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Comparative Evaluation of Effect of Salivary Contamination on Microleakage Using 8th Generation Bonding Agent with and Without Laser Application: An In Vitro Study.

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KEYWORDS

8 th Generation Bonding Agent, saliva Contaminant, Microleakage, Saliva,laser.

ABSTRACT:

Introduction: This study highlights that microleakage significantly affects the longevity of bonded restorations, especially in Class V cavities where moisture control is difficult. Salivary contamination reduces bond strength, leading to clinical failures. While advanced 8th-generation nano-adhesives improve adhesion, limited research exists on laser activation after contamination—making this study crucial in evaluating its effect on microleakage and bonding performance

Objectives:

The aim of this study is to evaluate the Microleakage after Salivary Contamination Using 8th Generation Bonding Agent with and without Laser Application.

METHOD AND MATERIALS:

Thirty freshly extracted human premolars were collected for the study. These teeth were randomly divided into three groups (n=10): Group 1 (control), Group 2 (salivary contamination without laser activation), and Group 3 (salivary contamination with laser activation).

A Class V cavity was prepared on the buccal surface of each tooth. The bonding agent was applied and light cured without contamination in Group 1. For Group 2, the teeth were contaminated with saliva before curing the bonding agent, while for Group 3, the teeth were contaminated with saliva, and laser activation was performed before curing the bonding agent. All groups were then restored with composite restoration.

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All samples underwent thermocycling and were prepared for dye immersion. The samples were immersed in 2% Methylene Blue dye for 24 hours and then sectioned buccolingually. Each half of the buccolingually sectioned samples was observed under a stereomicroscope at 40x magnification to evaluate microleakage.

STATISTICAL ANALYSIS:

Statistical analysis was conducted using the anova and post hoc Test to compare microleakage between the control and saliva-contaminated groups, as well as the effect of laser treatment on bonding agent.

RESULTS :

Results showed that samples treated with laser activation after salivary contamination exhibited significantly lower microleakage indicating a stronger seal compared to non-laser-treated samples.

Conclusion:

Laser activation enhances bonding effectiveness in contaminated conditions, reducing microleakage and supporting its use in challenging clinical settings.

1. Introduction

Effective marginal sealing is a key factor in determining the long-term success of bonded restorations. Microleakage, the movement of oral fluids, bacteria, molecules, and ions between cavity walls and restorative material, is often linked to postoperative failures.[1]

Therefore, Preventing microleakage and ensuring a strong bond between the cavity wall and restorative material are essential considerations when using bonding systems. [1]

Achieving strong adhesion in Class V cavity restorations is challenging due to the position of their cervical margin at the cemento-enamel junction (CEJ), where it is bordered by cementum. Maintaining the marginal quality of these restorations is the primary clinical concern. Microleakage at the tooth-restoration interface remains one of the greatest obstacles to achieving an ideal restorative material.

In clinical practice, various factors impact the adhesion and retention of restorative materials. Moisture, including gingival fluid, blood, handpiece oil, and especially saliva, can weaken bond quality, causing microleakage at the interface. This may result in restoration loss, recurrent caries, postoperative sensitivity, and discoloration.[2]

Human saliva is a complex mixture of oral fluids, primarily consisting of water, enzymes, electrolytes, and proteins, including mucoproteins, albumin, and gamma-globulin. Studies have shown that the protein content in saliva reduces adhesive bond strength when contamination with saliva occurs.[3]

Cervical restorations present significant challenges due to issues with moisture control, accessing caries, and the proximity to the gingival margin.[4]

However, many carious lesions that require dentin bonding are located in areas that are hard to isolate, particularly near or at the gingival margin, where salivary contamination is more likely.[2]

Recent years have seen extensive research focused on the development of improved adhesive restorative materials.[2]

Recently, a new class of dentin adhesives has emerged ,universal or multi-mode adhesives, which can be used either as etch-and-rinse or self-etch systems. Advancements in nanotechnology have also led to the creation of nano adhesives (eighth-generation bonding agents), which incorporate nanosized silica crosslinking particles. These new adhesives feature the addition of nanofillers (SiO2) with an average particle size of 12 nm, enhancing the penetration of resin monomers and

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increasing the hybrid layer thickness, thereby improving the mechanical properties of bonding systems.[5]

Lasers can be utilized for a range of procedures, including tooth surface treatment, cavity preparation, caries prevention, and teeth bleaching.[6]

In recent years, dental researchers have made several efforts to explore the effects of laser irradiation on adhesive systems applied to the dentin surface before being light-polymerized.[7]

Many studies in the literature has been conducted to check the effect of contaminants like saliva blood on sealing abilities of 5th 6th and 7th generation bonding agents[1,4,5]and different effects of laser irradiation on microleakage using different bonding agents also extensively studied[7],but there are very limited studies available in the literature which evaluates the significance effects of lasers on 8th generation bonding agent after salivary contamination.

Hence, through this study we have evaluated the effects of Salivary Contamination on microleakage in 8th Generation Bonding Agent with and without Laser Activation.

2. Objectives

To evaluate and compare the effect of Salivary Contamination on microleakage Using 8th Generation Bonding Agent with and without Laser Application.

To evaluate the effect of Salivary Contamination on microleakage Using 8th Generation Bonding Agent with and without Laser Application.

To compare the effect of Salivary Contamination on microleakage Using 8th Generation Bonding Agent with and without Laser Application.

.Methods

- 30 freshly extracted human premolars were collected for the study. The premolars with intact coronal tooth structure and without any structural defects like caries, attrition, abrasion, erosion or fracture were selected for the study.
- The samples were cleaned off debris and residual tissues with the help of an ultrasonic scaler and stored in the solution of 10% Formalin, until use.
- All the samples were randomly divided into three groups, with 10 samples in each group.

Group1(control):bonding agent application+curing.
Group2 (without laser application):bonding agent application+salivary contamination+curing
Group3(laser application):bonding agent application+salivary contamination+laser application+curing.

1.1. Tooth preparation:A Class V cavity was prepared on the buccal surface of each sample. The dimensions of the prepared cavity were as follows: 3mm width i.e. parallel to cementoenamel junction, 2mm height i.e. occluso-gingivally with 0.5 mm below CEJ and 1.5mm depth. The enamel margins were beveled (45°). After the tooth preparation was done, the cavity was thoroughly dried.(Figure 1)

1.2. Collection of The Contaminants: Fresh, unstimulated human saliva was collected in a sterile beaker.

1.3. Group1(Control):The 8th generation bonding agent(3M ESPE Single Bond Universal)was applied to the Class V cavity prepared on the samples according to the manufacturer's instructions. (Figure2)
the cavity was left undisturbed for 5-10 seconds, and then dried for 5 seconds, under Maximum air pressure.Then it was light cured using LED curing light (700mW/cm2) for 10 seconds, at a distance of less than 10mm.

1.4. Group2(without laser activation):After application of 8th generation bonding agent,the samples were contaminated with Fresh Collected Unstimulated Human Saliva,using a separate disposable applicator tip.The cavity was left undisturbed for 5-10 seconds, and then dried for 5 seconds, under Maximum air pressure.Then it was light cured using LED curing light (700mW/cm2) for 10 seconds, at a distance of less than 10mm.(Figure3)

- Group3(with laser activation): After application of 8th generation bonding agent, the samples were contaminated with Fresh Collected Unstimulated Human Saliva,using a separate disposable applicator tip. the cavity was left undisturbed for 5-10 seconds, and then dried for 5 seconds, under Maximum air pressure.Then subjected to

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940nm diode laser (Biolase Laser) irradiation for 15 sec with continuous wave mode,1W power, non contact tip of 400um. During irradiation laser tip was held perpendicular to buccal surface at a distance of 1mm. Later, then polymerized with LED curing light unit for 20 sec.(Figure 4)

- Restoration of the cavities: all the samples, after application of bonding agent, were then restored with Composite restorative material(3M ESPE Filtek Z350Xt).The final restoration was finished and polished(Shofu Inc., Japan).(Figure 5)
- The samples were then immersed in distilled water for 24 hours, at room temperature.
- After 24 hours, the samples were subjected to 500 cycles of thermocycling with the temperature range of 55°C ±5 to 5°C ± with a dwell time of 15 s.
- Preparation of the samples for dye immersion:The apical tip of all the samples were sealed with the help of the modelling wax.
- The entire tooth surface, except the restored cavity and margins of 1 mm surrounding it, was coated with nail varnish and allowed to dry.(Figure 6)
- The samples were then immersed in 2% Methylene Blue dye for 24 hours.
- After 24 hours the samples were removed from the dye solution and then rinsed under running water.
- Preparation of the samples for observation: The samples were sectioned buccolingually, using diamond sectioning disk, to observe the dye penetration at the occlusal and the gingival surface of the samples. (Figure 7)
- Each half of the buccolingually sectioned samples was observed under stereomicroscope under the power of 40x.(Figure8)
- Scoring criteria: 0 = No dye penetration

  1 = Dye penetration up to, but not beyond 1/2 to occlusal or gingival wall.

  2 = Dye penetration up to, but not contacting the axial wall.

  3 = Dye penetration along the axial wall

Figure Legends

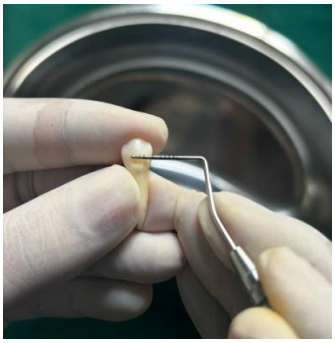

Figure 1:Class V cavity preparation

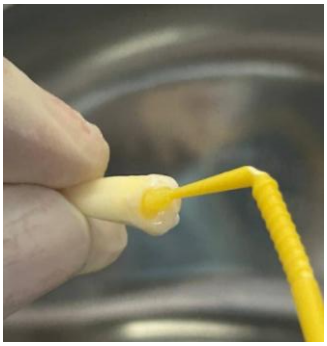

Figure2:Bonding agent application+Light curing

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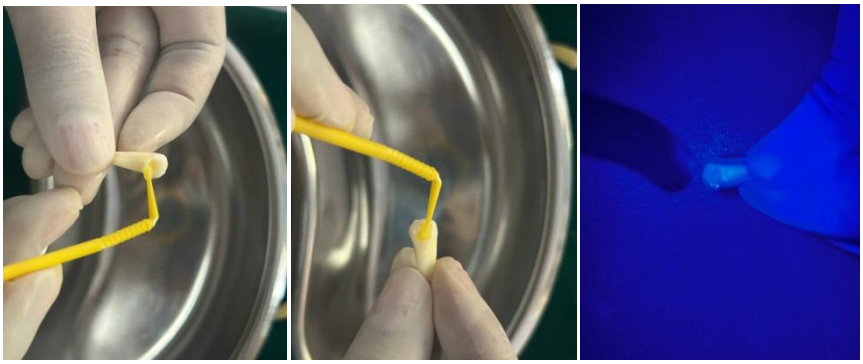

Figure3: Bonding agent application+Salivary contamination+Light curing

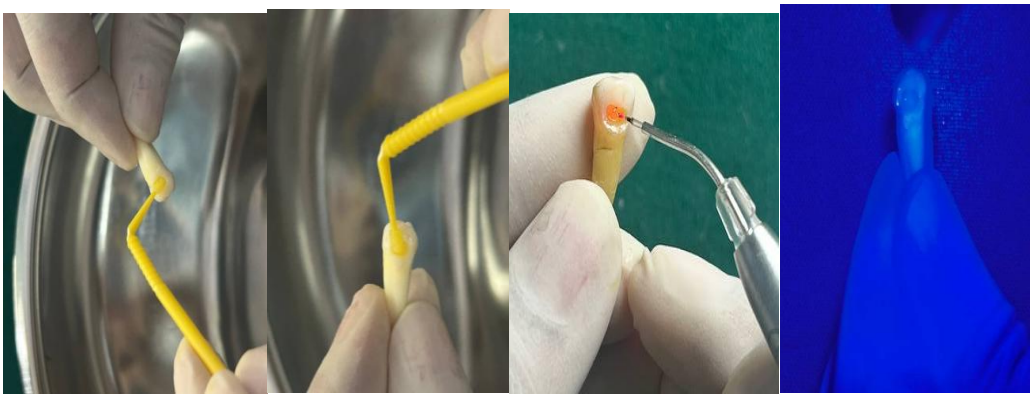

Figure4: Bonding agent application+Salivary contamination+Laser application+Light curing

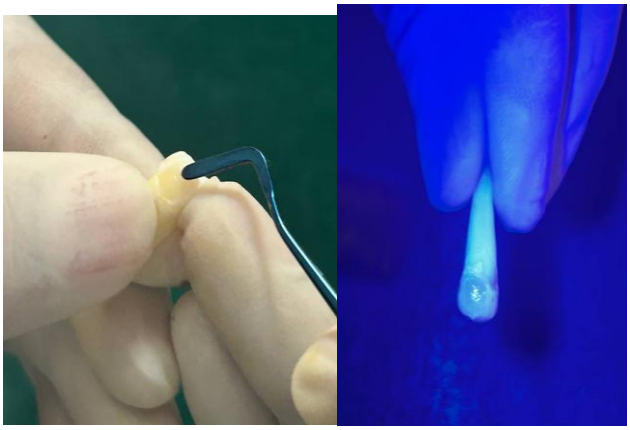

Figure 5: Restoration of the cavity with composite+Light curing

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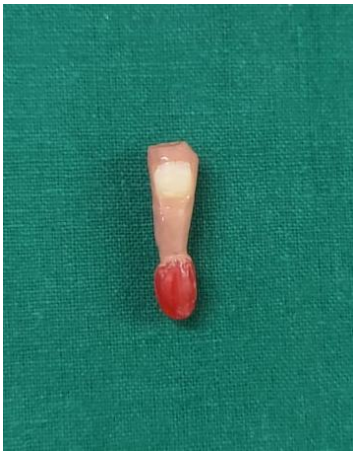
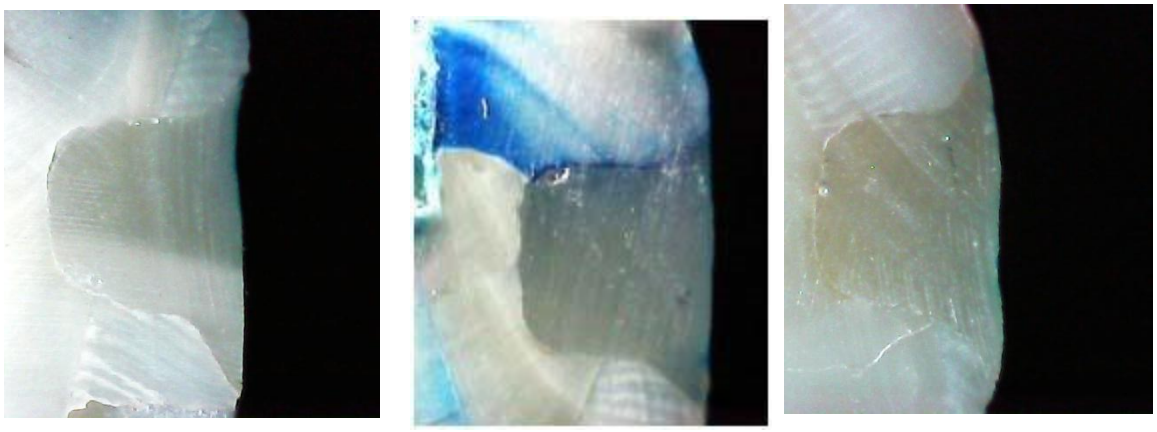


Figure 6: Preparation of the samples for dye immersion

Figure 7: The samples were sectioned buccolingually, using diamond sectioning disk



GROUP1(score 0) (control)

GROUP2(score 2) (without laser activation)

GROUP3(score 0) (with laser activation)

Figure 8: Samples observed under stereomicroscope under the power of 40x.

3. Results

Results showed that samples treated with laser activation after salivary contamination exhibited significantly lower microleakage scores as compare to non laser activated group, indicating a stronger seal compared to non-laser-treated samples.

In contrast, non-laser samples demonstrated increased microleakage, suggesting compromised bonding effectiveness due to salivary contamination. (Table1,2)

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Table 1: Comparison of microleakage score between three study groups at occlusal wallrespectively

Occlusal wall score	Score 0 N (%)	Score 1 N (%)	Score 2 N (%)	Score 3 N(%)
Group 1(Control)	**8 (80%)**	**2 (20%)**	**0 (0%)**	**0(0%)**
Group 2 (Contamination + without laser)	**1 (10%)**	**1(10%)**	**6 (60%)**	**2(20%)**
Group 3 (Contaminated + with laser)	**7 (70%)**	**3 (30%)**	**0 (0%)**	**0(%)**
Overall (1vs 2 vs 3)	**Chi =4.312, p=0.023* (statistical significance)**			
Group 1 vs Group 2	**Chi = 7.512,p=0.004* (statistical significance)**			
Group 1 vs Group 3	**Chi = 2.043, p=0.212 (no statistical significance)**			
Group 2 vs Group 3	**Chi = 5.067, p=0.037* (statistical significance)**			

p>0.05 – no significant difference *p<0.05 – significant difference

Table 2: Comparison of microleakage score between three study groups at gingival wallrespectively

Gingival wall score	Score 0 N (%)	Score 1 N (%)	Score 2 N (%)	Score 3 N(%)
Group 1(Control)	**9 (90%)**	**1 (10%)**	**0 (0%)**	**0(%)**
Group 2 (Contamination + without laser)	**1 (10%)**	**3 (30%)**	**5(50%)**	**1(10%)**
Group 3 (Contaminated +				

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with laser)	**6 (60%)**	**4 (40%)**	**0 (0%)**	**0(%)**
Overall (1 vs 2 vs 3)	**Chi = 14.09, p=0.005* (statistical significance)**			
Group 1 vs Group 2	**Chi = 24.05, p=0.002* (statistical significance)**			
Group 1 vs Group 3	**Chi = 8.96, p=0.094 (no statistical significance)**			
Group 2 vs Group 3	**Chi = 6.986, p=0.043* (statistical significance)**			

p>0.05 – no significant difference *p<0.05 – significant difference

Discussion

Microleakage is a major and persistent concern in restorative dentistry, as it can lead to problems like secondary caries, postoperative sensitivity, and ultimately, restoration failure.Therefore, reducing microleakage is crucial for ensuring long-term restoration durability. Bonding agents play a key role in minimizing microleakage by creating the bond between restorative materials and tooth structure.However,achieving a strong and reliable bond can be challenging, particularly in clinical settings where contamination is common. Saliva, one of the most problematic contaminants, can negatively impact the adhesive's ability to bond with the dental surface. This study aimed to investigate the comparative effects of salivary contamination on microleakage when using an 8th-generation bonding agent, with and without laser activation, in an in vitro model.

The findings indicate that laser activation of the bonding agent after exposure to saliva resulted in a significantly lower level of microleakage compared to the non-laser-activated group. This is an important discovery,suggesting that laser activation may have a therapeutic role in contaminated bonding situations, enhancing the bonding performance even in the presence of saliva. These results are consistent with previous research, which has demonstrated that laser irradiation can improve the overall effectiveness of bonding agents, enhancing adhesion strength, resistance to microleakage, and long-term durability.

Saliva is a complex biological fluid made up of various organic and inorganic substances, including mucins, enzymes, and electrolytes. When saliva contaminates the bonding surface, it interferes with the optimal conditions needed for adhesion by introducing these components, which can hinder proper resin infiltration and polymerization. Taneja S et al. found that saliva contamination of total-etch adhesives before polymerization significantly reduced dentin bond strength. This reduction was attributed to the interference of salivary proteins, which occluded open dentinal tubules and disrupted adhesion before curing. Additionally, an increase in the contact angle further weakened the bond strength. Salivary contamination after adhesive polymerization also led to a decrease in bond strength, as salivary glycoproteins deposited on the poorly polymerized adhesive layer created a physical barrier that hindered effective copolymerization. [8]

Group 2 (salivary contamination without laser activation):demonstrated significantly higher microleakage levels, The non-laser activated samples demonstrated higher levels of dye penetration, indicative of increased microleakage. These results suggest that, without laser activation, the bonding agent's effectiveness is notably compromised by salivary contamination, which inhibits proper resin infiltration and

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polymerization.supporting the hypothesis that saliva contamination impairs bonding effectiveness.

This observation highlights the critical need for strategies that can mitigate the negative impact of salivary contamination, especially in clinical scenarios where isolation may be difficult, such as bonding procedures in the posterior region or on patients with high salivary flow. By examining microleakage as an outcome measure, our study aligns with previous research in identifying the challenges associated with achieving optimal adhesion in the presence of contamination.

Laser activation of bonding agents has gained attention as a technique that may enhance the adhesive performance of dental materials. The laser's photothermal and photophysical effects are thought to play a crucial role in improving bonding outcomes by influencing the polymerization process and the formation of resin tags that anchor the adhesive to the tooth substrate. According to DE PAIVA GONÇALVES SE et al., laser systems can improve adhesion and reduce microleakage by creating a larger microtensile area free from smear layers. For this reason, some researchers have recommended the use of laser systems during the restoration process. Gonçalves et al. applied laser irradiation before the polymerization step.[9]

The reduction in microleakage observed with laser activation can be attributed to several mechanisms. Firstly, laser irradiation may enhance the penetration of the bonding agent into the dentin or enamel substrate by modifying its surface at a microscopic level. This effect can create a micro-retentive surface that improves mechanical interlocking between the adhesive and tooth surface, thereby enhancing bond strength.

Maenosono RM et al. found that the 810 nm diode laser is more readily absorbed by water and resin bonding materials than by hydroxyapatite. As a result, when laser irradiation is applied to a cavity with the bonding system, the unpolymerized adhesive absorbs the laser energy, causing an increase in temperature.[10]

Golbari N et al. propose that elevating the temperature of the adhesive system accelerates the evaporation of solvent components, leading to a reduction in porosity at the bonding interface due to the decreased solvent residue. This process helps improve the degree of polymerization and the overall quality of the hybrid layer. ND: YAG laser irradiation applied to unpolymerized adhesive raises the temperature of the bonding agent, which, in turn, enhances its physical and mechanical properties. Furthermore, the use of a 970 nm diode laser can increase the degree of conversion in simplified adhesives by facilitating the movement of free radicals, thereby strengthening the bond between enamel, dentin, and composite resin.[11.]

Furthermore, laser activation may promote the evaporation of water from the adhesive interface, which is beneficial for reducing microleakage. Excess moisture at the bonding interface is known to interfere with polymerization and can lead to incomplete curing of the adhesive.

In our study, the thermal energy from the laser likely created a more optimal polymerization environment by reducing moisture content, allowing for a more uniform cure. This is consistent with findings from Golbari N et al., who reported that laser irradiation lowers the residual water content in bonding agents, leading to enhanced bond stability and reduced microleakage..[11]

Group3 (salivary contamination with laser activation):- Samples treated with laser activation after salivary contamination showed significantly lower microleakage scores compared to those without laser activation. Quantitative analysis revealed that laser-treated bonding agents exhibited less dye penetration, indicating a more effective seal against microleakage. This finding underscores the positive impact of laser treatment on bonding reliability in the presence of salivary contaminants.

Microleakage testing is a widely used method to evaluate bonding effectiveness in restorative dentistry, as it provides insight into the adhesive's ability to prevent fluid and bacterial infiltration at the interface. In our study, the use of microleakage testing allowed us to directly compare the sealing effectiveness of laser-activated and non-laser-activated bonding agents under contaminated conditions. The significant reduction in microleakage in the laser group suggests that laser activation plays a pivotal role in

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achieving a more reliable seal, even when contamination is present.

The role of microleakage in clinical outcomes cannot be overstated, as it is closely associated with secondary caries development, postoperative sensitivity, and overall restoration failure. By reducing microleakage, laser activation of bonding agents may help prevent these adverse clinical outcomes, thus extending the lifespan of restorations. This finding aligns with the principles of modern adhesive dentistry, which prioritize techniques that minimize microleakage to enhance the durability and effectiveness of restorations over time.

Clinical Implications and Future Research:-

Our findings have practical implications for clinical practice, as they suggest that laser activation of bonding agents may be particularly useful in scenarios where contamination is difficult to control. In clinical settings, achieving complete isolation is not always feasible, especially in posterior restorations or on patients with high salivary flow. Laser activation provides a method for improving bond reliability under such conditions, offering a potential solution to the challenges posed by contamination.

Further clinical studies are warranted to validate these findings in vivo, as our study was conducted in an in vitro setting. In vivo studies would help determine the long-term effectiveness of laser-activated bonding agents in reducing microleakage and enhancing restoration longevity. Future research could also explore the specific parameters of laser activation, such as wavelength, energy output, and exposure time, to optimize its effects on different types of bonding agents and substrates.

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