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Bonding Strategies to Stabilize and Reinforce the Compromised Dentine Structure Using Cross Linking Agents

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Abstract:- Dentin is a critical component in adhesive restorations, and its bonding characteristics differ from those of enamel. Dentin organic tissue that contains collagen fibrils, hydroxyapatite crystals, and water. Unlike enamel, dentin bonding can be more challenging due to its composition and structure.

Several factors contribute to the deterioration of dentin bonding over time, such as the presence of water, collagen degradation, and enzymatic activity. Additionally, in clinical situations where caries affected dentin is present, the bond strength may be compromised due to changes in the structure of the dentin.

To address these challenges and enhance the durability of adhesive restorations on dentin, researchers and clinicians have explored various techniques, and one of them involves dentin biomodification with collagen cross-linking agents. Collagen cross-linking agents are substances that help stabilize and strengthen the collagen matrix within dentin. By enhancing the integrity of the collagen network, these agents aim to improve the bond strength and overall performance of adhesive restorations.

Common collagen cross-linking agents include glutaraldehyde, formaldehyde, and various other chemical agents. These agents work by forming covalent bonds between adjacent collagen molecules, thereby increasing the resistance of the collagen matrix to degradation.

It's important to note that while dentin biomodification with collagen cross-linking agents shows promise in improving bond strength and durability, the long-term clinical success and safety of these techniques need further research and validation. Dentistry is a dynamic field, and ongoing research may lead to advancements in adhesive techniques and biomodification strategies for better outcomes in restorative dentistry.

This study aimed Compare and evaluate the effect of two collagen cross linking agent cocoa seed extract and Glutaraldehyde on microshear bond strength of caries affected dentin – resin complex.

➢ ***Materials and Methods***

- **Thirty freshly extracted teeth with caries involving dentin were used. The roots of 30 molars were standardized and embedded in acrylic resin. Enamel was removed from all occlusal surfaces using a diamond saw disk in a high-speed handpiece under copious air-water spray. Acid etching with 37% phosphoric acid gel was performed for 15 seconds, followed by thorough rinsing with water. The samples were divided into two groups:**
- **Group 1 (n = 15): Treated with 6.5% cocoa seed extract.**
- **Group 2 (n = 15): Treated with 5% glutaraldehyde.**
- **Group 1: 6.5% cocoa seed extract was applied on caries-affected dentin for 10 minutes, rinsed with water, and blot dried.**
- **Group 2: 5% glutaraldehyde was applied for 10 minutes and blot dried.**

Composite was built up with a thickness of 4mm on caries-affected dentin.

Each sample was tested under a universal testing machine to measure micro-shear bond strength (μSBS). The recorded data was subjected to statistical analysis.

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➢ ***Results and Conclusion***

- **Within the limitations of the study, caries-affected dentin treated with 6.5% cocoa seed extract resulted in increased micro-shear bond strength (μSBS) when compared to 5% glutaraldehyde.**

I. INTRODUCTION

- Adhesive technology has gained popularity in modern dentistry due to the aesthetic benefits of composites and minimally invasive procedures[1].

➢ *Challenges with Dentin Bonding:*

- Dentin, being primarily composed of a wet tissue and tubular structure, tends to experience faster bonding deterioration over time compared to enamel.
- The use of collagen cross-linking agents in dentin biomodifications is explored to enhance the longevity of adhesive restorations, especially when dealing with caries-affected dentin in clinical settings.[2]
- The dentin organic matrix, composed of partially or completely demineralized collagen fibers, relies heavily on the infiltration of synthetic polymers from the adhesive system.
- The resulting dentin-polymer mixture is referred to as the hybrid layer.[3]

➢ *Differences in Caries-Affected Dentin:*

- Caries-affected dentin differs morphologically, chemically, and physically from normal dentin.
- The hybrid layer created in caries-affected dentin is thicker than that of normal dentin due to increased vulnerability to acid etching and partial demineralization.
- A deeper demineralized zone in caries-affected dentin makes it more challenging for resin monomers to penetrate, potentially reducing bond strength.[4]

➢ *Mineral Phase Changes in Caries-Affected Dentin:*

- The mineral phase of caries-affected dentin is mainly composed of carbonate-rich hydroxyapatite.
- Fourier-transform infrared imaging (FTIR) studies show that the mineral phase in caries-affected dentin is less crystalline and has a lower mineral content than normal dentin.[4]

➢ *Physical Characteristics of Caries-Affected Dentin:*

- Caries-affected dentin is softer than normal dentin, even with mineral depositions occluding dentinal tubules.
- The water content in caries-affected dentin is higher than that in normal dentin, ranging from 14% to 53%.
- The hardness and ultimate tensile strength (UTS) of caries-affected dentin are lower than those of normal dentin.[4]

➢ *Endogenous Proteases and MMPs:*

- MMPs, including MMP-2, MMP-3, MMP-8, MMP-9, and MMP-20, are endogenous proteases that play a role in the degradation of collagen matrices.[5]
- These enzymes are dependent on calcium and zinc and are involved in the pathophysiology of periodontal diseases and tooth caries.[6]
- MMPs identified in mineralized dentin include MMP-2 (gelatinase), MMP-8 (collagenase), MMP-9 (gelatinase), and MMP-20 (enamelysin).[5]

➢ *Activation of MMPs during Acid Etching:*

- Acid etching, particularly in the etch and rinse method using phosphoric acid, releases and activates pro-MMPs trapped in mineralized dentin.
- Acid etching lowers the pH, leading to the activation of pro-MMPs and causing hybridized dentin to exhibit collagenolytic and gelatinolytic activity.[7]

➢ *Release of MMPs during Dentin Formation:*

- MMPs, released by connective tissue cells such as fibroblasts, osteoblasts, and odontoblasts, are initially in a pro or inactive form during dentin formation.[8]
- In carious lesions, acidic conditions (pH 4.5 or lower) can catalyze the activation of pro-MMPs to active enzymes.[9]

➢ *Role of Organic Acids in MMP Activation:*

- Lactic acid produced by bacteria in carious lesions can activate pro-MMPs, contributing to the degradation of collagen matrix during the caries process.
- Organic acids found in food or created by plaque may also play a role in the disintegration of the hybrid connection.[10]

➢ *Inhibition of TIMP-MMP Complex:*

- Acid etching can cause MMPs to become active by inhibiting tissue inhibitors of metalloproteinases-1 (TIMP-1) in the TIMP-MMP complex.[11]

➢ *Objective of Dentin Biomodification:*

- Various strategies for dentin biomodification aim to enhance cross-linking between collagen fibers, improving biomechanical properties, and reducing biodegradation[3]. Collagen cross-linking agents, whether physical or chemical, are proposed as adjuvants in restorative procedures to strengthen dentin collagen's structural stability by adding intra- and intermolecular linkages.[12] Synthetic agents such as glutaraldehyde (GA) and carbodiimide, as well as natural bioflavonoids like proanthrocynidins (PAs), are used for collagen cross-linking.
- PAs, a class of bioflavonoids, can be found in naturally occurring sources such as grape seeds, cashew nut shells,

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green tea, cocoa seeds, pine bark, black tea, cinnamon, and cranberries.[13]

- PAs activate proline hydroxylase, an enzyme essential for collagen synthesis, when they attach to proline-rich proteins like collagen.
- The resulting hybrid layer exhibits resistance to dentin collagen degradation due to its cross-linking capacity and anti-collagenolytic properties.[14]
- GA is frequently used to cross-link collagenous biomaterials as a fixative.
- The amino groups of collagen polypeptide residues react with the aldehyde groups in GA.[15]
- Cross-linking agents like GA have been shown to increase the tensile strength and elastic modulus of demineralized dentin.
- They also enhance the durability of composite restorations by reducing proteolytic degradation within the hybrid layer caused by activated endogenous matrix metalloproteinases (MMPs).[16]
- The study aims to assess and compare the impact of two collagen cross-linking agents, cocoa seed extract, and glutaraldehyde, on the microshear bond strength between dentin and resin in caries-affected conditions.

II. MATERIALS & METHOD

- Thirty freshly extracted human carious molars were selected from a pool of extracted teeth.The roots of the selected 30 molars were embedded in acrylic resin for standardization. Enamel of all occlusal surfaces was removed using a diamond saw disk inserted into a high-speed handpiece under copious air-water spray, resulting in caries-affected dentin (CAD).
- Acid etching with 37% phosphoric acid gel was performed on the exposed dentin surfaces.

➢ *Collagen Cross-Linking Agents Application:*

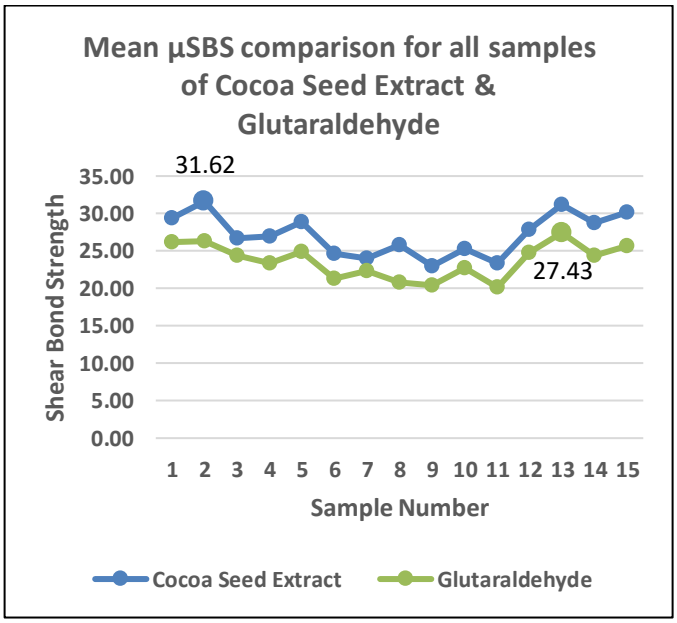
- Group 1 (n = 30): 6.5% cocoa seed extract was applied on CAD for 60 seconds using a microapplicator brush. The surface was then rinsed thoroughly with water for 10 minutes and blot dried.
- Group 2 (n = 30): 5% glutaraldehyde was applied for 10 minutes and then blot dried.
- After the completion of bonding, composite buildup was performed in all groups. For standardization of the samples for micro-shear bond strength (μSBS) testing, a composite build of 4mm in thickness was done on CAD.
- The composite was light-cured for 40 seconds.
- Each sample was fixed and mounted on the Universal Testing Machine.
- μSBS testing was conducted using a metal attachment placed as close as possible to the composite/dentin interface.

III. RESULTS

- One way ANOVA was done for inter group comparison

TABLE 1: MEAN AND STANDARD DEVIATION FROM MICRO SBS TEST

Group	n	Minimum	Maximum	Mean	SD
Cocoa	15	22.99	31.62	27.15	2.70
Glutar	15	20.14	27.43	23.67	2.30



GRAPH 1: INTERGROUP COMPARISON BETWEEN COCOA SEED EXTRACT AND GLUTARALDEHYDE

Graph showing intragroup comparisons in Group 1, the highest mean μSBS was of (31.62 MPa) followed by Group 2 (27.43 MPa).

IV. DISCUSSION

The challenges associated with bonding to dentin, highlighting various factors that make achieving effective adhesion a complex task.

➢ **Presence of Water and Organic Material:** Dentin is a complex tissue with a significant proportion of water and organic material, making it a challenging substrate for adhesion. Moisture control during bonding procedures is crucial to prevent the dilution of adhesives and to ensure proper bonding.

➢ **Smear Layer and Smear Plugs:** The mechanical and thermal processes involved in tooth preparation often create a smear layer on the dentin surface. This layer contains debris and can obstruct the penetration of adhesives. Efficient removal or modification of the smear layer is necessary for optimal bonding.

➢ **Degradation of Collagen Fibrils by MMPs:** Matrix metalloproteinases (MMPs) present in dentin can degrade

collagen fibrils over time. This enzymatic degradation can compromise the longevity of the bond. Inhibiting MMP activity or incorporating MMP inhibitors into adhesive systems is a strategy to address this issue.

- **Incomplete Infiltration of Resin Monomers:** Achieving complete infiltration of resin monomers into the demineralized dentin is crucial for a durable bond. Inadequate resin penetration can lead to gaps and weak interfaces. Optimizing adhesive formulations and application techniques is essential to improve resin infiltration.
- **Alteration in Dentin Structure:** Changes in dentin structure due to aging, caries, or pre-treatment with chemicals such as hydrogen peroxide (H2O2) and sodium hypochlorite (NaOCl) can affect the bonding properties. Adapting adhesive systems to accommodate variations in dentin conditions is necessary for successful and long-lasting bonds.[17]

Bond strengths to dentin affected by caries (CAD) are reported to be 20%–50% lower than those to sound dentin. This reduction in bond strength suggests that the altered composition and structure of dentin due to caries can significantly impact the adhesive performance. Dentin reactions to caries begin promptly after the enamel is affected. This implies that by the time restorative procedures are performed, the dentin may have undergone changes in composition and structure due to the carious process. Adhesive restorations in clinical settings are often bonded to dentin with varying degrees of alteration due to the progression of caries. The variability in the state of caries-affected dentin poses a challenge for relying on uniform bond strength values.

- **Limited Predictive Value of Bond Strengths to Sound Dentin:** The statement concludes that reported bond strengths to sound dentin have limited predictive value in clinical situations where caries-induced alterations are common. This underscores the need for a more nuanced understanding of adhesive behavior in the context of the specific conditions of caries-affected dentin.[18],[19]

MMPs are a family of over 20 host-derived proteolytic enzymes known for their ability to degrade various components of the extracellular matrix. In the context of dentistry, their presence is relevant to the degradation of collagen fibrils in dentin. The acid-etching procedure, commonly used in dentistry, may lead to the release and activation of pro-MMPs within mineralized dentin. This activation triggers collagenolytic and gelatinolytic activities within the hybridized dentin.

- **Impact on Resin-Dentin Bonds:** The activated host MMPs are responsible for the degradation of portions of hybrid layers formed during the bonding of resin to dentin. This degradation over time can lead to a decrease in bond strength, which is a concern for the long-term stability of dental restorations.

- **Collagen Cross-Linking Agents:** To address the issue of MMP-induced degradation and stabilize the hybrid layer, collagen cross-linking agents have been developed. These agents aim to prevent the breakdown of the resin-dentin bond over time.[20] The use of collagen cross-linking agents is supported by studies, such as the one mentioned by Asthana et al.[21]

In our study, we used 6.5% cocoa seed extract and 5% GA as primer for an application time of 10 mints, which is clinically acceptable. While making intragroup comparisons in Group 1, the highest mean μSBS was of (31.62 MPa) followed by group 2 (27.43 MPa). Proanthocyanidins, a type of compound found in cocoa seed extract, were found to interact with proteins and induce cross-links through various mechanisms (covalent, ionic, hydrogen bonding, and hydrophobic).

Proanthocyanidins were suggested to have a greater ability to interact with collagen compared to GA, leading to increased mechanical properties of dentin.[22]

- The application of 6.5% cocoa seed extract and 5% GA to caries-affected dentin resulted in increased microshear bond strength.
- The order of effectiveness was noted as cocoa seed extract > GA.
- The observed increase in microshear bond strength was attributed to the inactivation of matrix metalloproteinases (MMPs).
- Cocoa seed extract and GA were suggested to play a role in enhancing the durability of the resin-dentin bond through MMP inactivation. Within the tested concentrations, cocoa seed extract demonstrated a higher effectiveness in increasing microshear bond strength compared to GA.

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