Comparative evaluation of different surface pretreatment methods on the depth of penetration of adhesive resin in sandwich technique: A confocal laser scanning microscopy study

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Abstract

Aim: The aim of the study was to evaluate and compare the depth of penetration of adhesive resin in resin-modified glass ionomer cement (RMGIC) after different surface pretreatment methods using a confocal laser scanning microscope (CLSM).

Methods: Class I cavity preparation was done in 45 freshly extracted premolars and divided into three groups, according to the surface pretreatment of RMGIC which was placed in a thickness of 1 mm in the cavity. In the control group, no pretreatment was done, and other two groups were pretreated with acid etching and air abrasion (AA). The fifth-generation dentin bonding agent mixed with rhodamine B dye 0.1% was applied on RMGIC in all the samples and cured for 15 s. Samples were restored using composite resin and sectioned longitudinally. The depth of penetration of adhesive resin was evaluated using CLSM.

Results: Depth of penetration of adhesive resin was highest with AA (153.70 \pm 10.23), followed by acid etching (122.71 \pm 12.25) and control group (77.12 \pm 6.37).

Conclusion: Based on the findings of this research, AA enhances the depth of penetration of adhesive resin in the RMGIC surface. Thus, AA before placement of composite resin on the RMGIC can be effective in a clinical scenario.

Keywords: CLSM; depth of penetration; rhodamine dye

INTRODUCTION

The sandwich technique or "composite laminated restoration" has significantly reduced the postoperative sensitivity associated with the traditional procedure. It combines the qualities of glass ionomer cement (GIC)

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such as chemical bonding, sustained fluoride release, and superior esthetic properties of composite restoration.^[1] Resin modified glass ionomer cement (RMGIC) has enhanced properties in comparison to conventional GIC.^[2]

The bond between RMGIC and composite resin is crucial for the durability as it is a combination of both chemical and mechanical.^[3] Several factors including the tensile strength of GIC, viscosity, and wetting ability of the bonding agent, volumetric changes in the composite during curing, and the capacity to pack the composite without introducing

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voids, can significantly influence the bond. When RMGIC is placed, it reduces the configuration factor of the cavity, thus reducing the tensile stress created by the polymerization contraction of the composite.^[4] Composite restorations exhibit poor adhesion to the smooth surface of GIC. There are several approaches proposed to pretreat the GIC surface before applying the composite restoration. These techniques include air drying, grinding, acid etching, air abrasion (AA), photodynamic therapy, and laser.^[5] Following surface pretreatment, a rough surface is created on GIC, enhancing adhesion to the composite material. In addition, the use of adhesive further increases the bond strength between the two materials.^[6]

Bonding agents have advanced from multistep/two-step "etch and rinse" adhesive systems to self-etch systems. The one-step self-etch adhesive system is the simplest and recently developed adhesive systems; they have a shorter working time. For two-step technique, the adhesive and primer were combined into a single bottle, and its advantage is the complete removal of the smear layer.

According to the indexed literature, there are studies done to evaluate the bond strength of pretreated RMGIC surface but there are not many conclusions that are drawn to check the depth of penetration of adhesive on surface pretreated RMGIC using CLSM. Detection of the depth of penetration can be done by various approaches such as radioisotopes, dyes and neutron activation analysis, air pressure, pH changes, and scanning electron microscopy. Even though dye penetration has its limitations, they are still used, as they have the advantage of low cost, technique simplicity and ease of performance, absence of out-of-focus blur images, and the ability to direct noninvasive serial optical sectioning and the ability to create three-dimensional images, which provide more precise information than two-dimensional images. Hence, this study was undertaken to examine the depth of penetration of adhesive resin in sandwich technique using acid etching and AA as surface pretreatment on RMGIC using confocal laser scanning microscopy (CLSM).

MATERIALS AND METHODS

Sample preparation

After obtaining approval from the institutional ethics committee (DYPDCH/DPU/EC/582/141/2023), 45 intact freshly extracted permanent premolars were collected for the study. They were cleaned with ultrasonic scaler, autoclaved, and stored in 0.1% thymol solution.

Cavity preparation and placement of RMGIC

Class I cavity preparation was done using no. 245 round carbide bur, with a depth of 3 mm and 3 mm width. RMGIC was manipulated according to the manufacturer's instruction and placed in 1 mm thickness. Light curing of RMGIC was done for 20 s (woodpecker LED curing light) with an output intensity of 1000 m W/cm².

Surface pretreatment

The samples were randomly allocated into three groups (n = 15) according to the pretreatment done on RMGIC.

- Group A: Control group no surface pretreatment done
- Group B: Etching done with 37% phosphoric acid (PRIME, Dental) for 15 s
- Group C: AA (microetcher, Esthetrix) 50 μm for 10 s; 60–80 psi at 45° at a distance of 2 mm.

All the specimens were then rinsed with water for 30 s, and gently air dried with oil-free compressed air. Adhesive Adper Single Bond 2 (3M ESPE, St. paul, MN, USA) mixed with Rhodamine B 0.1% (HiMedia, Mumbai) was applied on the pretreated RMGIC with the help of a micro brush in a single stroke pattern and cured using an LED light-curing device (woodpecker) for 20 s at an intensity of 1000 mW/cm². The cavity was restored with Filtek Z250 composite resin (3 M-ESPE, St. Paul, MN, USA) and cured for 40 s. Finishing and polishing of composite restoration were done using a composite finishing kit (SHOFU, Japan).

Confocal laser scanning microscope analysis

Sectioning of all the samples was done with carborundum discs under continuous water cooling from the midline in buccolingual direction [Figure 1] in 1–1.5 mm thickness. The depth of penetration of the adhesive resin was noted using CLSM $10 \times$ (Carl Zeiss LSM 700) for all the groups.

Statistical analysis

Data were statistically analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistic for Window, version 21.0. Armonk, NY, USA: IBM Corp.) at 95% CI and 80% power to the study. The Kolmogorov–Smirnov and Shapiro–Wilk test was done to check the normal distribution of the data.



Figure 1: Longitudinal cut section of 1 mm thickness

Descriptive statistics was performed in terms of mean and standard deviation.

Analysis of variance followed by Tukey's *post hoc* test was applied to compare the depth of penetration between the groups.

Statistical significance was calculated at P < 0.05.

RESULTS

The findings demonstrated that the depth of penetration values in various RMGIC surface pretreatment varied significantly [Table 1]. Maximum depth of penetration was seen in surface pretreated with AA with a statistically significant difference in comparison with other groups [Table 2].

DISCUSSION

In clinical conditions, a restorative approach based on laminate technique using GIC with composite is regularly indicated. Practitioners can harness significant advantages of RMGIC through this technique, including its ability to absorb stress, release cations, compensate for shrinkage during various setting reactions, exhibit strong ionic adhesion, and possess a low modulus of elasticity.^[6] Laminating over RMGIC is more practical, as the bond strength of the resin composite to RMGIC is markedly higher compared to that of conventional GIC (C-GIC). This aligns with findings from previous reports.^[7,8] It has been proposed that enhanced bonding could not be seen due to their curing mechanism facilitated by the free-radical initiation.^[9,10]

Table 1: The mean depth of penetration

Depth of penetration								
	п	$Mean \pm SD$	Minimum	Maximum				
Group A	15	77.1280±6.37083	65.32	85.16				
Group B	15	122.7120±12.25429	100.13	138.87				
Group C	15	153.7060±10.23449	139.40	169.99				
Total	45	117.8487±33.25200	65.32	169.99				

It was observed that the maximum depth of penetration was seen in Group C (153.70 \pm 10.23) with a significant difference in the depth of penetration. SD: Standard deviation

In such cases, the bonding of RMGIC to dentin and RMGIC to composite is an important factor in the success of the restoration. The bonding is mainly achieved by the use of fifth-generation dentin bonding agent. Although the need for enamel and dentin pretreatment and the depth of penetration by adhesive has been well established in the literature, the need for depth of penetration of adhesive after surface pretreatment over RMGIC before composite resin lamination in sandwich restorations remains unanswered. The pretreatment of RMGIC creates the surface microtags. The bonding technique between RMGIC and composite is mainly by micromechanical retention caused by surface irregularities, roughness, and porosities.

The depth of penetration of adhesive resin between RMGIC and composite resin is undoubtedly crucial for both retaining the resin restoration and preventing microleakage. In past research, there was no need for acid etching of RMGIC to improve the bond.^[11-13] McLean *et al.* advocated etching for 60 s to achieve enhanced contact and mechanical interlocking between the bonding agent and the porosity created by acid etching of the cement. However, some researchers have opposed the acid etching procedure as it reduces the cohesive strength of the cement.^[14]

The depth of penetration between the surface pretreated with acid etching was significantly higher than control group. Researchers advocating for the etching procedure have not concluded standardizing the etching time. There were studies recommending 30 and/or 60 s of etching time for a desirable bonding effect.^[13,15,16] In the present study, etching was done for 15 s, as a study by Farshidfar et al.^[17] suggested that the use of 35% phosphoric acid for 15 s before the application of universal bonding agents improves the microtensile bond strength (µTBS) of GIC to resin composite. Acid etching of RMGIC cleans and moderately roughens the surface by creating a high surface energy.^[17] This process meets the demands for a highly bonded interface between RMGIC and composite resin.^[11] Based on the CLSM examination, an increase in surface irregularities of RMGIC was observed in the surface pretreated with acid etching [Figure 2] in comparison with the control group [Figure 1]. Otsuka et al.^[6] concluded that surface treatment on RMGIC had

Table 2: Depth of penetration in Group A in comparison with Group B and Group C

Groups	Sub groups	Mean difference $(I-J)$	SE	Р	95% CI	
					Lower bound	Upper bound
Control	Acid etching	-45.58400*	3.62399	<0.001*	-54.3885	-36.7795
	AA	-76.57800*	3.62399	<0.001*	-85.3825	-67.7735
Acid etching Cor AA	Control	45.58400*	3.62399	<0.001*	36.7795	54.3885
	AA	-30.99400*	3.62399	<0.001*	-39.7985	-22.1895
	Control	76.57800*	3.62399	<0.001*	67.7735	85.3825
	Acid etching	30.99400*	3.62399	<0.001*	22.1895	39.7985

*The mean difference is significant at the 0.05 level, *Statistical significance at P<0.05. More depth of penetration was observed in Group C compared to Group B with statistically high significant difference, respectively. (P<0.001). SE: Standard error, CI: Confidence interval, AA: Air abrasion

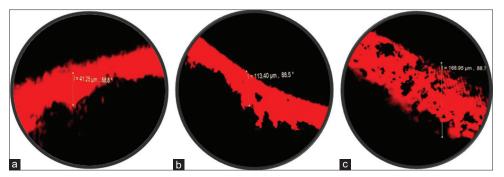


Figure 2: Confocal laser scanning microscopic (CLSM) images of the samples to check the depth of penetration of adhesive on the RMGIC surface. (a) CLSM image of the control group (Group I). (b) CLSM image of surface pretreated with acid etching (Group II). (c) CLSM image of surface pretreated with air abrasion (Group III)

a detrimental influence on shear bond strength (SBS), whereas surface treatment on CGIC encouraged stronger bond strength to resin composite. However, researchers have not concluded on the type and duration of surface treatment method for GIC. In contrast, a study by Munari *et al.*^[18] showed that acid etching on the GICs does not improve the μ TBS of the universal bonding agent. Some studies have shown that acid etching strengthens the bond between C-GICs and resin composite.^[18,19] Kermanshah *et al.*^[20] reported no discernible difference between resin composites bonded to etched and unetched GICs in terms of bond strength.

Surface pre-treatment of RMGIC with AA (Al₂O₂ particles) for 10 s at 60–80 psi at 90° at a distance of 2 mm) creates homogeneous microporosities on the surface, thereby increasing the surface energy, which allows the formation of resin microtags for the adhesive agent to penetrate resulting in increased bonding of composite resin.^[21,22] Based on the CLSM examination, there is a significant increased depth of penetration of adhesive observed in the surface pretreated with AA in comparison to that of acid etching and control groups. It could be due to the loss of the resin layer by Al₂O₂ particles in AA. Furthermore, this could be attributed to the elimination, either entirely or partially, of the polyacid chain of the methacrylate group of RMGIC responsible for its copolymerization with composite by AA.^[6,23] It has been seen that AA of RMGIC increases the SBS between composite resin and RMGIC.^[23] In correlation to that, the current study indicated that AA enhanced the sealing ability due to increased depth of penetration of the adhesive on the surface of RMGIC. On the contrary, a study by Ghubaryi et al.^[5] demonstrated that RMGIC treated with AA resulted in decreased SBS compared to conventional surface treatment techniques. This could be attributed to the destruction of the resin layer by Al₂O₂ particles from the AA process. Moreover, it is supposed that AA completely or partially removes residual or unreacted methacrylate groups in polyacid chain of RMGIC which is responsible for copolymerization with resin composite, hence compromising SBS.^[6,22]

Dental bonding agents have evolved from nonetch to total-etch (fourth and fifth generation), then self-etch (sixth, seventh, and eighth generation) systems.^[22] In this study, fifth-generation dentin bonding agent was used for bonding of composite resin to pretreated RMGIC. The primer and adhesive are in the same bottle. Its advantageous as it allows and helps in total removal of the smear layer. The RMGIC surface helps in creating the surface irregularities and gives higher penetration into the tags created by surface pretreatment. Rhodamine B, a fluorescent dye, has a smaller particle size and more surface-active molecules as compared to the most commonly used methylene blue dye. Hence, the adhesive resin was mixed with rhodamine dye (0.1%).

The current study has limitations due to its *in vitro* study methodology and the concentration of dye used. Future research should focus on the micromorphological study of the conditioned cement surface, as well as the influence of laser pretreatment on RMGIC and the depth of adhesive penetration on the same. Furthermore, the effect of acid etching on RMGIC may vary depending on the concentration and time of etching.

CONCLUSION

Within the constraints of this *in vitro* study, the results indicate that AA has the potential to be used as a pretreatment method before the placement of composite restoration in sandwich technique, and phosphoric acid etching of GIC before the placement of composite resin does not improve the sealing ability of sandwich restorations. Thus, AA before placement of composite onto RMGIC can be effective in a clinical scenario for increasing the depth of penetration of adhesive, which thereby increases the bond strength of sandwich restoration.

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Conflicts of interest

There are no conflicts of interest.

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