In vitro investigation and evaluation of dentin bonding agents

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Abstract: The development of dentin bonding agents has made the adhesion between composite restorative resins and dentin possible. Laboratory studies involving microleakage and bond strength tests are carried out to predict the longevity of composite resin restorations in the clinical situation. Microleakage at the restoration/tooth interface may result in breakdown at the margins of restorations, the development of secondary caries at the restoration/tooth interface and pulp pathology. The methods to evaluate microleakage and marginal gap formation at the dentin/restoration interface in laboratory studies are reviewed. The effects of polymerization shrinkage, differences in the coefficients of thermal expansion, and restorative techniques on these parameters are discussed. Tensile and/or shear bond strength tests are usually performed to predict the retention of composite restorations clinically. Some of these test methods routinely used in our laboratory are described. The shear bond strengths of dentin bonding agents to dentin are dependent on the test methodology. Both the laboratory microleakage and shear bond strength test vary tremendously. An appeal is made for the standardization of these test methods. Attempts should be made to establish a correlation between in vitro and in vivo evaluations.

Key words: Dentin bonding agents; restorative dentistry.

Adhesion to dentin

Adhesion as a phenomena is defined as the state in which two surfaces are held together by interfacial forces which may consist of primary or secondary chemical forces or mechanical interlocking forces or both. Until recently, none of the dental composite restorative resins adhered to mineralized tooth structure. It was demonstrated, however, that the adhesion of acrylic restorative resins to enamel could be increased significantly by acid-etching the enamel surface with orthophosphoric acid (\(\text{H}_3\text{PO}_4\)) prior to the placement of the restorative resin. Attempts were made to apply the acid-etch technique to dentin. Etching of dentin with \(\text{H}_3\text{PO}_4\) opens the dentinal tubules and the curing resin flows into the opened tubules. Despite the fact that the curing resin flows into the tubules, it was demonstrated that acid-etching of dentin did not enhance the bond strength of composite restorative resins to dentin. Using the vervet monkey as an experimental model, it was shown that the direct application of \(\text{H}_3\text{PO}_4\) elicited a severe pulpal response. Similar findings were reported in a subsequent study in which \(\text{H}_3\text{PO}_4\) was applied to human teeth in an in vivo study. It is therefore distressing that some of our colleagues advocate the removal of the dentin smear layer with \(\text{H}_3\text{PO}_4\).

Concentrated efforts were therefore made to develop dentin bonding agents. The dentin bonding agents are di- or multifunctional molecules which contain reactive groups that interact with dentin and the monomer of the restorative resin. The first generation dentin bonding agents included glycerophosphoric acid dimethacrylate, cyanoacrylates, polyurethanes and the adduct of N-phenyl glycine and glycidylmethacrylate (NPG-GMA). Problems encountered with the hydrolysis of the glycerophosphoric acid dimethacrylate in the oral environment, the difficulty in the bulk polymerization of the cyanoacrylates, and the instability of the NPG-GMA in solution, prevented the successful clinical use of these bonding agents.

The second generation bonding agents include: Scotchbond, Dentin Bonding Agent, Creation Bonding Agent, and Dentin-Adhesit. The tensile bond strengths of these bonding agents were determined recently. The results are presented in Table 1. The low bond strengths suggested that these bonding systems will not be retained in the clinical situation without providing mechanical retention.

The third generation dentin bonding agents include: Gluma (Lumifor), Tenure (Perfection), Scotchbond 2 (Silux), 4-Meta (Superbond), and Phenyl-P (Clearfil). Removal of the dentin smear layer is recommended with all these bonding agents. Gluma: 0.5 M EDTA (pH...
Table 1. Tensile bond strengths (MN.m$^{-2}$) of four dentin bonding restorative systems to dentin.

<table>
<thead>
<tr>
<th>Restorative System</th>
<th>Number of specimens</th>
<th>Number of specimens failed spontaneously</th>
<th>Tensile bond strength -MN.m$^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentin Bonding Agent Adaptic</td>
<td>12</td>
<td>2</td>
<td>3.01 3.38 9.69 0</td>
</tr>
<tr>
<td>Dentin Adhesit Isopast</td>
<td>12</td>
<td>7</td>
<td>0.11 0.14 0.34 0</td>
</tr>
<tr>
<td>Scotchbond P-10</td>
<td>12</td>
<td>8</td>
<td>0.10 0.18 0.56 0</td>
</tr>
<tr>
<td>Creation Bonding Agent Spectra-Bond</td>
<td>12</td>
<td>12</td>
<td>0 0 0 0</td>
</tr>
</tbody>
</table>

* Means linked by a vertical line were not significantly different.


A dentin surface ground wet on 600 grit silicon carbide paper is shown in Fig. 1. The grooves produced by the abrasive particles are clearly visible. The application of the Gluma Cleanser$^9$ effectively removes the smear layer and opens the dentinal tubules (Fig. 2). The application of the Tenure Dentin Conditioner$^6$ to a ground dentin surface also removes the smear layer and exposes the dentinal tubules (Fig. 3). The application of the Scotchprep Dentin Primer$^4$ to a ground dentin surface removes the smear layer but leaves the tubules partially occluded with debris (Fig. 4).
Microleakage

Microleakage is defined as the clinically undetectable passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it. Microleakage at the tooth restoration interface is a major factor which influences the longevity of dental restorations. It is suggested that microleakage may lead to staining at the margins of restorations, breakdown at the marginal areas of the restoration, secondary caries at the tooth restoration interface, and postoperative sensitivity and pulp pathology. The ingress of bacteria at the tooth restoration interface is believed to be responsible for the pulpal reactions.

The physical properties of restorative materials play an important role in determining the extent of microleakage at the restoration/tooth interface. These include:

1. Differences in the coefficients of thermal expansion of the tooth and the restoration.
2. Polymerization shrinkage of the restorative resin.
3. Water sorption of the restorative resin after exposure to the oral environment.

Several investigators have studied the effects of the physical properties of restorative resins and the results are summarized:

1. The more the composite resin shrinks on polymerization, the greater are the tensile stresses developed at the tooth/restoration interface.
2. The major part of the contraction stresses develop within 15 minutes after the initiation of polymerization.
3. Hygroscopic expansion is delayed and does not always compensate for polymerization shrinkage.
4. Polymerization stresses cause flow of the curing resin which partly compensates for polymerization shrinkage.

Several laboratory methods have been developed for the evaluation of microleakage. These include: dyes, radioisotopes, air pressure, neutron activation analysis, bacterial penetration, pH changes, scanning electron microscopy, both direct and indirect techniques. These methods have been reviewed recently.

Microleakage of the Tenure/Perfection restorative system was evaluated in a recent study. The Tenure Dentin Bonding System is based on a system developed by Dr. Raphael Bowen at the Paffenbarger Research Center of the American Dental Association Health Foundation at the National Bureau of Standards. It consists of Tenure Dentin Conditioner, an aqueous solution of aluminum oxalate, Tenure Powder A, a surface active comonomer which is the adduct of N-p-tolyl glycine and glycidyl methacrylate (NTG-GMA), and Tenure Powder B, a coupling agent which is the addition reaction product of pyromellitic dianhydride and 2-hydroxyethylmethacrylate (PMDM). Fresh solutions of NTG-GMA and PMDM were prepared in Tenure Liquid (acetone) according to the manufacturer's instructions. The bonding system was used in conjunction with Perfection light-cured composite resin.

Sixty extracted non-caries human permanent premolars free of defects were used in the study. After extraction, the teeth were cleaned on a dental lathe with flour of pumice and stored in 1% Chloramine solution. CI V cavities were made at the cemento-enamel junctions on the facial surfaces of the teeth with round FG-4 carbide burs with adequate water cooling. The preparations extended into enamel, cementum and dentin with a width of 5 mm mesiodistally, a length of 2 mm occluso-gingivally, and a depth of 1.5 mm. A new bur was used after 10 cavity preparations. At the cementum aspects of the preparations, a 90° cavosurface angle was avoided. The Tenure Dentin Conditioner was applied to dentin with a disposable brush for 60 seconds, rinsed with water for 30 seconds, and dried with oil-free compressed air. Using the round-handled dispenser, one load of Tenure Powder A was transferred to a dappen dish containing three drops of Tenure Liquid. After mixing with a disposable brush, the solution was brushed onto the dentin and allowed to evaporate for 60 seconds. The application was repeated and the solution again allowed to evaporate for 15 seconds. Then one drop of Tenure Liquid was applied to dentin and allowed to dry for 60 seconds. Using the square-handled dispenser, one load of Tenure Powder B was transferred to a dappen dish containing six drops of Tenure Liquid. After mixing with a disposable brush, the solution was applied to dentin and allowed to dry for 60 seconds. The dentin surface was air dried, the solution reapplied, and allowed to dry for 60 seconds. The low viscosity bonding resin, Visar Seal, was then applied to dentin overlapping the adjacent enamel. The resin was spread evenly over the surface and polymerized by exposure to visible light for 30 seconds. The enamel margins were then beveled with a Blue-White Diamond, FG-755 to an approximate 45° cavosurface bevel. The Etchant Gel was applied to the beveled enamel margins for 60 seconds, rinsed with water for 30 seconds, and air dried. Visar Seal was then applied to the entire area to be bonded and spread to a thin layer with compressed air. The Perfection restorative resin was transferred to the preparation with a syringe and held under firm pressure under a cervical matrix or Mylar strip while polymerizing with visible light for 60 seconds. The excess resin was removed with a FR7802 finishing bur. The restored teeth were stored in physiological saline and finished with Sof-Lex discs 15 minutes after curing and 24 hours after curing.

The restored teeth were then prepared for evaluation of microleakage. The root apices were removed with a diamond disc and CI V preparations were made with an inverted cone carbide bur. Two coats of Copalite varnish were then applied to the preparations and the cavities filled with amalgam restorations. The whole tooth except a 1 mm window around the composite restoration was covered with clear nail varnish. The restored teeth were then cycled in a thermocycling machine between 8°C and 50°C in a 0.5% basic fuchsin...
solution for either 250 or 500 cycles with a dwell time of 15 seconds. The nail varnish was removed and the teeth embedded in epoxy resin - 8 parts Epon-828 and 1 part triethylenetetramine. After curing overnight, at least four serial sections were cut through the restorations with an Isomet slow speed saw. Microleakage was evaluated at both the enamel and cementum margins of the restorations. A score of 0 to 4 was assigned to each margin depending on the extent of microleakage:

0 = No leakage.
1 = Leakage extending one third to the deepest point of the restoration.
2 = Leakage extending two thirds to the deepest point of the restoration.
3 = Leakage extending to the deepest point of the restoration.
4 = Leakage extending beyond the deepest point of the restoration.

The number of specimens exhibiting the various degrees of microleakage at the enamel and cementum margins of the Tenure/Perfection restorations are given in Table 2. Fifty-two of the restorations showed no leakage at the enamel aspects. Analysis of the data revealed that microleakage at the cementum aspects of the restorations was significantly greater than at the enamel aspects. Time after finishing and number of temperature cycles had no significantly different effect on microleakage.

The restorative procedures with the Tenure/Perfection restorative procedure took approximately 8 minutes to complete which is clinically unacceptable. The application of the Tenure Dentin Bonding System has now been shortened to make it more acceptable clinically.

Microleakage of the Gluma/Lumifor restorative system was evaluated in a recent study. The Gluma solution of 5% glutaraldehyde and 35% HEMA. The system was used in conjunction with Bayer Etching Gel for 60 seconds. The teeth were washed in running tap water and dried with oil-free compressed air. The Gluma Cleanser was applied to dentin with a cotton pellet using a rubbing action for 30 seconds. The teeth were washed in tap water for 30 seconds and dried with compressed air. The Gluma Dentin Bond was then applied to dentin for 30 seconds and then blown gently to a thin film. The Bayer Resin L was then applied over the Gluma Dentin Bond and the etched enamel and blown gently with compressed air to a thin film. The Lumifor composite was transferred to the preparation with a Mark III syringe and the restoration and finishing done as described for the Tenure/Perfection restorative system.

The teeth were prepared for microleakage evaluation as described previously. The results are presented in Table 3. Fifty-nine of the Gluma/Lumifor restorations showed no microleakage at the enamel margins while 16 showed no leakage at the cementum margins. Analysis of the data revealed that microleakage at the cementum aspects of the restorations was significantly greater than at the enamel aspects. Time after finishing and number of temperature cycles had no significantly different effect on microleakage.

**Marginal gap formation**

The effect of five adhesives on the adaptation of restorative resins in dentin cavities was determined. After extraction, the teeth were cleaned and stored in physiological saline for a maximum period of five weeks. The surfaces of the roots were ground flat and cavities with a 90° cavosurface angle were prepared to dentin. The cavity depth was 1.5 mm and the diameters ranged from 1.8 to 6.5 mm. The five dentin bonding agents Clearfil, Scotchbond, Bowen System, Superbond and Gluma were applied and the cavities were restored with Silux. The restorations were exposed to visible light for 60 seconds and the restored teeth placed in saline.

### Table 2. Microleakage at the enamel and cementum margins of the Tenure/Perfection restorative system.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of specimens</th>
<th>Enamel microleakage</th>
<th>Cementum microleakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Finish 15 min, 250 temp. cycles.</td>
<td>15</td>
<td>12 1 2 0 0</td>
<td>0 3 3 1 8</td>
</tr>
<tr>
<td>B: Finish 15 min, 500 temp. cycles.</td>
<td>15</td>
<td>14 1 0 0 0</td>
<td>0 3 0 4 8</td>
</tr>
<tr>
<td>C: Finish 24 h, 250 temp. cycles.</td>
<td>15</td>
<td>12 2 1 0 0</td>
<td>0 8 1 2 4</td>
</tr>
<tr>
<td>D: Finish 24 h, 500 temp. cycles.</td>
<td>15</td>
<td>14 1 0 0 0</td>
<td>0 1 2 5 7</td>
</tr>
</tbody>
</table>
solution. After 10 minutes, the restored root surfaces were ground wet to remove 0.1 mm of the dentin and the restorations. The surfaces were micro-polished and the largest gaps at the peripheries were measured. The authors reported that the marginal contraction gaps increased with an increase in cavity diameter.

The maximum contraction gaps in cavities with a 4 mm diameter are presented in Table 4. Munksgaard et al. concluded that:

1. For a given adhesive, the width of the contraction gap was found to increase with the diameter of the cavity.
2. The main determinant of the size of the contraction gaps was the ratio of the volume of the restoration to the area of the walls of the cavity; the higher the ratio the larger the contraction gap.

We may conclude that:

1. Increasing the cavity depth does not influence the marginal contraction gap at the tooth/restoration interface.
2. A two-phase application technique with oblique layers reduces the marginal gap by 25%.
3. An increase in the cavosurface angle results in a significant reduction in marginal gap dimensions.
4. Functional articulation has a major influence on marginal adaptation.

Shear bond strength tests
Shear bond strength tests are determined in laboratory studies in an attempt to predict the retention of dentin bonding restorative systems in the clinical situation. Each investigator uses his own test system for determining the shear bond strengths of dentin bonding systems to dentin because no guidelines are available for these tests.

The shear bond strength of the Gluma/Lumifor® restorative system has recently been determined in our laboratory. Freshly extracted human permanent teeth were obtained from the Department of Oral and Maxillofacial Surgery at the University of Alabama School of Dentistry and stored in 1% Chloramine solution immediately after extraction. The test specimens for shear bond strength determination were prepared within 24 hours after extraction. The teeth were embedded with cold-cure acrylic in brass tooth cups and stored in 1% Chloramine. Immediately prior to the preparation of a test specimen, the exposed tooth surface was ground wet on a polishing machine on 600 grit silicon carbide paper to expose the dentin surface. The exposed dentin surface was treated as previously described for the microleakage study. The tooth cup with the embedded tooth was then assembled in a specially constructed device (Fig. 5). A split teflon mold with a circular hole was positioned in the device (Fig. 5A), and the tooth cup (Fig. 5B) elevated to make firm contact with the teflon mold. A thin layer of Bayer Resin L* was brushed onto the exposed surface and cured by exposure to visible light for 60 seconds. The Lumifor® composite resin was transferred to the opening in the teflon mold and firmly compressed prior to exposure to visible light for 2 minutes. The test specimens were disassembled after 15 minutes and allocated to one of five groups:

- **Group A:** Shear bond strength determined immediately after disassembly.
- **Group B:** The test specimens were stored in physiological saline at 37°C for 24 hours prior to testing.
- **Group C:** The test specimens were stored in physiological saline at 37°C for 24 hours and then subjected to 250 complete temperature cycles between 5°C and 55°C in a temperature cycling machine with a dwell time of 30 seconds.
- **Group D:** The test specimens were stored in physio-
logical saline at 37°C for 4 weeks prior to testing.

**Group E:** The test specimens were stored in physiological saline at 37°C for 4 weeks and then subjected to 250 complete temperature cycles.

Fifteen test specimens were prepared in each group. The tooth cup with the bonded composite resin was then placed in a special device which was positioned on the compression load cell of an Instron testing machine. A shear load was applied at a crosshead speed of 0.02 inch. min⁻¹. The shear bond strengths were calculated and expressed in MN.m⁻².

The mean ± SD, range and coefficient of variation of the shear bond strengths are presented in Table 5. Analysis of the data by ANOVA indicated that the shear bond strengths were not significantly different. Both temperature cycling and duration of storage in physiological saline had no adverse effect on bond strength.

The shear bond strength of the Tenure/Perfection restorative system to dentin was determined in a recent study. The dentin surfaces of the embedded teeth were treated as for the microleakage study. The same test methodology as described for the Gluma/Lumifor system was employed. The results are presented in Table 6.

In a recent study the shear bond strength of the Scotchbond 2/Silux² restorative system to dentin was determined. The system consists of Scotchbond Dentin

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### Table 5. Shear bond strengths (MN.m⁻²) of the Gluma/Lumifor restorative system to dentin.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of specimens</th>
<th>Shear bond strength (MN.m⁻²)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: 15 min</td>
<td>15</td>
<td>8.9 ± 2.3</td>
<td>6.2-13.5</td>
</tr>
<tr>
<td>B: 24 h</td>
<td>15</td>
<td>10.8 ± 3.0</td>
<td>8.4-20.4</td>
</tr>
<tr>
<td>C: 24 h              Temperature cycle</td>
<td>15</td>
<td>10.8 ± 2.3</td>
<td>7.0-14.8</td>
</tr>
<tr>
<td>D: 4 weeks</td>
<td>15</td>
<td>10.8 ± 2.1</td>
<td>8.2-15.3</td>
</tr>
<tr>
<td>E: 4 weeks Temperature cycle</td>
<td>15</td>
<td>10.3 ± 1.4</td>
<td>8.1-12.5</td>
</tr>
</tbody>
</table>

### Table 6. Shear bond strengths (MN.m⁻²) of the Tenure/Perfection restorative system to dentin.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of specimens</th>
<th>Shear bond strength (MN.m⁻²)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: 15 min</td>
<td>15</td>
<td>5.5 ± 1.5</td>
<td>3.7-8.5</td>
</tr>
<tr>
<td>B: 24 h</td>
<td>15</td>
<td>5.8 ± 2.9</td>
<td>3.0-13.1</td>
</tr>
<tr>
<td>C: 24 h              Temperature cycle</td>
<td>15</td>
<td>6.1 ± 3.2</td>
<td>2.9-13.1</td>
</tr>
<tr>
<td>D: 4 weeks</td>
<td>15</td>
<td>4.7 ± 2.0</td>
<td>1.8-9.1</td>
</tr>
<tr>
<td>E: 4 weeks Temperature cycle</td>
<td>15</td>
<td>4.4 ± 1.7</td>
<td>1.9-7.6</td>
</tr>
</tbody>
</table>
Primer, an aqueous solution of maleic acid and HEMA, Scotchbond 2 light-cured dental adhesive, a mixture of hydrophilic monomer (HEMA), and a hydrophobic monomer (Bis-GMA), and Silux, a light cure composite resin.

Extracted human permanent molars which were stored in 70% ethanol were used in the study. The teeth were embedded with cold cure acrylic resin in brass tooth cups as previously described. Immediately prior to the preparation of a test specimen, the exposed surfaces were ground wet on 600 grit silicon carbide paper on a polishing machine. The embedded tooth was washed in running tap water and dried with an air syringe. The Scotchprep Dentin Primer was applied to the dentin surface with a brush and coverage maintained for 60 seconds. The primed surface was dried with an air syringe and a single coat of Scotchbond 2 light-cured adhesive applied to the dentin surface. The adhesive was spread to a thin even layer with an air syringe, exposed to visible light for 30 seconds, and the tooth cup with the embedded tooth placed in a redesigned assembly apparatus (Fig. 6). A finger spring (Fig. 6A) was disengaged and the platform (Fig. 6B) elevated so that the dentin surface in the tooth cup (Fig. 6C) made firm contact with the lower surface of the split teflon mold.

The shear bond strengths of the Scotchbond 2/Silux® restorative system to dentin are presented in Table 7. Analysis of the data showed that the shear bond strengths were not significantly different. Temperature cycling and duration of storage in physiological saline, therefore, has no significant adverse effect on the shear bond strength.

The optimal shear bond strengths of dentinal bonding systems to dentin that would result in gap-free restorations are not known. To resolve this question, Gluma®-treated dentin was covered by seven different resins before a microfilled composite was applied. A highly significant inverse correlation (r = -0.85) was established between shear bond strength and the marginal contraction gap in μm. The authors suggested that a system must promote a shear bond strength of approximately 17 MN.m⁻² if gap-free fillings in dentin cavities are to be achieved by a system. The shear bond strengths of some of the third generation bonding systems are approaching this value.

**CONCLUSIONS**

Laboratory studies are often conducted in an attempt to predict the performance of dentin bonding restorative systems in the clinical situation. As far as microleakage tests are concerned, unfortunately no clear correlation between in vitro and in vivo microleakage studies has

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of specimens</th>
<th>Shear bond strength (MN.m⁻²)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>A: 15 min</td>
<td>15</td>
<td>6.9 ± 2.3</td>
<td>4.9-13.9</td>
</tr>
<tr>
<td>B: 24 h</td>
<td>15</td>
<td>8.5 ± 1.6</td>
<td>7.1-13.5</td>
</tr>
<tr>
<td>C: 24 h</td>
<td>15</td>
<td>8.5 ± 1.6</td>
<td>7.0-12.1</td>
</tr>
<tr>
<td>Temperature cycle</td>
<td>15</td>
<td>8.4 ± 1.9</td>
<td>5.7-12.1</td>
</tr>
<tr>
<td>D: 4 weeks</td>
<td>15</td>
<td>8.1 ± 1.2</td>
<td>6.5-10.7</td>
</tr>
<tr>
<td>E: 4 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Shear bond strengths (MN.m⁻²) of Scotchbond 2/Silux restorative system to dentin.
been established. If a restorative system exhibits microleakage under simulated in vitro studies, it is likely that the system will also leak in the oral environment. The development of secondary caries at the tooth/restoration interface is but one manifestation of microleakage.

The direct extrapolation of the results of in vitro studies to the oral environment is also not possible. However, if a dentin bonding restorative system exhibits low bond strengths in in vitro studies, the chances that these restorations will be retained in the clinical situation without mechanical retention are negligible.

It is impossible to compare the laboratory results of microleakage and bond strength studies because of the tremendous variations in the test methodologies employed. An appeal is made for the standardization of these laboratory methods.

REFERENCES