Original Article

A comparative evaluation of the effect of matrix metalloproteinase inhibitor on the fracture resistance of endodontically treated teeth restored with Everstick-reinforced composite resin: An *in vitro* study

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Abstract

Aim: To evaluate the effect of applying a matrix metalloproteinase (MMP) inhibitor on the fracture resistance of root-filled teeth restored with Everstick fiber-reinforced composite resin.

Subjects and Methods: After the selection of 60 freshly extracted human mandibular first molar, root canal access and standard uniformly sized mesio-occluso-distal (MOD) cavities were made and the teeth were randomly assigned into three groups ($n = 20$ each): Group I, the MOD cavity was first lined with flowable composite resin and then restored with composite resin. In Group II, Everstick fiber was placed into the bed of flowable composite in buccal–pulpal–lingual direction before the composite restoration was placed. In Group III, after etching of the cavity, a 2% chlorhexidine MMP inhibitor was applied. Then, the MOD cavity was restored same as group II. A universal testing machine was employed to compressively load the teeth at a cross-head speed of 0.5 mm/min till fracture. The maximum fracture loads were recorded in Newtons (N) and data were analyzed with one-way ANOVA and *post-hoc* Tukey tests.

Results: Group III exhibited significantly higher fracture resistance compared to all other groups ($P < 0.001$), whereas Group I demonstrated the lowest fracture resistance.

Conclusion: The utilization of Everstick glass fiber, combined with MMP inhibitor treatment, yielded the greatest fracture resistance. Hence, this method may be prioritized over conventional restoration techniques for strengthening root canal-treated teeth with structurally compromised crowns.

Keywords: Chlorhexidine; composite resin; Everstick fiber; fracture resistance; matrix metalloproteinase inhibitor; universal testing machine

INTRODUCTION

During endodontic treatment, access preparation frequently causes significant coronal damage.[1] Therefore, it is crucial to restore teeth intracoronally with suitable

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material, particularly in posterior teeth, to prevent fractures caused by occlusal stresses on unprotected cusps.[2] Cavity preparation and endodontic treatment can lead to increased stress concentrations in dentin compared to vital teeth; however, appropriate restoration techniques can help reduce internal stresses.[2]An ideal restoration after endodontic treatment should focus on maintaining the remaining tooth structure, ensuring both aesthetic qualities and functionality, while effectively preventing any microleakage.[3]

The conventional approach to restoring non-vital teeth involves using a post and core restoration, a full crown, or a cast inlay, all of which help protect the cusps.[4] The current trend towards minimally invasive techniques and modern access cavity designs, along with the development of newer and more dependable restorative materials and methods, has encouraged clinicians to reevaluate the necessity of conventional restorative techniques. This change aims to preserve natural tooth structure, adopt conservative restoration options, and utilize advanced materials and technologies for the best possible outcomes.

An additional concern regarding endodontically treated teeth involves issues with coronal microleakage and bacterial contamination, which can lead to treatment failure and the need for retreatment if not promptly restored. Bonded restorations, like direct composites, offer a conservative approach by preserving tooth structure with minimal preparation. In addition, resin-bonded restorations are cost-effective and quicker to place compared to indirect restorations, which involve additional laboratory time and expenses.[5]

The introduction of fibers into composite resin systems represents a recent innovation designed to address the limitations of conventional composite resins, such as brittleness, ease of crack propagation, and polymerization shrinkage.[6] Everstick (Everstick C and B, GC Corp., Tokyo, Japan), which utilizes an E glass fiber system, comprises 4000 unidirectional fibers coated with epoxy resin. The Everstick system is composed of calcium–aluminum–borosilicate fibers.[7]

Dentin bonding remains challenging due to its organic composition. Unlike enamel, dentin is less mineralized and contains abundant organic material.[8] Acid etching exposes collagen below the hybrid layer, resulting in weakened dentin susceptible to long-term degradation. Thus, preventing collagen degradation is essential for preserving adequate bond strength.[9]

Recent research has emphasized that matrix metalloproteinase (MMP) activity contributes significantly to the degradation of collagen fibers within the hybrid layer, which in turn compromises bond strength at the interface between the tooth and restoration. Inhibiting MMPs is beneficial for maintaining the stability of the resin–hybrid layer complex, thereby enhancing bond durability.[10]

Chlorhexidine is frequently used as an antimicrobial agent, especially for disinfection before restoring dental work. It is well recognized for its ability to inhibit matrix metalloproteinase (MMPs), the enzymes that break down collagen. MMP inhibitors are commonly integrated into adhesive, etchant, or primer formulations. The proposed mechanism involves CHX chelating with calcium and zinc ions present in MMPs, facilitating bonding to dentinal surfaces via electrostatic interactions.[11]

Considering these factors, the present study aims to assess whether the fracture resistance of endodontically treated teeth is enhanced using Everstick fibers and incorporating a MMP inhibitor layer into the adhesive process. The null hypothesis examined was that the fracture resistance would be unaffected by the fiber-reinforced composite (FRC) and application of MMP inhibitor.

SUBJECTS AND METHODS

Sample size

Sixty noncarious mandibular first molars, extracted for orthodontic or periodontal reasons within the last month, were chosen. The inclusion criteria include sound teeth, with nearly similar crown sizes and no cracks under transillumination and magnification. Teeth with visible cracks, restorations, caries, or developmental anomalies were excluded. The teeth were cleaned with a hand scaler, and stored in 0.5% chloramine T (Narsipur Chemicals Pvt. Ltd., Navi Mumbai, Maharashtra, India). Sixty teeth were randomly assigned to four groups, with each group containing twenty teeth.

Sample preparation

The teeth were prepared with a standard mesio-occluso-distal (MOD) cavity, ensuring that the buccal and lingual wall thickness remained at 2.5 ± 0.2 mm at the height of the contour on each surface. In addition, the gingival cavosurface margin was placed 1.5 mm coronal to the cementoenamel junction (CEJ).

For the endodontic procedure, access was made using an Endo-Access Bur (Dentsply Maillefer) with a high-speed hand piece (NSK, Japan), followed by the location of canals. A size 10 K-file was placed into each canal until it reached the apical foramen. The working length was determined by subtracting 1 mm from this measurement. Biomechanical preparation was done with ProTaper Universal rotary files, along with irrigation consisting of normal saline, 3% sodium hypochlorite, and ethylenediaminetetraacetic acid paste as directed. Canal preparation continued until reaching size F2 or F3 as necessary. Following drying with paper points,

gutta-percha cones (Dentsply Maillefer) were selected and then obturated with AH Plus root canal sealer.

Any excess sealer was removed with a cotton pellet dipped in 95% ethanol. Selective etching was carried out with 37% phosphoric acid gel for 20 s. The gel was subsequently rinsed thoroughly, and the structure was gently air dried.

Sample grouping and restoration

The total specimen (n = 60) were randomly divided into three groups.

- Group I: Flowable composite + direct resin composite
- Group II: Flowable composite + Everstick fibers + direct resin composite
- Group III: MMP inhibitor + flowable composite + Everstick fibers + direct resin composite.

Group I: Flowable composite + direct resin composite

Once the cavity surfaces were etched, two layers of bonding agent, single bond universal (3M ESPE, St. Paul, MN, USA) were applied and cured. The cavity surfaces were then coated with a layer of flowable composite resin, Filtek™ Z350 XT (3M ESPE, USA) on buccal, lingual walls and the pulpal floor. Incremental build up was done with direct resin composite (3M Filtek Z 350). Finishing and polishing was done using composite finishing kit and discs (Shofu Inc, Kyoto, Japan).

Group II: Flowable composite + Everstick fibers + direct resin composite

After etching and bonding, cavity surface received a coated with FCR. A 10 mm long and 3 mm wide piece of Everstick fiber (Everstick C and B, GC Corp., Tokyo, Japan) was prepared by coating it with adhesive resin. After removing excess material, the fiber was embedded into the flowable composite [Figure 1]. Following a 20-s curing period, the cavities were restored using composite material, following the procedure outlined in Group I.

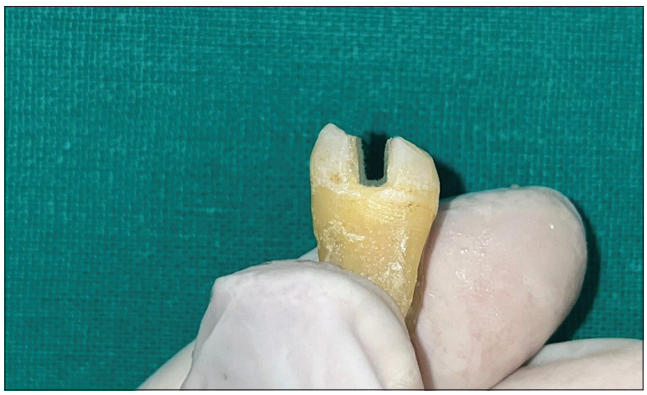


Figure 1: Everstick fiber placed on buccal–pulpal–lingual wall of MOD cavity.

Group III: Matrix metalloproteinase inhibitor + flowable composite + Everstick fibers + direct resin composite

After etching, the cavities received treatment with a 2% CHX solution (Globus Medisys, Vadodara,Gujarat, India). The solution was applied using a microbrush with gentle rubbing for 60 s. Any excess CHX was then removed using an absorbent paper. They were subsequently restored using everstick fiber and composite resin following the procedure outlined in Group II.

Finally, the roots of all samples were treated with polyvinyl siloxane 1 mm below the cemento-enamel junction (CEJ) to simulate the periodontal ligament. The teeth were then mounted in self-curing acrylic resin using custom-made molds.

Measuring the fracture resistance

The acrylic block, with the sample secured, was positioned on a custom-made base to ensure it was perpendicular to the horizontal surface. It was subsequently moved to a universal testing machine (Instron, Buckinghamshire, England). Using a 2 mm diameter round bar, a compressive force was applied centrally on the occlusal surface of the teeth, parallel to their long axis, at a strain rate of 0.5 mm/min. The force necessary to fracture each tooth was documented in Newton (N). Following the ANOVA *F*-test, *post hoc* data analysis was performed using Tukey's multiple comparison test.

RESULTS

Mean fracture resistance (N) and standard deviations for all groups are listed in Table 1. Pairwise comparisons using Tukey's *post hoc* test are shown in Table 2. One-way ANOVA revealed significant differences between the groups ($P < 0.001$). Group III demonstrated the highest fracture resistance, significantly surpassing all other groups ($P < 0.001$), whereas Group I had the lowest. The order of increasing fracture resistance was no fiber < Everstick fiber only < Everstick fiber + MMP inhibitor [Graph 1].

DISCUSSION

Mandibular molars frequently need endodontic treatment. Teeth with MOD cavities are more susceptible to fractures because the absence of mesial and distal walls increases stress on the remaining tooth structure.[12] Furthermore, the decreased width of the tooth structure postpreparation increases the susceptibility to cuspal fractures, making MOD cavities the most vulnerable in terms of fracture resistance. Therefore, mandibular molars with MOD cavities were chosen for the study.

Table 1: Comparative statistics of fracture resistance (N) of all three groups, respectively, using one-way ANOVA *F*-test

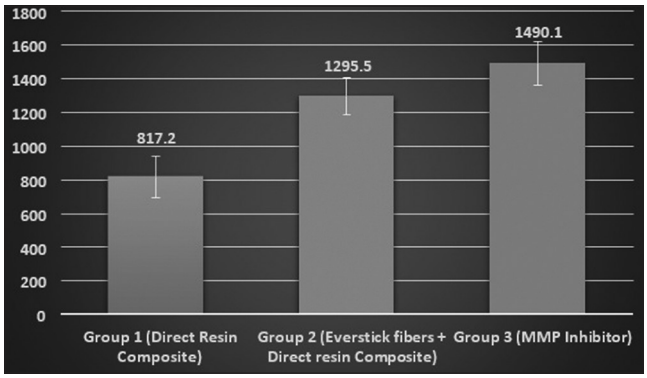
Flowable composite additions	Mean±SD	One-way ANOVA *F*-test value	*P* value, significance
Group I (direct resin composite)	817.2±121.2	161.91	<0.001**
Group II (Everstick fibers + direct resin composite)	1295.5±111.4		
Group III (fiber reinforced composite with MMP inhibitor)	1490.1±131.63		

**$P<0.001$ highly significant. $P>0.05$ no significant difference. MMP: Matrix metalloproteinase, SD: Standard deviation

Table 2: Pairwise comparative statistics of fracture resistance (N) of all three groups using Tukey's *post hoc* test

Group	Comparison group	Mean difference	*P*
Group I (direct resin composite) versus	Group II (Everstick fibers + direct resin composite)	478.3	<0.001**
	Group III (fiber reinforced composite with MMP inhibitor)	672.9	<0.001**
Group II (Everstick fibers+direct resin composite) versus	Group III (fiber reinforced composite with MMP inhibitor)	194.6	<0.001**

**$P<0.001$ highly statistical difference, ^ *P* value calculated using Tukey's *post hoc* test. MMP: Matrix metalloproteinase



Graph 1: Comparison for Fracture resistance (N) among all three groups

Based on our study findings, we have rejected the null hypothesis. Applying a layer of 2% CHX MMP inhibitor after etching, along with incorporating Everstick glass fibers, significantly improved the fracture resistance of root-filled molars with MOD cavities. Statistical analysis showed a significant difference in the mean force needed to fracture teeth between Group I and both Group II and Group III. Group I displayed the lowest fracture resistance, whereas Group III exhibited the highest.

Indirect crown restoration is routinely recommended for weakened cusps of pulpless teeth, but it can be costly and time-consuming.[13] An alternative strategy involves providing patients with immediate FRC permanent restorations which offer benefits such as preventing microleakage, preserving tooth structure, and being cost-effective.[7] The introduction of fiber-reinforced composite (FRC) technology has brought a new material into the field of metal-free, adhesive aesthetic dentistry. E glass and S glass fibers are frequently used to strengthen dental composites, with products like Everstick employing the E glass system.[14] The network structure formed by these fibers acts like small stitches across bonded fractured surfaces, improving cohesion and preventing early separation under load.[6]

Everstick fibers show a uniform interdiffusion layer between precured FRC and newly applied monomers, indicating a robust bond. This bond modifies stress dynamics at the interface between the tooth and resin, thereby preventing crack propagation.[15] Moreover, incorporating these fibers decreases the amount of composite resin required in the cavity, which helps minimize polymerization shrinkage. The fibers ability to withstand dimensional changes or deformation is also critical in preventing restoration failure.[16] An alternative interpretation of the result proposes that combining fiber with resin enhanced fracture strength by stabilizing cusps. Extending fibers into buccal or lingual walls maintained cusp positions, while improved bonding boosted resistance, particularly in root-filled teeth with MOD cavities.[17]

Sadr *et al*. recommended saturating fibers with resin before placement to ensure seamless integration into the polymer matrix, preventing voids and oxygen entrapment that can compromise resin polymerization and reduce strength.[18] Therefore, in this study, Everstick fibers were embedded within a layer of flowable resin.

In this study, mean fracture resistance (N) and standard deviation of Group II are more than Group III. This result would be justified by synergistic effect of both FRC and MMP inhibitor.

Dentin, a mineralized tissue, comprises inorganic apatite crystals, a rich supply of Type I collagen fibers, and diverse enzymes. Among these enzymes, matrix metalloproteinases (MMPs) have recently become prominent due to their potential roles in different physiological and pathological structures within dentin.[19] MMPs are endogenous Zn- and Ca-dependent enzymes capable of degrading essentially all extracellular components. The identified MMPs in human teeth are MMP-2, 3, 8, 9, and MMP-20.[20]

MMPs are pivotal in breakdown of the hybrid layer, resulting decrease in the strength of the resin–dentin bond and directly influencing the durability of adhesive

restorations.[21] Consequently, inhibiting MMP activity may be essential for addressing issues related to dentin adhesion.

Numerous MMP inhibitors, both endogenous and exogenous, have been identified to reduce MMP activity. Endogenous inhibitors arise from human cells, while exogenous inhibitors are frequently employed therapeutic agents.[19] These inhibitors function by chelating calcium or zinc ions at the functional area or interacting with the propeptide fragment. They may also prevent MMP entry or coat the substrate to inhibit its action. Tested MMP inhibitors for preventing bond breakdown include chlorhexidine (CHX), galardin, tetracycline, benzalkonium chloride, and quaternary ammonium methacrylates.[9] These MMP inhibitors aid in preserving dentin bonds, reducing collagen degradation, prolonging the durability of dental restorations, and improving their mechanical properties.

Chlorhexidine (CHX) is the most extensively tested MMP inhibitor and considered the gold standard. Previous study have demonstrated that when treated as a preconditioner or integrated into adhesive systems, CHX effectively maintains the structural integrity of demineralized collagen and mitigates degradation of resin–dentin bond strength for long time.[22] In this study, CHX digluconate was chosen based on its ability to maintain bonding stability of resin to dentin without affecting solubility, water sorption, or the degree of conversion of adhesive systems and resin blends, as demonstrated in prior research.[21,22]

Various concentrations of CHX have been examined for their efficacy. Gendron *et al*. discovered that even at low concentrations, CHX effectively inhibits MMP activity. For instance, MMP-2 was completely inhibited by a concentration as low as 0.0001%, whereas MMP-8 was inhibited by concentrations of 0.01% and 0.02%, and MMP-9 was completely inhibited by 0.002%.[23] Currently, a 2% CHX solution applied for 60 s is extensively employed before primer application in adhesive systems. This concentration effectively inhibits MMP activity and helps maintain bond strength stability. Therefore, in our study, we chose to use 2% CHX for 60 s based on these established benefits

Carrillo et al. performed an in vitro study demonstrating that applying 2% chlorhexidine after acid-etching effectively preserves the durability of the hybrid layer and bond strength for up to 14 months.[22] This preservation is likely due to the inhibition of matrix metalloproteinase (MMPs) bound to the dentin matrix, which helps reduce the degradation of collagen fibrils in both the hybrid layer and the underlying sub-hybrid layer.[23] Ricci *et al*. (2014) noted that higher concentrations of CHX, like 2%, were more effectively absorbed into porous caries-affected dentin.[24] Loguercio *et al*. used Raman spectroscopy to demonstrate CHX molecules persisted within the hybrid layer even after 5 years.[25] Gendron *et al*. highlighted CHXs' direct inhibition of MMP-2, MMP-9, and MMP-8.[23]

CHX exhibits specific characteristics, including a robust positive ionic charge, facile binding to phosphate groups, and a strong attraction to the cavity surface, particularly following acid etching.[25] These attributes contribute significantly to its effectiveness in enhancing resin–dentin bond strengths in etch-and-rinse adhesive systems. In addition, CHX increases the surface-free energy of enamel and dentin and exhibits substantivity, which further enhances its beneficial effects. In our study, the application of 2% CHX resulted in the highest and statistically significant improvement in fracture resistance.

Clinical fractures and those induced by *in vitro* testing appliances differ significantly. Masticatory forces vary in magnitude, speed, and direction intraorally, unlike the constant speed and direction in laboratory tests. Replicating clinical conditions like thermal changes and fatigue from repeated stresses is challenging. Longer-term clinical trials are needed to evaluate resin composite restoration performance *in vivo* and assess bonding stability with MMP inhibitors over time, as adhesive bond failure may occur in clinical situations.

CONCLUSION

According to the findings from this laboratory investigation, it was established that the inclusion of Everstick fibers and the application of an MMP inhibitor (2% CHX) after etching substantially enhanced the fracture resistance of endodontically treated teeth with MOD cavities.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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