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Antimicrobial Activity of Hydrophilic Sealants incorporated with Green Synthesized Silver nanoparticles - An invitro Study

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Abstract **Background:** Dental caries formation is highly prevented by applying pit and fissure sealant but was associated with microleakage and secondary caries formation. **Aim and Objectives:** To synthesize novel pit and fissure sealant with green synthesized silver nanoparticles and evaluation of antibacterial activity against Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans. **Materials and Methods:** Green synthesis of silver nanoparticles was done using beetroot, characterized using FTIR (Fourier transform infrared), EDAX (Energy Dispersive X-ray Analysis), and Scanned Electron Microscope Analysis. A novel pit and fissure sealant was made by incorporating synthesized Ag nanoparticles and hydrophilic nature from Ultra seal XT Hydro. Using amoxicillin as a control, an agar well diffusion method was used to conduct an antimicrobial test for a new sealant against Staphylococcus aureus, Streptococcus mutants, Enterococcus faecalis, and Candida albicans in Muller Hinton Agar ($n = 3$) for each organism. **Result:** Zone of inhibition was measured, showing statistically significant antimicrobial activity against Streptococcus mutans with the ZOI value 21mm $\pm$ 2mm at $100\mu l$ concentration of Ag nanoparticles in sealant material. **Conclusion:** Novel Hydrophilic pit and fissure sealant with Ag nanoparticles are potentially antibacterial against the cariogenic microorganism. Thus, it can be utilized for the better efficiency of topical pit and fissure sealant preventive therapy.

Key Words Hydrophilic sealant, Pit and fissure sealant, Antimicrobial additives

1. Introduction

Dental caries is a multifactorial disease associated with various etiological factors such as microbial, genetic, immunological, behavioral, and environmental that interact altogether, ultimately leading to dental caries onset and development [1], [2]. The human oral cavity is an incredibly diverse, dynamic, and unique ecosystem with the instability of its ecological conditions [3]. It has been found that a different ecological niche exists in different oral cavity sites, constituting different groups of microorganisms [4]. The onset and progression of dental caries are caused by a change in the oral microbiota's makeup from normal to aciduric and acidogenic bacteria. This shift is found to be mainly due to the frequent consumption of sugary or carbohydrate intake that can be fermented with the production of acid and reducing the local pH around the tooth, causing demineralization [5].

The typical oral cavity flora most often contains the Gram-positive bacteria Streptococcus mutans, as reported in many epidemiological studies [6], [7]. Its capacity to metabolize various sugar carbs has led to reports that it is a main cariogenic bacterial pathogen [8]. Also, most of the carious lesions arise in the pit and fissures of the occlusal surfaces of the teeth, rendering tooth brushing incomplete in eliminating the cariogenic organisms [9], [10].

Most prior research findings indicate that 56-70% of caries in children aged 5-17 happen in pits and fissures [11], [12]. Pit and fissure sealants are regarded by the World Health Organisation (WHO) as one of the least intrusive and most efficient methods for guaranteeing the whole occlusal protection against carious phenomena [13], [14]. In addition to preventing caries, pit and fissure sealants help prevent caries from progressing to cavities.

Since the placement of most of the resin-based sealant are hydrophobic and strictly in need of isolation from salivary moisture contamination for longer clinical efficacy, a novel substance called Ultra Seal XT hydroTM was introduced; it is hydrophilic, self-adhesive, light-cured, acrylate-based, and moisture-tolerant. It bonds well to wet enamel surfaces and can be used when patient compliance, isolation, and moisture control are difficult. It has been stated that this substance functions well and chases moisture [15]. Nanotechnology has been applied to dental materials as a novel idea for creating materials with improved qualities and antibacterial potential. To regulate the development of cariogenic oral biofilms, functional materials or structures at the nanoscale (0.1-100.0 nm) can be employed: nanoparticles can transport bioactive substances and antibiotics [16], [17].

Apart from S. mutans, Enterococcus faecalis has been connected to oral infections, such as caries, endodontic infections, periodontitis, and peri-implantitis [18]. Staph. Aureus is a major pathogenic microorganism causing a variety of infectious diseases. The dental caries initiation process favors the plaque accumulation around the teeth by the production of the polysaccharide intercellular adhesin (PIA) [19]. Numerous in vitro investigations have demonstrated that Candida albicans increases S. mutans' adherence, suggesting a potential method of facilitation during their connection in which the bacteria may employ the yeast cells as support for adherence [20], [21].

Since no trial has been conducted on the antibacterial activity of nanoparticles that have been incorporated in the hydrophilic pit and fissure sealant, the aim of the current study involves the biosynthesis of silver nanoparticles, its incorporation into the hydrophilic pit and fissure sealant and evaluation of its antimicrobial activity against Strep. Mutans, Staph. Aureus, E. faecalis and C. albicans.

2. Methodology

Nanoparticles Green Synthesis

20.5 grams of beetroot were boiled for 4 hours in 100 milliliters of distilled water to extract the beetroot extract. 50 milliliters of distilled water and 0.016 grams of powdered silver nitrate were then added. After being in an orbital shaker for 32 hours, the final mixture was centrifuged for 20 minutes at 10,000 rpm. A Petri plate was filled after separating the concentrated solution from the supernatant solution. The solution in the petri dish was heated to a high temperature for a full day, producing powdered silver nanoparticles. The hydrophilic pit and fissure sealer are combined with this powdered silver nanoparticle mixture at a ratio of 1:4. (Figure 1)

Nano particles Characterization

Energy dispersive spectroscopy (Figure 2) and scanning electron microscopy (SEM) (Figure 3) both confirmed the presence of silver nanoparticles, which are round and rectangular particles with a size of less than 100 nm set against a backdrop of sugars with a green origin. Figure 4 illustrates the phytochromes that synthesise nanoparticles using Fourier-transform infrared spectroscopy.

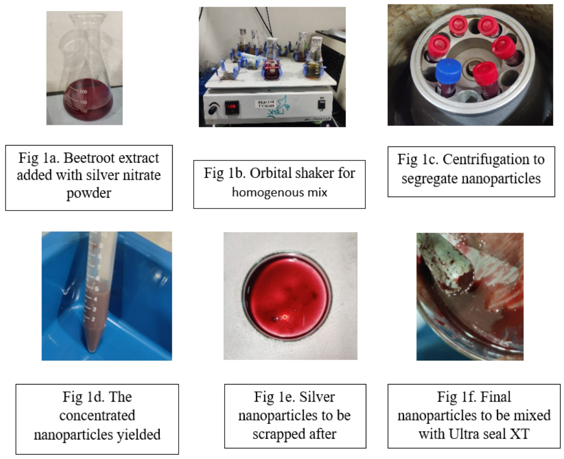

Figure 1: Nano particles misxtures

Antimicrobial test assessment

By employing the agar well diffusion method with Mueller-Hinton agar (MHA), the antibacterial efficacy of hydrophilic pit and fissure sealing material combined with silver nanoparticles was assessed. Then, 0.25 mL of molten MHA was used to inoculate the microorganisms S. mutans, S. aureus, E. faecalis, and C. albicans into petri plates (n=3). Uniformly sized wells (6 mm) were formed on the hardened agar. The experimental test material was impregnated into discs with a diameter of 6 mm and placed on inoculated agar at concentrations of 25, 50, and 100 μL per disc. The positive reference for microorganisms is amoxicillin (500 mg/ml). A blank disc served as the negative control. The inhibition was measured to assess the antimicrobial activity. After allowing the solutions to permeate the MHA for an hour at room temperature, the plates were incubated for the entire night at $37°C$. Ultimately, the experiments were run in duplicate and independently three times, with the zones of inhibition being measured from the plate bases (Figure 5).

Statistical Analysis

Statistical analyses were done in SPSS software. Kruskal wallis test was used to compare the antimicrobial activity of the test material between the different microorganisms. Friedman test was used to compare the antimicrobial activity by different concentration of test material against the microorganisms.

3. Results

Zone of inhibition measured for different pathogenic bacteria Strep. Mutans, Staph. Aureus, E. faecalis, and C. albicans and control antibiotic (Cap. Amoxicillin 500 mg) are shown in Table 1 with mean and standard deviation ($p =< 0.001$). The highest antimicrobial activity was observed against streptococcus mutans at the concentration of 100μl, and the least was noticed against all the microorganisms at 25 μl. The overall value differed significantly with the antibiotic control

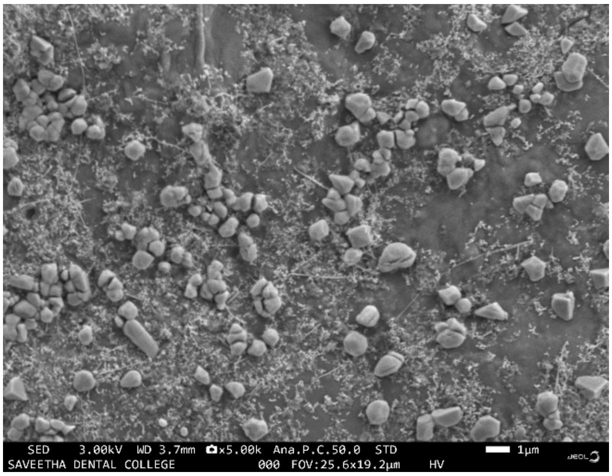

Figure 2: Scanned electron microscope

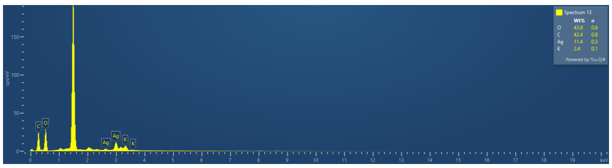

Figure 3: EDAX image

except for Streptococcus mutans at 100 μl. The graphical representation was also given, Figure 6.

4. Discussion

Caries development has always been associated with bacterial plaque formation in which streptococcus mutans forms a major etiological part. However, the caries prevention strategy, such as pit as a fissure sealant, plays a successful role; most of the time, microleakage and retention following a restoration or sealant placement are challenging, leading to the failure of the preventive strategy [22]. It is also well known from the literature that the interaction between the restorative material and oral microorganism is very important for the outcome and effectiveness of the restoration, which makes the antibacterial activity the important property of the sealant material [23], [24].

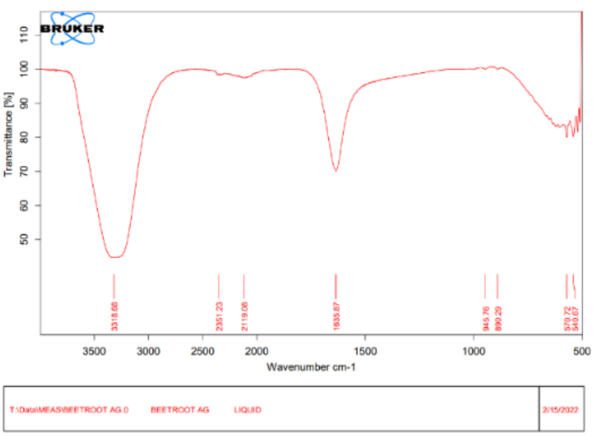

Figure 4: FTIR image

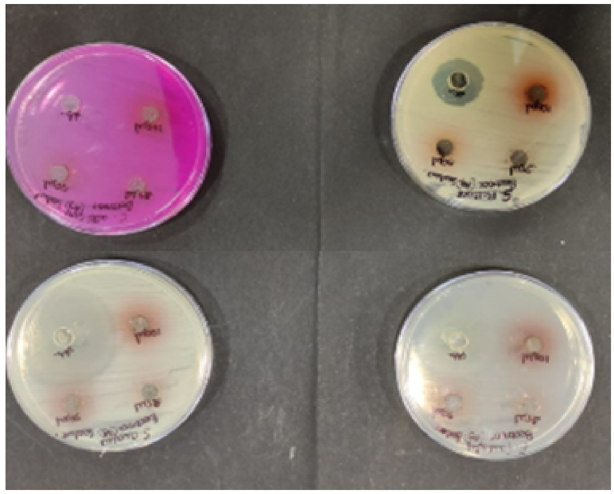

Figure 5: Zone of inhibition against S. mutans, S. aureus, E. faecalis, C. albicans at different concentration

Test concentration of sealant	Mean ±SD Zone of inhibition in mm			
	S. aureus (n=3)	S. mutans (n=3)	E. faecalis (n=3)	C. albicans (n=3)
25μl	9.00± 1.00 mm	9.00 ± 0.50mm	9.00 ±1.00 mm	9.00 ± 1.20mm
50μl	13.00± 1.00 mm	17.00 ±2.00 mm	18.00 ± 1.50mm	9.00 ± 1.00 mm
100μl	19.00 ±1.00 mm	21.00 ±1.50 mm	19.00 ± 1.00 mm	16.00 ± 1.00 mm
Antibiotic (Control)	45.00± 1.00 mm	22.00 ±2.00 mm	45.00 ± 1.00 mm	9.00 ± 1.00 mm
P value	0.004	0.001	0.005	0.005

Table 1: Zone of inhibition at different concentration against pathogenic microbes. Each value is expressed as mean ±SD (n=3)*p value < 0.001 with control.

Because of its strong efficacy against a wide range of pathogens, including bacteria, viruses, and fungus, as well as its broad-spectrum antimicrobial capabilities, silver nanoparticles have been the subject of extensive research and exploration among other nanoparticles. According to reports, AgNPs cling to cell walls and membranes, causing oxidative stress that destroys intracellular biomolecules and structures and establishing the antibacterial action [25], [26]. Addition-

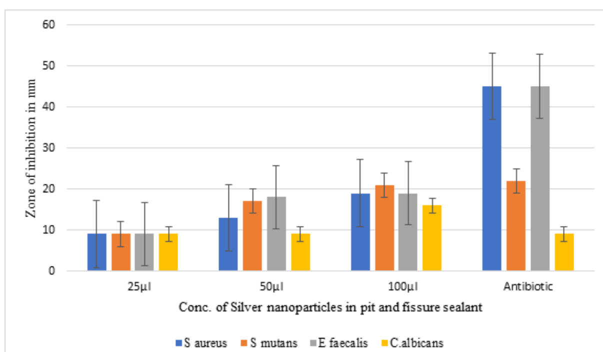

Figure 6: : Zone of inhibition against S. mutans, S. aureus, E. faecalis, C. albicans at different concentration

ally, because silver is a Lewis acid, it frequently reacts with Lewis bases, such as biomolecules that contain phosphorus and sulphur, which are important building blocks of proteins, DNA bases, and cell membranes [27]–[31]. It creates an insoluble substance that prevents cell division and reproduction when it reacts with biomolecules like DNA, RNA, and peptides [30], [31]. AgNPs can also build up on the cell wall and membrane, resulting in clear morphological changes that can be seen with transmission electron microscopy (TEM): membrane detachment, multiple electron dense pits, cytoplasmic shrinkage, and ultimately a disrupted membrane [32]–[35].

Numerous studies have demonstrated the significant antibacterial properties of silver nanoparticles, which are caused by the particles' oxidative disintegration in the presence of oxygen [36]–[39]. In addition to streptococcus mutans, enterococcal surface protein (esp), gelatinase (gelE), aggregation substance (asa1), cytolysin B (cylB), and endocarditis-specific antigen A (efaA) gene are among the virulence factors linked to E. faecalis. Other factors include ArgR family transcription factor (ahrC), endocarditis and biofilm-associated pili (ebpA), enterococcal polysaccharide antigen (epal), epal and OG1RF_11715 (epaOX), and (p)ppGpp-synthetase/hydrolase (relA) genes [40], [41].

Staph aureus is also found to place a role in the accumulation of cariogenic biofilm and thus favouring the development of dental caries. The antibacterial activity against this bacterium helps in preventing the cariogenic biofilm prevention that forms the smart preventive strategy in caries prevention [19].

Apart from bacterial species, C. albicans is a fungus that has been reported in many in vitro studies that it increases the adherence of S. mutans [20], [21].

This current study aims to overcome the disadvantages of pit and fissure sealants, such as microleakage and secondary caries formation, by incorporating silver nanoparticles into the hydrophilic pit and fissure sealant to possess an antimicrobial property.

Previous studies have green synthesized silver nanoparticles from leaf extract Rosmarinusofficinalis, [42]. Thus, this study has utilized beetroot to green synthesize silver nanoparticles to avoid the disadvantages associated with the chemical synthesis of nanoparticles; the bacterial resistance of the synthesized nanoparticles incorporated sealants was determined with the Agar well diffusion method, in which it was observed that the bacterial resistance power was variable according to the concentration of silver nanoparticles incorporated into the sealant. At 100 μl concentration, Ag nanoparticles incorporated sealant produced a maximum zone of inhibition for Streptococcus mutans, comparable to antibiotic control.

[37] found the antibacterial activity of biosynthesized Ag nanoparticles from spirulina against the similar three types of bacteria Strep. Mutans, Staph. Aureus and E. faecalis. They have found the zone of inhibition of 12 mm, 12 mm, and 13 mm for Streptococcus mutans, Staphylococcus aureus, and Enterococcus faecalis, respectively, which were lesser than the results of the present study. [38] conducted a study to evaluate the antibacterial activity in which they found staph. Aureus had a 36mm inhibition zone at 0.002 mg of silver nanoparticles. [39] found 8mm ZOI for Staph. Aureus at 50 μl concentration, which was also lesser than the obtained result of our current study. Matalon et al. [43] measured the halo zone in the agar diffusion plate against the Streptococcus mutans by the Ultra seal XT, which failed to develop the zone of inhibition. This is in contrast with the results of our current study. The incorporation of Ag nanoparticles into the Ultra seal XT might have added the antibacterial activity of the sealant material. Similar better antimicrobial activity was also noted by Zn and Cu nanoparticles against S. mutans in a previous study [44], [45].

However, many of the previous studies evaluating the antimicrobial activity used the agar diffusion method as a standard assay. It is mainly based on measuring the activity of only soluble and diffusible components in the surrounding aqueous media. Since most of the components of the restorative material are low soluble, this method of antibacterial assessment is inadequate. This forms the limitation of the study.

Another drawback is that there is no follow-up period to find the antibacterial activity after a certain period. The result of the current study cannot be considered for promising the prevention of caries development since dental caries is a multifactorial disease. Hence, further in vivo follow-up trials are needed to conclude the effectiveness of the Ag nanoparticles incorporated hydrophilic pit and fissure sealant.

5. Conclusion

A green synthesis method was used for the synthesis of Ag nano particles that was incorporated into hydrophilic pit and fissure sealant which showed an effective antibacterial activity especially against the streptococcus mutans.

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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