ORIGINAL PAPER

COMPARATIVE EVALUATION OF TENSILE BOND STRENGTH IN HEALTHY DENTIN AND CARIES-AFFECTED DENTIN, PRE-TREATED WITH EITHER GRAPE SEED EXTRACT OR RIBOFLAVIN: AN *IN VITRO* STUDY

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ABSTRACT

Introduction: Advances in composite resins have changed the way restorative dentistry is now performed. The objective of restorative dentistry is to replace lost hard tissue with restorative material that has a good prognosis and longevity. In spite of the great improvements in the field of adhesive dentistry, hybridization of caries-affected dentin has been broadly avoided by clinicians and researchers. The loss of mineral content during a caries process is further compounded by action of matrix metalloproteinases (MMPs) on collagen of dentin. Recently, inhibition of MMPs has been the focus of research.

Objectives: To compare the effect of MMP inhibitors on the bond strength of healthy dentin and caries-affected dentin. MMP inhibitors selected for this study were grape seed extract and riboflavin.

Material and methods: Thirty-six extracted teeth were divided into two groups: healthy dentin and caries-affected dentin (CAD). Samples were grounded to expose dentinal surfaces. CAD samples were exposed to 10% citric acid for 5 minutes to simulate caries. The exposed dentinal surfaces of samples in each group were then treated, either with grape seed extract or riboflavin, followed by application of eighth-generation bonding agent and restoration with a composite resin, standardized to a specific height and diameter. This assembly was then subjected to testing in a universal testing machine to evaluate tensile bond strength (TBS) of adhesive layers.

Results: The average TBS of healthy dentin treated with grape seed extract was found to be the highest (34.28 MPa) amongst the groups. The lowest TBS was found in the group with caries-affected dentin treated with riboflavin. There was a marked increase in TBS obtained in CAD after the application of MMP inhibitors.

Conclusions: There is a significant difference between the bond strengths of healthy and caries-affected dentins. GSE and riboflavin both enhanced the bonding performance of eighth-generation bonding agent in both healthy dentin and CAD samples.

Key words: dentin, caries-affected dentin, matrix metalloproteinases, bond strength, adhesive dentistry, grape seed extract.

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INTRODUCTION

Adhesive dentistry is a rapidly evolving field, relying on the formation of a hybrid layer, in which the adhesive systems impregnate etched tooth substrate and provide retention through a micro-mechanical method. The resultant hybrid layer is a collagen resin interface that is most vulnerable, and tends to show stress build-up leading to failure [1, 2].

Both in the coronal and radicular portions of the tooth, it is the dentin that forms the major portion. Dentin is a composite of minerals, organic components, and water, with an extremely complex arrangements of these parts. The organic component of dentin is mainly fabricated of collagen type I and several non-collagenous proteins. The fibers of collagen in dentin form a meshwork to accommodate hydroxyapatite crystals. This arrangement is such that both the stability and strength of the dentinal fibers are optimized. The terms "collagen fibers" and "collagen fibrils" should not be used interchangeably [2, 3]. Upon etching, collagen fibrils are exposed, and excessive air drying could lead to a collapse of this meshwork and prevent adequate infiltration of the adhesive. Therefore, it is vital that the exposed collagen network maintains in its mesh-like state, to allow permeation of the adhesive and avoid adhesive's failure. This sensitive situation is further aggravated by the action of matrix metalloproteinases (MMPs), which could hydrolyze the collagen framework, causing its breakdown and failure of adhesive restoration. Ammar *et al.* [3] reported on actual chemical modification of the collagen fibrils with the application of MMP inhibitors, such as glutaraldehyde and grape seed extract.

With the development of MMP inhibitors (external collagen cross-linkers), there has been an improvement in the inter- and intra-molecular collagen cross-linking, which ultimately enhance the structural stability of adhesive interface. The use of collagen cross-linkers has been proven to be an effective additional treatment to prevent deterioration of dentinal collagen. Various MMP inhibitors, such as GSE (which is rich in proanthocyanidins), riboflavin (UVA), can be used to serve the purpose [4, 5].

Hybridization of CAD is lower as compared with normal dentin because of de-mineralization and loss of hydroxyapatite crystals secondary to the carious process. In CAD, de-mineralization occurs leading to the loss of hydroxyapatite crystals and leaving collagen fibrils open to chemical onslaught and physical collapse of the fibrillar network. As a consequence, the bond strength achieved in CAD is significantly less than that of healthy dentin. Even if the primary aim of a clinician is not to conserve and hybridize CAD, it is unlikely that the tooth preparation done in a carious tooth would result in floors and walls consisting entirely of healthy dentin [6-8].

OBJECTIVES

This study attempted to explore the effect of MMPs on the bond strength achieved when an MMP was used on CAD prior to hybridization within the adhesive resin. Also, the purpose of the current study was to evaluate and compare the tensile bond strengths of caries-affected dentin and healthy dentin, pre-treated with either GSE or riboflavin.

MATERIAL AND METHODS

A total of thirty-six permanent teeth were randomly allocated into two primary groups (n = 18 teeth in each group: healthy dentin and CAD), and sub-grouping of each of these groups was done by random allocation (n = 9 teeth in each sub-group), depending on MMP inhibitor used: in total, 4 groups, including normal dentin (with GSE and riboflavin) and caries-affected dentin (with GSE and riboflavin).

Occlusal one-third of each tooth was grounded to expose dentin, and the roots were embedded in acrylic blocks. All the teeth were etched with a 37% orthophosphoric acid for 30 seconds, followed by air drying. CAD group was exposed to 10% citric acid for 5 minutes to simulate caries prior to etching with phosphoric acid [9]. Each group was further divided into 2 sub-groups as per the type of MMP inhibitor used (either GSE or riboflavin). One gram of riboflavin in the form of powder was collected from a capsule and dissolved in 100 ml of distilled water. The dentin surface was pre-treated with 1% riboflavin for 5 minutes, and photo-activated by conventional light curing unit for 20 sec., 1,500 mW/cm^2 [1]. 6.5 gram GSE powder was dissolved in 100 mL of distilled water. Specimens were pre-treated with 6.5% GSE solution for 5 min., and rinsed with distilled water [1].

After pre-treatment, a bonding agent (G-Premio Bond CG, 8th generation bonding agent) was applied to all the specimens, and they were restored with composite resin (Tetric N-Flow Bulk Fill composite, Ivoclar™) using dimensions of 4 mm diameter and 4 mm height build-up, with the help of plastic straws, and bonded to the exposed pre-treated dentin. Evaluation of tensile bond strength was performed using a universal testing machine at a cross-head speed of 1 mm/min.

STATISTICAL ANALYSIS

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) for Windows, version 28.0. (IBM Corp.; Armonk, NY, USA). Confidence interval was set at 95%, and a p-value ≤ of 0.05 was considered statistically significant. One-way ANOVA test was applied to compare maximum tensile load and tensile strength among different groups. Post-hoc test was done to find significance in different groups.

RESULTS

The tensile bond strength and maximum load in normal and caries-affected dentins using grape seed extract and riboflavin are shown in Table 1. The average TBS of normal dentin treated with grape seed extract was found to be the highest (34.28 MPa) in all the groups. This was followed by the normal dentin riboflavin group, with an average value of 30.82 MPa. The lowest TBS was found in caries-affected dentin in the group treated with riboflavin.

The result shows a comparison of maximum load and TBS between all the groups (Tables 1-3). The mean maximum load was 212.25 (92.6), and the mean tensile bond strength was 24.50 (8.3). The inter-group comparison was done using one-way ANOVA test for both maximum load and TBS, and the result was statistically significant, hence Tukey's HSD post-hoc test was applied to observe significant mean differences **between** the groups (Table 2). A comparative understanding of TBS after the application of MMPs to normal dentin and CAD is shown in Table 3 and Figure 1.

TABLE 1. Comparison of tensile strength in different groups

Groups	Mean ± SD	*F*-value	*p*-value
Healthy dentin treated with GSE	34.16 ± 1.33	526.903	0.001*
Healthy dentin treated with riboflavin	30.82 ± 1.36		
Caries-affected dentin treated with GSE	17.86 ± 0.97		
Caries-affected dentin treated with riboflavin	15.35 ± 1.17		

*One-way ANOVA. *Statistical significance.*

Healthy dentin demonstrated statistically higher tensile bond strength compared with caries-affected dentin. Grape seed extract and riboflavin both enhanced the bonding performance of the eighth-generation bonding agent when compared with the base-level values of bonding in healthy dentin and caries-affected dentin treated with grape seed extract.

DISCUSSION

Dentin is a substrate, to which adhesive procedures are performed during restorative dentistry. The ultramicrostructure of dentin is extremely complex [9]. In bonding to dentin, clinician have to deal with both inorganic and organic components. Additionally, this bonding is further confounded by the presence of dentinal fluid in vital teeth. The dentin is essentially composed of dentinal tubules surrounded by inter-tubular dentin, which is a meshwork of collagen fibrils, enmeshed

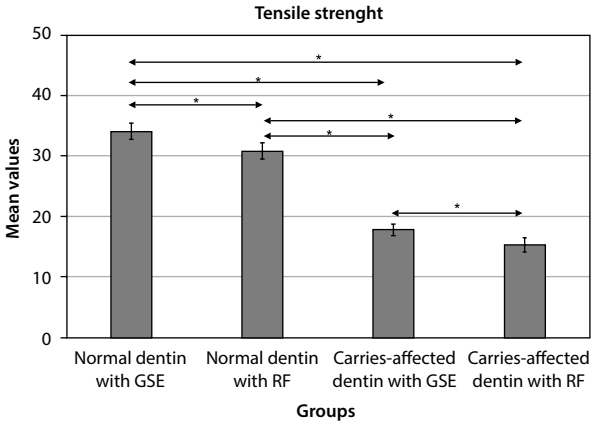

FIGURE 1. Comparison of tensile strength in different groups

TABLE 2. Post-hoc analysis to compare the significance in different groups

Groups	Healthy dentin treated with GSE	Healthy dentin treated with riboflavin	Caries-affected dentin treated with GSE	Caries-affected dentin treated with riboflavin
Healthy dentin treated with GSE	–	0.001*	0.001*	0.001*
Healthy dentin treated with riboflavin	0.001*	–	0.001*	0.001*
Caries-affected dentin treated with GSE	0.001*	0.001*	–	0.001*
Caries-affected dentin treated with riboflavin	0.001*	0.001*	0.001*	–

*Tukey's post-hoc analysis. *Statistical significance.*

TABLE 3. Comparative presentation of TBS after application of MMPs to healthy dentin and CAD

	Healthy dentin (no intervention)	Healthy dentin + GSE	Healthy dentin + riboflavin	CAD (no intervention)	CAD + GSE	CAD + riboflavin
TBS (MPa)	27.78	34.28	30.82	10.56	17.86	15.35
% change		23.3 ↑	10.9 ↑		69.0 ↑	45.0 ↑

with hydroxyapatite crystals. Adhesive dentistry relies on the removal of inorganic content of the dentin and enamel substrate, and the infiltration of these empty spaces with an adhesive resin. The depth of penetration of adhesive resin along with its capability to infiltrate laterally, is the primary cause of success/failure of all dentinal bonding procedures [10].

Matrix metalloproteinases (MMPs) are a part of collagen fibrils, and are responsible for the degradation of collagen fibrils. This degradation of collagen fibrils leads to a collapse of the collagen meshwork, thus compromising the penetration of dental adhesive. Collagen fibrils that are not adequately impregnated by adhesive resin are the leading cause of failures in adhesive dentistry. MMPs that cause degradation of the collagen fibrils are liberated prolifically when collagen is damaged. This damage to collagen fibrils can occur during tooth preparation procedures and by carious dentin [11, 12].

Damage at the cellular level is irreversible, but it can be contained by the application of MMP inhibitors to an etched tooth surface prior to the application of bonding agent. This will ensure that the degradation of collagen is arrested in the hybrid layer that is formed. In dental caries, de-mineralization of inorganic part and a disintegration of organic part occur. The organic part of the dentin is mainly type 1 collagen. Collagenases contribute to proteolytic degradation of extra-cellular matrix of dentin. MMP-20 (enamelysin) has been found to contribute to the progression of dental caries [13].

The hybrid layer formed between the adhesive layer and dentin substrate is unstable in aqueous media, and is subject to hydrolytic degradation of both the resin adhesive and collagen [14]. Polyphenolic compounds, such as proanthocyanidins, are known to behave as MMP inhibitors. They act by the inhibition of MMP production and activity, and are known to inactivate close to 90% of MMP-2, MMP-8, and MMP-9. MMP inhibitors competitively bind to metal ions, such as zinc and calcium, thereby making them unavailable for MMPs. By inhibiting the availability of these metal ions, polyphenols lead to an inhibition of the catalytic activity of these proteases (MMPs) [15].

Almahdy in 2012 investigated the effect of MMP inhibitors incorporated into dental adhesive on MMPs within the dentin, micro-tensile bond strength of the assembly, and micro-permeability of adhesives. The incorporation of MMP inhibitor led to a positive result in all the three properties [16]. Zheng i Chen [17] in 2017 reported that the application of MMP inhibitors to the exposed dentin prior to application of dental adhesive, led to significant improvement in the bond strength and uniform formation of a dense dentin hybrid layer. This positive effect of the application of MMP inhibitor on healthy dentin has been well-documented by another researcher also. Adhesive dentistry has permitted clinicians to be more conservative in removal of diseased dentin; in tandem, as per principles of minimum intervention dentistry, it is often practiced that while infected dentin is removed, affected dentin is left behind [6].

In a tooth preparation for intra-coronal restoration, it is rare to find the presence of solely healthy dentin, infected dentin, or caries-affected dentin (CAD) [6]. Therefore, it is wise to consider the effect of various bonding procedures on healthy dentin and CAD. In their studies, Xuan *et al.* [11], Milia *et al.* [18], and Hass *et al.* [6] have demonstrated the formation of a hybrid layer in CAD. Xuan *et al.* [11] and Hass *et al.* [6] were both able to establish that a poorly formed hybrid layer leads to compromised physical strength.

MMP inhibitors have been shown to positively impact the bond strength when included in the adhesive system or applied on the etched surface of substrate [16, 18].

The present study examined the effect of two MMP inhibitors, including grape seed extract and riboflavin. All the bond strengths were achieved in both healthy dentin and CAD samples [18-21].

The grape seed extract (GSE) is a naturally occurring compound that has a four-pronged interaction with proteins. It has a chemical composition of $C_{11}H_{30}O_{11}$. These four mechanisms include covalent interaction, ionic interaction, hydrogen bonding interaction, and hydrophobic interaction. Application of GSE to the etched substrate causes a positive increase in the bonding strength [3]. Riboflavin, also known as vitamin B_2, is widely found in nature, and in plant- and animal-based food. Riboflavin is capable of producing strong free radicles, such as O_2 and O_{2-}. These free radicals form covalent cross-links with adjacent collagen molecules. Riboflavin has been identified chemically as $C_{17}H_{20}N_4O_6$. The ability of riboflavin to act as a collagen cross-linker is known to enhance the mechanical stability of etched dentin; it also delays the hydrolytic degradation of collagen [1]. GSE and riboflavin were included in the study as collagen cross-linkers because they are known to positively influence the bonding strength in dentin. Kshirsagar *et al.* [9] demonstrated the simulation of dental caries. Adopting a similar methodology accelerated the collagen fibrils to the intervention with etching and bonding.

An inter-group comparison between healthy dentin and CAD showed higher bond strength figures for healthy dentin (Tables 1 and 2). This was true, irrespective of whether GSE or riboflavin were applied. In both healthy dentin and CAD, GSE outperformed riboflavin. The bond strengths obtained in CAD were significantly lesser than those obtained in healthy dentin. This was in accordance with studies conducted by Xuan *et al.* [11] and Hass *et al.* [6]. Bonding strengths achieved in CAD, being lesser than that in healthy dentin, are expected due to disruption of the ultrastructure of dentin at microscopic level.

TBS showed an appreciation of 20% after the application of GSE to healthy dentin, whilst the application of riboflavin to healthy dentin resulted in a 10% increase in TBS (Table 3). This increase was far more when MMP

inhibitors were used on CAD, prior to testing for TBS. The increase was almost 70% and 45%, respectively, when GSE and riboflavin were applied to CAD. These results are in line with the observations reported by Hass *et al.* [6] in 2010. Although the authors did not work on TBS, they did report a marked increase in the thickness of hybrid layer. The resin tags formed in the hybrid layer were shorter but thicker, and this could explain the result obtained in this study. The application of MMP inhibitors would have prevented degradation of the fibrils, whilst at the same time, the space created when crystals were lost from the collagen lattice (due to dental caries) would permit an increase of TBS. Whether this increase in TBS can sustain over a period of time in CAD is not shown. In fact, Milia *et al.* [18] in 2012 reported a pooling of the adhesive at the bottom of CAD. This would initially raise TBS, but be unable to sustain it over long period of time.

Even though the bonding strength achieved by treatment of CAD with collagen cross-linkers does not bring up the bonding strengths to a similar level as that in healthy dentin, they are still clinically relevant.

In a study, Srinivasulu *et al.* [22] demonstrated the use of proanthocyanidin, and stated that the stability of collagen fibrils depends upon inter-molecular and intramolecular bond strengths. The use of a significant strategy of MMP used in their study significantly increased its bonding strategy on the substrate. Follok *et al.* [23] conducted a study using multi-mode adhesive on caries-affected dentin, and concluded that neither of the etching strategies influenced strength on healthy dentin or caries-affected dentin. Jawale *et al.* [24] performed a study among various groups using GSE, and concluded that GSE was the best in pH cycling model, and had a firm re-mineralization potential due to its natural cross-linking ability.

Nandakumar *et al.* [25] discussed re-mineralization using GSE, and stated that the availability of gallic acid helps in deposition of minerals, which further adds to the re-mineralization protocol when used. Minimally invasive dentistry can be performed considering the fact that tooth preparation from all caries excavation will result in a part of cavity wall, cavity floor, of fractions of healthy dentin and caries-affected dentin. Clinicians is able to enhance the prognosis of restorative procedure, even though CAD is included in the dentinal substrate and forms an interface with dentin adhesive.

CONCLUSIONS

Within the limitations of this study, a significant difference was observed between the bond strengths of healthy dentin and caries-affected dentin, both with and without the application of the MMP inhibitors. Therefore, treating CAD with MMP inhibitors could clinically improve the longevity of restorations in cavities where CAD forms a part of the cavity walls or floor. Examining the durability of the bond formed, remains an area to be further investigated.

DISCLOSURES

1. Institutional review board statement: The study was approved by the Regional Ethics Committee of the Dr. D.Y. Patil Ethics Committee, with approval number: DPU/1184/32/2019.
2. Assistance with the article: None.
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