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Original Research Article

Efficacy of dentin bio-modification with quercetin-bromelain on shear bond strength of teeth restored with MICR technique

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ABSTRACT

Context: Dentin biomodification with natural extracts is done to improvise the bond strength with chemomechanical caries removal technique**.**

Aim: To evaluate and compare the efficacy of dentin bio-modification with quercetin-bromelain on SBS of teeth restored with Minimal Invasive Caries Removal (MICR) technique

Materials and Methods: 48 extracted teeth with class I dentinal caries were divided into 2 followed by subdivided into 2 subgroups in each group (n=12).

Group1: Without dentin biomodification

Caries removal done with No.4 round bur (1a) & V-carie-solve (1b)

Group2: With dentin biomodification (Quercetin-Bromelain)

Caries removal done with No.4 round bur (2a) & V-carie-solve (2b) followed by dentin biomodification

After composite restoration, samples were subjected to immediate SBS analysis. Two samples were examined under SEM from each subgroup.

Statistical analysis: Kruskal Wallis test with Bonferroni's correction for multiple tests was used to assess the mean difference between the groups.

Results: Dentin biomodification with mechanical and MICR technique showed better bond strength. Dentin biomodification with mechanical caries removal showed highest SBS.

Conclusion: Dentin bio-modification with Quercetin-Bromelain could improve bond strength with MICR technique.

1. Introduction

Dentin bonding plays a crucial role in aesthetic resin restoration.[1] Because dentin has a heterogeneous structure and content, resin-dentin bonds are typically less durable than resin-enamel bonding. Microleakage, staining, recurrent caries, and post-operative sensitivity are the outcomes of resin-dentin bond failure, and the combination of these conditions might hasten the bond's degradation.[2] Enhancing the hybrid layer's collagen fibrils' mechanical and chemical resilience may be crucial from a therapeutic standpoint to prolong the longevity of the resin-dentin link. Dentin biomodification can be used to stabilize dentin collagen using biocompatible cross-linking agents to improve mechanical qualities and reduce enzymatic degradation using matrix metalloproteinase inhibitors (MMPI).[2]

Matrix metalloproteinases(MMPs) are present in the dentin substrate as an inert form. The pH adjustments can modulate the activation and expression of MMPs that cause the degradation of the collagen fiber.[3] Both synthetic (carbodiimide, glutaraldehyde, CHX, tetracyclines,

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quaternary ammonium compounds, ascorbic acid) and natural (quercetin, baicalein, catechins, oligomeric proanthocyanidins, genipin, hesperidin, and other polyphenols) products can be used to biomodify dentin through collagen cross linking and MMP inhibition.[2] As a member of the flavanol family, quercetin is frequently found in red wine, onions, apples, and tea. Antioxidant, anticarcinogenic, anti-inflammatory, anti-aggregatory, and vasodilating properties are only a few of the many roles quercetin plays.[4] Proteases, such as bromelain, are a class of enzymes that break down proteins. They are derived from the pineapple fruit and its stem (Ananas comosus) and are sold commercially. It functions as a deproteinizing and collagen cross-linker.[5]

Recently, definite studies have evaluated the effects of deproteinization with bromelain enzymes on the improvement of the shear bond strength of restorative materials to dental structure.[6] Chemomechanical Caries Removal (CMCR) technique involves the application of solutions or gels that selectively removes the softened, infected dentin, which aids in enhancing the ease of manual caries excavation. This technique provides a patient-friendly aspect to the treatment of dental caries, which can be a windfall for anxious, medically compromised patients and children.[7] Habib et al., used Sodium hypochlorite for the removal of carious dentine.[8]

CMCR method was first based on few studies by Goldman and Kronman with the introduction of GK-101, followed by GK-101E in 1980, which was marketed as a Caridex system.[9] Recently V-Caries Solve was introduced by VISHAL DENTAL, INDIA which is a papain enzyme based gel formulation that allows the atraumatic chemomechanical caries removal and allows the maximum preservation of healthy dental structures. V-CARIE- SOLVE contains a refined enzyme derived from the plant “Caprica Papaya” (papaya) along with added benefit of therapeutic essential oils. This gel system is specially designed for eradicating the carious dentin by using only the hand instruments and minimizing the need of rotary instruments.

Majority of the studies used Quercetin and Bromelain individually as primer after mechanical caries removal. None of the studies evaluated the effect of Quercetin and Bromelain suspension biomodification on the chemo-mechanical caries removed dentin. So, the aim of the study was to evaluate the efficiency of Quercetin-Bromelain biomodification on shear bond strength of Chemo-mechanical Caries removed dentin. Here, the Null hypothesis stated that there is no effect of Quercetin-Bromelain biomodification on bond strength.

2. Materials and Methods

The study was conducted after assimilating ethical approval from Institutional Ethical Committee (IES) with reference No. KIDS/IES/2022/21. Forty-eight freshly extracted human premolars and molars with carious lesions involving enamel and dentin were selected for the study which was extracted attributable to periodontal reasons (Figure 1a). This study did not include teeth with restorations, abrasions, cracks, or attrition. The samples were cleaned with an ultrasonic scaler and then thoroughly cleaned with pumice slurry and a rubber prophylactic cup rotating slowly in the contra-angled micromotor handpiece. They were then kept at room temperature in a solution containing 0.1% thymol (p H 7) in distilled water until the experiment started. In order to guarantee that an occlusal surface was installed parallel to the acrylic resin with the cementoenamel junction (CEJ) 2 mm higher than the acrylic resin surface, the samples were then cleaned, softly dried, and embedded in acrylic resin.

Teeth were randomly apportioned into 2 experimental groups which were further divided into 2 subgroups congenial to the caries removal techniques and dentin biomodification.

Group1: Control group

Subgroups: 1a(n=12) - Caries removal done with No.4 round carbide bur – using highspeed handpiece and coolant (Figure 1b)

1b(n=12) - Caries removal done with V-carie-solve (Vishal Dentocare, India) (CMCR). The V-Carie solve solution was placed on the carious lesion, left for 60seconds and excavation was done with spoon excavator (HU-FRIEDY)(Figure 1e). Caries indicator dye (Caries Detector, Kuraray Co, Ltd) was used according to manufacturing instructions to ascertain if any caries was left behind. If required, reapplication of the CMCR solution was done for another 60seconds and further caries excavated. Later etching and bonding was done accordingly and composite restoration was done using incremental technique.

Group2: Dentin bio-modification group (Quercetin-Bromelain ethanol suspension)

Preparation of Quercetin and Bromelain with Ethanol for Surface Pre-Treatment: Quercetin- 500mg per capsule and Bromelain-3000 GDU per capsule (HEALTHVIT, INDIA) were collected (Figure 1c&d). The capsules in powder form were mixed in the ratio of 6:1 and 10ml of 99% ethanol was added since Quercetin and Bromelain is barely soluble in water. It was heated at 60 degree centigrade to prepare 1% QUERCETIN-BROMELAIN ethanol suspension as explained in the previous article.[1]

2a(n=12)- Caries removal done with No.4 round carbide bur (dentin biomodification)- After complete Caries removal, 37% phosphoric acid etching (EAZETCH, ANABOND) was done for 15seconds and rinsed with water for 20-30seconds. Biomodification was done with the application of Quercetin-Bromelain ethanol solution (1%wt) with an applicator tip by rubbing on acid-etched dentin surface for 1 min and gently dried with filter paper before application of the adhesive. Bonding agent(ONE

COAT, Coltene) was applied using all over the cavity and light cured for 20seconds and cavity was filled with nano-composite (BRILLIANT NG, Coltene) with incremental technique.

2b. Caries removal done with V-carie-solve (dentin biomodification)- After the CMCR as mentioned in Group 1b, dentin biomodification was done with the application of Quercetin-Bromelain ethanol solution (1%wt) with an applicator tip by rubbing on acid-etched dentin surface for 1 min and gently dried with filter paper before application of the adhesive. Restoration was done as explained in 2a group.

In a Universal testing equipment (MICRO MACH TECHNOLOGIES S1300, Nanowatts Technologies, Bengaluru), the immediate (24-hour) shear bond strength of every sample from every subgroup was assessed. The jig was used to position and stabilize the specimens before applying a straight knife edge rod (2.0 mm) at a cross head speed of 0.5 mm/min to the tooth-restoration contact. The sample was loaded up until the point of restoration failure. By dividing the load at failure by the specimen's surface area, the force was converted to shear bond strength (MPa).

2.1. SEM analysis

Dentin blocks were obtained by sectioning two samples, one for each sub-group, across the coronal and root portions. The pre-treatment and bonding steps were the same as those used to prepare the Shear Bond Strength (SBS) sample. The whole area of the dentin that was exposed was restored using composite material. After being fixed for 24 hours in a 10% formalin solution, the samples were further decalcified for 30 seconds in 6 N HCl. The samples were decalcified, rinsed in distilled water, and then deproteinized by immersing them in 1% NaOCl for 10 minutes. Finally, they were rinsed in distilled water once more. After that, samples were coated with gold using a gold sputtering unit, and the resin-dentin interfacial adaptation was examined using a scanning electron microscope (LEO 430, England).

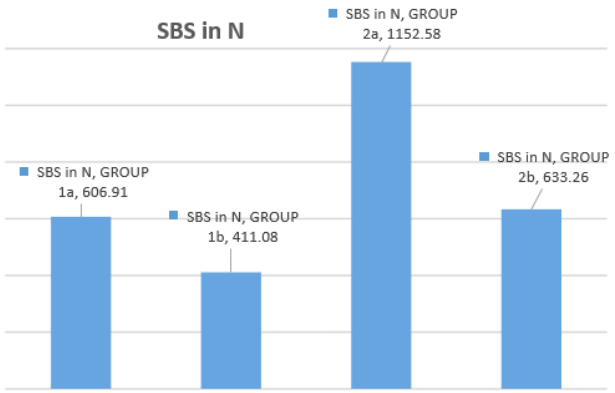
2.2. Statistical analysis

The data were described using descriptive statistics like mean and standard deviation. A non-normal data distribution was shown using the Shapiro-Wilk test, which was used to evaluate normality. For multiple tests, the Kruskal Wallis test with Bonferroni's correction was employed to assess the mean difference between the study groups. SPSS v25 software was used for all analyses, with a significance level of 5%.

3. Results

3.1. Shear bond strength analysis

Mean SBS values (MPa) of all the groups are presented in Graph 1. The Quercetin-Bromelain pretreatment biomodification groups (group 2A and 2B) showed higher bond strength and significant difference than groups without pretreatment/biomodification (group 1a and 1b). Caries removal done with No.4 round carbide bur and Quercetin-Bromelain dentin biomodification group (group 2a) showed the highest SBS values. Group 2b (CMCR with dentin biomodification) showed better SBS than control group (1b) with significant difference. Mechanical caries removal groups (1a&2a) showed significant higher SBS compared to CMCR groups (1b&2b). There was no statistical significant difference between Group 1a and Group 2b (Table 1).



Graph 1: Mean SBS values (MPa) of experimental groups

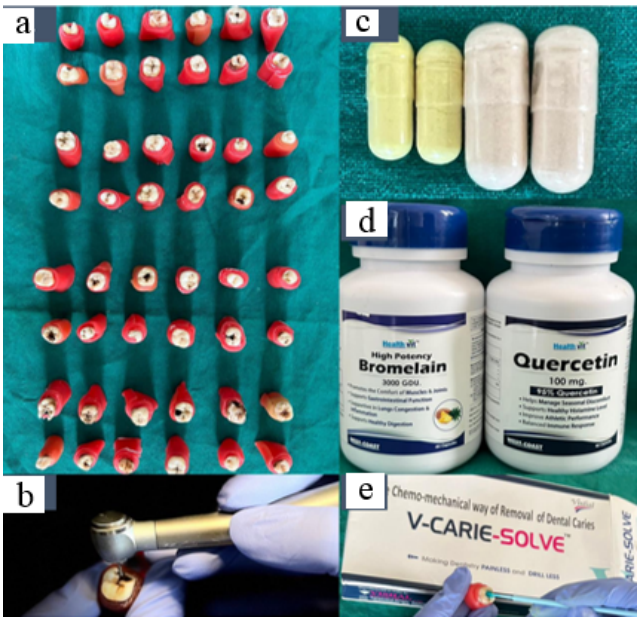


Figure 1: a: Samples (48 extracted teeth); **b:** Caries removal with size 4 round carbide bur; **c:** & **d:** Quercetin and Bromelain capsules; **e:** Chemo-mechanical caries removal with V-Carie solve

Table 1: Comparison of mean SBS values (in MPa) of four subgroups.

(I) Group	(J) Group	Mean Difference (I-J)	Test statistic	p-value
1a	**1b**	211.083	2.938	**.020***
	2a	-530.417	-3.230	**.007***
	2b	15.250	-.029	1.000
1b	**2a**	741.500	6.168	**<.001***
	2b	-195.833	-2.967	**.018***
2a	**2b**	545.667	3.201	**.008***

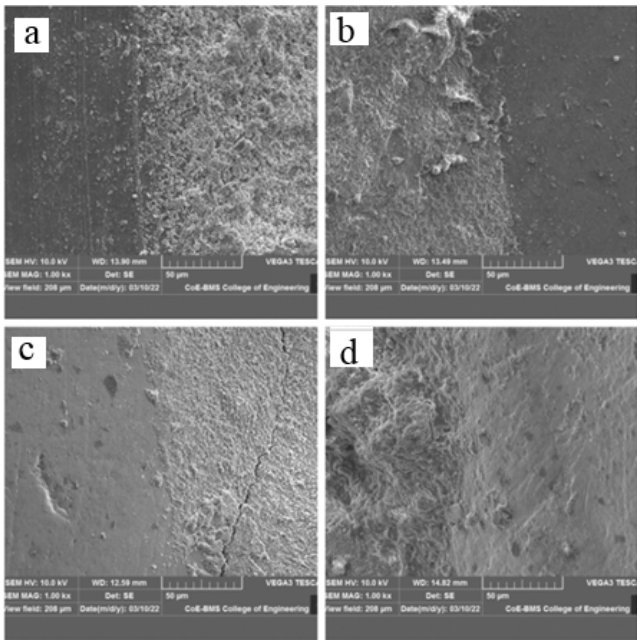


Figure 2: SEM Analysis:Without dentin biomodification; **a:** (No.4 round diamond bur) & **b:** (V-carie-solve). With dentin biomodification; **c:** (No.4 round diamond bur) & **d:** (V-carie-solve)

3.2. SEM analysis

Exhibited better resin-dentin interface in groups with dentin biomodification Quercetin-Bromelain (Figure 2). The morphology of residual dentin in the CMCR group showed a rough and irregular surface with a smear layer. SEM images of the mechanical caries removal group showed less irregularity and a thin smear layer (Figure 2). In some areas, a cracked surface was observed.

4. Discussion

Salivary enzymes may hasten the degradation of the hybrid layer, which is thought to be the primary cause of insufficient bonding stability in adhesive restorations. Enzymes that break down the collagen in the hybrid layer may make the exposed resin matrix more prone to hydrolysis. In addition to external enzymes such as bacterial collagenase, indigenous enzymes such as MMPs are also crucial in the degradation of dentin collagen.[1] Newer hybrid layer stabilization techniques that will stop the resin–dentin bond from deteriorating over time are needed to solve this issue. By applying artificial or natural cross-linking chemicals to the demineralized dentin, it is possible to inactivate the enzymes' catalytic site and enhance the mechanical characteristics of the dentin collagen, thereby stabilizing the hybrid layer. As a result, the resin-dentin interface is more stable and cross-linked dentin collagen is less susceptible to enzymatic destruction by collagenases.[10]

Chemo-mechanical caries removal (CMCR) is a non-invasive method that uses a chemical substance to remove diseased soft dentine. This technique removes soft carious structure without drilling by using a chemical agent and an atraumatic mechanical force.[11] It was first used in dentistry as a substitute for burs and local anesthesia in the removal of dental caries. It is primarily recommended to avoid these problems, resulting in less pain for patients and the preservation of healthy tooth structure, in line with the principles of minimally invasive dentistry (MID).[12]

V-CARIE-SOLVE (VISHAL DENTO CARE), a pure enzyme derived from the plant "Caprica Papaya" (papaya), is the chemo-mechanical caries elimination agent utilized in this study. It also contains preservative colors, clove oil, chloramine, and papaya extract (Papain). The primary component of V-Carie Solve, papain, is an enzyme with bacteriostatic and bactericidal qualities that resembles human pepsin. Since A1 antitrypsin, a plasmatic anti-protease that is only found in healthy tissues and inhibits protein digestion, is absent from infected tissues, Elindt's research showed that papain only operates on infected tissues. Papain can break partially damaged molecules because diseased soft dentin lacks this A1 antitrypsin enzyme.[13]

By causing bacteria to dissolve dentine minerals, papain interacts with exposed collagen to soften diseased dentine, facilitating its removal with non-cutting tools and avoiding the need for burs and local anesthetic.[14] Patients who are nervous, have large cavities, carious lesions that are encroaching on the pulp, and have teeth that need indirect pulp capping can benefit from it.[15] Caries Affected Dentin (CAD) bonding is always regarded as extremely difficult.[16] According to reports, the bond strengths to CAD are 20%–50% lower than those to sound dentin.[17]

Better outcomes in the removal of soft carious dentin were observed in earlier investigations with PAPACARIE,

a CMCR compound including papaya extract, chloramine, and toluidine blue.[16] Therefore, in order to assess the SBS and SEM analysis, a novel CMCR approach (V-CARIE-SOLVE) was employed in this study in contrast with a classic caries removal method (round carbide bur) with dentin biomodification.

In this study, carious molars with cavities that extended over the middle third of the dentin were chosen, and there was still 1.5–2 mm of dentin available for bonding. In order to replicate a clinical setting, caries excavation was restricted to removing the outermost layer of severely denatured and infected dentin while leaving the innermost layer of caries-affected dentin, which still has a high potential for remineralization.[16]

Collagen fibrils become unprotected and susceptible to degradation by endogenous matrix metalloproteinases (MMPs) and cathepsins due to reduced resin monomer penetration in acid-etched dentin and subsequent resin leaching from hydrolytically unstable polymeric hydrogels in the hybrid layers, according to earlier research. Thus, two approaches can be combined in an effort to stabilize the organic matrix. First, exogenous MMP silencers/inhibitors are introduced to block the proteolytic activity and enzymatic destruction of the organic matrix by the MMPs. Secondly, employing cross linkers to enhance the strength of the collagen fibrils' connection can help preserve the collagen by hiding its active sites of cleavage and strengthening its resistance to enzymatic degradation.[18] Therefore, it was thought that using exogenous MMP inhibitors, such as quercetin and bromelain in a 6:1 ratio, might improve the durability of adhesive restorations.

The additive impact of pretreating adhesive–dentin bonding with a quercetin–bromelain/ethanol solution was assessed in this work. The outcomes demonstrated that the bonding capacity of carious damaged dentin was enhanced by pretreatment with quercetin-bromelain/ethanol solutions. Consequently, the null hypothesis was disproved.

The most likely explanation for the increased bond strength is that quercetin inhibits MMP 2 and MMP9, which are found in dentin and may be exposed and activated by acid etching.[1] In order to maintain the collagen fibrils extended before adhesive application, it also serves as a collagen cross-linker. Furthermore, quercetin possesses hydrophobic properties that may alter the dynamics of water in the dentin matrix through interactions with collagen that heighten hydrophobicity and produce variations in water content.[1] By effectively removing the collagen network from acid-etched dentin, bromelain functions as a deproteinizing agent. This increases the potential for monomer diffusion into the undamaged dentin, reducing nano-leakage.[5]

Vijayababu and colleagues used an in situ zymography test to assess quercetin's capacity to inhibit MMP. According to the findings, quercetin at 1.0 weight percent reduced MMP activity in the hybrid layer. Quercetin has hydrophobic properties, cross-linking actions, and MMP inhibition, which provide it a promising primer in preserving dentin bond strength and hybrid layer integrity.[1]

Dayem and Tameesh evaluated the bromelain enzyme's deproteinizing capacity. The application of bromelain enzyme was shown to result in the elimination of the collagen network from acid-etched dentin and a considerable reduction in the adhesive system's global leakage scores.[19]

Dentin biomodification using the CMCR approach demonstrated a stronger connection than the group that did not use dentin biomodification; this could be attributed to the previously mentioned synergistic effects of a 1% pretreatment of quercetin and bromelain. The findings align with the research conducted by Govil et al., which involved treating carious-affected dentin with 6.5% grapeseed extract (Quercetin), 2% green tea extract, and 5% glutaraldehyde, leading to an increase in μSBS.[17]

In comparison to other groups, the dentin biomodification group (quercetin-bromelain 1%) using a bur and mechanical caries removal technique demonstrated the strongest connection. Because there was carious damaged dentin in the chemomechanical caries removal groups, the shear bond strength was lower than in the mechanical caries removal groups. The most likely explanation is that V-Caire solves only soft dentin removal. Because dentin tubules affected by caries often contain acid-resistant mineral casts made of b-tricalcium phosphates (whitlockite), which are more challenging to remove with a bur, the surfaces of these tubules are often rougher and more difficult for resin monomers to penetrate.[16] In terms of completely removing cavities, Kitsahawong K said that CMCR was just as effective as drilling; however, it took longer to excavate and produced less microhardness.[20]

In our work, we used SEM analysis to assess the resin-dentin interface subsequent to dentin biomodification and CMCR method (Figure 2).

The results of our investigation do not support the findings of other research that found that the traditional mechanical caries removal technique was inferior to CMCR with papain-containing gel in terms of shear bond strength.[21] Therefore, we recommend conducting additional research to enable the drawing of more trustworthy findings.

5. Limitations

As this is an in-vitro study, actual clinical conditions could not be simulated. Further in vivo studies over longer periods are needed to substantiate the same.

6. Conclusion

Dentin biomodification using Quercetin-Bromelain ethanol suspension outperformed the control groups in terms of

resin-dentin interface and shear bond strength, within the parameters of the study. Compared to the chemo-mechanical caries removal procedure, mechanical caries removal with bur demonstrated a better bond strength.

7. Source of Funding

None.

8. Conflict of Interest

None.

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