Effect of Different Collagen Cross-Linking Agents on Shear Bond Strength of Composite to Dentin – An In Vitro Study

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Abstract:
Objectives: The aim of this in vitro study was to evaluate the shear bond strength of composite resin to deep dentin after pretreatment with different collagen cross linking agents i.e proanthocyanidin and glutaraldehyde.

Study design: 30 freshly extracted molars were collected. The occlusal surfaces of the teeth were ground flat to expose the deep dentin. After acid etching of the dentin surface with 37% phosphoric acid, all the specimens were randomly divided into three groups based on the pretreatment to be done. Group I (control, n=10, no surface pretreatment), Group II (n=10, pretreatment with 6.5% proanthocyanidin), Group III (n=10, pretreatment with 30% glutaraldehyde). Following bonding agent application and light curing, a 4 mm composite build up was done using a tube with an internal diameter of 3 mm. All the specimens were tested for shear bond strength using universal testing machine.

Results and conclusion: Both the collagen cross linking agents significantly increased the shear bond strength of composite to dentin when compared to the control group (p value = 0.002). There was no statistically significant difference between the two experimental groups (p value = 0.007).

Keywords: Collagen cross linking agents, Glutaraldehyde, Proanthocyanidin, Shear bond strength.

Introduction
Adhesive dentistry is a rapidly emerging discipline of dentistry¹. Since years, there have been many attempts to achieve adequate and predictable adhesion of resin composite to tooth structure, as reliable bonding ensures restoration stability, high strength and less microleakage.

In recent years, highly amphiphilic monomers have been incorporated into the adhesive systems² due to which bonding of resin composites to the dentin surfaces have greatly improved³. A layer of collagen is exposed on the dentinal surface followed by acid etching which causes subsequent penetration of the resin composite into the demineralized collagen-rich dentin⁴. The resin-impregnated layer which is thus created, is also called the hybrid layer⁴, and this is considered to be an important factor in dentin adhesion⁵. According to Gwinnet, only one-third of the shear bond strength of composite resin to dentin is because of the resin infiltration and resin tag formation into the dentinal tubules, whereas two-thirds of the bond strength is provided by the interaction of primers and adhesive resins with the partially demineralized dentin collagen⁶.

In many clinical situations complex cavity designs are necessitated. They not only include exposed enamel surfaces and superficial dentin, but also include deep dentinal surfaces⁷. Out of the many adhesive systems that are available today, it is advisable to use a system that produces high and uniform bond strength to all of these dental hard tissues.

Despite significant improvement in the adhesive systems, the bonded interface formed by the combination of dentin organic matrix, resin monomers and residual hydroxyapatite crystallites still remains the weakest area of the adhesive restoration⁷.

The dentin organic matrix has been reported to consist of 90% fibrillar type I collagen and only 10% is made up of non-collagenous proteins such as phophoproteins and proteoglycans⁸. In restorative dentistry, high mechanical properties of collagen are desirable. The resin-dentin interface should also have a lower biodegradation rate so as to provide longevity to the restoration. The formation of exogenous collagen cross-linkage has been reported in dental literature as a mechanism to enhance the mechanical stability and physico-mechanical properties of dentin collagen⁹. The formation of predictable intra and intermolecular as well as intermicrofibrillar cross-links can enhance the mechanical properties of dentin collagen. Improvement of the mechanical properties of collagen can be achieved by the use of different collagen cross-linking agents. Dentin surface pretreatment by these agents prior to the bonding procedures can aid in increasing the bond strength values. Several naturally occurring (proanthocyanidin, genipin and others) as well as synthetic (glutaraldehyde, carbodimides and others) collagen cross linking agents may induce exogenous collagen cross-linking and thus improve the mechanical properties of the collagen-dentin interface.

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Endodontology, December 2015;27(2):97-100
cross-links and also improve the mechanical properties\textsuperscript{11}.

Proanthocyanidin (PA), a preferred cross-linking agent, is a naturally occurring bioflavonoid found in high concentrations in grape seed extracts, cranberries, lemon tree bark, pine bark, and hazel nut tree leaves\textsuperscript{12}. Proanthocyanidins are considered to be one of the most important classes of secondary metabolites. Chemically, they are oligomeric flavonoids and show interaction with proline-rich proteins such as collagen thus resulting in a cross-linking effect. Recent studies have shown that PA-based cross linking agents increase the mechanical properties of demineralized dentin matrix and improve the resin-dentin bond strength after pretreatment of the demineralized dentin surface for 1 hour\textsuperscript{13}.

Glutaraldehyde, which is an organic compound with a formula of CH\textsubscript{2} (CH\textsubscript{2}CHO\textsubscript{2}) is a pungent colorless, oily liquid used to sterilize medical and dental equipment, for industrial water treatment and also as a preservative. Additionally it also has its use in biochemistry as a collagen cross linker.

The aim of this in vitro study was to evaluate the effect of different collagen cross linking agents i.e proanthocyanidin and glutaraldehyde on the shear bond strength of composite to deep dentin.

Materials and Method

30 freshly extracted intact human molars were collected from the Department of Oral and Maxillofacial Surgery and were stored in thymol solution until use. The teeth were ultrasonically cleaned to wash off the debris and teeth with fractures, craze lines, developmental anomalies or caries were excluded from the study.

The occlusal surfaces of the selected samples were ground flat perpendicular to the long axis of the tooth using a slow speed diamond disc, for exposure of the deep dentin. All the prepared occlusal surfaces were acid etched with 37\% phosphoric acid (Etching gel, Dentsply DeTrey GmbH, Germany) for 15 seconds following which the teeth were randomly divided into three groups based on the pretreatment to be done.

**Group I** (control group, n = 10): no surface pretreatment

**Group II** (PA group, n = 10): dentin surface pretreated with 6.5\% proanthocyanidin

**Group III** (GD group, n = 10): dentin surface pretreated with 30\% glutaraldehyde

A Total etch Adhesive System (Prime and Bond NT, Dentsply DeTrey GmbH, Germany) was then applied on the prepared occlusal surfaces of all the samples in the control group (Group I) and light curing was done for 20 seconds following which a 4 mm composite build up was done using a transparent hollow tube with an internal diameter of 3 mm. 2 mm increment build up technique was used and light curing was done for 40 seconds for each increment.

In group II and group III, the prepared occlusal surfaces were pretreated with 6.5\% proanthocyanidin and 30\% glutaraldehyde respectively, following which bonding and build up procedure was done following the same technique as group I.

All the specimens were then stored in water for 24 hours. Shear bond strength of composite resin to the deep dentin surface was then tested for all the specimens using an universal testing machine. The data collected was statistically analyzed using one way ANOVA and Post hoc Bon Ferroni test.

**Results**

As analyzed by one way ANOVA and Post hoc Bon Ferroni test, higher shear bond strength values were associated with the proanthocyanidin group (Group II) when compared to the control group (p value = 0.002). Glutaraldehyde group (Group III) also showed higher shear bond strength values when compared to the control group (p value = 0.002). Mean shear bond strength values of proanthocyanidin was observed to be higher than that of Glutaraldehyde. However, the values were not found to be statistically significant (p value = 0.007).

| Table 1: Comparison of shear bond strength between Group I, Group II and Group III using one way ANOVA |
|-----------------|-----------------|-----------------|
| **Group**      | **Number of samples** | **Mean and Standard deviation (MPa)** |
| I (control)    | 10              | 17.3 ± 2.5      |
| II (PA group)  | 10              | 26.4 ± 3.4      |
| III (GD group) | 10              | 23.5 ± 3.2      |

P value ≤ 0.005 was taken as significant
Table 2: Pairwise comparison between Group I, group II and Group III using Post Hoc Bonferroni Test

<table>
<thead>
<tr>
<th>Group pair</th>
<th>Difference of the Means</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II – Group I</td>
<td>9.1</td>
<td>0.002*</td>
</tr>
<tr>
<td>Group III – Group I</td>
<td>6.2</td>
<td>0.002*</td>
</tr>
<tr>
<td>Group II – Group III</td>
<td>2.9</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*Statistically significant

Discussion

Dentin tissue has been symbolized as a biologic composite of collagenous matrix. Marshall et al reported dentin to be composed of nanometerised calcium deficient, carbonate rich apatite crystallites dispersed between parallel micron-sized hypermineralized, collagen poor, hollow cylinders (dentin tubules containing peritubular dentin). The structure and properties of the dentin changes with increasing depth. The superficial dentin contains fewer dentinal tubules and is composed mainly of intertubular dentin, whereas the deep dentin contains mainly larger dentinal tubules and lesser amount of intertubular dentin matrix. Kinney et al reported that the intertubular dentin is softer when the deeper layer of dentin is tested. In addition, the water content of superficial dentin is lower when compared to deep dentin. The physical and mechanical properties of the collagen of acid demineralized superficial and deep dentin play a major role in determining the bond strength and its durability.

The present study showed a higher shear bond strength values of composite resin to deep dentin after pretreatment with 6.5% proanthocyanidin and 30% glutaraldehyde as compared to the control group. The enhanced mechanical properties and dentin collagen stability, obtained by the use of these collagen cross linking agents may be considered to be responsible for the increase in the bond strength.

Proanthocyanidin primarily forms a hydrogen bond between the protein amide carbonyl and the phenolic hydroxyl groups thus subsequently forming cross links with the dentin collagen. Increased shear bond strength of composite to deep dentin may be attributed to the specificity of Proanthocyanidin to facilitate the enzyme proline hydroxylase, which catalyzes the hydroxylation of proline, which is an essential step in collagen biosynthesis. Proteins and proanthocyanidin have been shown to interact in any of the four different ways: (i) covalent interactions, (ii) ionic interactions, (iii) hydrogen bonding interactions, or (iv) hydrophobic interactions. Castellan et al showed that demineralized dentin, produced by acid etching when treated with Proanthocyanidin, results in improved mechanical properties gained by the use of exogenous cross-linking agents. The results of the present study corroborated these findings. Proanthocyanidin resists the enzymatic degradation and hence proanthocyanidin treated dentin collagen matrix has increased stability of bond. Han et al reported proanthocyanidin to have some properties of tissue fixation due to collagen cross linking. However, it is less cytotoxic than other similar agents. Srinivasulu et al also reported that proanthocyanidin significantly increased the shear bond strength to deep dentin.

Glutaraldehyde, on the other hand is a synthetic fixative agent and is also used as a cross linking agent. Like proanthocyanidin, glutaraldehyde also has been reported to improve the mechanical properties of various collagen based tissues. Studies suggest that the presence of exogenous cross-links induced by glutaraldehyde is sufficient enough to improve the mechanical properties of the exposed dentin collagen matrix and consequently increases the bond strength. Glutaraldehyde reacts primarily with the e-amino groups of lysyl (or hydroxylysyl) residues by using its aldehyde functional groups. Results of the present study suggest that glutaraldehyde treated samples showed increased shear bond strength which is in accordance with the results of the study by Al-Ammar A et al. However despite its ability to induce exogenous cross-links in collagen, the primary disadvantage of glutaraldehyde is its cytotoxicity.

Conclusion

Within the limitation of the present study it can be concluded that dentin surface pretreatment with collagen cross-linking agents like 6.5% proanthocyanidin and 30% glutaraldehyde significantly improved the bond strength of resin composite to deep dentin when compared to the control group. However there was no statistically significant difference observed between results obtained with 6.5% proanthocyanidin and 30% glutaraldehyde.

Conflict of interest and sources of funding statement: Nil

References

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