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Comparative evaluation of Penetration Depth, Marginal Adaptation and Microleakage of Conventional, Hydrophilic and Bioactive Pit and Fissure sealants in extracted third molars – an in-vitro study

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Abstract

Introduction: Dental caries predominantly affects the occlusal pits and fissures of posterior teeth due to their plaque-retentive anatomy. Pit and fissure sealants provide a protective barrier; however, their effectiveness depends on penetration, marginal adaptation, and microleakage. Hydrophilic and bioactive sealants have been developed to overcome limitations of conventional resin-based materials. **Objectives:** To compare the penetration depth, marginal adaptation, and microleakage of conventional, hydrophilic, and bioactive pit and fissure sealants. **Methods:** This in vitro study included 81 sound extracted third molars, randomly allocated to three groups (n=27): Conseal F (conventional), UltraSeal XT Hydro (hydrophilic), and BioCoat (bioactive). Following sealant placement and thermocycling (250 cycles, 5–55°C), penetration depth, marginal adaptation, and microleakage (n=9/group) were assessed using stereomicroscopy, scanning electron microscopy, and confocal laser scanning microscopy, respectively. Data were analysed using ANOVA, Tukey's post hoc, and Chi-square tests. **Results:** Conseal F demonstrated the highest complete penetration (33.3%; $p=0.01$). BioCoat showed the greatest mean penetration depth (583.33±125 µm; $p<0.03$), superior marginal adaptation (66.7% smooth margins; $p<0.001$), and least microleakage (55.6% with no dye penetration; $p<0.001$). UltraSeal XT Hydro showed the highest microleakage ($p=0.007$). **Conclusion:** BioCoat demonstrated superior marginal adaptation and sealing ability, suggesting potential for enhanced fissure protection. Further long-term clinical studies are warranted. **Clinical Significance:** BioCoat, with its bioactive properties and favourable sealing characteristics, may be a preferable option for preventive occlusal caries management by limiting microleakage at the sealant tooth interface. Its potential to preserve tooth structure and support minimally invasive care highlights a promising biologically orientated approach to caries-preventive sealants.

Keywords: pit and fissure sealants; bioactive; scanning electron microscopy; confocal microscopy; dental marginal adaptation; micro leakage

1. Introduction

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth characterized by demineralization of the inorganic component and destruction of the organic matrix, often leading to cavitation [1]. It is also defined as an infectious microbiologic disease that results in localized destruction of the calcified dental tissues [2]. Dental caries remains one of the most prevalent infectious diseases worldwide, affecting individuals across all age groups [3]. It develops due to an imbalance between commensal and cariogenic microorganisms. Cariogenic bacteria such as Actinomyces species, Lactobacillus, and Streptococcus mutans metabolize carbohydrates to produce acids that demineralize enamel [4]. This acidic environment lowers the pH below 5.5, resulting in hydroxyapatite dissolution and caries formation [5].

Although occlusal surfaces account for only about 13% of the total tooth surface area [6], they contribute to 80–90% of dental caries in permanent teeth [7]. Approximately 90% of carious lesions in permanent posterior teeth originate in pits and fissures [8]. Ash defined a pit as a small depression at the junction or termination of developmental grooves, whereas Orban described fissures as deep clefts between adjoining cusps [9]. Their complex anatomy promotes plaque retention and hinders effective cleaning, making them highly susceptible to caries. Pits and fissures account for nearly 56–70% of dental caries among school children aged 5–17 years [10, 11].

The high caries susceptibility of posterior occlusal surfaces is primarily attributed to bacterial accumulation within the intricate morphology of pits and fissures [12]. Their morphology is determined during tooth development by the internal enamel epithelium [13] and varies considerably among teeth [14]. Pit and fissure depths range from 120 to 1050 μm [15]. Nagano classified fissures into V, U, I (Y1), and IK (Y2) types based on morphology [16].

The American Dental Association (ADA), Council on Scientific Affairs (CSA), and the American Academy of Pediatric Dentistry have recognized pit and fissure sealants as more effective than fluoride varnish or no treatment in preventing and arresting occlusal caries in primary and permanent molars [17]. Simonsen defined pit and fissure sealants as materials placed into susceptible pits and fissures to form a micromechanically bonded protective barrier that blocks cariogenic bacteria from nutrient sources [18]. Sealants prevent food impaction and plaque accumulation and can reduce caries incidence by up to nine times compared with unsealed teeth [19].

Despite their effectiveness, sealants may exhibit microleakage, inadequate marginal adaptation, and insufficient penetration depth, which can compromise their long-term success [20]. Their clinical performance depends largely on retention on the occlusal surface [21]. Partial or complete sealant loss may create stagnation areas that favor caries development, emphasizing the need for improved materials [22]. Conventional resin-based fluoridated sealants such as Conseal F provide fluoride release and good retention but are hydrophobic and highly technique-sensitive, requiring strict moisture control [23]. To overcome these limitations, hydrophilic sealants such as UltraSeal XT hydro have been developed to bond effectively to slightly moist surfaces, thereby enhancing retention and reducing microleakage [24].

Recent advancements have led to the development of bioactive sealants, although only a limited number are commercially available. Their caries-preventive effect is attributed to antimicrobial activity and ion release in response to pH changes [25]. BioCoat is a bioactive pit and fissure sealant containing rechargeable microcapsules loaded with fluoride, calcium, and phosphate ions that provide sustained protection [26]. To the best of our knowledge, no previous study has compared the penetration depth, marginal adaptation, and microleakage of BioCoat with a conventional resin-based fluoridated sealant (Conseal F) and a hydrophilic sealant (UltraSeal XT hydro). Therefore, this study aimed to compare and evaluate these three sealants with respect to penetration depth, marginal adaptation, and microleakage.

2. Methodology

2.1. Sample Preparation

This in vitro experimental study was approved by the Institutional Ethics Committee of Madha Dental College and Hospital, Tamil Nadu, India (Approval No. MDCH/IEC/2022/18). The study involved anonymised extracted third molars obtained as discarded clinical material from the dental institution. No personal or identifiable patient information was collected, accessed, or recorded. The consent requirement for the use of anonymised extracted teeth has been clarified with the Institutional Ethics Committee. The sample size was calculated using the mean and standard deviation of outcome parameters from a previous study by Prabakar et al. (2020) [27] using G*Power 3.1.2 software with a power of 95%, an α error of 0.05, and an effect size of 0.92. The estimated sample size was 27 teeth per group, and the final sample size was determined to be 81 teeth, which were equally distributed into three groups. Group I consisted of 27 samples treated with conventional sealant (Conseal F), Group II consisted of 27 samples treated with hydrophilic sealant (UltraSeal XT hydro), and Group III consisted of 27 samples treated with bioactive sealant (BioCoat).

2.2. Procedure

Anatomically and morphologically well-defined maxillary and mandibular third molars were selected for the study. The inclusion criteria consisted of recently extracted, non-carious third molars with deep pits and fissures, whereas teeth with dental fluorosis or developmental defects were excluded. Freshly extracted maxillary and mandibular third molars that met the selection criteria were cleaned by soaking in 3% sodium hypochlorite to remove blood and tissue debris and were subsequently stored in 10% formalin until further use [27]. For sealant placement, the occlusal surfaces of the teeth were etched with 37% orthophosphoric acid for 30 seconds, followed by thorough rinsing with water and drying with a three-way syringe until a frosty white enamel appearance was obtained. The respective sealants—Conseal F for Group I, UltraSeal XT hydro for Group II, and Premier BioCoat for Group III—were then applied according to the study

grouping and light-cured for 30 seconds. The treated samples were stored in artificial saliva until further evaluation.

2.3. Evaluation of Penetration depth

The penetration depth of the sealant was evaluated in nine samples from each group. The samples were thermocycled for 250 cycles between 5 °C and 55 °C with a dwell time of 30 seconds. The apices of the samples were sealed with sticky wax, and the coronal surfaces (except the occlusal surfaces) were double coated with nail varnish, leaving a 2 mm margin around the sealant. The samples were then immersed in 5% methylene blue dye for 24 hours, after which they were washed thoroughly to remove excess dye and mounted in acrylic resin. Buccolingual sections were made through the sealant using a high-speed straight handpiece and a fine diamond disc under water spray. The sectioned samples were examined under a stereomicroscope at 10× magnification [28]. Penetration depth was assessed using the three-point scoring system described by Barbara Kane et al. [29],

Score 1 - Sealant penetrated 1/3 the total length of the fissure,

Score 2 - Sealant penetrated 1/2 the total length of the fissure,

Score 3 - Sealant penetrated the total length of the fissure.

2.4. Evaluation of Marginal Adaptation

Marginal adaptation of the sealants was evaluated in nine samples from each group. The samples were subjected to thermocycling between 5 °C and 55 °C for 250 cycles with a dwell time of 30 seconds to simulate oral conditions. The apices were sealed with sticky wax and the coronal surfaces (except the occlusal surfaces), were double coated with nail varnish, leaving a 2 mm margin around the sealant. The samples were then stored in artificial saliva for 15 days prior to evaluation. Following this, the samples

were embedded in acrylic resin, the roots were removed, and the crowns were trimmed from the sectioned side to obtain a final thickness of 4 mm. The specimens were then evaluated under a Scanning Electron Microscope (SEM) [30]. Marginal adaptation was assessed using the two-point scoring system described by Barbara Kane et al. [29],

Score 1 - Smooth adaptation. Sealant flows with enamel. No ledges,

Score 2 - Sealant is not well adapted. Ledge may be present.

2.5. *Evaluation of Microleakage*

Microleakage evaluation was also performed on nine samples from each group. The sealed molars were thermocycled between 5 °C and 55 °C for 250 cycles with a dwell time of 30 seconds. The apices of the samples were sealed with sticky wax, and the coronal surfaces (except the occlusal surfaces), were double coated with nail varnish, leaving a 2 mm margin around the sealant. The samples were immersed in 0.1% rhodamine B isothiocyanate fluorescent dye for 24 hours at 37 °C and then washed thoroughly to remove excess dye. The samples were mounted in acrylic resin and sectioned longitudinally. The sectioned samples were evaluated for microleakage using a Confocal Laser Scanning Microscope (CLSM). Microleakage scoring was carried out according to the criteria described by Övrebö RC and Raadal M [31],

Score 0 - No dye penetration,

Score 1 - Dye penetration restricted to the outer half of the sealant,

Score 2 - Dye penetration to the inner half of the sealant,

Score 3 - Dye penetration into an underlying fissure.

2.6. *Statistical Analysis*

Data was entered into a Microsoft Excel spreadsheet and analyzed using the Statistical Package for Social Sciences (SPSS version 27.0 for Windows, SPSS Inc., Chicago, USA). Descriptive statistics, including mean and standard deviation, were calculated for all groups. ANOVA was used to assess mean differences in continouous data among the three Pit and Fissure sealant groups. A Post Hoc Tukey HSD test was used to compare Penetration depth, Marginal adaptation, and Microleakage among the groups. The Chi square test was used to compare the proportions among the different groups.

3. Results

Table 1 shows the Penetration depth of the study groups viewed under a stereomicroscope. Penetration depth was categorized into three scores: Score 1 (penetration of 1/3 of the fissure length), Score 2 (½ of the fissure length), and Score 3 (complete penetration to the base of the fissure). The high-viscosity hydrophilic sealant (Ultraseal XT hydro) demonstrated the worst performance, in 66.7% of samples with Score 1 and only 33.3 % Score 2. The Bioactive sealant (BioCoat) also had poor penetration depth, with 33.3% and 66.7% of the samples receiving scores of 1 and 2, respectively. By way of comparison, the Conventional sealant (Conseal F) showed the best results, where no samples showed limited penetration (Score 1) 66.7% showed moderate penetration (Score 2), and 33.3% showed full penetration (Score 3). The results are illustrated in [Figure 1]. These differences were statistically significant with p value ($p = 0.01$), ($X^2 = 13.2$).

Table 2 shows the Marginal adaptation of various study groups of the present study viewed under a scanning electron microscope. Bioactive sealant (BioCoat) was observed to perform best with 66.7% samples depicted smooth adaptation (Score 1), followed by both conventional (Conseal F) and hydrophilic (Ultraseal XT hydro) sealants with 33.3% smooth adaptation. In the rest of the samples of these two groups (66.7%) were observed worse adaptation with existence of possible ledge (Score 2). The results are illustrated in [Figure 2]. However, the difference was not statistically significant with p value (p = 0.26) and Chi square value (X^2 = 2.7).

Table 3 shows the Microleakage scores of the study groups viewed under Confocal Laser Scanning Microscope with scores ranging from 0 (no leakage) to 3 (maximum leakage). Best results were seen with the Bioactive sealant, where 55.6 % of the samples (5 out of 9) were scored as no dye penetration (Score 0) and only 22.2 % as Score 3. Widest leakage areas were observed in Hydrophilic sealant which showed 55.6 % and 33.3 % of the samples scoring 2 and 3 respectively while Hydrophilic sealant was found to have the lowest sealing ability. The Conventional sealant presented an intermediate performance, with 44.4% of surfaces rated 1 and 2 each and 11.1% rated 3. The results are illustrated in [Figure 3]. The difference was statistically significant with p value (p = 0.007) and Chi square value (X^2 = 17.7).

Table 4 and graph 1 shows the mean differences in Penetration depth between Conventional, Hydrophilic, and Bioactive Pit and Fissure sealant groups viewed under Stereomicroscope. The Conventional group showed a mean penetration depth of 521.37 µm (no deviation, SD = 0) and Hydrophilic group had a mean penetration depth of 500

μm (no deviation, SD = 0). The Bioactive sealant, however, exhibited a greater average penetration depth of 583.33±125.00 μm with significant difference ($p = 0.03$) and ($F = 4$).

Table 5 and graph 2 shows the Post Hoc comparison of Penetration depth between the groups viewed under Stereomicroscope. There was a significant difference between the Conventional and Bioactive groups (mean difference -83.33 μm, $p = 0.05$) and Hydrophilic and Bioactive groups (mean difference 83.33 μm, $p = 0.05$). But there was no statistically significant difference between the Conventional and Hydrophilic groups with no mean difference ($p = 1.00$).

Table 6 and graph 3 shows the mean differences in Marginal adaptation between Conventional, Hydrophilic, and Bioactive Pit and Fissure sealant groups viewed under a Scanning Electron Microscope. The highest mean value (10866.67±278.39 μm) was recorded for the Bioactive sealant, revealing better adaptation to enamel. The Conventional sealant series exhibited the next highest mean value of 10533.33±132.29 μm with the Hydrophilic sealant showing the lowest mean value of 10366.67±50.00 μm. This difference was statistically significant ($p < 0.001$) and ($F = 17.9$).

Table 7 and graph 4 shows the Post Hoc comparison of Marginal adaptation between the groups viewed under a Scanning Electron Microscope. There was also a significant difference between the Bioactive and the other groups by marginal adaptation, compared to both the Conventional (mean difference = -333.33 μm, $p = 0.002$) and the Hydrophilic (mean difference = -500.00 μm, $p = 0.001$) sealants. But a statistical significant difference was not observed between the Conventional and Hydrophilic groups (Mean difference=166.67 μm, $p = 0.14$).

Table 8 and graph 5 shows the mean differences in Microleakage between Conventional, Hydrophilic, and Bioactive Pit and Fissure sealant groups viewed under Confocal Laser Scanning Microscope. The Bioactive sealant had the highest mean microleakage of 424.42±14.15 µm, followed by the Conventional sealant with 385.11±7.62 µm, and the lowest mean microleakage was observed in Hydrophilic sealant with 264.11±43.99 µm. The difference was statistically significant ($p < 0.001$) and ($F = 85.9$).

Table 9 and graph 6 shows the Post Hoc comparison of Microleakage between the groups viewed under Confocal Laser Scanning Microscope. A significant mean difference of 120.99 µm ($p < 0.001$) was observed between Conventional and Hydrophilic sealants, indicating higher microleakage in the Conventional group. The Bioactive sealant showed significantly lower microleakage compared to the Hydrophilic sealant with a mean difference of -160.31 µm ($p < 0.001$). A smaller but significant difference was also noted between Bioactive and Conventional groups (- 39.32 µm, $p = 0.01$).

4. Discussion

Pits and Fissures are more prone to enamel caries due to the complex form and the reduced access of saliva for the cleansing of these sites [32]. Consequently, the most effective measure to protect against caries in these regions is sealing [33]. Pit and fissure sealants have long been considered a primary intervention to reduce pit and fissure caries especially in children and adolescents [17]. This study was conducted to assess and compare the clinical effectiveness of a newly introduced bioactive sealant (BioCoat) in comparison with a conventional sealant (Conseal F) and a hydrophilic sealant (Ultraseal XT hydro) by assessing the penetration depth, microleakage, and marginal

adaptation by Stereomicroscope, Scanning Electron Microscope and Confocal Scanning Laser Microscope respectively.

4.1. Penetration Depth

The present study demonstrated significant differences in the penetration depth of the three pit and fissure sealants ($p = 0.01$). Conseal F showed the best penetration, likely due to its low viscosity and favorable flow properties, whereas UltraSeal XT hydro exhibited the least penetration, possibly because its higher filler content increased viscosity and reduced flow into fissures. BioCoat demonstrated intermediate penetration, indicating satisfactory adaptation to fissure morphology. These findings suggest that material viscosity and filler content play an important role in sealant penetration.

The present findings are partially supported by Selecman et al. (2007) [34], who reported no significant differences in penetrability or microleakage among five sealants, including Conseal F. However, Conseal F demonstrated a balanced combination of penetration and sealing ability, supporting its clinical effectiveness.

Analysis of mean penetration depth revealed that BioCoat exhibited the greatest penetration, followed by Conseal F and UltraSeal XT hydro, with significant differences between BioCoat and the other two groups ($p = 0.03$). The enhanced penetration of BioCoat may be attributed to its improved flow characteristics and bioactive composition, which may facilitate better adaptation to fissure morphology and potentially improve sealant retention.

In contrast, Prabakar et al. (2023) [35] reported superior penetration of UltraSeal XT hydro compared with the conventional sealant Clinpro. However, UltraSeal XT hydro demonstrated the least penetration in the present study, possibly due to its higher filler content and differences in study methodology.

4.2. Marginal Adaptation

The present study demonstrated that BioCoat exhibited superior marginal adaptation compared with Conseal F and UltraSeal XT hydro. Although the Chi-square analysis did not reveal a statistically significant difference ($p = 0.26$), SEM analysis showed significantly greater marginal adaptation for BioCoat than both conventional and hydrophilic sealants ($p < 0.001$). Post hoc analysis confirmed significant differences between BioCoat and Conseal F ($p = 0.002$) as well as UltraSeal XT hydro ($p = 0.001$), whereas no significant difference was observed between Conseal F and UltraSeal XT hydro ($p = 0.14$). The improved adaptation of BioCoat may be attributed to its favorable flow characteristics and bioactive ion releasing properties.

These findings differ from those of Fernandes et al. (2019) [36], who reported intermediate marginal adaptation with Conseal F. While Conseal F demonstrated acceptable sealing ability in the present study, BioCoat achieved superior adaptation, possibly due to advancements in material composition and flow properties.

Similarly, Ghadge et al. (2024) [37] reported better marginal adaptation of Conseal F than UltraSeal XT hydro, attributing this to its lower filler content and the use of a bonding agent. In the present study, although Conseal F performed better than UltraSeal XT

hydro, BioCoat demonstrated the highest marginal adaptation, suggesting its potential for improved clinical sealing performance.

4.3. Microleakage

The present study demonstrated significant differences in microleakage among the three pit and fissure sealants ($p = 0.007$). BioCoat exhibited the best sealing ability, with the highest proportion of samples showing no dye penetration and the lowest mean microleakage. In contrast, UltraSeal XT hydro showed the greatest microleakage, while Conseal F demonstrated intermediate performance. These findings suggest that the bioactive composition of BioCoat may enhance marginal sealing and reduce microleakage.

Mean microleakage analysis further confirmed the superior performance of BioCoat, with significantly lower leakage than both Conseal F and UltraSeal XT hydro. Post hoc analysis demonstrated the greatest difference between BioCoat and UltraSeal XT hydro ($p < 0.001$), indicating the superior sealing efficacy of the bioactive sealant.

These findings contrast with those of Prabakar et al. (2020, 2023) [27,35], who reported lower microleakage with the hydrophilic sealant UltraSeal XT hydro than with conventional sealants. Similarly, Güçlü et al. (2016) [38] observed improved sealing of UltraSeal XT hydro when used with laser and acid etching. The discrepancy may be attributed to differences in study design, surface conditioning protocols, and testing methods. The superior sealing performance of BioCoat observed in the present study

suggests that its bioactive composition may provide improved seal integrity and reduce microleakage, making it a promising material for long-term clinical success.

Despite the significant findings of the present study, certain limitations should be acknowledged. As an in vitro investigation, the study could not fully replicate the complex intraoral environment, including occlusal forces, salivary contamination, and masticatory stresses. Therefore, further long-term in vivo studies are warranted to comprehensively evaluate the performance of these sealants with respect to penetration depth, marginal adaptation, microleakage, and caries-preventive efficacy.

5. Conclusion

Based on the results and the limitations of the present in vitro study, the bioactive pit and fissure sealant (BioCoat) demonstrated significantly superior penetration depth, marginal adaptation, and reduced microleakage compared with the conventional sealant (Conseal F) and the hydrophilic sealant (UltraSeal XT hydro). Its enhanced performance may be attributed to its bioactive ion-releasing properties, which promote improved sealing and adaptation. Conseal F exhibited good fissure penetration, likely due to its low viscosity and favorable flow characteristics, but showed moderate performance in marginal adaptation and microleakage. UltraSeal XT hydro demonstrated the least favorable performance, possibly due to its higher filler content, which may have reduced its flowability despite its hydrophilic nature. These findings suggest that bioactive sealants have promising potential for long-term caries prevention. However, further long-term in vivo studies are required to validate these findings under clinical conditions.

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Authors' contributions

Conceptualization – PA Elias, Z Abdullah; NB Iyenkani; Methodology – PA Elias, NB Iyenkani; Formal analysis – PA Elias, Investigation – PA Elias, data curation – PA Elias, Z Abdullah, AK Sundaragopal; Writing—original draft preparation – PA Elias, Z Abdullah, NB Iyenkani, AK Sundaragopal; Writing—review and editing - PA Elias, Z Abdullah, NB Iyenkani, AK Sundaragopal, R Chakkarapani, C Sandra; Visualization – PA Elias, Supervision – PA Elias, Project administration - PA Elias, All authors have read and agreed to the published version of the manuscript.

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Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to data privacy but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee of a Private Dental College and Hospital, Chennai, India (IEC No. MDCH/IEC/2022/18). Extracted human third molars obtained following routine clinical extraction were used for the study. The teeth were anonymized, and no personal or identifiable patient information was collected. The study was conducted in accordance with the ethical principles applicable to research involving human derived biological materials.

Consent for publication

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

SEM – Scanning Electron Microscopy; CLSM – Confocal Laser Scanning Microscopy; SPSS – Statistical Package for the Social Sciences; ANOVA – Analysis of Variance; SD - Standard deviation; IEC - Institutional Ethics Committee; CI - Confidence interval; µm - Micrometre

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TABLES AND GRAPHS

TABLE 1: PENETRATION DEPTH OF THE STUDY GROUPS VIEWED UNDER STEREOMICROSCOPE

GROUPS	PENETRATION DEPTH				Chi square	p value
	Score 1 n (%)	Score 2 n (%)	Score 3 n (%)	Total N (%)		
GROUP I	0	6 (66.7%)	3 (33.3%)	9 (100%)	13.2	**0.01***
GROUP II	6 (66.7%)	3 (33.3%)	0	9 (100%)		
GROUP III	3 (33.3%)	6 (66.7%)	0	9 (100%)		
TOTAL	9 (33.3%)	15 (55.6%)	3 (11.1%)	27 (100%)		

There is a statistically significant difference in penetration depth of the study groups (p value = 0.01*)

Chi square test; **p ≤ 0.05 is considered statistically significant.**

GROUPS	MARGINAL ADAPTATION			Chi square	p value
	Score 1 n (%)	Score 2 n (%)	Total N (%)		
GROUP I	3 (33.3%)	6 (66.7%)	9 (100%)	2.7	0.26
GROUP II	3 (33.3%)	6 (66.7%)	9 (100%)		
GROUP III	6 (66.7%)	3 (33.3%)	9 (100%)		
TOTAL	12 (44.4%)	15 (55.6%)	27 (100%)		

TABLE 2: MARGINAL ADAPTATION OF THE STUDY GROUPS VIEWED UNDER SCANNING ELECTRON MICROSCOPE

There is no statistically significant difference in the Marginal adaptation of the study groups. (p value = 0.26)

Chi square test; p ≤ 0.05 is considered statistically significant*

TABLE 3: MICROLEAKAGE OF THE STUDY GROUPS VIEWED UNDER CONFOCAL LASER SCANNING MICROSCOPE

GROUPS	MICROLEAKAGE					Chi square	p-value
	Score 0 n (%)	Score 1 n (%)	Score 2 n (%)	Score 3 n (%)	Total N (%)		
GROUP I	0	4 (44.4%)	4 (44.4%)	1 (11.1%)	9 (100%)	17.7	**0.007***
GROUP II	0	1 (11.1%)	5 (55.6%)	3 (33.3%)	9 (100%)		
GROUP III	5 (55.6%)	2 (22.2%)	0	2 (22.2%)	9 (100%)		
TOTAL	5 (18.5%)	7 (25.9%)	9 (33.3%)	6 (22.2%)	27 (100%)		

There is a statistically significant difference in Microleakage of the study groups (p value = 0.007*)

Chi square test; p ≤ 0.05 is considered statistically significant*

TABLE 4: MEAN DIFFERENCES IN PENETRATION DEPTH BETWEEN THE STUDY GROUPS VIEWED UNDER STEREOMICROSCOPE

GROUPS	N	Mean±SD (μm)	F value	p value
GROUP I	9	521.37±0.00	4	**0.03***
GROUP II	9	500.00±0.00		
GROUP III	9	583.33±125.00		

There is a statistically significant difference in mean difference of Penetration depth between the study groups viewed under Stereomicroscope (p value = 0.03*)

One way ANOVA; p ≤ 0.05 is considered significant*

GRAPH 1: MEAN DIFFERENCES IN PENETRATION DEPTH BETWEEN THE STUDY GROUPS VIEWED UNDER STEREOMICROSCOPE

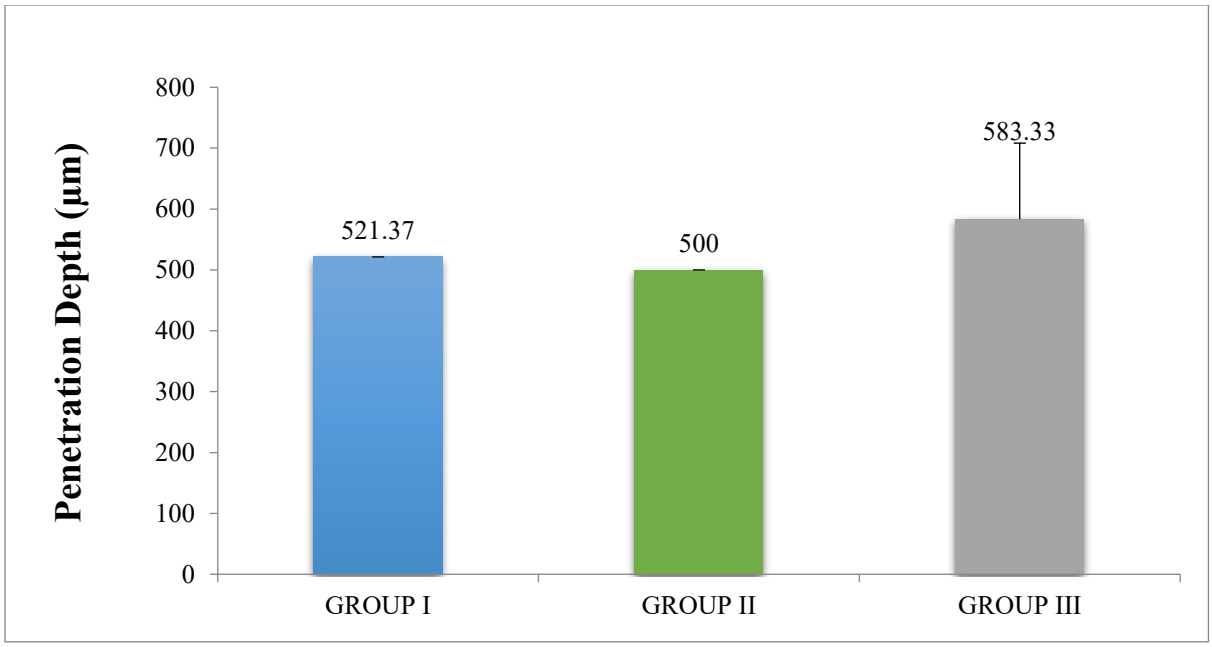


TABLE 5: POST HOC COMPARISON OF PENETRATION DEPTH BETWEEN THE STUDY GROUPS VIEWED UNDER STEREOMICROSCOPE

COMPARISON	Mean Difference (µm)	Std. Error	p value
GROUP I VS GROUP II	0.00	34.02	1.00
GROUP I VS GROUP III	83.33		**0.05***
GROUP II VS GROUP III	-83.33		**0.05***

There is a statistically significant difference in the Post Hoc comparison of Penetration depth between Group I VS Group III (p = 0.05*) and Group II VS Group III (p = 0.05*) viewed under Stereomicroscope.

Post Hoc Test – Tukey HSD; p ≤ 0.05 is considered significant*

GRAPH 2: COMPARISON OF PENETRATION DEPTH BETWEEN THE STUDY GROUPS VIEWED UNDER STEREOMICROSCOPE

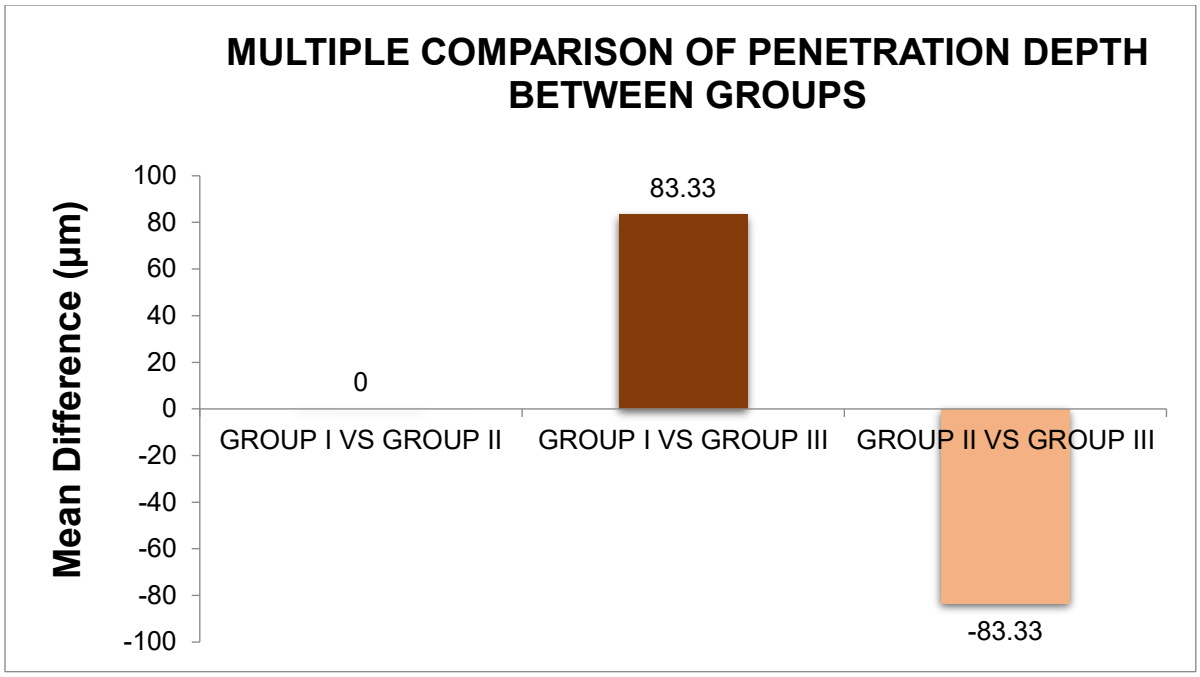


TABLE 6: MEAN DIFFERENCES IN MARGINAL ADAPTATION BETWEEN THE STUDY GROUPS VIEWED UNDER SCANNING ELECTRON MICROSCOPE

GROUPS	N	Mean±SD (µm)	F value	p value
GROUP I	9	10533.33±132.29	17.9	**<0.001***
GROUP II	9	10366.67±50.00		
GROUP III	9	10866.67±278.39		

There is a statistically significant difference in mean difference of Marginal adaptation between the study groups viewed under Scanning Electron Microscope (p value <0.001*)

One way ANOVA; p ≤ 0.05 is considered significant*

GRAPH 3: MEAN DIFFERENCES IN MARGINAL ADAPTATION BETWEEN THE STUDY GROUPS VIEWED UNDER SCANNING ELECTRON MICROSCOPE

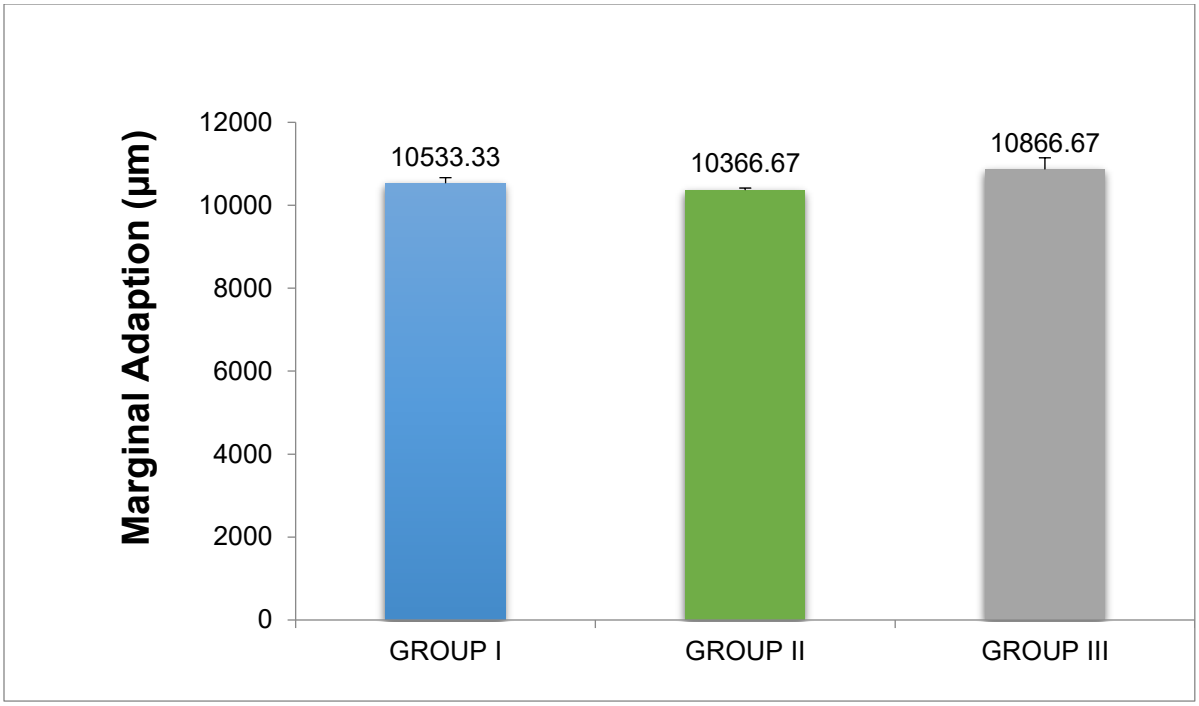


TABLE 7: POST HOC COMPARISON OF MARGINAL ADAPTATION BETWEEN THE STUDY GROUPS VIEWED UNDER SCANNING ELECTRON MICROSCOPE

COMPARISON	Mean Difference (µm)	Std. Error	p value
GROUP I VS GROUP II	166.67	84.98	0.14
GROUP I VS GROUP III	-333.33		**0.002***
GROUP II VS GROUP III	-500.00		**0.001***

There is a statistically significant difference in the Post Hoc comparison of Marginal adaptation between Group I VS Group III (p = 0.002*) and Group II VS Group III (p = 0.001*) groups viewed under Scanning Electron Microscope.

Post Hoc Test – Tukey HSD; p ≤ 0.05 is considered significant*

GRAPH 4: COMPARISON OF MARGINAL ADAPTATION BETWEEN THE STUDY GROUPS VIEWED UNDER SCANNING ELECTRON MICROSCOPE

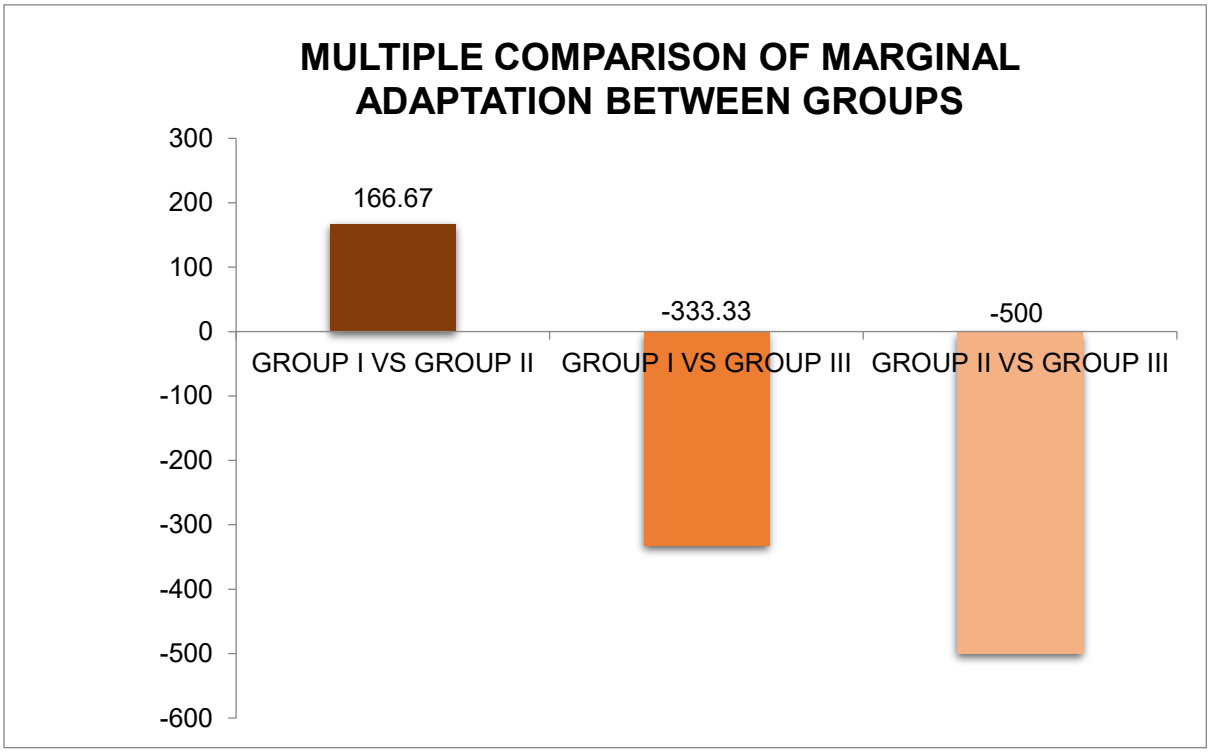


TABLE 8: MEAN DIFFERENCES OF MICROLEAKAGE BETWEEN THE STUDY GROUPS VIEWED UNDER CONFOCAL LASER SCANNING MICROSCOPE

GROUPS	**N**	**Mean±SD** (µm)	**F value**	**p-value**
GROUP I	9	385.11±7.62	85.9	**<0.001***
GROUP II	9	264.11±43.99		
GROUP III	9	424.42±14.15		

There is a statistically significant difference in mean difference of Microleakage between the study groups viewed under Confocal Laser Scanning Microscope (p value <0.001*)

One way ANOVA; p ≤ 0.05 is considered significant*

GRAPH 5: MEAN DIFFERENCES IN MICROLEAKAGE BETWEEN THE STUDY GROUPS VIEWED UNDER CONFOCAL LASER SCANNING MICROSCOPE

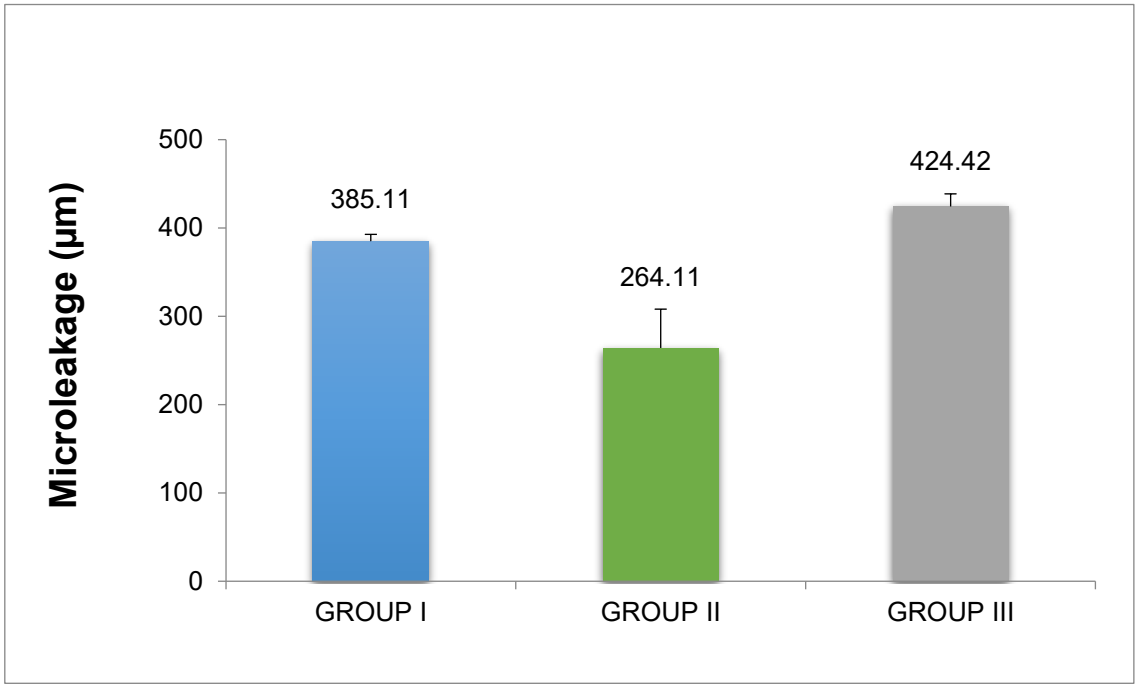


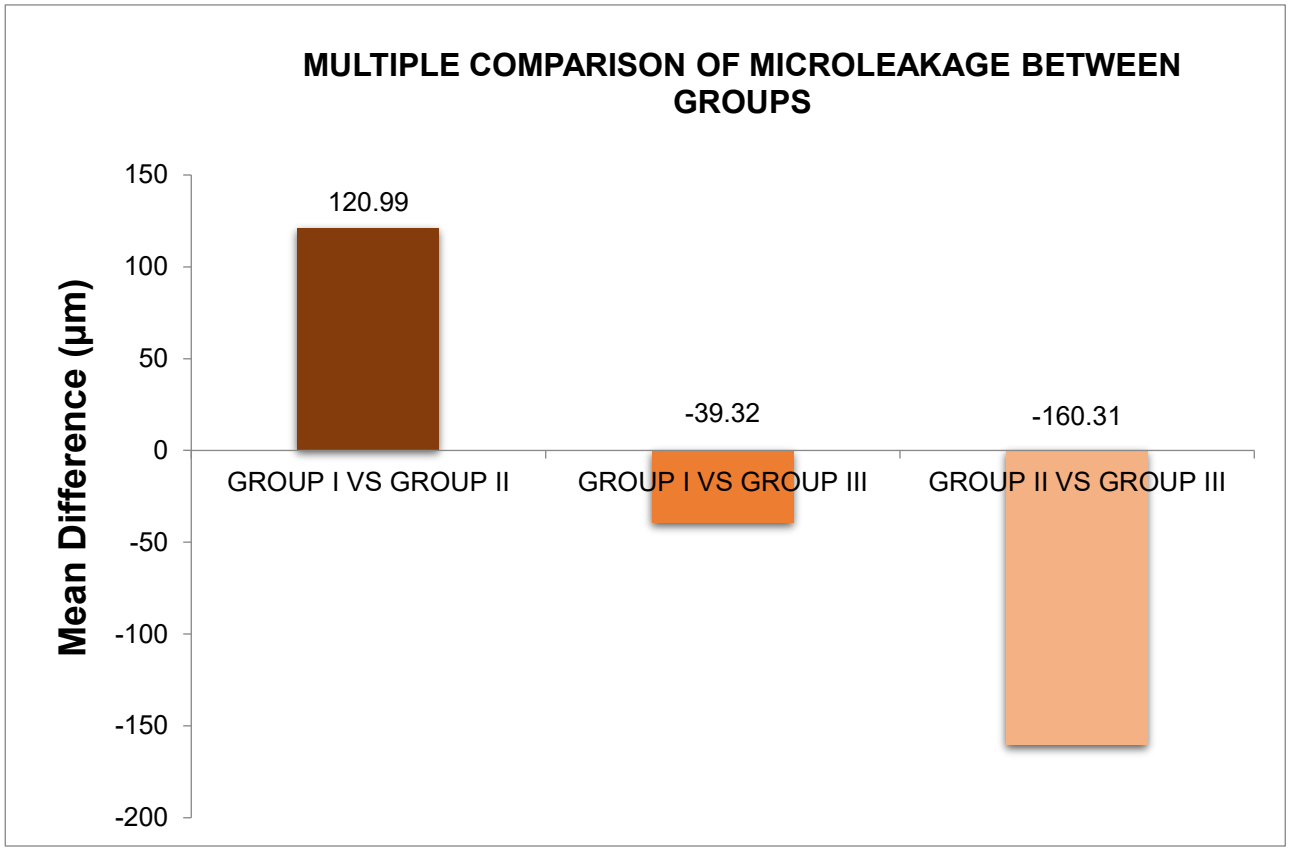
TABLE 9: POST HOC COMPARISON OF MICROLEAKAGE BETWEEN THE STUDY GROUPS VIEWED UNDER CONFOCAL LASER SCANNING MICROSCOPE

COMPARISON	Mean Difference (µm)	Std. Error	p value
GROUP I VS GROUP II	120.99	12.75	**<0.001***
GROUP II VS GROUP III	-160.31		**<0.001***
GROUP I VS GROUP III	-39.32		**0.01***

There is a statistically significant difference in the Post Hoc comparison of Microleakage between Conventional VS Hydrophilic ($p < 0.001$*); Hydrophilic VS Bioactive ($p < 0.001$*) and Conventional VS Bioactive ($p = 0.01$*) groups viewed under Confocal Laser Scanning Microscope.

Post Hoc Test – Tukey HSD; p ≤ 0.05 is considered significant*

GRAPH 6: COMPARISON OF MICROLEAKAGE BETWEEN THE STUDY GROUPS VIEWED UNDER CONFOCAL LASER SCANNING MICROSCOPE



FIGURES

FIGURE 1: STEREOMICROSCOPIC IMAGES OF SAMPLES SHOWING PENETRATION DEPTH SCORES UNDER 10x MAGNIFICATION.

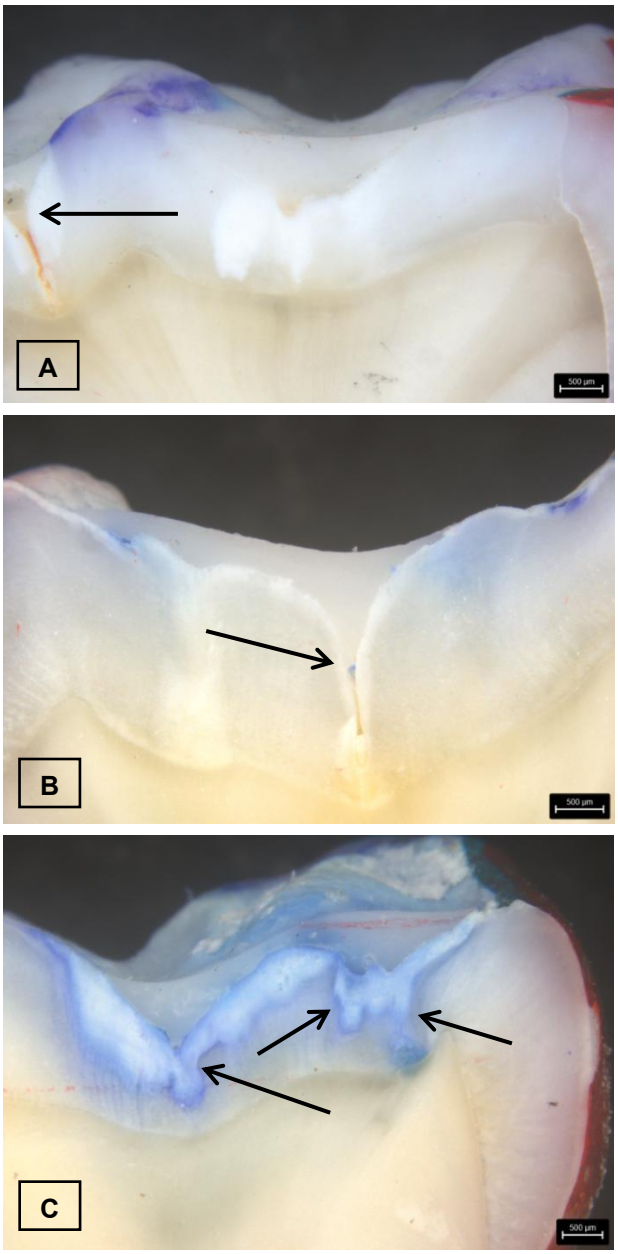
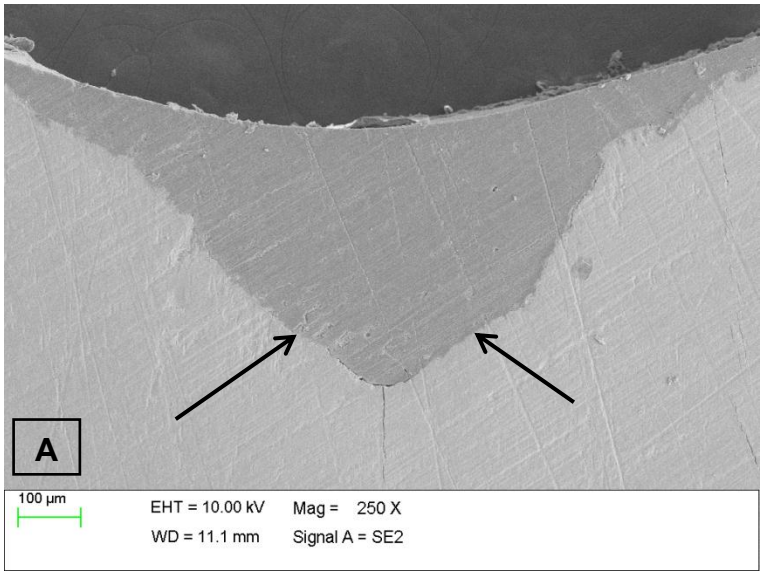


Figure 1 (A) Score 1 in Group II, Black arrow indicates Sealant penetrated 1/3 of total length of the fissure; Figure 1 (B) Score 2 in Group I, Sealant penetrated ½ of total length of the fissure; Figure 1 (C) Score 3 in Group I, Black arrow indicates Sealant penetrated the total length of fissure.

FIGURE 2: SCANNING ELECTRON MICROSCOPIC IMAGES OF SAMPLES SHOWING MARGINAL ADAPTATION SCORES UNDER 250x, 200x MAGNIFICATION.



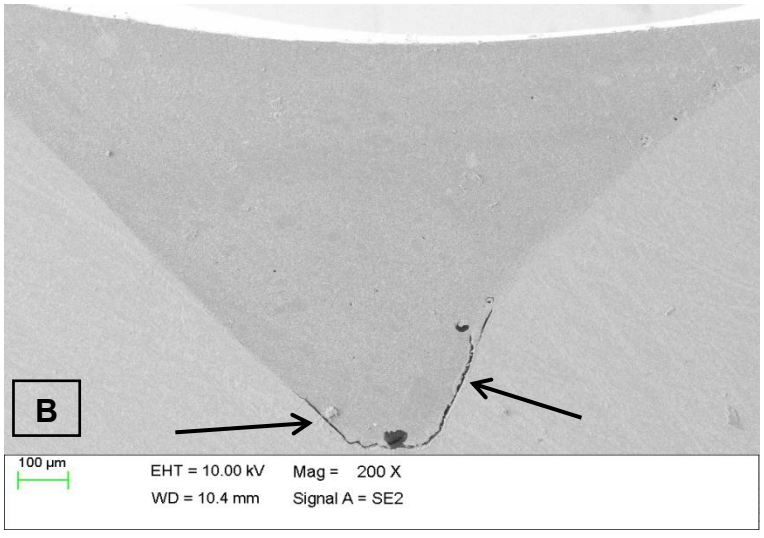


Figure 2 (A) Score 1 in Group III, Black arrow indicates the well adapted smooth margins without ledges; Figure 2 (B) Score 2 in Group I, Black arrow indicates the not well adapted smooth margins with legdes.

FIGURE 3: CONFOCAL LASER SCANNING MICROSCOPIC IMAGES OF SAMPLES SHOWING MICROLEAKAGE SCORES UNDER 250x, 200x MAGNIFICATION.

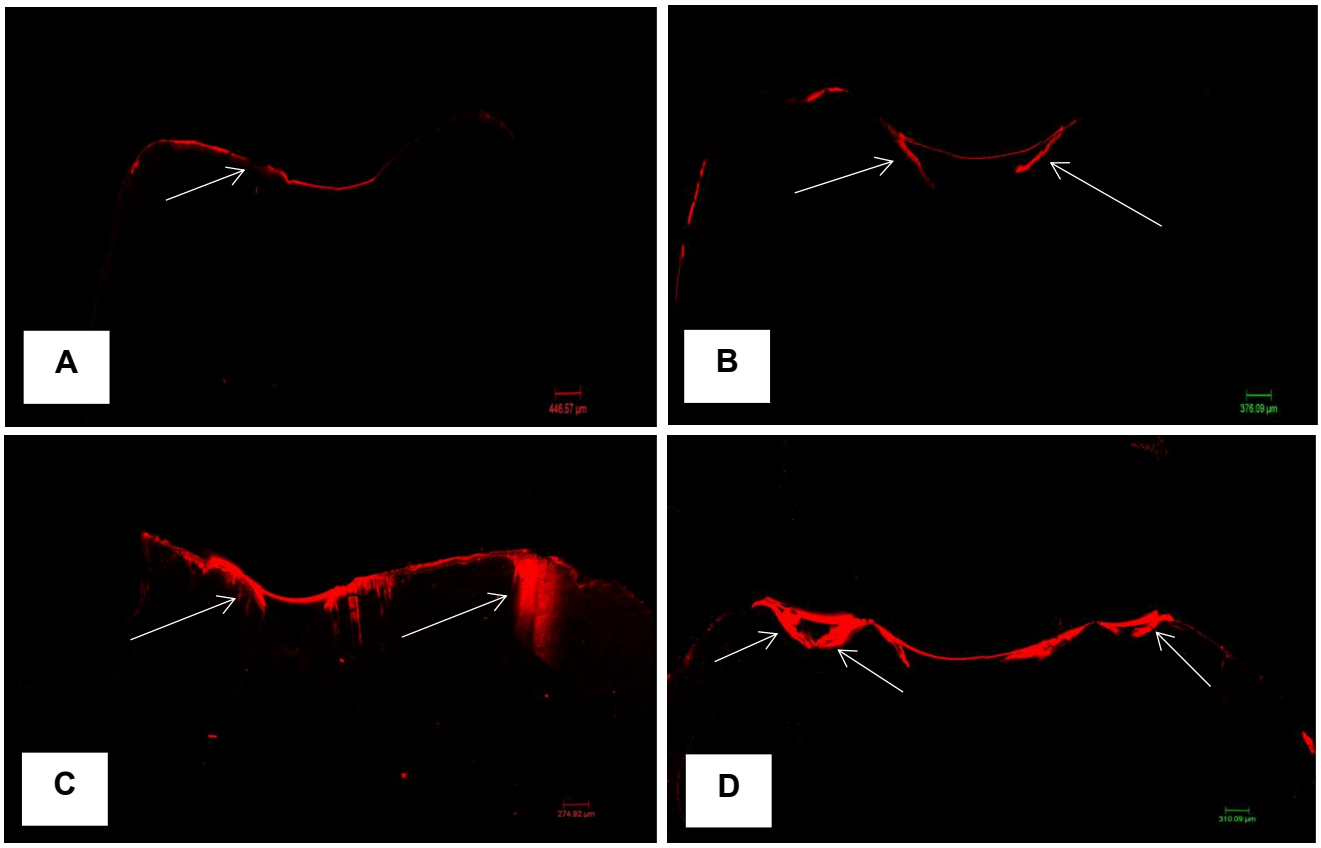


Figure 3 (A) Score 0 in Group III, White arrow indicates no dye penetration; Figure 3 (B) Score 1 in Group I, White arrow indicates dye penetration restricted to the outer half of the sealant; Figure 3 (C) Score 2 in Group II, White arrow indicates the dye penetration to the inner half of the sealant; Figure 3 (D) Score 3 in Group II, White arrow indicates dye penetration into the underlying fissure.

Supplementary Files

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