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Microleakage Assessment of Hydrophilic Pit and Fissure Sealants Fortified with Green Synthesized Silver Nanoparticles-An in Vitro Study

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Abstract **Background and Aim:** Pit and fissure sealants play a significant role in preventive dentistry. The sealant's physical properties lend a notable contribution to its long-term success. The invention of nanoscience has led to breakthrough discoveries in improving the mechanical properties of sealants. The current work aimed to evaluate the microleakage of Hydrophilic sealant fortified using silver nanoparticles compared to the Hydrophilic sealant and conventional sealant on permanent molars. **Materials and methods:** Silver nanoparticles were green synthesized using beetroot extract and incorporated into hydrophilic Ultraseal XT Hydro sealant. A sample of extracted third molars, thirty in number, were allocated randomly to three different groups as follows; Group I: conventional Clinpro sealant, Group II: hydrophilic Ultraseal XT hydro, and Group III: Ultraseal XT hydro fortified with silver nanoparticles. Microleakage assessment was performed using a stereomicroscope. The Kruskal-Wallis test was employed to compare the microleakage values of the three groups. **Results:** Group III had the lowest values about mean microleakage score (0.60±0.548), and the highest scores were noted for Group I (1.40±0.548). A statistically significant difference was observed in comparing the three groups employing the Kruskal-Wallis test. **Conclusion:** The results of the current study have established that the microleakage was the lowest in fortified UltraSeal XT Hydro (Group III) when compared to Ultraseal XT Hydro (Group II) and Clinpro sealant (Group I).

Key Words Fluoride releasing sealants, Minimal invasive dentistry, Primary prevention

1. Introduction

Most dental caries tend to originate from occlusal pits and fissures. This is because these are anatomically inconspicuous regions that lack exposure to salivary flow, its remineralizing properties, and the effects of thorough brushing [1]. Regarding the total area constituted by tooth surfaces, occlusal surfaces contribute only 12.5% and yet succumb to a more significant percentage of the total caries prevalence observed in children [2]. For this reason, dental caries should be intervened at the very beginning to prevent serious complications [3]–[5].

In light of this fact, various approaches are sought to reduce the probability of dental caries formation, including the habit of flossing, fluoride prophylactic agents such as rinses and varnish, and the regular use of fluoride toothpaste. However, more than routine brushing may be required concerning deep pits and fissures, given that brush bristles cannot reach the inaccessible depths of these areas [6]. To overcome this limitation, dental sealants could serve as an optimal treatment of choice by protecting tooth regions that are more vulnerable to plaque and bacterial accumulation by transforming them into smoother self-cleansing areas [7].

Despite advancements in restorative dentistry, contemporary sealant materials are restricted by their bioinert nature and negligible interaction with the oral environment [8]. This calls for formulating innovative and bio active materials with added valuable physical and functional properties to manage and treat dental caries efficiently [8], [9]. This could be done by harnessing the potential of nanotechnology. Nano technology is a scientific field that handles matter on a nanoscale (diameter less than 100nm) that displays pioneering physical, optical, chemical, and biological characteristics [10].

The nano particle synthesis process can be carried out by various chemical, physical, and biological techniques. As the chemical method is limited by the production of by-products that are hazardous to the environment, there exists a demand for more eco-friendly methods [11]. This has led to the invention of "green nanotechnology," which uses a biological approach wherein microorganisms and plants are involved in the process of extracellular and intracellular nano particle synthesis [12]–[14].

In this study, the potential of beetroot extract as a reducing agent was explored and used to formulate silver nano particles. Beetroot is a readily available vegetable rich in folate content. Moreover, it is a powerful antioxidant that contains Vitamins A, B6, and C [15].

The science behind sealant synthesis has widened its branches to incorporate the attribute of moisture resistance. Hydrophilic sealants with moisture-tolerant capabilities are now at a drawing level with conventional hydrophobic sealants. Being readily miscible with water, they flow into etched enamel coated with moisture, thus forming a retention-enhancing strong bond [16]. This is highly relevant as sealing pits and fissures are technique-sensitive and rely on effective moisture control for their long-term success.

Sealants that tolerate moisture, such as Ultraseal XT Hydro, have been introduced to combat this. This light-cured sealant exhibits excellent thixotropic properties apart from being fluoride-releasing. Moreover, an amalgamation of benefits of both hydrophobic and hydrophobic sealants is noted in this material. The hydrophilicity of the sealant ensures its uninterrupted flow into the depths of the fissures, even in the presence of saliva [17].

Though many studies [17], [18] have been done to assess the properties of hydrophilic sealants, very few have been done concerning sealants modified with silver nano particles. In the present study, we aimed to assess the microleakage of Ultraseal XT hydro sealant fortified with silver nanoparticles compared to the Ultraseal XT hydro sealant and conventional Clinpro sealant on permanent molars.

2. Materials and Methods

This in vitro study was conducted to compare three different sealant materials. The sample for the study was constituted of permanent molars. A study by Prabakar J et al. was used to arrive at the sample size [18] employing G*Power 3.1.2 software with a power of 0.95 and ($p \leq 0.05$). A sample size of 10 teeth per group was derived, resulting in a total of 30 teeth. Only teeth that were extracted for surgical or orthodontic reasons, with intact occlusal surfaces, were included in the study. Teeth exhibiting caries or developmental anomalies were excluded. The scientific review board of Saveetha University granted its approval before initiating the study. Following computer-generated randomization, ten molars were allotted to each of the three groups, which were Clinpro sealant (Group I), Ultraseal XT hydro (Group II), and Ultraseal XT hydro fortified with silver nanoparticles (Group III).

Green Synthesis of Silver Nano Particles using Beetroot Extract

After washing the beetroots with clean drinking water to remove impurities, they were diced and left to dry at room temperature. The dry pieces were added into a grinder mixer and powdered. In a beaker containing 100mL of distilled water, 20 grams of the beetroot powder was incorporated and brought to a boil, after which it was filtered to form the beetroot extract. 50 ml of the beetroot extract was added to a beaker containing a mixture of 0.0169 grams of 1 millimole silver nitrate and 50 ml of distilled water. This mixture was then kept for 32 hours in an orbital shaker, which was then distributed and poured into six centrifugation tubes. The tubes were placed in the centrifugation machine and centrifuged at 10,000 rpm for 20 minutes.

The supernatant liquid was disposed of from each tube, and the remaining material present at the bottom of each tube (pellet solution) was transferred to a single tube and then refrigerated for future use. The pellet solution was laid out on a petri dish, placed in the hot air oven, and subjected to a temperature of 100 degrees Celsius for 8 hours. The petri dish, now containing dry solution, was scraped out to yield silver nano particles.

Addition of Silver Nano Particles into the Sealant

Following a proportion of 1:10 by weight, the silver nano particles were added to Ultraseal XT hydro. This mixture was then subjected to mixing in an amalgamator for a period of 10 minutes.

Placement of Sealant

The teeth sample were first etched for 30 seconds using 37% orthophosphoric acid. Next, the teeth were rinsed with water and dried thoroughly with the help of a three-way syringe to make the enamel surface appear white and frosty for Clinpro sealant. For Groups II and III, the sample teeth were dried gently and left in moderate wetness to yield a shiny appearance. The sealants allocated for each group were placed on the teeth, followed by light-curing for 30 seconds.

Microleakage Assessment

A solution of 5% sodium hypochlorite was used to soak the teeth with sealant placed on it. Any residual periodontal tissue or calculus particles were cleaned and removed thoroughly. Following this, all the teeth were scrutinized under the microscope to rule out the presence of caries, cracks, or defects. The specimens not fulfilling the inclusion criteria were rejected, while those fulfilling were stored in 10% formalin solution until further use.

After the placement of sealants, molars were immersed inverted in 0.1% silver nitrate solution at 37°C for 8 hours, followed by placement in developer solution for 4 hours. All the teeth were then exposed to a thermocycling procedure for 30 seconds, with temperature ranges between 5°C and 55°C. Molars were sliced buccolingually [Figure 1], and the tooth sections were evaluated for microleakage using a

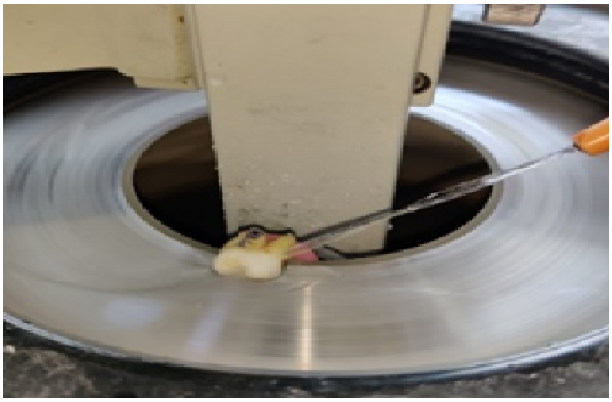

Figure 1: Buccolingual sectioning of the tooth specimen

Figure 2: Microleakage assessment using stereomicroscope

stereomicroscope (LEICA M205C) [Figure 2,3] and scored by an examiner who was blinded to the study. Ovrebo and Raadal [19] guidelines were used to assess the microleakage and the interpretation of the scores.

3. Results

Table 1 shows the mean microleakage scores for Group I, II, and III, ranging from 1-2, 0-2, and 0-1, respectively.

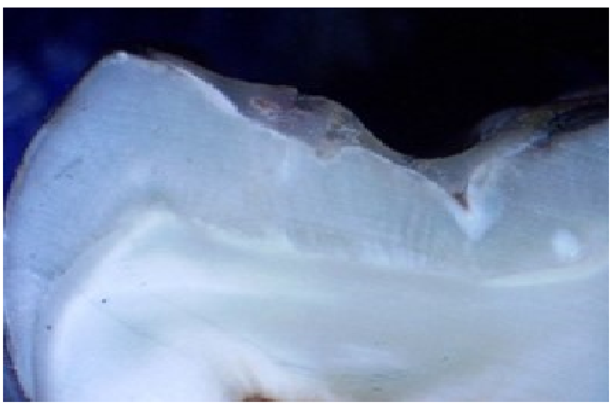

Figure 3: Silver nitrate dye penetration as seen through the stereomicroscope

Descriptive Statistics	Group I	Group II	Group III
Number of samples	10	10	10
Mean microleakage scores	1.40	1.00	0.60
Standard deviation	0.548	0.707	0.548
Median	1.00	1.00	1.00
Minimum	1	0	0
Maximum	2	2	1

Table 1: Mean microleakage scores of Groups I, II, and III

Groups	N	Mean Rank	Kruskal Wallis Score	Significance
I	10	10.40		
II	10	8.00	3.733	0.04*
III	10	5.60		

Table 2: Mean difference in microleakage score between Group I, II, and III

The median value of all three groups is 1.0. The mean microleakage scores were found to be the lowest for Group III (0.60 ± 0.548) and highest for Group I (1.40 ± 0.548). Kruskal Wallis test exhibited a significant difference between the three groups regarding microleakage. Hence, group 3 was found to be superior to the other groups (Table 2).

4. Discussion

Nanotechnology aids in the treatment of dental caries in two ways. The first approach is remineralization, which employs nano-materials capable of releasing fluoride and calcium. The method includes using antibacterial nanoparticles such as silver, quaternary ammonium polyethylene amine, and zinc oxide [20].

Because of their increased surface-to-volume ratio and bioavailability toward cells and tissues, nanosized systems have unique characteristics [21]. Increased surface area leads to improved mechanical interlocking of nanoparticles to the resin matrix [22]. Because of the minimization in stress concentration areas, there is superior crack propagation resistance and increased fatigue strength [23].

Silver nanoparticles are broad-spectrum antimicrobial agents that can be used to prevent dental caries [24]. Because of the particles' high surface area, they adhere to the outer cell membrane of bacteria, influencing the bacteria's permeability and cell structure [25].

Recent investigations have shown that a dental sealant containing Ag NPs can be more effective than a traditional sealant in preventing enamel caries in first permanent molars [26]–[31].

In the current study, silver nanoparticles were green synthesized from beetroot extract, incorporated into Ultraseal XT hydro, and subjected to microhardness and microleakage testing. In the current study, the mean microleakage scores for Clinpro, Ultraseal XT Hydro, and reinforced Ultraseal XT Hydro were 1.40 ± 0.548, 1.00 ± 0.707, 0.60 ± 0.548 respectively. Though there was no significant difference in the microleakage ($p > 0.05$) of the three sealants, Clinpro exhibited the highest microleakage scores, and reinforced Ultraseal XT Hydro exhibited the lowest (Borsatto et al.

reported similar findings [32]). Clinpro sealant had a higher level of microleakage than its glass ionomer counterpart. Under saliva contamination, the article has demonstrated that the glass ionomer offered better marginal sealing than the resin-based Clinpro sealant.

This is in contrast to a study by Kusgoz A et al. [33], in which Fuji Triage had significantly higher microleakage scores than Clinpro and Grandio Seal. This result is by Ganesh and Shobha's results [34], and Riratt Tanapong et al. [35], which revealed that the resin-based sealant outperformed the Fuji VII GIC Sealant in terms of sealing ability (in dry conditions). However, it contradicted the findings of Ashwin and Arathi, who found no difference in microleakage score between Fuji Triage and resin-based pit and fissure sealant [36]. In a study by Khairy et al. [37], silver nanoparticle-added Clinpro sealant demonstrated lower microleakage than conventional Clinpro sealant, though the difference was not statistically significant. The author attributes this difference to the enhanced thermal stability of the resultant nano-polymer caused by AgNPs. This may reduce marginal defects between the material and the tooth substrate, especially when subjected to thermal cycling. The results agreed with Morales-Quiroga et al. [38], who found no significant difference in primary molars between conventional Clinpro and Clinpro modified with silver nanoparticles.

Although the current study sought to simulate clinical conditions by thermocycling to simulate aging, one potential limitation is that extracted teeth lack the pulp pressure and intertubular fluid pressure found in natural teeth. This may affect tooth moisture levels, resulting in microleakage at the tooth restoration interface. Another limitation of the study was the easy control of moisture as it was an in vitro study.

5. Conclusion

Within the limitations of the present study, we concluded that reinforced UltraSeal XT Hydro (Group III) showed the lowest level of microleakage than Ultraseal XT Hydro (Group II) and Clinpro sealant (Group I). As a result, microleakage is a significant issue and factor in pit and fissure sealant longevity and clinical outcomes because a carious mechanism can be orchestrated and sustained beneath the sealant. As a result, the lower the microleakage, the better the sealant's retention for a prolonged timeframe and cariostatic action.

Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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