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Antibacterial Activity of Mouthwash Incorporated with Silica Nanoparticles against *S. aureus*, *S. mutans*, *E. faecalis*: An *in-vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MDB, SDK and SR designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MDB conducted the study. Authors MAI and RPK contributed in editing and correction of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Silica based nanoparticles are used in various fields of medical sciences to diagnose, control disease, for genetic disorders, owing to their size, surface area, biocompatibility and low toxicity. In dentistry, silica nanoparticles have been used as dental filler, teeth whitening agent but limited evidence is there regarding antimicrobial activity against oral pathogens. Therefore, the current study was conducted to assess the anti-bacterial activity of mouthwash incorporated with silica nanoparticles against oral pathogens. Tetraethoxysilane, ammonium hydroxide, absolute ethanol were used and centrifuged to obtain the silica nanoparticle pellet. XRD analysis was done to

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confirm the characterization of the thus obtained silica nanoparticle. The mouthwash was prepared with the synthesized silica nanoparticle as the main constituent. Agar well diffusion method was used to assess the antimicrobial activity against *S. mutans, S. aureus and E. faecalis.* The XRD analysis confirmed the amorphous nature of the synthesized silica nanoparticles. The zone of inhibition was found to increase as the concentrations increased mainly for *S. aureus and E. faecalis*. The synthesized nanoparticles incorporated mouthwash showed good potential as antimicrobial agents against strains of gram positive bacteria. Further animal studies/in vivo research should be conducted to validate the above findings.

Keywords: Nano-dentistry; gram positive bacteria; silica nanoparticles; antibacterial; antimicrobial.

1. INTRODUCTION

Dental caries is an irreversible disease of the oral cavity, which is multifactorial in nature but mostly driven by a high sugar diet and it manifests as phasic demineralization and remineralization of the hard tissues [1]. It affects people of all ages, with the prevalence rate being 50%, 52.5%, 61.4%, 79.2%, and 84.7% in 5, 12, 15, 35-44, and 65-74 year old, respectively according to the National Oral Health Survey report 2002-2003 which makes it a potentially high morbidity related diseases and that's why it has been an important area of focus for dental professionals [2]. Among the main hypothesis for the etiology of dental caries, the specific plaque hypothesis states certain bacterial strains are mainly responsible for dental caries, where bacteria like *S. sanguinis* were associated with health whereas *Lactobacillus, S.mutans, S.salivarius* were associated with caries [3]. Previous landmark studies have shown the demineralization of enamel and dentin by the action of bacteria found in oral cavity [4], caries formation in rats due to cariogenic diet [5], considering these factors the use of antibiotics or antimicrobial agents is an effective strategy for the prevention and treatment of dental caries [6].

In recent times, antibiotic resistance has become one of the most important worldwide problems, due to overuse of broad spectrum antibiotics, unnecessary prescription, improper use of antibiotics and unfinished antibiotic prescription. The ability of different strains of bacteria to withstand the effects of common antibiotics had led to finding varying strategies for treatment of antibiotic resistance bacteria. Research has shown nanoparticles containing antibiotics have many tremendous advantages; in fact nano particles have started being considered as nano-antibiotics because of their enhanced antimicrobial activities [7–9]. Among the nano-materials, special interest has been directed at silicon nanoparticles because of its excellent drug delivery capability. It is also biodegradable in nature, has reduced toxicity and stimulates macrophages [10]. Silica has been known as an abrasive agent used mostly in dentifrices, other functional versatility includes its use as a dentin blocking agent used for desensitization, teeth whitening, remineralization of teeth [11]. Previously, we have successfully completed numerous epidemiological and in vitro studies for the betterment of our community [12–30]. Since, very little evidence is there, where silica nanoparticles have been assessed for their antimicrobial effects against oral pathogens, this study was conducted to assess the anti-bacterial activity of mouthwash incorporated with silica nanoparticles against oral pathogens.

1.1 Objective of the Study

The objective of the study is to synthesis mouthwash incorporated with silica nanoparticles and to assess its antibacterial activity against *S. Aureus*, *S. Mutans*, *E.faecalis.*

2. MATERIALS AND METHODS

2.1 Study Design

In-vitro study.

2.1.1 Chemicals used

1. Tetraethoxysilane (TEOS) - Used as a sol-gel precursor to generate three dimensional silica based hybrid organic - inorganic networks.
2. Absolute ethanol - Used as the solvent.
3. Ammonium hydroxide - Used as a catalyst for hydrolysis and condensation of TEOS, thus accelerating the rate of the reaction.
4. Distilled water - Used as a vehicle.
5. Sucralfate - Aluminum salt of sucrose, used as a surface coating agent.
6. Sodium benzoate - Used as a preservative.

7. Clove oil - Used as a flavoring agent.
8. Sodium dodecyl phosphate - Used as a foaming agent.

2.1.2 Preparation of silica nanoparticles

Tetraethoxysilane (Sigma-Aldrich), absolute ethanol (Arima Chemicals Pvt Ltd), ammonium hydroxide (Sigma-Aldrich), were used in the ratio of 1:2:1 In a clean vessel, 2 ml of ammonia hydroxide was added to 5 ml of water. The mixture was stirred for 5 minutes and 4 ml of triethoxysilane and 40 ml of ethanol was added to the mixture and stirred for 1 hour (Fig. 1 above). The silica nanoparticle was recovered by centrifugation at 10,000 rpm for 30 minutes. The pellet was dried in a hot air oven at 60 degree Celsius for 8 hours.

2.1.3 Preparation of mouthwash solution

The mouthwash to be prepared using silica nanoparticles, ethanol, distilled water, sucrose, sodium benzoate, clove oil, Sodium dodecyl Phosphate. Silica nanoparticles are the main constituent, ethanol acts as a solvent to solubilize the ingredients. Sodium benzoate acts as a preservative and clove oil acts as a flavoring agent.

2.1.4 Microorganisms to be tested for

S. Aureus, S. Mutans, E. faecalis.

2.2 Study Methods

2.2.1 Materials characterization

For characterization of the synthesized silica nanoparticles, X-Ray Diffraction (XRD) analysis using X'Pert PRO machine was done. XRD shows the plot of intensity of X-rays scattered at different angles by a sample. From the XRD pattern, the crystalline phases of the sample can be determined, as well as presence or absence of any amorphous material can be assessed.

2.2.2 Antibacterial activity analysis

Agar well diffusion method was used to determine the antibacterial activity of different concentrations of SiNPs against oral pathogens such as *S. Aureus, S. Mutans, E.faecalis.* Secondary cultures of microbial suspension were dispersed evenly on the surface of Muller Hinton agar and rose Bengal agar plates using a sterile spreader. Different concentrations of nanoparticles (25, 50 & 100 µl) were incorporated through a sterile micropipette into the wells created on the agar plate using sterile cork borer. The plates were then incubated at 37°C for 24 h to 48 h. Commercial antibiotic ampicillin (50 mg/ml) was used as positive control for S. Aureus, S. Mutans, E.faecalis . The zone of inhibition (mm) was recorded for each plate and compared with control. All the tests were replicated in triplicate for analysis.

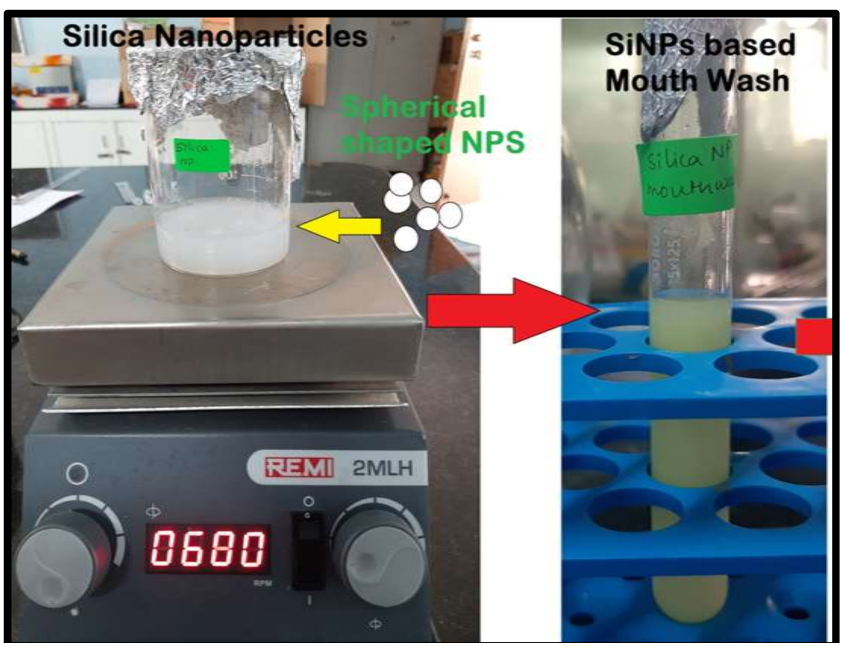

Fig. 1. Synthesis of silica nanoparticle incorporated mouthwash

3. RESULTS

3.1 Characterization of the Silica Nanoparticles

In Fig. 2, the XRD pattern shows distinct diffraction peaks and Braggs reflections are also observed. The formulated powder has the characteristic peaks at 2θ regions of 26°, 27°, 31°, 45°, 56°, 75°, 84°. The peaks represent the purity of the silica nanoparticles. These Braggs reflections clearly indicated the presence of sets of lattice planes and further on the basis that they can be indexed as face-centered-cubic (FCC) structure [31] of silica nanoparticles formed in this present synthesis are crystalline in nature.

3.2 Minimum Inhibitory Bactericidal Concentration

Agar well diffusion method was used to determine the antibacterial activity of different concentrations of SiNPs against *S. mutans, E. faecalis and S. aureus* (Fig. 3). Antimicrobial efficacy of silica nanoparticles in different concentrations has been shown in Fig. 4. The

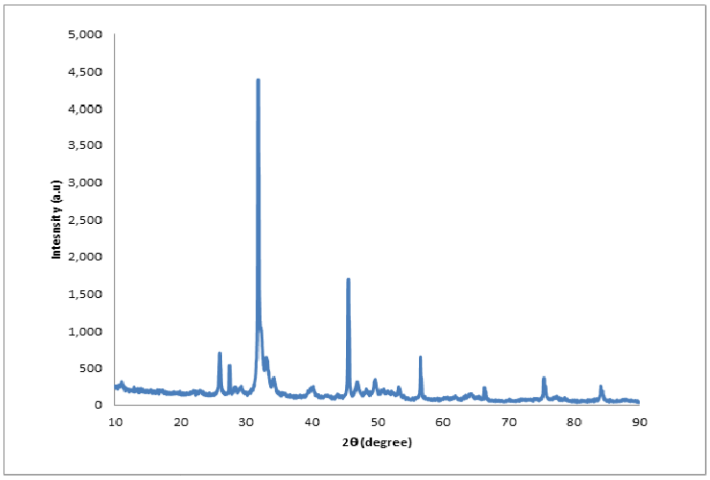

Fig. 2. XRD analysis of silica nanoparticles. the x axis represents the 2θ degree region, y axis represents the intensity. the formulated silica nanoparticle has the characteristic peaks at 2θ regions of 26°, 27°, 31°, 45°, 56°, 75°, 84°, which were found to be consistent with the standard hap phases. the diffraction peaks are markedly broader suggesting the prepared powder particles were in nano size

Fig. 3. Antimicrobial activity of silica nanoparticles incorporated mouthwash against *S.mutans, E. faecalis, S. aureus*

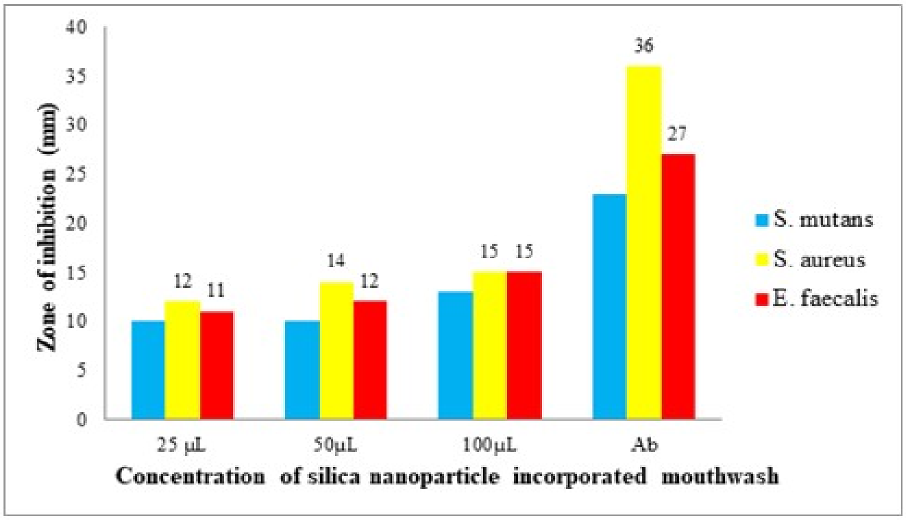

Fig. 4. Zone of inhibition of silica nanoparticles incorporated mouthwash against *S. Mutans, S.aureus, E.faecalis.* X axis represents the different concentrations of the silica nanoparticle incorporated mouthwash, Y axis represents the zone of inhibition against the oral pathogens. According to the finding, as the concentrations increased, the zone of inhibition also increased

mean zone of inhibition (ZOI) was found to increase as the concentration of SiNPs increased, however the maximum was found for ampicillin/cycloheximide. At 100 µl concentration of the SiNP, produced a maximum zone of inhibition for *S.aureus, E.faecalis*, however better results were observed along with ampicillin/cycloheximide

4. DISCUSSION

In an attempt to assess the feasibility of imparting long term antimicrobial effect to the oral cavity, various antimicrobial agents have been used. The modification of the existing agents like oral dentifrices, mouthwash with natural agents, inorganic elements, has been assessed in previous studies [32]. Silica, also known as silicon dioxide is an inorganic element found in nature and in living organisms. Silica nanoparticles, due to their size, surface area, low toxicity have a significant role in nanotechnology [33] . In the field of dentistry, silica has been used as a dental filler [34], for teeth polishing [35], for dentin hypersensitivity. The important factors in regard to the antimicrobial activity of silica are stability, adhesion and good dispersion of silica particles in organic matrix [36]. Silica in the form of nanoparticles has found acceptance in the field of nanotechnology, as their efficacy is primarily related to the fact that they reduce bacterial resistance.

The current study was conducted to assess the anti-bacterial activity of mouthwash incorporated with silica nanoparticles against oral pathogens. Silica nanoparticles were synthesized using the sol-gel method. In sol-gel reaction, there is hydrolysis and condensation of Tetra-ethoxy silane, where it is first hydrolyzed to silicic acid, followed by condensation reaction leading to the formation of Si-O-Si bonds, similar process has been used in previous studies [37,38] and this method is considered to be one of the most widely used methods for synthesis of nanoparticles. The synthesized silica nanoparticles were then characterized using XRD in order to confirm the nature of the nanoparticles. The XRD findings confirmed the amorphous nature of the synthesized silica nanoparticles. Followed by characterization, the antimicrobial activity was assessed against the gram positive bacteria S. mutans, S. aureus and E. faecalis. The bacterial resistance of the synthesized nanoparticles was determined with the Agar well diffusion method, where it was observed that the bacterial resistance power was variable according to the concentration used. At 100 µl concentration of the SiNP, produced a maximum zone of inhibition for S.aureus, *E. faecalis*, however it was comparatively lesser than that of ampicillin/cycloheximide. The concentration of the Silica nanoparticles used in the current study were lesser compared to that of the commercial antibiotics, which could justify the lesser zone of inhibition, as it was intended to use minimum concentration to observe the effect however, it also shows a good potential for silica nanoparticles as antimicrobial agents. The presence of singlet oxygen and other reactive oxygen species on the silica nanoparticles leads to oxidative damage to bacterial membranes and further death of bacteria, is considered a possible mechanism for its antimicrobial action [39]. The current study showed potential antimicrobial activity of the synthesized silica nano-particles in mouthwash against gram positive bacteria, similar to various other studies [36,40,41], in studies silica nano-particles have shown good antimicrobial action around implants as well [42].

Silica nanoparticles incorporated mouthwash show a good potential as an antimicrobial agent that maintains control of biofilm, preventing initial colonization of bacteria. However, the current study had certain limitations, it was not tested against gram negative bacteria. Hence, further research should be conducted to gather evidence regarding the potential of silica nanoparticles and its antimicrobial activities. Previously the metal nanoparticles were widely used in the antimicrobial applications and its based products [43-46] and in our study the polymer nanoparticles shows good activity.

5. CONCLUSION

A comprehensive sol-gel method was used to synthesize silica nanoparticles incorporated mouthwash. The formulation was effective against strains of *S.mutans, S.aureus and E.faecalis*. Silica nanoparticles even in minute concentrations showed good potential against gram positive bacteria.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Prior to the start of the study, ethical approval (IHEC/SDC-PHD-1801/19/170) was obtained from the Scientific Review Board, Saveetha Dental College, SIMATS.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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