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EVALUATION OF MICROTENSILE BOND STRENGTH AT THE COMPOSITE-DENTINE INTERFACE WITH MODIFIED PHOSPHORIC ACID ETCHANTS –AN IN-VITRO STUDY

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Conflicts of Interest: Nil

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Abstract:

Aim of the study: To evaluate the micro tensile bond strength at the dentin – resin interface after using different etchants.
Materials and methods: Twenty four extracted caries free molars are collected, cleaned of debris and stored in a hypotonic solution. Occlusal surfaces of the teeth were flattened, which eliminates the pits and grooves, thereby the surface of the Enamel was standardized.Teeth will be randomly divided into four groups of 6 teeth each depending on the etchant used.Group I : 37% phosphoric acid (Control),Group II : 37% phosphoric acid+2% chlorexidine digulconate, Group III: 37% phosphoric acid+2% EDTA, Group IV: 37% phosphoric acid+20% tannic acid. To test the microtensile bondstrength test specimens should be sectioned through centre of the restoration (both buccoingually and mesiodistally).
Results: Stable micro tensile bond strength values were observed only for the modified phosphoric acids (CHX,EDTA,TA).
Conclusion: Compared to the control, the phosphoric acid etchants which contained protease inhibitors (CHX,EDTA,TA) promoted the stability of composite-dentin microtensile bond strength.
Clinical significance: Providing an effective way to prolong the stability of the composite-dentin bonds without an additional bonding step.

Keywords: Chlorexidine, EDTA, Micro-tensile bond strength, Phosphoric acid, Tannic acid

Introduction

Revolutions in dental adhesive technology have allowed clinicians to perform increasingly esthetic restorations. Preconditiong of dentin before application of bonding agent enhances the procedure of bonding which removes the smear layer.This produces a deeper demineralisation of dentin surface.The bond strength is increased due to enhanced resin infiltration. However, studies still report that the **durability of composite-dentin bonds is not stable**.[1]

The degradation of the dentin bonding interface usually occurs through several simultaneous processes.[2]

The **first stage** of degradation begins when dentin is acid etched to remove the smear layer, exposing the underlying collagen fibril matrix for hybrid layer formation.

The **second stage** involves leaching of the resins that infiltrated the dentin matrix via water-filled nanometer-sized voids within the hybrid layer.[3]

The **third stage** involves the enzymatic attack on the exposed collagen fibrils by host-derived matrix metalloproteinases (MMPs) and cysteine cathepsins, leading to their degradation over time.[4]

Several approaches to inhibit the activities of these proteases have been proposed with **the aim of prolonging the clinical lifetime of the bonding interface.** Therefore this in vitro study was designed to evaluate the effect of different substances added to phosphoric acid (CHX,EDTA,TA) vs a conventional phosphoric acid on the immediate(24h) and 1 year composite-dentin microtensile bond strength.

AIMS & OBJECTIVES:

To evaluate and compare the effects of three different MMP inhibitors in combination with 37% Phosphoric acid on the microtensile bond strength of composite resins to dentin.

MATERIALS & METHO:

Twenty four extracted caries free Mandibular molars collected, from were used for the study. Inclusion criteria non carious mandibular molars with intact crown and root. Teeth with Caries, Fractures, Craze lines, Development defects are excluded. Teeth collected were cleaned of calculus, soft tissue and debris by scaling.

Occlusal surfaces of the teeth were flattened, in order to eliminate the pits and grooves. Standardised class I cavities were prepared on occlusal surfaces of tooth by Using **ISO size (NO.001) round bur and ISO size (NO.112) straight fissure,** under copious water coolant in a high speed hand piece with following dimensions.**4**mm-wide(bucco-lingual),**3**mm-deep (occluso-gingival), **4**mm-long (mesio-distal).

Teeth were randomly divided into four groups of 6 teeth each depending on the etchant with three different MMP inhibitors used for 15 secs.

Group I: 37% phosphoric acid (Control), Group II : 37% phosphoric acid+2% chlorexidine digulconate, Group III: 37% phosphoric acid+2% EDTA, Group IV : 37% phosphoric acid+20% tannic acid.

After etching, the surfaces were rinsed off with distilled water for 30s, blot dried for 5s.Application of three different MMP inhibitors in their respective groups for 2minutes and blot dried for 5s.Application of the dentin bonding agent (Fusion Bond 5; 5thgeneration) and light cured for 10-15 secs. After bonding procedure, **composite restoration (Ivoclar) was placed** in increments (i.e, 1.5mm) in all the prepared teeth. Each increment was light cured for **40 seconds** using a light curing unit. To test the microtensile bondstrength test specimens should be sectioned through centre of the restoration (both buccoingually and mesiodistally).

All the specimens were mounted in acrylic tube or mold (20 mm height &10 mm diameter) with an auto-cure acrylic resin. The root is positioned at the centre of the acrylic tube vertically with only crown portion exposed and allow the acrylic to set completely before doing the test.

In each group half of the specimens per tooth are tested immediately (24hrs) and the other half are subjected to thermocycling.[6]

Measurement of Bond strength:

The specimens were subjected to the micro tensile bond strength test after their respective storage periods.

The micro tensile bond strength was measured with a Universal Testing Machine and values of the specimens was calculated and expressed in MPa.

All the values are subjected to statistical analysis

STATISTICAL ANALYSIS:

Two way ANOVA test and Post hoc Tukeys test were done for statistical analysis.

	GroupI (CONTROL) in Mpa	Group II (CHX)in Mpa	Group III (EDTA)in Mpa	Group IV (TANNIC ACID) in Mpa
Immediate (24h)	37.6 ± 4.7A	41.4 ± 3.2A	42.5 ± 4.9A	44.2 ±3.9A
1 Year	28.9 ± 4.1B	36.4 ± 3.4A	38.4 ± 4.7A	40.2 ± 3.4A

Groups with same capital letter are not significantly different (Tukey's test, p > 0.05).

Results:

The highest microtensile bond strengths were observed for the immediate groups. However, after 1 year Group I showed maximum decreases in the microtensile bond strengths followed by Groups II,III and IV.

Group I **>** Group II **>** Group III **>** Group IV

Group IV showed the maximum bond strength followed by Groups III, II and I.

Group IV **>** Group III **>** Group II **>** Group I

DISCUSSION:

CHX has been recently studied as a protease inhibitor to preserve the hybrid layer through the inhibition of MMPs [4] and cysteine cathepsins.[7]Dentinal collagen fibrils may undergo degradation by MMPs if they are not fully enveloped by resin.[8] The use of CHX-containing aqueous primer results in the preservation of dentin bond strengths and the integrity of the hybrid layer with time, [9,10] similarly to when CHX is included in the composition of commercial phosphoric acid etchants, [11] or incorporated into the composition of some bonding resins.[12] However, in spite of the **demineralized dentin being able to bind more CHX than mineralized dentin,** rinsing with water easily removes CHX, as opposed to ethanol or even HEMA, which do not remove as much CHX from demineralized dentin.[13] The dissolution with water may preclude the future use of CHX in the composition of phosphoric acid gels.

EDTA - self-limiting decalcifying agent, decalcifies smear layer-covered dentin superficially. EDTA acts as a chelating agent, reacting with calcium ions from dentin hydroxyapatite and forms soluble calcium salts.[14] As EDTA is an effective Zn2+ and Ca2+ chelator,[15] it might inhibit MMP activity. In fact, EDTA has inhibitory effect against human dentin MMP-2 and MMP-9 when applied for 1 to 5 minutes. [16] The use of EDTA has been suggested as a dentin pretreatment for dentin adhesives. The results showed an increase in dentin bond strength, as compared with phosphoric acid and other types of dentin-conditioning agents. EDTA also preserved the dentin-adhesive interface in longevity tests.[17,18,19] One drawback is that EDTA is removed from dentin by extensive rinsing with water. There may be no residual EDTA left to inhibit the activity of MMPs.[16,20] It has also been showed that collagen in EDTA-demineralized dentin was as susceptible to MMP degradation as collagen in dentin etched with phosphoric acid.[21] Therefore, it is not clear if the preservation of hybrid layer from EDTA is a result of a shallow dentin demineralization or from MMP inhibition. Future studies are needed to test this hypothesis. Intermolecular crosslinks are the basis for the stability, tensile strength, and viscoelasticity of the collagen fibrils.[22]

Several synthetic and natural chemicals have the ability to increase the number of covalent inter- and intra-molecular collagen cross-links and affect its properties.[23]

Tannic acid, a commercial form of condensed tannin, is a naturally occurring polyphenol with weak acidity that has the ability to modify collagen chemically.[24] Alterations to the collagen, mostly by modification in the number of crosslinks, provide the collagen matrix with enhanced mechanical properties and lower rates of enzymatic degradation. These two properties are desirable for dentin collagen as a component of tooth restorations. Tannic acid is a naturally occurring collagen cross-linking agent consisting of a complex mixture of polygalloylglucose esters. Increased mechanical properties of collagen films after the use of TA has been reported.[25]

Resistance to collagenase digestion is a crucial property of a TA-collagen matrix, since it indicates an increase in stability and possibly a protection mechanism against degradation over a long period of time. Structural and dynamic changes in TA-collagen interactions have been characterized by infra-red spectroscopy in TA-stabilized pericardial tissue. Hydrogen

bonds are formed between amide NH groups from collagen and hydroxyl groups from TA.[24]

It can be expected that changes to dentin matrix would be similar to other type I collagen-based tissues; therefore, the formation of hydrogen bonds by TA is most likely accountable for the changes to dentin modulus of elasticity. TA treatment inhibited the action of collagenase, either by masking the cleavage sites or decreasing the enzymatic activity. Bacterial collagenase is a potent enzyme that has the ability to cleave collagen molecules at different sites. We speculated that the TA hydrogen-bonded to multiple sites on collagen molecules, and reduced possible cleavage sites and stabilized the TA-dentin matrix complex. In all demineralized dentin matrices, collagenolytic matrix metalloproteinases are bound to collagen. These endogenous MMPs may also be inhibited by TA. Hence, clinically exposed collagen would be subjected to degradation by endogenous MMPs which act in specific sites of the collagen molecule.[10,26]

The ability of TA-dentin matrix complexes to increase the mechanical properties of dentin, reduce enzymatic degradation, and increase resin-dentin bond strength demonstrate the utility of incorporating tissue-engineering/biomimetic approaches into restorative dentistry.

The use of biologically inspired mechanisms such as increased collagen stiffness by the presence of collagen cross-links and hydrogen bonds is possible by the use of naturally occurring products, such as tannic acid.

CONCLUSION:

Within the limits of this study, it was found that the use of 20% Tannic acid as a dentin priming solution after acid etching would prove as an effective alternative of the other MMP inhibitors tested in the present study, so it serves as an alternative to retarding the degradation of resin-dentin interfaces.

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