Evaluation of Bioflavonoids on the Immediate and Delayed Microtensile Bond Strength of Self-etch and Total-etch Adhesive Systems to Sound Dentin

Abstract

Aim: This study aims to evaluate the effect of two bioflavonoids (epigallocatechin-3-gallate [EGCG] and catechin) and a protein inhibitor (chlorhexidine [CHX]) on the immediate and delayed microtensile bond strength of self-etch and total-etch adhesive systems to sound dentin. Materials and Methods: The occlusal surfaces of 96 mandibular human third molar teeth specimens were ground after removal of the excess tissues, to expose the middle dentin. The dentin specimens were randomly allocated into four groups, each consisting of 24 teeth (n = 24) according to the application of the enzyme inhibitor. The adhesive system used in this study was Adper easy bond, a self-etch adhesive system, and Adper Single Bond 2, a total-etch adhesive system. Microtensile bond strength testing was conducted using thermocycler 2000, Heto-Holten A/S. Results: All the three enzyme inhibitors increase the bond strength values of the resin–dentin interphase when used during dentin bonding. The EGCG enzyme inhibitor has shown the highest immediate bond strength to dentin when used with both the adhesive systems. Keywords: Adhesive systems, bioflavonoids, microtensile bond strength

Introduction

An indestructible, resilient bonding of composite resin with dentin is a considerable long-term challenge in restorative procedures.[1–3] Many studies have confirmed the role of extrinsic factors on contemporary dentin adhesives such as oral fluid and water sorption with subsequent polymer swelling, leading to dentin bond failure.[4–7] In addition, endogenous matrix metalloproteinases (MMPs) have also been proved to play a role in the separation of the bonding layers.[8,9] The dentin matrix contains MMPs, a family of zinc-activated, calcium-dependent endopeptidases, and is involved in the development of dentition and dental caries.[10,11] Less aggressive self-etching adhesives carry the property of releasing and activating endogenous MMPs during dentin bonding and further leads to the gradual loss of collagen fibrils from the partially infiltrated hybrid layers of aged, bonded dentin.[9,12] The susceptibility of dentin bonding to endogenous MMPs can be altered either through the application of protease inhibitors or by stabilizing the dentin collagen by the application of collagen cross-linkers both of which come under the bioflavonoids.[13–16] The objective was to evaluate the effect of two bioflavonoids (Epigallocatechin 3 gallate [EGCG] and catechin) and a protein inhibitor (CHX) on the immediate and delayed microtensile bond strength of self-etch and total-etch adhesive systems to sound dentin.

Materials and Methods

Specimen preparation

The study was approved by Institutional Ethics Committee of the Sri Ramachandra University of Chennai, India. Ninety-six mandibular human third molar teeth specimens were stored in 0.9% NaCl solution and 0.05% sodium azide at 4°C–7°C. After thorough rinsing with distilled water and removal of the excess tissues, the occlusal surfaces were ground flat, to expose the middle dentin. A 600-grit abrasive paper (Buehler, Lake Bluff, IL, USA) was used to obtain a uniform smear layer.

The dentin specimens were randomly allocated into four groups, each consisting of...
of 24 teeth \( n = 24 \) according to the application of the enzyme inhibitor. The enzyme inhibitors used in each group were as follows: Group I – control group (phosphate buffer solution, PBS); Group II – dentin treated with 0.02M 5\% v/v EGCG (E4143-50MG Lot #080M1690V ≥95\%, from green tea, Sigma-Aldrich, India); Group III – dentin treated with 2\% CHX (Dенточлор, Ammdent, PB, India); and Group IV – dentin treated with 0.02M catechin (C0692-1MG Lot # 120M1173V analytical standard, ≥99.0\%, Fluka, Sigma-Aldrich, India). Each of these groups was further subdivided according to the adhesive system used into subgroup A (self-etch adhesive) and subgroup B (total-etch adhesive). Each one of these subgroups was further subdivided based on the aging protocol into immediate (I) and delayed (D).

For all the groups, the teeth randomized under subgroup A (self-etch adhesive) were immersed in their respective solutions (Group I: PBS, Group II: EGCG, Group III: 2\% CHX, and Group IV: catechin) for about an hour before dentin bonding. The teeth randomized under subgroup B (total etch adhesive) were treated with 37\% phosphoric acid for 20 s and then thoroughly rinsed with water for about a minute and further immersed in their respective solution similar to subgroup A, for about an hour before dentin bonding.\(^{[17]}\)

**Restorative procedures**

The adhesive system used in this study was Adper easy bond, a self-etch adhesive system (3M/ESPE, St. Paul, MN, USA) and Adper Single Bond 2, a total-etch adhesive system (3M/ESPE, St. Paul, MN, USA). The manufacturer’s instructions were followed for all the adhesive systems that were used. A composite resin block (Filtek Z 250, A1 color, batches #5AY and #6YN, 3M ESPE, St. Paul, USA) was placed over the bonded surfaces incrementally with a total thickness of 5 mm to allow for gripping during the microtensile bond strength testing. Each increment thickness limited to 2 mm, and curing was accomplished for 40 s per increment (Demetron LC, SDS K, USA). All the specimens were stored in distilled water for 24 h.

**Microtensile bond strength testing**

During this process, 16 out of the 96 beams were lost due to technical difficulties. The total sample used for this study is 80 beams. One beam of 1 mm thickness from each of the 80 beams, consisting resin composite in the upper half and dentin in the lower half was sectioned perpendicular to the bonded surface using hard tissue microtome under cool water. A universal instron testing machine on which the beams were mounted were run at a cross speed of 0.5 mm/ min to record the tensile load at which the fracture occurred. Forty beams (10 for each subgroup TE/SE) of the total of eighty beams, randomized for delayed bond strength, were subjected to thermocycling process (thermocycler 2000, Heto-Holten A/S) for 10,000 cycles between 5°C and 55°C. Care was taken to avoid dehydration of the samples throughout the process. The dwelling time at each temperature was 30 s. The remaining 40 specimens were kept in desiccators at room temperature for 24 h before testing for immediate bond strength.

**Statistical analysis**

The one-way analysis of variance (ANOVA) and Tukey’s Honest Significant Difference (HSD) post hoc tests for multiple comparisons were used for the immediate and delayed bond strength values for different groups with two different adhesive systems.

**Results**

Tables 1 and 2, Figure 1 show the descriptive statistics, one-way ANOVA results, and Tukey’s HSD post hoc pairwise comparison analysis for the immediate bond strength values for different groups with two different adhesive systems, respectively. Tables 3 and 4, Figure 2 show the descriptive statistics, one-way ANOVA results, Tukey’s HSD post hoc pairwise comparison analysis for the delayed bond strength values for different groups with two different adhesive systems, respectively. Among the various groups and subgroups in the immediate bond strength values, the groups that performed statistically significant include EGCG self etch immediate specimens (IIAI) which showed the highest mean bond strength (57.92 MPa) followed by EGCG total etch adhesive system (IIIB) with a mean bond strength value (56.35 MPa). The total-etch 2\% CHX (IIIB) group showed a bond strength value of 51.83 MPa. However, the mean bond strength values of self etch 2\% CHX (47.40MPa) and the cross linker catechin in both total etch (46.06MPa) and self etch

**Table 1: Descriptive statistics for immediate bond strength (IBS) between different groups and system**

| Group     | System    | Mean | Std. Dev |
|-----------|-----------|------|----------|
| Control (I) | Self (IIAI) | 37.59 | 5.27     |
|           | Total (IIA) | 41.56 | 4.56     |
| EGCG (II)  | Self (IIIAI) | 57.92 | 3.34     |
|           | Total (IIIB) | 56.35 | 6.40     |
| 2\% CHX (III) | Self (IIIAI) | 47.40 | 7.41     |
|           | Total (IIIB) | 51.83 | 3.04     |
| Catechin (IV) | Self (IVAI) | 47.24 | 3.38     |
|           | Total (IVBI) | 46.06 | 9.78     |

**Table 2: One Way ANOVA to compare the mean Immediate Bond Strength (IBS) between different groups and system**

| Time point | Sum of Squares | DF | Mean Square | F | Sig. |
|------------|----------------|----|-------------|---|------|
| IBS (1)    |                |    |             |   |      |
| Between Groups | 1684.350      | 7  | 240.621     | 7.081 | <0.001 |
| Within Groups | 1087.381      | 32 | 33.981      |     |      |
| Total      | 2771.731       | 39 |             |    |      |
The control group (Group I) showed the least bond strength value for both the adhesive systems. Among the 1-year aged groups, EGCG self-etch delayed specimens (IIA_D), showed the highest mean bond strength (56.46 MPa) followed by 2% CHX self-etch delayed specimens (IIA_D) 45.81 MPa. The EGCG total-etch delayed specimens (II B_D) group showed the next highest bond strength value (43.73 MPa). The total-etch 2% CHX and both the self-etch and total-etch catechin had lower bond strengths. The least bond strengths were observed in the controls.

Discussion
The quality of hybridization plays a vital role in increasing the bond strength of adhesives to the dentin.\(^\text{[18]}\) The stability of collagen fibrils within the hybrid layer is important for the bonding effectiveness.\(^\text{[19]}\) The existence of host origin MMP-2 and MMP-9 in mature dentin plays a contributory role in the degradation of the organic matrix of teeth in the process of caries progression as well as in the resin-penetrated dentin interfaces.\(^\text{[10,11]}\)

The enzyme inhibitor EGCG has shown the highest mean bond strength value both in the self-etch and total-etch adhesive systems. This could be probably because EGCG involves itself with higher noncovalent hydrogen bonding interaction with a different set of proteins and have greater contact surface and surplus more number of hydroxyl groups that can act as hydrogen acceptors and donors.\(^\text{[12]}\) The reason for immediate and effective self-etchings of EGCG in both the self-etch and total-etch adhesive systems may be the presence of water, ethanol, and 2-Hydroxylethyl methacrylate (HEMA)-rich component present in both the adhesive systems that were selected.

The possible reason for enzyme inhibitor catechin not being statistically significantly higher than the control groups in both the self-etch and total-etch adhesive systems may be due to the lesser contact surface and lesser number of hydroxyl groups in catechin to involve in hydrogen bonding interaction with various functional proteins.\(^\text{[12]}\)

The control group in both the self-etch and the total-etch adhesive systems have showed lowest bond strength values due to the degradation of denuded collagen within the hybrid layer. In addition to the inherent reasons of the absence of dissolution of unreacted monomers, water sorption, polymer swelling, resin hydrolysis, and enzymatic activity destroy the exposed Type I collagen fibrils located at the base of the hybrid layer. The absence of enzyme inhibitors in the control group could have resulted in low bond strength.

All the specimens showed similar results with the self-etch and total-etch adhesive systems. This can be explained by the reason that the presence of water in their composition.

| Table 3: Descriptive Statistics for Delayed Bond Strength (DBS) between different groups and system |
| --- |
| Group | System | Mean | Std. Dev |
| Control (I) | Self (IAD) | 28.29 | 4.89 |
| Total (IBD) | 28.67 | 1.46 |
| EGCG (II) | Self (IIAD) | 56.46 | 12.79 |
| Total (IIBD) | 43.73 | 1.96 |
| 2% CHX (III) | Self (IIIAD) | 45.81 | 5.56 |
| Total (IIIBD) | 34.70 | 7.65 |
| Catechin (IV) | Self (IVAD) | 35.70 | 5.07 |
| Total (IVBD) | 34.22 | 4.93 |

| Table 4: One Way ANOVA to compare the mean Delayed Bond Strength (DBS) between different groups and system |
| --- |
| Time Point | Sum of Squares | DF | Mean Square | F | Sig |
| DBS (2) | Between Groups | 3224.848 | 7 | 460.693 | 11.074 | 0.000 |
| Within Groups | 1331.289 | 32 | 41.603 |
| Total | 4556.136 | 39 | | | |

Figure 1: Mean immediate bond strength of control and study samples in different groups and system

Figure 2: Mean delayed bond strength of control and study samples in different groups and system
causes penetration in the acid-conditioned substrate and makes them less vulnerable to overwetting or overdrying condition, which is inherent in the operator performance.

CHX self-etch delayed specimens have shown statistically significantly higher microtensile bond strength values than the EGCG total-etch specimens.[13] The resin tags that occlude the dentinal tubules in addition to the adhesive resin coating collagen fluids and the adhesive layer overlaying have been speculated to sequestrate the CHX-soaked demineralized matrix from the interstitial fluids. This in turn causes prolonged retention of CHX and thereby that MMPs are inhibited. As long as the CHX remained bound to the matrix, the MMPs remain dominant. The HEMA is unable to displace CHX from the dentin matrix and hence may remain bound to demineralized dentin after bonding.[14] This may be the reason for the better performance of 2% CHX in the delayed samples.[14] The length of time that the CHX remains with the hybrid layer has to be further investigated.

The bond strength values of the EGCG and CHX self-etch group remain stable even after thermocycling, thereby indicating that they have the ability not only to inhibit the breakdown collagen within the hybrid layer but also to retain the durability of dentin bonding over a time as is evident from the sustained microtensile bond strength value.

**Conclusion**

Within the limitations of our study, it has been shown that all the three enzyme inhibitors increase the bond strength values of the resin–dentin interface when used during dentin bonding.

The EGCG enzyme inhibitor has shown the highest immediate bond strength to dentin when used with both the adhesive systems and has also inhibited its durability over time when used with the self-etch adhesive system.

The durability of the bond has shown to definitely deteriorate over time statistically significantly with the total-etch adhesive system in all the groups.

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**Conflicts of interest**

There are no conflicts of interest.

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