Effect of casein phosphopeptide-amorphous calcium phosphate, Riva Star, and *Moringa oleifera*-based nano-silver fluoride on caries affected dentin remineralization bonded to composite resin

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Abstract. – OBJECTIVE: **This study aims to assess the effects of the most recent remineralizing agents, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), CO_2 laser irradiation + topical fluoride (CO_2 laser + TF), and Nanosilver fluoride - *M. oleifera* (NSF-MOLE), on the shear bond strength (SBS) and bond failure between resin composite and remineralized caries affected dentin (CAD).**

MATERIALS AND METHODS: Fifty human molars with occlusal caries reaching approximately halfway through the dentin were immersed in a 4% thymol solution. The infected dentin was removed using an excavator and the CAD surface was exposed. The sample was allocated into five groups (n=10) based on the remineralizing agent applied. Group 1: no remineralizing agent, group 2: CPP-ACP, group 3: Riva Star, group 4: NSF:MOLE, and group 5: (CO_2 laser + TF). The shear bond testing procedure was conducted utilizing a universal testing machine and a stereo-microscope was used to study the failure pattern. The researchers utilized a one-way analysis of variance. The Tukey post hoc test was conducted for multiple comparison tests.

RESULTS: Group 4 (NSF-MOLE) (13.77±1.94 MPa) treated testers recognized the highest bond values of tooth color restoration to the CAD surface. Nonetheless, group 1 test samples with no mineralization unveiled the minimum outcome of bond integrity (9.12±1.14 MPa). Intergroup comparison exploration showed that group 2 (CPP-ACP), group 4 (NSF-MOLE) (13.77±1.94 MPa), and group 5 (CO_2 laser + TF) established comparable values of SBS. Furthermore, group 3 (Riva Star) displayed better SBS than group 1 but lower than group 2, group 4, and group 5.

CONCLUSIONS: Remineralization of CAD using modern regimes (CPP-ACP, NSF-MOLE, and CO_2 laser + TF has the potential to be used to enhance the bond strength of CAD to composite restoration.

Key Words:
Riva Star, Shear bond strength, Nanosilver fluoride, Carious affected dentin.

Introduction

Minimally invasive dentistry is characterized by employing conservative techniques for the removal of caries, aiming to preserve dental hard tissue that can potentially undergo remineralization[1,2]. After removing infected dentin, the remaining carious tooth structure is a combination of regular dentin around the edges and caries-affected dentin (CAD) at the lesion's center[3,4]. The mineral distribution on the surface of CAD shows significant variation, and the adhesion of restorative materials to CAD results in lower shear bond strength (SBS) compared to regular dentin. However, unlike denatured caries-infected dentin, the collagen matrix of CAD presents cross-banding patterns when examined under transmission electron microscopy (TEM)[5]. This collagen matrix in CAD has the potential to undergo physiological remineralization[3,6].

Biomimetic remineralization is a highly effective approach involving the elimination of diseased dental tissue, followed by the introduction of mineral content to mimic the characteristics of healthy tissue[7]. The emergence of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) as a remineralizing agent marked a significant advancement in treating the remineralization of CAD surface[8]. In contemporary dentistry, the combination of silver diamine fluoride (SDF) and potassium iodide (KI), marketed as Riva Star, has gained extensive acceptance. SDF is recognized for its efficacy in preventing and treating dental

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caries. It possesses both the bactericidal properties of silver and the remineralization benefits of fluoride[9]. The addition of KI is to safeguard dentin from potential SDF-induced staining. However, there is currently an absence of data regarding the impact of Riva Star on the bond strength of composite restorations adhering to CAD[10]. Further investigation in this area is warranted.

In modern dentistry, there has been a significant increase in the use of lasers. Previous experimental studies[11,12] have shown that applying CO_2 laser irradiation triggers a process of melting followed by recrystallization. This process enhances the dental hard tissues' resistance to acid. Furthermore, laser irradiation has been suggested as a potential method to enhance the effectiveness of various fluoride solutions. This includes improving the uptake of fluoride in enamel and facilitating the conversion of hydroxyapatite to fluorapatite[13]. Likewise, the medicinal plant *Moringa oleifera*, commonly known as Moringa, has garnered attention from researchers due to its proven effectiveness in promoting remineralization of enamel and dentin[13]. A novel formulation called *Moringa oleifera*-based nano-silver fluoride (NSF-MOLE) combines silver nanoparticles and fluoride, offering antimicrobial and preventive properties[14]. However, despite these advancements, no studies have yet been conducted to assess how these agents, acting as remineralizing agents, might impact the bond strength of composites to affected dentin[11].

Based on the existing literature, it can be asserted that there is a lack of sufficient data regarding the impact of various contemporary remineralizing agents (CPP-ACP, Riva Star, NSF-MOLE, and CO_2 laser + TF) on the SBS of tooth color adhesive restorative material to the CAD. Therefore, it is probable to propose a hypothesis that there will be no significant difference in bond values of composite restorations that are bonded to remineralized CAD using CPP-ACP, Riva Star, NSF-MOLE, and CO_2 laser + TF when compared to a group that does not utilize any remineralizing agent. Hence, the objective of the present study is to evaluate the impact of various remineralizing agents on the adhesive strength between resin composite and remineralized CAD surface.

Materials and Methods

Sample Preparation

Fifty human molars with occlusal caries reaching approximately halfway through the dentin were collected for the current study after obtaining written consent from the patients. The research was carried out in strict adherence to the guidelines outlined in the Declaration of Helsinki by the World Medical Association. The teeth underwent a cleaning procedure using an ultrasonic scaler. Subsequently, they were immersed in a 4% thymol solution (Sigma-Aldrich) at room temperature for 2-3 weeks before the experimentation[15].

After tooth disinfection, the infected dentin was removed using an excavator. This was then followed by flattening the tooth surface with a water-cooled low-speed cutting saw (Mecatome T201 A, Presi, Grenoble, France) by placing it perpendicular to the long axis of the teeth. CAD surface was confirmed with the help of tactile sensation and visual examination. The surface that exhibits discolored dentin and displays resistance to the excavation was classified as CAD. The diagnosis was further verified using a caries detector solution (Kuraray Co.) that was applied to the dentin surface according to the instructions provided by the manufacturer. This application was performed by a second operator who specializes in restorative procedures. Subsequently, the specimens were mounted within a polyethylene mold filled with self-cured acrylic resin (Acropars, Marlik Co.)[16].

Preparation of NSF-MOLE

Fresh *M. oleifera* (MOLE) plant was removed from the stems, rinsed with distilled water, and air-dried. The extract was prepared by adding 100 mL of deionized water to 5 g of fresh leaf in a 250 mL beaker, and the mixture was boiled for 15 minutes. The extract was cooled at room temperature, filtered, and utilized to synthesize silver nanoparticles. Afterward, 10 mL plant extract was added to 100 mL 1 mM $AgNO_3$ (Thomas Baker Pvt Ltd, India). The mixture was stirred and heated using a hotplate magnetic stirrer (Labinco, the Netherlands) for 20 minutes at 70°C. The aqueous solution turned yellowish-brown, indicating silver nanoparticle production. After the reaction, sodium fluoride (NaF; Riedel-de Haën, Germany; 10.104 ppm) was added to enhance stability and cariostatic efficacy. Stirring continued overnight and the final NSF-MOLE solution was kept in the dark until usage[14].

The allocation of the samples into five groups (n=10) was performed arbitrarily based on the remineralizing agent applied on the CAD surface.

Group 1: no remineralization

In this group, the CAD surfaces were left untreated with no application of remineralizing agent.

Group 2: CPP-ACP

In this group, a 35% phosphoric acid gel was smeared onto the CAD surface. It was then applied with CPP-ACP (MI paste; GC Corp, Tokyo, Japan) paste by vigorously brushing it for 3 minutes. Subsequently, distilled water was used to rinse the surface.

Group 3: Riva Star

35% phosphoric acid gel was smeared onto the CAD surface, which was followed by SDF application. The substrate was then treated with the immediate application of a substantial quantity of KI solution (Riva star, SDI Limited, Australia) using a micro brush. The application continued until the creamy white precipitate transformed into a clear state, after which the area was gently dried[9].

Group 4: NSF-MOLE

Samples were treated with 35% phosphoric acid gel followed by NSF-MOLE application. The surface was then washed with distilled water.

Group 5: CO_2 laser + TF

Samples were treated with 35% phosphoric acid gel followed by topical fluoride (TF) solution application, specifically Nupro Neutral™ (Johnson and Johnson, Inc., New Brunswick, NJ, USA), which contained 2.0% NaF (9,047 ppm). After 4 mins, the fluoride gel was thoroughly removed from the tooth surface using tissue paper. The surface was then irradiated with a CO_2 laser (PC 015-D CO_2 Laser System, Shanghai JueHua Laser Tech, Development Co., Ltd., Shanghai, China). The laser was operated in ultra-pulsed mode with an average power of 0.5 W, an energy density of 0.44 J/cm^2, pulse durations of 100s, and a 0.001-sec interval between pulses. The surface was then washed with distilled water[13].

Bonding Procedure

Following the remineralization protocol, a bonding agent (3M ESPE, Saint Paul, MN, USA) was applied and subsequently cured for 20 seconds using light curing equipment. A plastic tube with dimensions of 3 mm (internal diameter) and 2 mm (height) was positioned on the CAD surface. A 2 mm composite restoration (Filtek Z350; 3 M ESPE) was built using a plastic tube and successively subjected to light exposure for polymerization. The specimens were incubated in a humid environment at 37°C for 24 hours. Afterward, all samples were placed in an automated Thermal Cycler (manufactured by Applied Biosystems, CA, USA) to subject the specimens to a total of 8,000 cycles at temperatures of 5 and 60°C, with a dwell duration of 30 seconds.

SBS Analysis and Failure Mode Analysis

The shear bond testing procedure was conducted utilizing a universal testing machine (UTM) (Instron, Z020; Zwick Roell). The specimens were positioned within a jig, and a force was exerted on each sample in a direction parallel to the bonded interface. This force was applied at a crosshead speed of 1 mm/min until the point of failure was reached. The SBS was determined using the TestXpert II software (Zwick Roell). The stereo-microscope was used to study the failure pattern at 40x magnification. The fracture modes seen in the de-bonded testers were divided into three groups i.e., adhesive cohesive, and admixed[17,18].

Statistical Analysis

The normality of the distribution and homogeneity of variance of the values were assessed using the Kolmogorov-Smirnov test. The researchers utilized a one-way analysis of variance (ANOVA) and Tukey post-hoc test to perform multiple comparisons. The statistical calculations were conducted using SPSS software version 21 (IBM Corp., Armonk, NY, USA), with a significance level set at 0.05.

Results

Means and SD of bond strength values in MPa among different trial groups are presented in Table I and Figure 1. The outcomes of the study advocated that group 4 (NSF-MOLE) (13.77±1.94 MPa) treated study samples recognized the highest bond values of tooth color restoration to CAD surface. Nonetheless, it was detected that group 1 specimens with no remineralization protocol unveiled the minimum outcome of bond integrity (9.12±1.14 MPa). Intergroup comparison exploration showed that group 2 (CPP-ACP) (12.93±1.35 MPa), group 4 (NSF-MOLE) (13.77±1.94 MPa), and group 5 (CO_2 laser0 + TF) (12.25±1.26 MPa) established the comparable values of SBS (p>0.05). Furthermore, it was also detected that group 3 (Riva Star) (10.69±1.13 MPa) displayed better SBS than group 1 but lower than group 2, group 4, and group 5.

Failure modes among experimental groups are presented in Table II and Figure 2. It was observed that CPP ACP, CO_2 laser + TF, and NSF-MOLE displayed a cohesive type of failure predominantly. Riva Star and control demonstrated all three types of failure modes.

Table I. Shear bond strength of composite restoration after treating CAD with different remineralization protocol.

Remineralizing pretreatment	Mean	SD	*p*-value[I]
Group 1: No reminerlizng agent	9.12[c]	1.14	<0.05
Group 2: CPP-ACP	12.93[b]	1.35	
Group 3: Riva star	10.69 [b]	1.13	
Group 4: NSF-MOLE	13.77[b]	1.94	
Group 5: CO_2 laser + TF	12.25[b]	1.26	

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), CO_2 laser irradiation + Topical fluoride CO_2 (laser + TF), Nanosilver fluoride-*M. oleifera* (NSF-MOLE). The different small letters denote statistically significant differences. [I]Showing significant differences among study groups (ANOVA) (Tukey multiple comparison test).

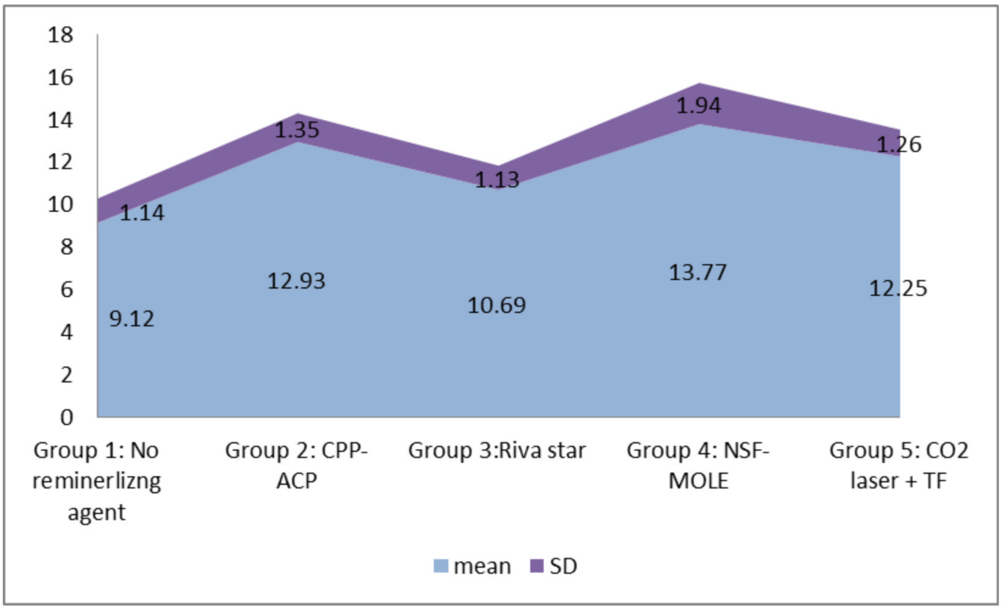

Figure 1. Variations in SBS of composite restorations subsequent to the treatment of CAD specimens with distinct remineralization protocols.

Discussion

The recent investigation was predicated on the assumption that there would be negligible disparities in the bond strength of composite restorations that adhere to remineralized CAD using different agents, namely, CPP-ACP, Riva Star, NSF-MOLE, and CO_2 laser combined with thermal cycling when compared to a group where no remineralizing agent was employed. However, the anticipated hypothesis was unequivocally refuted, as all the examined groups exhibited superior bond integrity of composite restorations compared to the non-remineralization group. The Universal Testing Machine (UTM) serves as the primary tool for assessing the mechanical attributes of both metallic and non-metallic materials. It finds extensive applicability across diverse sectors, including industrial and mining enterprises, scientific research institutions, universities, and other pertinent departments.

Based on the available data, it can be asserted that this study is the first laboratory-based trial designed to evaluate the effectiveness of NSF-MOLE as a remineralizing agent for CAD on SBS of composite restorations. The results obtained from this research indicated that the application of NSF-MOLE resulted in the highest SBS compared to all other groups under investigation. This observation is consistent with the findings of a meta-analysis conducted by Gao et al[19]. Moreover, the current study adopted a biological approach to develop NSF-MOLE. This green synthesis technique draws inspiration from the research conducted by Kadhem and Al Haidar[14], which, instead of conventional chemical synthesis methods, employs

Table II. Failure analysis after different remineralizing agents.

Study Groups	Adhesive (%)	Cohesive (%)	Admixed (%)
Group 1: No remineralizing agent	70	20	10
Group 2: CPP-ACP	/	90	10
Group 3: Riva star	60	20	20
Group 4: NSF-MOLE	-	90	10
Group 5: CO_2 laser + TF	10	80	10

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), CO_2 laser irradiation + Topical fluoride CO_2 (laser + TF), Nanosilver fluoride- *M. oleifera* (NSF-MOLE).

Moringa oleifera leaf extract to synthesize silver nanoparticles[14,20]. This technique is straightforward and environmentally friendly. The positive outcomes can be attributed to the fluoride content incorporated into the NSF-MOLE formulation, which is primarily responsible for enhancing the remineralization potential of caries-affected dentin.

Likewise, the utilization of CPP-ACP as a remineralizing agent yielded similar outcomes in terms of bond integrity when compared to NSF-MOLE. CPP-ACP, derived from casein milk protein, is a calcium phosphate complex[21]. It is noteworthy that this complex lacks fluoridation and crystallinity. Through its interaction with plaque and hydroxyapatite, CPP-ACP enhances the localization and availability of essential components such as calcium, phosphate, and fluoride[7]. Prior investigations[22,23] have substantiated the efficacy of CPP-ACP in significantly augmenting the microhardness of early enamel and root surface caries lesions. Moreover, the results also unveiled that the combined application of CO_2 laser irradiation with thermal cycling TF yields a bond integrity improvement on par with CPP-ACP and NSF-MOLE. Earlier research[24,25] has provided supporting evidence for the present study's findings and has expounded upon the synergistic impact achieved through the combination of laser irradiation and fluoride application. This combination effectively restrains enamel demineralization while facilitating the transformation of hydroxyapatite into fluorapatite.

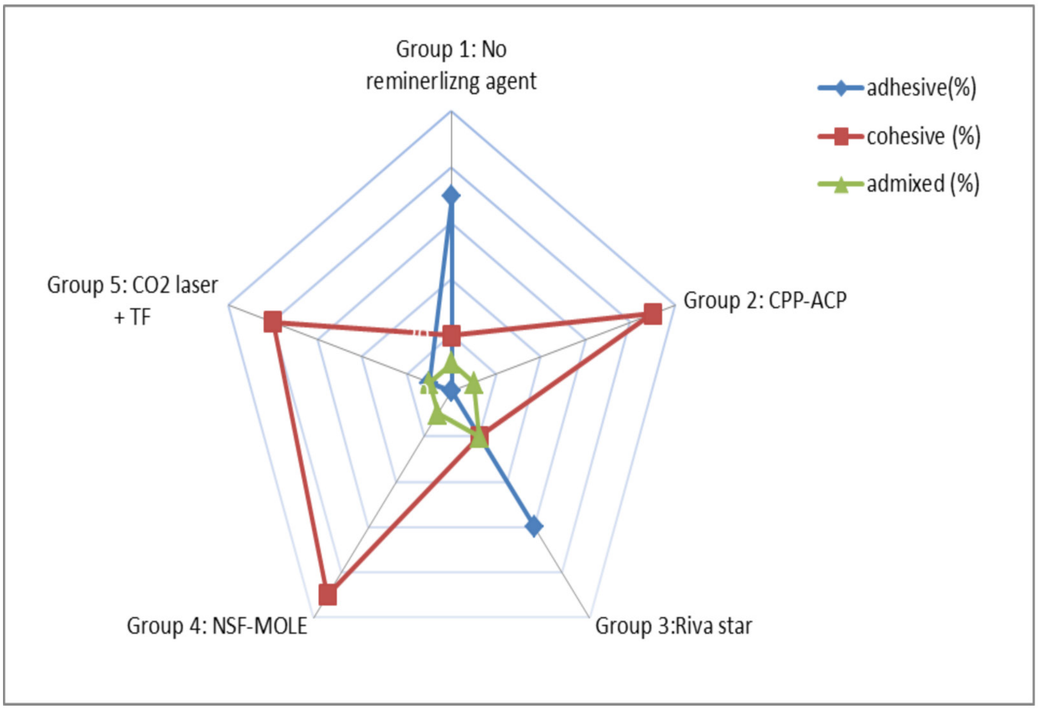

Figure 2. Analysis of failures following the application of various remineralizing agents.

Regarding Riva Star, it was observed that the tested samples displayed significantly lower outcomes of SBS than CPP ACP, CO_2 laser + TF, and NSF-MOLE but significantly higher than the control. A study by Abdullah et al[9] reported that KI possesses no antibacterial activity as compared to SDF and SDF/KI. Furthermore, they have also stated that SDF and SDF/KI possess potent antibacterial efficacy without any statistically significant difference between the two. Chhattani et al[26] and Yu et al[27] and coworkers investigated the effect of SDF vs. Riva Star on the microhardness of carious and sound dentin in permanent teeth. They explained that both display comparable outcomes of remineralization. However, data related to the effect of Riva Star is scarce and needs investigation.

Regarding failure mode, it was observed that CPP ACP, CO_2 laser irradiation + TF, and NSF-MOLE displayed the cohesive type of failure predominantly. Cohesive failure within the materials can occur due to factors such as strong material properties, uniform composition, effective adhesion, compatible mechanical properties, uniform stress distribution, and synergistic effects of combined treatments[28,29]. These factors collectively contribute to a cohesive bond that resists failure at the interface, whereas Riva Star and control demonstrated adhesive failure type.

This study has certain limitations. Notably, the challenge of accurately replicating the intricate oral environment, including elements like salivary biofilm and oral flora. Furthermore, the study overlooked the wide spectrum of salivary components, individual dietary patterns, and oral hygiene habits. Consequently, it is advised to undertake additional research, both in controlled laboratory conditions (*in vitro*) and real-world situations (*in vivo*), to comprehensively explore the potential effectiveness of these new regimens in dental restorative procedures. Moreover, it is crucial to extend the analysis of CAD surfaces that have undergone remineralization pretreatment. This thorough assessment should utilize advanced methodologies like the Atomic Force Microscope (AFM) and Scanning Electron Microscope (SEM).

Conclusions

Remineralization of CAD using modern regimes (CPP-ACP, NSF-MOLE, and CO_2 laser +TF) has the potential to be used to enhance the bond strength of CAD to composite restoration.

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Ethics Approval
The Ethics Committee of Specialist Practice and Research Center reviewed and approved the study protocol (UD-CRC-FC-8997-3).

Conflict of Interest
The authors declare no conflict of interest.

Authors' Contributions
Conceptualization, methodology, software, validation, formal analysis, investigation data curation, writing-original draft preparation, and writing-review and editing, visualization, supervision, and project administration, funding acquisition performed by Fahad Alkhudhairy, Yasser AlFawaz.All authors have read and agreed to the published version of the manuscript.

Informed Consent
All patients gave consent for their participation.

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