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# Comparative Evaluation of Penetration Depth of Nano-Silver Reinforced Pit and Fissure Sealant: Scanning Electron Microscope Study

**Rand Hasan[1], Abdulkareem Almezuori[2]**

[1]Department of Paediatric and Preventive Dentistry, College of Dentistry, University of Duhok, Iraq/Kurdistan, Duhok. Email: rndbarwari9@gmail.com

[2] Department of Conservative Dentistry, College of Dentistry, University of Duhok, Iraq/Kurdistan, Duhok*

Corresponding author: Email: Email: rndbarwari9@gmail.com

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## ABSTRACT

**Background:** Recently developed nano-silver reinforced pit and fissure sealants have been introduced to increase their antimicrobial properties. However, the addition of silver nanoparticles may influence the viscosity and flow of the material. Therefore, evaluating penetration performance of nano-silver reinforced pit and fissure sealant is essential to determine clinical effectiveness.

**Objective**: This study aims at comparing and evaluating the penetration depth of Nano-silver reinforced pit and fissure sealant to other pit and fissure sealants.

**Materials and methods:** A total of 60 extracted premolars for orthodontic purposes were divided into four groups (n = 15 per group). Group I Nano-silver reinforce sealant, Group II Unfilled sealant, Group III Filled sealant Group IV Nano-filled flowable composite sealant. Standard occlusal fissure preparations were conducted, and each fissure sealant was applied according to the manufacturer's instructions. Teeth were then sectioned buccolingually. The sections were prepared for scanning electron microscope (SEM) evaluation and the penetration was measured using ImageJ software. Data were analyzed using ANOVA followed by post-hoc testing to compare differences among groups.

**Results:** Among all four groups, unfilled resin-based sealant showed the highest penetration depth. followed by the filled resin-based sealant. Flowable composite exhibited moderate penetration, while the nano-silver reinforced sealant demonstrated the lowest penetration depth. **Conclusion:** The nano-silver reinforced sealant recorded the least penetration depth.

**Key words**: *Fissure sealant, Depth of penetration, scanning electron Microscope, Nanosilver reinforced sealant.*

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## INTRODUCTION

Dental caries is a multifactorial disease initially caused by alteration in the bacterial biofilm; this disturbs the balance between demineralization and remineralization processes, causing carious lesions in both primary and permanent teeth. It significantly affects the children's quality of life and their caregivers which in consequence results in issues in their behaviour and increase the dental care costs. Occlusal surfaces of posterior teeth are mainly susceptible to caries development because of their morphology, which makes oral hygiene more complicated. Although smooth surface caries has decreased thanks to fluoride use, occlusal caries persists. Historically, protective methods for pits and fissures have evolved, starting from fissurotomy and silver nitrate application to the more effective prophylactic restorations introduced by Hyatt in 1923 [1]. A pivotal advancement was the introduction of acid etching in 1955, enhancing resin adhesion and paving the way for modern sealants [1]-[3]

Sealants act as barriers against plaque accumulation and are particularly indicated for high-risk individuals such as children with deep fissures or cognitive impairments [2].

Different types of sealant materials have been developed, with resin-based sealants being the most prominent. Based on their polymerization methods, they are categorized into generations, with the advances that introduce fluoride for additional caries prevention. Glass ionomer sealants are also commonly used because of their ch`1emical bond with enamel and dentin, by providing various viscosity options [4],[5].

Adding nanofiller particles to improve physical properties, antibacterial efficacy, and remineralization abilities of the pit and fissure sealants is the main focus of the recent studies. Silver nanoparticles are known for their antibacterial activity and have been incorporated into sealants. However, some research indicates that incorporating nanosilver can lead to increased viscosity, promote particle aggregation, reduce its ability to penetrate fissures, or show no significant enhancement in sealing effectiveness [6]-[7]. In 2023, the first nano silver-reinforced bioactive sealant was introduced commercially by Kids-e dental company in India.

The performance of sealants in caries prevention is particularly linked to their physical features and retention rates within the oral cavity. Deep penetration into fissures reduces biofilm accumulation and enhances restoration durability. Therefore, a reliable deep sealant penetration is essential for maintaining adhesion to tooth structures and avoiding the progression of caries [8],[9]. So, this study was conducted to evaluate and compare the penetration depth of the recently developed nano-silver reinforced pit and fissure sealant.

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## MATERIALS AND METHOD

### Sample collection

A total of sixty maxillary sound human premolar teeth extracted for orthodontic and therapeutic purposes were collected. The sample size was established based on comparable in-vitro research on pit and fissure sealants, which typically use 30 to 60 teeth to ensure dependable statistical analysis of gap width and microleakage results [10]-[11].

After extraction, teeth were cleaned with water and polished with a soft bristle brush to remove soft tissue on the tooth surface. Teeth were assessed with a dental loupe and verified through periapical x-ray to ensure their structural integrity. Inclusion criteria consisted of sound maxillary premolars that were free from caries, restorations, cracks, and developmental anomalies. And exclusion criteria included teeth with cavities, restorations, fractures, wear, enamel hypoplasia, and any other teeth with damaged crown [12]-[14].

All teeth were stored in normal saline containing 0.1% thymol crystals at room temperature in a glass bottle with weekly water changes to avoid bacterial contamination [14].

Before use, teeth were washed with running water and polished with pumice and were cleaned with spray water with a sharp explorer tip passed through all pits and fissures to remove all the remaining pumice. Then embedded in cold-cure resin up to 1mm below the cement-enamel junction using polyvinyl chloride (PVC) molds (2cm in diameter and 2.5cm height) [15].

Teeth were randomly divided into four groups of 15 teeth each (*n*= 15) to apply the four fissure sealants; which are Group I Silver reinforced resin-based fissure sealant, Group II unfilled resin-based fissure sealants, Group III filled resin-based sealant and Group IV flowable composite.

### Group I Silver reinforced resin-based sealant (Kids-E-Sealant, Kids-E-dental)

The occlusal surfaces were etched with 37% phosphoric acid gel for 30 seconds and were rinsed with water for 20 seconds and dried until the characteristic white frosty chalky appearance was obtained. Then the material was injected homogenously to the pits and fissures using a syringe needle tip. A periodontal probe was used to eliminate bubbles and voids and the excess was removed by an applicator. Then the sealant was cured with a light curing unit for 20 seconds (according to manufacturer instructions).

### Group II Unfilled resin-based sealant (Clinpro, 3M)

Similar etching, rinsing and drying protocols were followed, with sealant applied via syringe needle tip, then cured for 20 seconds [16].

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### Group III Filled resin-based sealant (Helioseal F Plus, Ivoclar)

Similar etching, rinsing and drying protocols were followed, with direct application of the material using a syringe needle tip. After curing the sealant for 20 seconds using a LED light the material changed color from pink to opaque white [17].

### Group IV Flowable composite (Filtek Supreme, 3M)

Etching, rinsing and drying were performed similarly to previous groups followed by application of sealant material with a syringe tip, and finally cured similar to previous groups [18].

### Scanning electron microscope evaluation (SEM)

Samples were sectioned in buccolingual direction up to the cemento-enamel junction using a hard tissue microtome with water coolant and was performed in which the disc was parallel to the long axis of the tooth. Each tooth was given two buccolingual cuts to make a section of 2mm which gives a precise SEM image of sealant-enamel interface [19], [20]. After sectioning, all specimens were immediately and individually placed in sealed bags with a small moist gauze as a short-term storage prior to microscopic evaluation. The sealed bags were kept at temperature of 37C until fixation to preserve a saturated 100% humidity and prevent dehydration [21],[22].

Specimens were rinsed and allowed to dry for 24 hours, and then mounted on SEM specimen stubs. Each specimen was desiccated and sputter coated with thin film of pure gold alloy. The stubs were then placed in the chamber of SEM. Finally, the prepared specimen was evaluated under a scanning electron microscope at different magnifications (10X to 750X) [15].

Micrographs were collected for a quantitative evaluation of the sealant's internal penetration into the fissure. Penetration was then determined by drawing a straight line from the fissure entrance to the deepest point reached by the sealant in micrometers (μm) using ImageJ software [23].

A reference line was established from the occlusal entry of the crack to the deepest visible point reached by the sealant as shown in Fig.1. This indicates the material's actual linear penetration into the fissure according to the measurement protocol described for restorative and fissure-sealing materials under SEM [24].

The total fissure depth was determined as well by measuring the fissure length from the occlusal opening to the anatomical base, in order to establish the degree of penetration in relation to fissure shape. Then the following formula was utilized to calculate the percentage of penetration: **Fig. 1** Microscopic image of a sectioned tooth with Nanosilver reinforced sealant applied illustrating the measurements made to check the penetrability.

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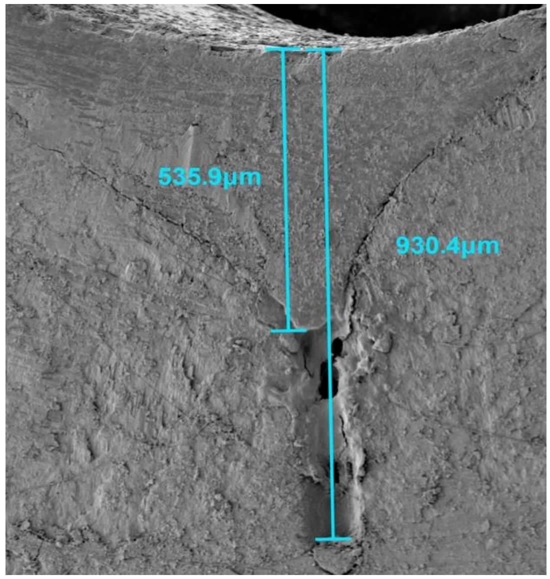


## Data management and Statistical analysis

The data were collected, coded and uploaded to Statistical Package for Social Sciences (SPSS) version (25) for statistical analysis. Descriptive statistics provided mean value, standard deviation (SD), standard error (SE), and range for each group giving an overview of the overall variability in the data. The inferential statistics included one-way ANOVA followed by the post-hoc LSD test. The significance level was established at <0.05 at 95% confidence intervals [18].

## RESULTS

Table 1 shows descriptive statistics for penetration depth percentages among the four groups. Group 2 (Unfilled sealant) exhibited the greatest mean penetration value (91.4432 ± 7.36722 µm), followed closely by Group 3 (Filled sealant) (88.9169 ± 8.00842 µm). Group 4 (Composite resin) displayed a moderate mean penetration (79.18 ± 13.96), while Group 1 (Nano-silver reinforced sealant) had the lowest mean penetration value (69.2931 ± 9.99494 µm), indicating relatively poorer penetration capacity. One-way ANOVA test revealed a statistically significant difference between the groups (p= 0.000).

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**Table 1.** Assessment of mean penetration depth percentage of the sealant material into pit and fissure.

| Groups | N | Mean | Std.Deviation | Std.Error | Minimum | Maximum | p Value |
|---|---|---|---|---|---|---|---|
| Group 1 | 15 | 69.29 | 9.99 | 2.58 | 53.64 | 93.13 | |
| Group 2 | 15 | 91.44 | 7.36 | 1.90 | 78.23 | 99.37 | 0.000* |
| Group 3 | 15 | 88.91 | 8.00 | 2.06 | 72.86 | 99.31 | |
| Group 4 | 15 | 79.18 | 13.96 | 3.60 | 42.78 | 95.65 | |

*p<0.05; significant

The LSD post-hoc analysis illustrated in Table 2 indicated that Group 1 (Nano-silver reinforced sealant) had a statistically significant difference in penetration depth compared to Group 2 (Unfilled sealant), Group 3 (Filled sealant) and Group 4 (Flowable Composite resin), (p= 0.000), (p =0.000), (p =0.010) respectively. Additionally, Group 2 (Unfilled sealant) showed a significant difference when compared to Group 4 (p= 0.002). A similar significant difference was found between Group 3 (Filled sealant) and Group 4 (Flowable Composite resin) (p= 0.011). However, no significant difference was found between Group 2 (Unfilled sealant) and Group 3 (Filled sealant) (p= 0.499), suggesting that their penetration performance was similar.

**Table 2.** Multiple comparisons of penetration depth between all four study groups by LSD post-hoc test.

| (I) Factor | (J) Factor | Mean Difference (I-J) | Std. Error | P Value |
|---|---|---|---|---|
| **Group 1** | Group 2 | 22.15 | 3.71 | 0.000* |
| | Group 3 | 19.62 | 3.71 | 0.000* |
| | Group 4 | 9.89 | 3.71 | 0.010* |
| **Group 2** | Group 3 | 2.52 | 3.71 | 0.499 |
| | Group 4 | 12.25 | 3.71 | 0.002* |
| **Group 3** | Group 4 | 9.73 | 3.71 | 0.011* |

*Significant, p< 0.05

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## DISCUSSION

The prevention efficacy of pit and fissure sealants is determined by their penetration depth into the occlusal pits and fissures. Because if the sealant fails to penetrate into the fissures unsealed areas becomes susceptible to bacterial colonization. Mechanically, deeper sealant penetration improves retention and durability. Sealants that extend into the fissures completely have an increased contact with the enamel surface that enhances the micromechanical interlocking after polymerization [1],[2],[27].

Nano-filled sealants use nanoparticles to improve mechanical strength, increase wear resistance and minimize polymerization shrinkage. In this manner, nanotechnology enhances both the biological and mechanical justifications. To confirm these advancements, accurate evaluation techniques are essential, and scanning electron microscopy (SEM) offers the required resolution and three-dimensional (3D) imaging to analyze penetration depth and material compatibility. SEM allows the examination of the interactions between sealants and fissure walls, identification of voids or micro-gaps, and verification of continuous penetration. Considering the essential impact of penetration and material properties on the performance of sealants, it is important to analyze the effectiveness of recent formulations such as nano-silver incorporated sealants in relation to conventionally used sealants [28].

Therefore, this study was designed to evaluate and compare the penetration depth of nano-silver reinforced sealant against conventional sealant materials using a scanning electron microscope.

The results of the present study demonstrated that the nano-silver reinforced pit and fissure sealant exhibited the lowest penetration depth among all groups (69.29 ± 9.99 µm). This can be clarified by the increase in viscosity due to the incorporation of nano-silver particles. But it can also be attributed to a variety of interrelated components and structural factors. The incorporation of silver nanoparticles typically reported in the nanometer range (approximately 20–80 nm) introduces antimicrobial property and may enhance physical properties, yet it also increases the viscosity of the resin matrix by limiting polymer chain mobility and promoting particle to particle interactions even at relatively low nanoparticle concentrations (0.5–2 wt%) nanoparticles possess a high surface area to volume ratio, which intensifies resin–filler interactions and can substantially alter rheological behavior. As a result, the ability of the sealant to flow into narrow and complex pit and fissure morphologies may be compromised. [29]-[32].

Beyond viscosity-related effects, the surface chemistry and nanoscale size of silver particles (20–50 nm) may influence the wettability and surface energy of the sealant. Altered wettability can lead to non-uniform spreading on etched enamel surfaces, reducing capillary action and limiting penetration depth [7],[27]. Naimi et al [33] come in line with this in which they examined silver and zinc oxide incorporated resin-base sealants, with particles ranging approximately from 20 to 50nm.

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While the primary outcome was not penetration depth, the study reported increased viscosity after the incorporation of nanoparticles that suggested limitations in penetrating into fissures [33].

Memarpour et al. [34] assessed the penetration depth of filled resin-based sealants and reported significantly reduced penetration compared with unfilled sealants or other conventionally used sealants. Although the fillers in their study consisted of silica-based particles in the micrometer range (approximately 0.7–1 µm) rather than silver nanoparticles, their results agree with the present study by demonstrating that the presence of fillers may increase the materials viscosity there by limiting the flow and penetration into deeper fissures [34].

In contrast, varies studies have reported findings that do not line with the current study. Quiroga et al. [30] reported no significant difference in penetration with a silver nanoparticle sealant, which partially disagrees with the present study, this difference may be due to lower nanoparticle concentration or enhanced distribution, which may have prevented viscosity increase. Also, Koohpeima et al. [7] observed that including silver nanoparticles sized around 10-30 nm in low viscosity systems results in improved penetration. These findings contrast with the current study, which can be explained by the different methods of application. When nano particles are used as pretreatments or in low viscosity adhesive, they tend to improve surface energy and resin infiltration. However, incorporating nanoparticles directly into the main sealant material could raise the viscosity and reduce penetration [30],[32].

In the present study unfilled and filled resin sealants documented the highest penetration depth values among the evaluated sealants (91.4432 ± 7.36722 µm) (88.9169 ± 8.00842 µm) respectively. While the two materials recorded different mean values, their performance was not statistically significant, indicating that their effectiveness was closely comparable. Multiple studies by Reddy [35] Bagheiry et al. [36] and Rajashekar Reddy et al. [37] consistently compared unfilled and filled resin-based sealant and found no statistically significant differences in penetration depth. Moreover, Garg et al. [38] reported a penetration depth of approximately 78.26% for unfilled resin sealant and 74.89% for filled resin sealant with no statistically significant differences between the two groups, which also suggesting comparable penetrating ability similar to the present study. Nevertheless, it is essential to mention that other investigations such as Fernandes et al. [39] and Al-Mutairi et al. [40] reported statistically significant differences, as the unfilled sealant showed considerably better penetration depth than filled sealant, due to their lower viscosity, which allows for deeper penetration [35]-[40].

Flowable composite, noted moderate average penetration (79.18 ± 13.96), rating inferior to both resin-based sealants but superior to the nano-silver reinforced sealant. Despite that flowable composite is less viscous than traditional composite but still contains high loads of filler compared to pit and fissure sealants, which naturally increases the viscosity and consequently reduces the flow into the fissure system [32]. Additionally, surface wettability and tension play a crucial role in how deeply the resin material can penetrate into the fissure system. Improved wetting properties

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facilitate the spreading of the material on the etched enamel, which encourages deeper infiltration. On the other hand, polymerization shrinkage of composite materials results in more significant volumetric contraction during the curing process generating shrinkage stresses that can pull the material and in turn reduce the depth of penetration [41],[44].

In agreement with the present study, Gillet et al. [42], Khatri et al. [43] and Arastoo et al. [32] independently assessed the penetration behavior and consistently found that conventional pit and fissure sealants penetrate deeper than flowable composites. They attributed the superior penetration of sealant materials to their lower viscosity, and improved capillary flow, which aids in the narrow complex anatomy of pits and fissures. In contrast, Singh et al. [44] observed that flowable composites exhibited superior performance. Nevertheless, Patel et al. [41] determined that there were no significant statistical differences in penetration depth comparing flowable composite to resin sealants, indicating factors that like fissure morphology and application method could impact penetration results [32], [42]-[44].

## CONCLUSION

The nano-silver reinforced sealant recorded the least penetration depth. These findings highlight that; conventional unfilled and filled sealants are the more effective for fissure infiltration.

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## Conflict of interests.

There are non-conflicts of interest.

## Ethics

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Medical Ethics Committee.

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