of placement and obtaining optimum properties of the restorative materials. However, isolation seems to be challenging in cases with intrasulcular class II or class V cavities where rubber dam isolation may prove to be inadequate to provide a clean and dry field for bonding procedures.

Various chemical agents have been used for control of bleeding before placing restorations and obtaining a clear field such as adrenaline, aluminium sulphate, aluminium chloride, ferric sulphate. Among them, use of aluminium chloride in concentration 5-20% and 20% ferric sulphate are widely used. It is preferred as it has no systemic effects, along with the property of precipitating proteins, cause constriction of blood vessels and also extract fluid from tissues.<sup>1</sup> However, ferric sulphate has an ability to cause discolouration due to its iron content as a disadvantage. However, it has been reported that these haemostatic agents are acidic in nature.

They can remove smear layer and some amount of demineralisation may be caused after their application for longer period of time which may also lead to removal of some peritubular dentin.<sup>2</sup> Their effect as reduced bond strength to dentin has thus been a matter of conflict in restorative dentistry.

Hence, rinsing of this haemostatic agent residue has to be performed prior to applying any adhesive system which may not be completely effective when water is used. Various cleansing agents for the same have been tested before such as ethylene diamine tetraacetic acid, phosphoric acid and chlorhexidine.<sup>3</sup> Chlorhexidine is also tested as cavity disinfectant agent due to its antibacterial property which can reduce the number of residual bacteria after cavity preparation.<sup>4</sup>

The newer generation of bonding agent, the seventh generation, is a self etch system which does not require additional etching with phosphoric acid. Thus it reduces the additional steps of rinsing and drying which may desiccate the dentin.<sup>5,6</sup> It is better than total etch technique, in that the resin penetration occurs throughout the exposed collagen fibrils unlike the total etch technique in which resin is not completely

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penetrated in the demineralised collagen network. Thus it has reduced post operative sensitivity and ease of placement. The present study aimed to investigate the effect of two haemostatic agents, 20% aluminium chloride and 20% ferric sulphate as haemostatic agents on shear bond strength of a seventh generation bonding agent after the use of 0.2% Chlorhexidine solution as a cleansing agent. The null hypotheses tested were: 1) There is no effect of contamination of two haemostatic agents on shear bond strength of composite to dentin using a seventh generation bonding system. 2) There is no effect of 0.2% Chlorhexidine rinse on shear bond strength of composite bonded to dentin contaminated with haemostatic agents using a seventh generation bonding system.

**MATERIALS AND METHOD**

The occlusal enamel of each sixty caries-free mandibular molars was removed using diamond disc and their midcoronal dentin was exposed. The specimens were then mounted in autopolymerizing resin 1mm apical to CEJ. Then the specimens were randomly divided into 5 groups (n=10):

**In group 1**, Optibond All in One (Kerr) was applied as per manufacturer’s instructions and light cured for 10 seconds.

**In group 2**, One drop of Hemostal (H) was applied directly to the dentin surface for 2 minutes, rinsed with distilled water for 30 seconds and air-dried. The next steps were similar to those in group 1.

**In group 3**, One drop of the H was applied directly to the dentin surface for 2 minutes, rinsed with distilled water for 30 seconds and air-dried. After that, rinsing with 0.2% chlorhexidine (CHX) solution was performed with a syringe of 50 mL of solution and dried using absorbent paper. The next steps were similar to those in group 1 (H+CHX+OB).

**In group 4**, One drop of Viscostat (Ultradent) (V) was applied directly to the dentin surface for 2 minutes, rinsed with distilled water for 30 seconds and air-dried. The next steps were similar to those in group 1. (V+OB).

**In group 5**, One drop of the V was applied directly to the dentin surface for 2 minutes, rinsed with distilled water for 30 seconds and air-dried. After that, rinsing with 0.2% chlorhexidine (CHX) solution was performed with a syringe of 50 mL of solution and dried using absorbent paper. The next steps were similar to those in group 1. (V+CHX+OB).

In all the groups, a composite resin (Herculite Precise, Shade A2, Kerr) rod was built up on the dentin surface of each tooth using a split plastic mold with an internal diameter of 4 mm and a height of 2 mm and light-cured with an LED light-curing unit for 20 seconds. Then the specimens were stored in distilled water at 37°C in an incubator for 24 hours. Subsequently, the specimens were tested in shear mode using a chisel-shaped rod of a universal testing machine at a crosshead speed of 1 mm/min. The force at failure was recorded in Newtons (N); then, shear bond strength values were calculated in MPa.

**Statistical analysis**

Mean and standard deviations were calculated for all groups. Data was analyzed in SPSS (24.0 version IBM USA) using two way ANOVA test and multiple comparison was done using Post-hoc Tukey’s test. (p<0.05)

**RESULTS**

Statistically significant difference was seen between Group 1 (control OB) and all other groups(p<0.05). Statistically significant difference was seen between Group 2 (H+OB) and Group 3 (H+CHX+OB) (p<0.05). Statistically significant difference was seen between Group 4 (V+OB) and Group 5 (V+CHX+OB) (p<0.05). On comparison, no significant difference was seen between Group 2(H+OB) and Group 4 (V+OB) as (p>0.05). Whereas Group 3 and Group 5 also did not show any significant difference (p>0.05).

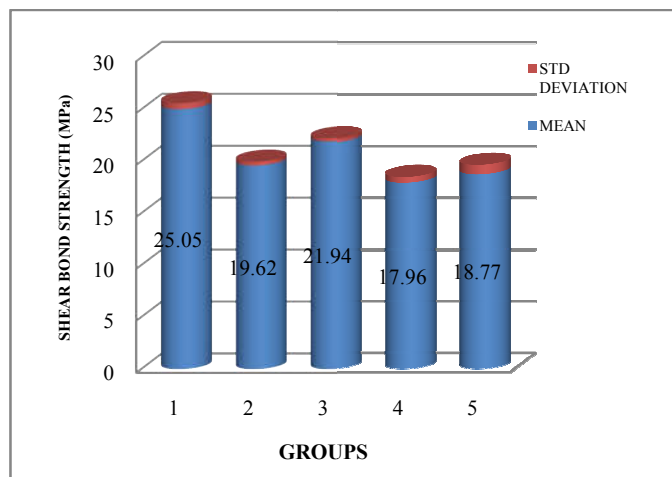
**Table 1** Materials tested in the study.

Material	Commercial brand
20% aluminium chloride	Hemostal (Prevest DenPro, India) in liquid form
20% Ferric sulphate	ViscoStat (Ultradent Product Inc., USA) in syringe form
Self etch adhesive system	Optibond All-In-One(Kerr, USA)
0.2 % Chlorhexidine	Hexidine (ICPA Health Products Ltd, India)

**Table 2** Mean shear bond strength values

GROUPS	Shear bond strength (MPa) MEAN ± STD. DEV
GRP 1 (OB)	25.05± 0.56 <sup>a</sup>
GRP 2 (H+OB)	19.62± 0.36 <sup>b</sup>
GRP 3 (H+CHX+OB)	21.94± 0.30 <sup>c</sup>
GRP 4 (V+OB)	17.96± 0.55 <sup>b</sup>
GRP 5 (V+CHX+OB)	18.77± 0.91 <sup>d</sup>

(different letters indicate statistically significant difference)



**Graph 1** Comparison of shear bond strength of all groups.



**Figure 1** Representative image of prepared specimen for shear bond strength testing



Figure 2 Specimen mounted and shear bond strength testing done on a Universal Testing machine.

## DISCUSSION

As an inherent nature, haemostatic agents have a low pH (0.7-3). They are also hydrophilic in nature which may increase their affinity to dentin. Thus they may affect the quality of hybrid layer formed and affect the bond strengths to dentin. This is evident from the result of the present study which showed that Group 1 (Control) without any contamination with haemostatic agent showed significantly higher bond strength than other groups. Ayo-Yusuf *et al* have stated that haemostatic agents have the ability to dissolve smear layer and obturate the dentinal tubules. They also form an amorphous layer of granular precipitate on the surface.<sup>7</sup> The self etch systems utilize the exposed collagen fibres and modified smear layer for bonding. The removal of smear layer by haemostatic agents and also the dentinal tubules undergoing partial occlusion have been stated by as the reason why bonding in self-etch systems gets affected.<sup>8</sup> Kuphasuk *et al* in a similar study on aluminium chloride has previously stated the reason for this as the residual aluminium remaining on dentin after application of haemostatic agent.<sup>8</sup> This aluminium replaces the calcium in hydroxyapatite in enamel and dentin and inhibits its demineralisation. Thus there was inhibited penetration of adhesive resin into the collagen structure<sup>9</sup> and that affected the shear bond strength of contaminated dentin. The results of our study are similar to Arlsan *et al*,<sup>10</sup> Harnirattisai *et al*,<sup>2</sup> Ajami *et al*,<sup>3</sup> Kuphasuk *et al*<sup>8</sup> and Sharafeddin *et al*<sup>11</sup> who concluded that shear bond of both total etch and self etch adhesives is affected by contamination with haemostatic agents.

It is evident that it is essential to remove all surface contaminants in self-etch system in order to achieve a proper bond. The manufacturer of Viscostat recommends rinsing with water prior to bonding as it is a water soluble gel. Aluminium chloride was also rinsed with water owing to its hydrophilic nature in our study. A study by O'Kefee *et al* showed increased bond strength of dentin contaminated with ferric chloride and aluminium sulphate after rinsing when compared to non rinsed specimens.<sup>12</sup> For further rinsing, 0.2% Chlorhexidine was used which has proved to be beneficial owing to significantly increased bond strength compared between Group 2 and Group 3 and also significant difference was observed between Group 4 and Group 5. Better microtensile bond strength after thermocycling were demonstrated by Chang and Shin in their study with

Chlorhexidine treated groups. In their study on microtensile bond strength, Brechi *et al* reported that irrespective of the concentration of Chlorhexidine used (0.2 vs 2%), both increased the microtensile bond strength values.<sup>13</sup> Similar results have been shown after use of Chlorhexidine in an in vitro study conducted by Carvalho *et al* to check microtensile bond strength of dentin after use of Chlorhexidine.<sup>14</sup> Chlorhexidine has a cationic detergent activity which may have resulted in better rinsing of haemostatic agents.

It is known that degradation of adhesive bond occurs due to elution of resin and alteration of the exposed collagen fibrils.<sup>15</sup> Application of chlorhexidine seemed to partially recover the bond strength due to its property of inhibiting matrix metalloproteinases.<sup>14</sup> Matrix metalloproteinases are enzymes that proteolytic, tissue- destructive and responsible for degradation of various types of collagen.<sup>16</sup> The matrix metalloproteinases enzymes are present inherently in host dentin and saliva and get activated on acid etching. This results in drop in pH and presence of metallic ions like  $Zn^{2+}$  and  $Ca^{2+}$  which cause degradation of exposed collagen which is not penetrated by the resin affecting the bond.<sup>17</sup> Chlorhexidine slows down the degradation of bond interface between resin and dentin and stabilising the bond in various studies.<sup>18</sup> Hebling *et al* states that Chlorhexidine can arrest the self-destruction of collagen.<sup>19</sup>

In the present study, the lowest bond strength values were demonstrated by samples contaminated with ferric sulphate. This may be attributed to difficulty in removal of the agent due to the viscous nature of Viscostat in gel form. Similar result was obtained by Harnirattisai *et al*,<sup>2</sup> the reason stated was because of the weak acidity of primer present in bonding system, complete dissolution of ferric sulphate could not be achieved which rendered penetration of bonding agent in deeper areas impossible.<sup>2</sup>

In the present study we have used shear bond strength as opposed to microtensile bond strength in various studies. Microtensile bond strength is a technique sensitive method and demands perpendicular alignment of the bonded surface in direction loading of tensile force. Otherwise it may develop bending stresses.<sup>20</sup>

The limitation of this study was that clinical conditions like aging and fatigue were not simulated by thermo cycling. The results represent immediate bond strength after storage in distilled water over a short period of one day. Further investigations must be carried out in this direction to consider the effect of these factors.

## CONCLUSION

Within the limitations of this study, we conclude that contamination with haemostatic agent decreases the shear bond strength of self etching adhesive to dentin. Chlorhexidine as rinsing agent partially recovers the shear bond of contaminated dentin.

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