ENHANCED ANTI-MICROBIAL EFFICACY OF THIOFLAVIN-DERIVED SILVER NANOPARTICLES AGAINST CARIOGENIC MICROORGANISMS

Sarah Cherian [1], K. Yuvaraj Babu [2], Taniya M [3] and M Sundaram K [4]*

[1,2,3,4] Department of Anatomy, Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University,
Poonamalle High Road, Velappanchavadi, Chennai.
*Corresponding Author Email: meenakshisundaram.sdc@saveetha.com

DOI: 10.5281/zenodo.12592287

Abstract

This study investigates the synthesis, characterization, and antimicrobial properties of Thioflavin-Derived Silver Nanoparticles (Th-AgNPs) against cariogenic microorganisms. Certain bacteria are primary contributors to dental caries. Thioflavin's biofilm-targeting capabilities, combined with the potent antimicrobial effects of silver nanoparticles, offer a novel approach for caries prevention and treatment. Th-AgNPs were synthesized and characterized using UV-Vis spectroscopy and FTIR to confirm size, shape, and stability. Antibacterial efficacy was assessed through assays, showing significant inhibition of bacteria. Molecular docking studies were performed to understand the interactions between Th-AgNPs and bacterial enzymes involved in biofilm formation. Results indicated strong binding affinities of Th-AgNPs to glucosyltransferases and lactate dehydrogenase, critical for bacterial virulence and survival. These interactions suggest that Th-AgNPs inhibit enzyme activity, impairing bacterial metabolism and biofilm development. This study highlights the synergistic effects of thioflavin and silver nanoparticles, providing a foundation for developing advanced therapeutic strategies against dental caries through targeted experimental methods and bacterial inhibition.

Keywords: Thioflavin-Derived Silver Nanoparticles, Streptococcus Mutans, Dental Caries, Antimicrobial Properties, Molecular Docking.

1. INTRODUCTION

Dental caries, commonly known as tooth decay, is a widespread oral health issue affecting a significant portion of the global population(Peres, Macpherson et al. 2019, BABU and MOHANRAJ 2020). This multifactorial disease results from the demineralization of tooth enamel due to the acid production by cariogenic microorganisms. These microorganisms, particularly *Candida albicans*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli, Staphylococcus aureus* colonize the oral cavity, forming biofilms on tooth surfaces(Rodrigues, Gomes et al. 2019). Candida albicans, a fungus commonly residing in the mucosal niches of the human body, is often identified in the biofilms that form on the teeth of toddlers suffering from severe childhood caries(Krzyściak, Jurczak et al. 2014). This condition, characterized by rampant tooth decay, represents a significant global public health issue. The presence of *Candida albicans* in dental plaque exacerbates the progression of caries by enhancing biofilm resilience and contributing to the acidic environment that promotes enamel demineralization(Palaniappan, Mohanraj et al. 2021). Understanding the role of *Candida albicans* in these biofilms is crucial for developing targeted strategies to prevent and treat severe childhood caries, aiming to mitigate its impact on affected populations(Akshaya and Ganesh 2022). Specific types of acid-producing bacteria, particularly *Streptococcus mutans*, colonize the dental surface and initiate damage to the hard tooth structure when exposed to fermentable carbohydrates like sucrose and fructose(Soro, Lamont et al. 2024).

These bacteria metabolize these sugars, producing acids as byproducts, which lower the pH in the oral environment(Ambika, Manojkumar et al. 2019). The acidic conditions lead to the demineralization of tooth enamel, creating cavities and advancing the progression of dental caries. The ability of *Streptococcus mutans* to thrive in such environments and form resilient biofilms makes it a primary contributor to tooth decay, underscoring the importance of controlling dietary sugar intake and maintaining oral hygiene to prevent caries. The primary cause of endodontic failure is the presence of certain bacterial species within the root canal system, notably *Enterococcus faecalis*(Senthil, Sundaram et al. 2022). These bacteria exhibit high resistance to common disinfection agents, leading to persistent intra-radicular or extra-radicular infections(Yuvaraj, Sangeetha et al. 2020, Biagio, de Magalhães Silveira et al. 2022). *E. faecalis* can survive in harsh conditions, including nutrient-depleted environments, and often forms resilient biofilms that protect it from antimicrobial treatments. This persistence contributes to the failure of root canal therapy, necessitating advanced and targeted disinfection strategies to effectively eradicate these resilient bacterial populations and ensure successful endodontic outcomes.

Escherichia coli, typically associated with fecal contamination, is not a resident but rather a transient member of the oral microbiota(Cox, Aloulou et al. 2020). Its occasional presence in the oral cavity may indicate environmental exposure to contaminated sources. The isolation of *E. coli* in the oral cavity raises concerns about potential fecal-oral transmission routes for pathogenic organisms. This transient colonization underscores the importance of maintaining oral hygiene and preventing oral ingestion of pathogens that could lead to gastrointestinal infections(Martinson and Walk 2020). Monitoring and preventing the presence of E. coli in the oral environment are crucial for mitigating health risks associated with microbial contamination. *Staphylococci aureus* is commonly acknowledged as part of the oral flora, although their exact role in oral health and disease remains a subject of debate and ongoing research. While traditionally associated with skin and nasal carriage, staphylococci can transiently colonize the oral cavity through various environmental exposures. Their presence in the oral microbiota suggests a potential influence on oral health, yet their specific contributions—whether beneficial, neutral, or potentially harmful—are still not fully understood(Sundaram, Bupesh et al. 2022).

The biofilms protect the bacteria from environmental stresses and antimicrobial agents, making them challenging to eradicate. As a result, innovative approaches to prevent and treat dental caries are necessary, particularly those that can effectively target and disrupt biofilms while minimizing the risk of antibiotic resistance(Anbarasu, Vinitha et al. 2024). In recent years, silver nanoparticles (AgNPs) have gained attention for their potent antimicrobial properties. AgNPs are effective against a broad spectrum of microorganisms, including bacteria, fungi, and viruses(Raj, Martin et al. 2024). Their antimicrobial action is primarily attributed to their ability to disrupt microbial cell membranes, generate reactive oxygen species (ROS), and interfere with microbial DNA and protein functions(Divya Sri, Vishnu Priya et al. 2020). These mechanisms collectively contribute to the bactericidal effects of AgNPs, making them a promising candidate for combating dental caries. Thioflavin is a benzothiazole dye known for its amyloid-binding properties, which have been extensively used in the detection and study of amyloid fibrils in neurodegenerative diseases. In this study, we explore the use of thioflavin to enhance the antimicrobial efficiency of silver nanoparticles. Thioflavin-Derived Silver Nanoparticles (Th-AgNPs) are synthesized to combine the biofilm-targeting capabilities of thioflavin with the potent antimicrobial effects of AgNPs(Alam, Rauf et al. 2018). This novel combination aims to provide a more effective strategy for preventing and treating dental caries by targeting the biofilms formed by cariogenic microorganisms. Dental caries is primarily caused by cariogenic microorganisms that adhere to the tooth surface, form biofilms, and produce acids that demineralize the enamel. Streptococcus mutans are the primary contributors to this process. S. mutans is particularly adept at adhering to the tooth surface and producing glucans from dietary sugars, which facilitate the formation of a robust biofilm(Dehvari and Ghahghaei 2018, Sasikumar, Gayathri et al. 2020).

The antimicrobial action of silver nanoparticles is well-documented. AgNPs exert their effects through multiple mechanisms: they disrupt microbial cell membranes, leading to cell lysis; they generate ROS, which cause oxidative stress and damage to cellular components; and they interfere with microbial DNA and protein functions, inhibiting replication and metabolic processes. When combined with thioflavin, the targeting and binding efficiency of AgNPs to microbial biofilms is potentially enhanced. Thioflavin's ability to bind to amyloid-like structures within biofilms can facilitate the delivery of AgNPs directly to the biofilm matrix, improving their antimicrobial efficacy. To understand the interaction between Th-AgNPs and key bacterial enzymes involved in biofilm formation and maintenance, molecular docking studies were conducted. These studies focus on enzymes such as glucosyltransferases and lactate dehydrogenase, which play critical roles in the virulence and survival of cariogenic bacteria. Glucosyltransferases are responsible for synthesizing extracellular polysaccharides that form the structural matrix of the biofilm, while lactate dehydrogenase is involved in the metabolic pathways that produce lactic acid, contributing to the acidic environment that demineralizes tooth enamel(Rezaei, Mehramouz et al. 2023).

Molecular docking results indicated strong binding affinities of Th-AgNPs to these enzymes. By binding to glucosyltransferases, Th-AgNPs can inhibit the synthesis of the biofilm matrix, preventing the establishment and maintenance of biofilms(Kovacs 2019). Binding to lactate dehydrogenase can disrupt the metabolic processes of the bacteria, reducing acid production and thereby mitigating enamel demineralization. These interactions suggest that Th-AgNPs not only exert direct antimicrobial effects but also interfere with critical bacterial functions necessary for biofilm formation and maintenance(Sadeghinejad 2016). The primary objective of this study is to evaluate the enhanced antimicrobial efficacy of Th-AgNPs against cariogenic microorganisms. Specifically, the study aims to assess the ability of Th-AgNPs to inhibit biofilm formation and reduce microbial viability. By combining the biofilm-targeting capabilities of thioflavin with the antimicrobial properties of silver nanoparticles, we seek to develop a more effective strategy for preventing and treating dental caries. This study holds significant implications for dental health, particularly in the context of developing new preventive and therapeutic strategies for dental caries. Current treatments often fall short in effectively targeting biofilms and are increasingly limited by the growing issue of antibiotic resistance. Th-AgNPs offer a promising alternative, providing a multi-faceted approach to combating cariogenic microorganisms. By enhancing the targeting and antimicrobial efficiency of silver nanoparticles, Th-AgNPs could lead to more effective treatments that address the limitations of current therapies and reduce the incidence and severity of dental caries. In conclusion, the synthesis, characterization, and evaluation of Th-AgNPs represent a novel approach to tackling dental caries. Through both experimental and computational analyses, this study aims to demonstrate the potential of Th-AgNPs as a powerful antimicrobial agent capable of disrupting biofilms and inhibiting cariogenic bacteria. This research not only contributes to the understanding of Th-AgNPs' mechanisms of action but also lays the groundwork for future developments in dental caries prevention and treatment(Wang, Wang et al. 2019, Jalan, Gayathri et al. 2020).

2. MATERIALS AND METHODS

2.1 Synthesis of Thioflavin-Derived Silver Nanoparticles (Th-AgNPs)

To synthesize Thioflavin-Derived Silver Nanoparticles (Th-AgNPs), a silver ion solution was prepared by dissolving 0.1 mM silver nitrate ($AgNO3$) in deionized water, and separately, a 0.1 mM thioflavin T solution was also prepared. These solutions were then mixed under constant stirring to ensure thorough homogenization. Subsequently, a freshly prepared 0.1 M sodium borohydride solution was added dropwise to the mixture while vigorously stirring to initiate the reduction of silver ions, leading to the formation of Th-AgNPs. Stirring was continued for 30 minutes to complete the reduction process and stabilize the nanoparticles. The resulting nanoparticle solution was then centrifuged at 10,000 rpm for 20 minutes to separate the Th-AgNPs from any unreacted materials and by-products. After discarding the supernatant, the nanoparticles underwent multiple washes with deionized water to eliminate residual reactants, ensuring the purity and stability of the synthesized Th-AgNPs.

2.2 Characterization of Th-AgNPs

Following the synthesis of Thioflavin-Derived Silver Nanoparticles (Th-AgNPs), characterization involved several analytical techniques. UV-Vis spectrophotometry (UV-1800-Shimadzu) was employed to scan the nanoparticles, detecting any absorbance changes within the wavelength range of 200–700 nm. The particle size of Th-AgNPs was calculated using the Debye–Scherrer equation, where λ represents the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the Bragg's angle Fourier transform infrared spectrometry (FTIR) using KBr pellets in the 500–4,000 cm^{-1} range identified functional groups present in the Thioflavin extract responsible for reducing silver ions to nanoparticles. These characterization techniques collectively provided comprehensive insights into the structural, morphological, and chemical properties of Thioflavin-Derived Silver Nanoparticles(Izumi 1989, Tayyeb, Priya et al. 2024).

2.3 Evaluation of Antimicrobial Efficacy by antimicrobial assay

Using a disc diffusion assay, the antimicrobial efficacy of Thioflavin-Derived Silver Nanoparticles (Th-AgNPs) was evaluated against *Candida albicans*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli, Staphylococcus aureus* bacterial and fungal strains. Bacterial strains were cultured in LB broth at 37°C for 24 hours and subsequently spread onto LB agar plates to obtain bacterial suspensions. Fungi were cultured on potato dextrose agar at 25°C in darkness. Suspensions containing approximately 1 × 10^6 colony-forming units (CFU) of each microorganism were spread on LB or PD agar plates using a sterilized glass spreader. Sterile filter paper discs (6 mm diameter) were loaded with fixed concentrations of Th-AgNPs, while sterile water served as the negative control and standard antibiotics as positive controls. Plates were then incubated at 37°C for 24 hours. After incubation, the diameter of the inhibitory zones formed around the discs loaded with different concentrations of Th-AgNPs was measured to assess their antimicrobial activity. All experiments were performed in triplicate to ensure reliability and reproducibility of the results(Velumani, Arasu et al. 2023).

2.4 Molecular Docking Studies

A molecular docking study employing the AutoDock method was conducted to investigate the interaction between Thioflavin-Derived Silver Nanoparticles (Th-AgNPs) and the protein receptor Thioflavin, extracted from the RCSB Protein Data Bank (PDB:2G9H). FabH plays a crucial role in bacterial fatty acid biosynthesis(Prathap and Jayaraman 2022). The crystallographic information file (CIF) of Th-AgNPs was obtained and converted into PDB format for use as a ligand in the docking simulations. Before initiating the simulations, Th-AgNPs and the 2G9H receptor were prepared by assigning Gasteiger partial charges, Kolman charges, and adding polar hydrogen atoms. The Lamarckian genetic algorithm was employed for the docking process. The autogrid parameters were adjusted to generate a comprehensive grid map covering the entire surface of the 2G9H protein. The docking simulations aimed to identify the optimal binding mode and binding sites of Th-AgNPs with 2G9H(Azam and Abbasi 2013). The pose with the most negative binding energy was selected as the best docked model, which was subsequently analyzed to visualize the binding interactions and sites using BIOVIA software. This approach provided insights into how Thioflavin-Derived Silver Nanoparticles interact with2G9H, potentially affecting bacterial fatty acid metabolism(Vijesh, Isloor et al. 2013).

3. RESULTS

Thioflavin-Derived Silver Nanoparticles (Th-AgNPs) were synthesized using a method involving the reduction of silver ions by Thioflavin, resulting in a distinctive yellow-brown color change in the reaction mixture. Studies have identified Thioflavin as a benzothiazole dye with amyloid-binding properties. The synthesis process of Th-AgNPs incorporates the antimicrobial efficacy of silver nanoparticles (AgNPs) with Thioflavin's biofilm-targeting capabilities, potentially enhancing their effectiveness against cariogenic microorganisms such as *Candida albicans*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli, Staphylococcus aureus* bacterial and fungal strains. Characterization studies using UV-Vis spectroscopy confirmed the formation of Th-AgNPs, exhibiting absorbance peaks characteristic of silver nanoparticles.

The binding interactions and mechanisms of Th-AgNPs with bacterial biofilms were further explored through molecular docking studies, elucidating their mode of action at the molecular level. Overall, Thioflavin-Derived Silver Nanoparticles represent a promising approach in combating dental caries and other microbial infections, leveraging the synergistic properties of Thioflavin and silver nanoparticles for enhanced therapeutic outcomes.

3.1 UV-Vis spectroscopy analysis

Biogenic Thioflavin-Derived Silver Nanoparticles (Th-AgNPs) were characterized using UV-Visible spectroscopy, which identified a distinct exciton band at 377 nm. This absorption peak closely resembles the bulk exciton absorption of Th-AgNPs (373 nm), indicating the formation of spherical Th-AgNPs with an average size range of

40–60 nm. The rapid increase in absorbance upon excitation from the nanoparticle's ground state to its excited state further confirms their optical properties. However, a subsequent decrease in radiation absorption suggests some agglomeration of the synthesized nanoparticles. The bandgap energy (Eg) of the Th-AgNPs was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscore the successful synthesis of biogenic Th-AgNPs nanoparticles and their promising optical characteristics for various applications.

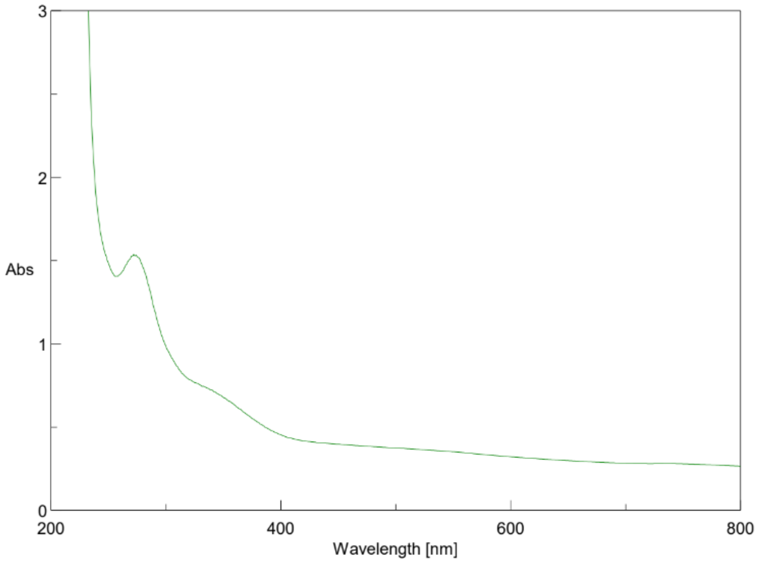

Figure 1: UV-Vis absorption spectra of Thioflavin-Derived Silver Nanoparticles

3.2 FTIR analysis

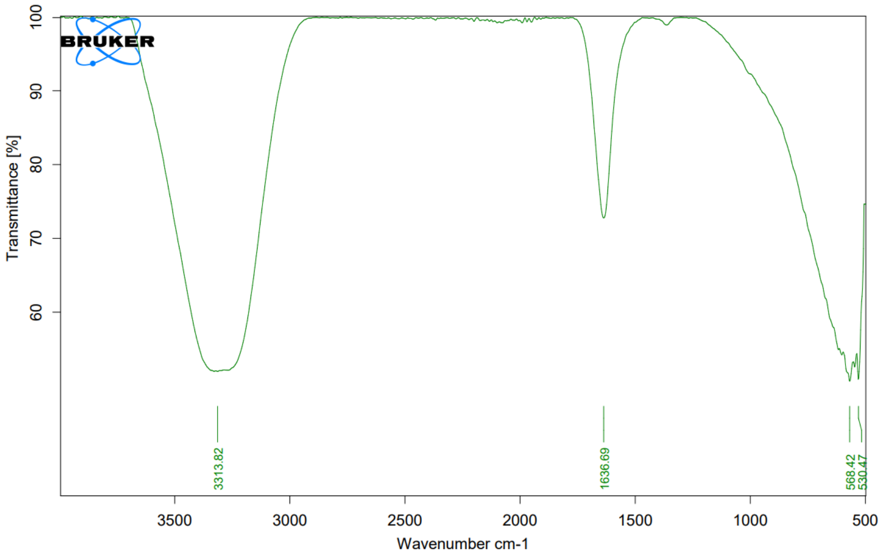

Figure 2: FTIR spectra of Thioflavin-Derived Silver Nanoparticles

The FTIR analysis of biosynthesized thioflavin-derived AgNPs was utilized to confirm putative functional groups of extracts and to involve potential bioactive compounds for the reduction of Ag+ to Ag0 and the capping and stability of bio-reduced Th-AgNPs manufactured using extract. As can be seen from Figure 3 of the IR spectrum, a broad peak at 3,371 cm−1 could be assigned markedly to O–H stretching vibration of the alcohol functionality, whereas a broad peak with low strength in the IR spectrum of AgNPs compared to the FTIR of extract was found to be around 3,400 cm−1, indicating the participation of bioactive compounds with OH groups in the formation of AgNPs. Other informative peaks were found at 2,890 and a slightly split peak at 1,639 cm−1 that can be attributed to C–H, and C═C fused with C═O, stretching vibration of alkane groups and ketones, respectively. The prominent peak about 499 cm−1 in the FTIR spectrum of AgNPs matching to metal–oxygen (M–O) supports the formation of NPs. Spectral analyses of the extract revealed that phytochemicals such as phenol, terpenes, and flavonoids may play an active role in the reduction of metal ions to metal.

3.3 Antimicrobial potential of Thioflavin-Derived Silver Nanoparticles

The table presents the inhibitory effects of Streptomycin (50 µg/ml), Compound A (50 µg/ml), and Compound B (100 µg/ml) on various microorganisms, as measured by the diameter of the inhibition zone (in mm) with standard deviations provided. Streptomycin and Compound B have similar inhibitory effects on E. coli, with Compound B being slightly more effective, while Compound A has a lower inhibitory effect compared to both. For E. faecalis, Compound B shows the highest inhibitory effect, significantly more than Streptomycin and Compound A, which have similar effects with Streptomycin being slightly more effective. Against S. aureus, Compound B is the most effective, followed by Compound A and then Streptomycin, with all three compounds demonstrating effectiveness. For S. mutans, Compound B is significantly more effective compared to Streptomycin and Compound A, with Streptomycin showing moderate inhibition and Compound A the least. Regarding C. albicans, Streptomycin and Compound B have similar inhibitory effects, with Compound B being slightly less effective, while Compound A shows the least inhibition among the three. Overall, Streptomycin (50 µg/ml) shows moderate inhibitory effects across all tested microorganisms, while Compound A (50 µg/ml) generally has the lowest inhibitory effects, except against S. aureus where it performs better than Streptomycin. Compound B (100 µg/ml) exhibits the highest inhibitory effects on most microorganisms, indicating strong antimicrobial properties, particularly against E. faecalis, S. aureus, and S. mutans. This data suggests that while Streptomycin is a standard antibiotic with moderate efficacy, Compound B at a higher concentration (100 µg/ml) tends to be more effective against the tested microorganisms, making it a potentially more potent antimicrobial agent. Although less effective, Compound a still shows activity, especially against S. aureus. Therefore, Compound B appears to be a superior antimicrobial agent in this study, showcasing significant potential for treating infections caused by the tested microorganisms.

Table 1: Antimicrobial activity of Thio-Ag NPs against different pathogens

Microorganism	Streptomycin (50µg/ ml)	Thio-AgNPs (50µg/ ml)	Thio-AgNPs (100 µg/ ml)
E. coli	12.77± 0.5	10.73± 0.5	13.4± 0.5
E. faecalis	13.90± 0.4	12.97± 0.4	16.9± 0.6
S. aureus	13.23± 0.7	14.00± 0.25	15.5± 0.45
S. mutans	11.00± 0.6	7.83± 0.3	16± 0.65
C. albicans	12.33± 0.5	7.00± 0.37	12.03± 0.84

ISSN 1462 2815

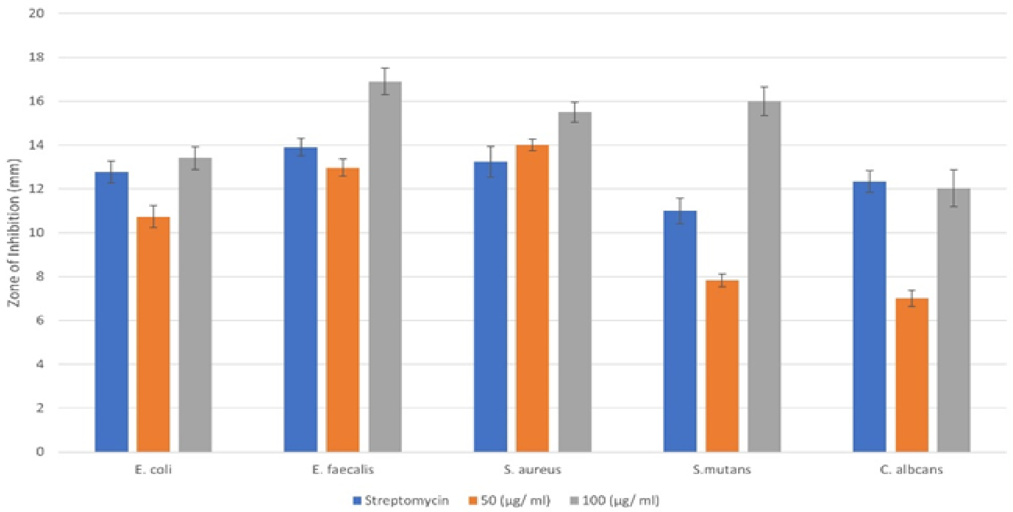

Figure 4: Antimicrobial activity of Thio-Ag NPs against different pathogens.

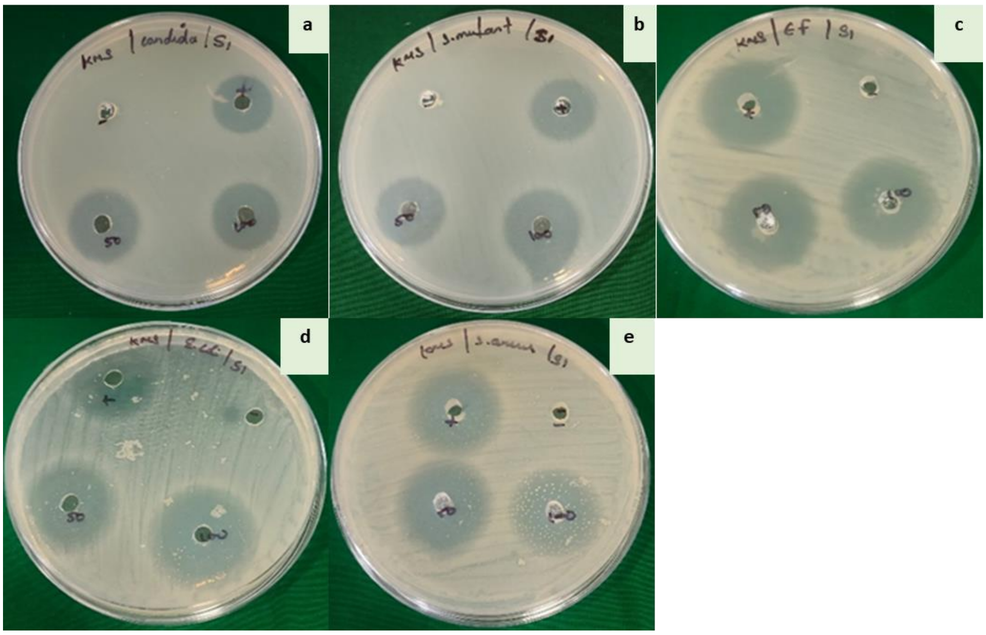

Figure 3: Antimicrobial activity of Thioflavin-Derived Silver Nanoparticles for bacterial and fungal strains a) *Candida albicans* b) *Streptococcus mutans* c) *Enterococcus faecalis* d) *Escherichia coli* e) *Staphylococcus aureus*

3.4 Molecular docking analysis

A catalytic triad tunnel composed of Val (309), Lys (310), and His (105) is found in the active site of 2G9H (PDB: 2G9H) (Figure 4). The catalytic activity of an enzyme can be dramatically influenced, inhibited, or even stopped by affecting these amino acid residues. Additionally, the active site residues of the Thioflavin derived silver nanoparticle receptor are conserved across Gram-positive and Gram-negative bacteria, making 2G9H protein a promising therapeutic target for the development of innovative and broad-spectrum antimicrobial drugs as selective and nontoxic 2G9H inhibitors. To predict the in vitro efficiency of Th-AgNPs, the ligand- 2G9H model was used to perform molecular docking study. Docking of Th-AgNPs into a modelled receptor named 2G9H was done to investigate proper nanoparticle orientation within the receptor and obtain useful information for the active mechanism, including noncovalent interactions between the active site of the receptor and Th-AgNPs, which could lead to the development of new drugs for further biological research.

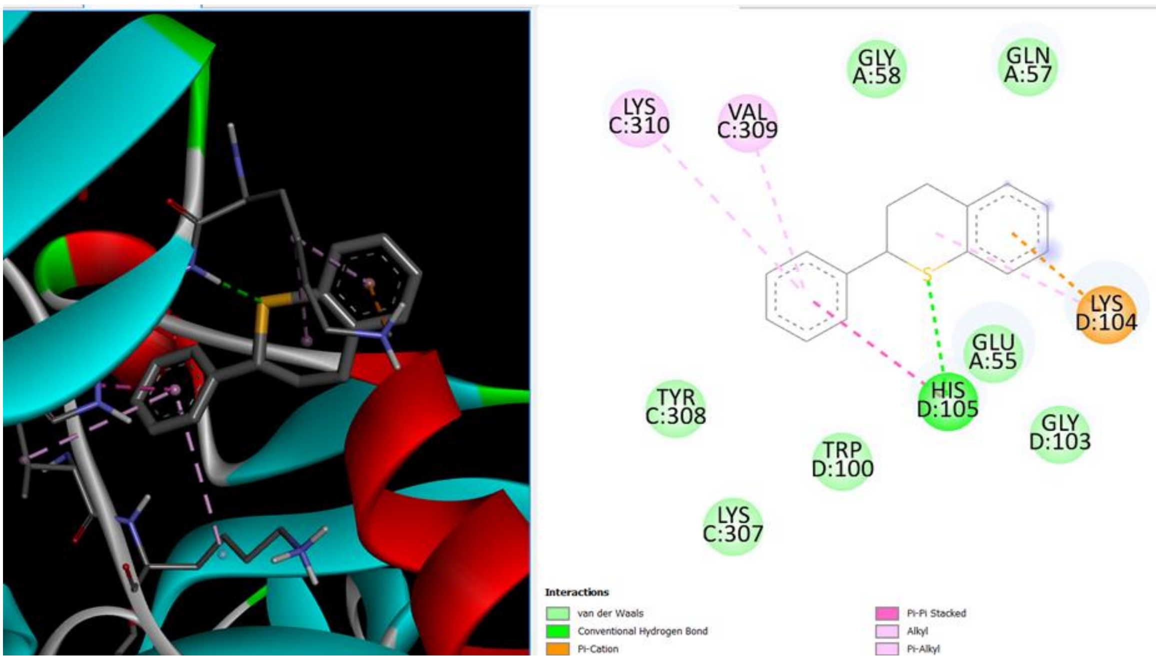

Figure 4: Molecular docking study of receptor, ligand (Thioflavin) best docking pose and various Thioflavin-Derived Silver Nanoparticles interactions with amino acids contribute to cavity formation

4. DISCUSSION

Dental caries, a prevalent oral health issue caused by bacterial activity, has driven the search for novel antimicrobial agents to combat antibiotic-resistant pathogens (Belibasakis, Lund et al. 2020). Traditional treatments often face limitations due to bacterial resistance, prompting the need for innovative solutions. In this context, the use of nanoparticles (NPs) has emerged as a promising approach (Mantravadi, Kalesh et al. 2019). Thioflavin-derived silver nanoparticles (Th-AgNPs), in particular, exhibit significant potential due to their unique physicochemical properties and broad-spectrum antimicrobial activity(Haque, Sartelli et al. 2019). The incorporation of thioflavin-derived AgNPs into dental care formulations could revolutionize the management of dental caries. Th-AgNPs possess several advantageous properties that make them suitable for this application. Their small size and large surface area-to-volume ratio enhance their antimicrobial activity, allowing for better penetration and interaction with bacterial cells. This increased surface reactivity enables Th-AgNPs to disrupt bacterial cell membranes, leading to the release of silver ions which can bind to cellular components, interfere with metabolic pathways, and ultimately cause cell death(Roberts and Mullany 2010, Ravikumar, Marunganathan et al. 2024).

Additionally, Th-AgNPs have shown effectiveness against biofilms, which are complex bacterial communities that adhere to surfaces and are highly resistant to conventional treatments. Biofilms play a significant role in the progression of dental caries, as they protect cariogenic bacteria like Streptococcus mutans from the effects of antimicrobial agents. The ability of AgNPs to penetrate and disrupt biofilms offers a significant advantage in dental caries prevention and treatment. The photo oxidizing and photocatalytic properties of Th-AgNPs also contribute to their antimicrobial efficacy. When exposed to light, Th-AgNPs can generate reactive oxygen species (ROS), which have been shown to damage bacterial cell walls, DNA, and other cellular components. This light-activated antimicrobial action can be particularly useful in dental applications, where controlled light exposure can enhance the effectiveness of Th- AgNPs containing treatments. Furthermore, the biocompatibility of thioflavin-derived Th-AgNPs is an important consideration for their use in dental products. Studies have shown that these nanoparticles can be incorporated into dental materials such as composite resins, adhesives, and sealants without compromising their mechanical properties or causing adverse effects on surrounding tissues. This makes them suitable for a range of dental applications, including fillings, coatings, and oral hygiene products. The antimicrobial efficiency of thioflavin-derived AgNPs against common oral pathogens a major contributor to dental caries, underscores their potential in dental applications. By inhibiting biofilm formation and bacterial proliferation, these nanoparticles could significantly reduce the incidence of dental caries and improve overall oral health(Ram, As et al. 2020, Giridharan, Chinnaiah et al. 2024).

5. CONCLUSION

Thioflavin-derived silver nanoparticles represent a promising advancement in the fight against dental caries. Their ability to target conserved enzymatic sites like 2G9H, coupled with their potent antimicrobial properties, positions them as effective agents for dental applications. Future research should focus on optimizing their formulation and delivery in dental products to harness their full potential in preventing and treating dental caries. As part of an integrated approach to oral health, Th-AgNPs could play a crucial role in reducing the burden of dental caries and combating antibiotic-resistant oral pathogens. Thioflavin-Derived Silver Nanoparticles exhibit enhanced antimicrobial and anti-biofilm properties against cariogenic microorganisms. These findings suggest that Th-AgNPs could play a significant role in developing new preventive and therapeutic strategies for dental caries. Further research is warranted to fully realize their clinical potential.

References

1) Akshaya, A. and K. Ganesh (2022). "Knowledge, prevalence, and impact on social distancing on coronavirus (COVID-19) infection spread in young, adult, and aged population: a survey." ECS Transactions **107**(1): 14063.

2) Alam, M. T., et al. (2018). "Green synthesis of silver nanoparticles, its characterization, and chaperone-like activity in the aggregation inhibition of α-chymotrypsinogen A." International journal of biological macromolecules **120**: 2381-2389.

3) Ambika, S., et al. (2019). "Biomolecular interaction, anti-cancer and anti-angiogenic properties of cobalt (III) Schiff base complexes." Scientific reports **9**(1): 2721.

4) Anbarasu, M., et al. (2024). "Depolymerization of PET Wastes Catalysed by Tin and Silver doped Zinc oxide Nanoparticles and Evaluation of Embryonic Toxicity Using Zebrafish." Water, Air, & Soil Pollution **235**(6): 433.

5) Azam, S. S. and S. W. Abbasi (2013). "Molecular docking studies for the identification of novel melatoninergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines." Theoretical Biology and Medical Modelling **10**: 1-16.

6) BABU, B. V. and K. G. MOHANRAJ (2020). "Knowledge, frequency and health hazards of overuse of mobile phone among Chennai population-A questionnaire based survey." International Journal of Pharmaceutical Research (09752366) **12**(4).

7) Belibasakis, G. N., et al. (2020). "Healthcare challenges and future solutions in dental practice: assessing oral antibiotic resistances by contemporary point-of-care approaches." Antibiotics **9**(11): 810.

8) Biagio, B., et al. (2022). "Persistent lesions in endodontics." Research, Society and Development **11**(14).

9) Cox, E., et al. (2020). "The intriguing interaction of Escherichia coli with the host environment and innovative strategies to interfere with colonization: a summary of the 2019 E. coli and the Mucosal Immune System meeting." Applied and environmental microbiology **86**(24): e02085-02020.

10) Dehvari, M. and A. Ghahghaei (2018). "The effect of green synthesis silver nanoparticles (AgNPs) from Pulicaria undulata on the amyloid formation in α-lactalbumin and the chaperon action of α-casein." International journal of biological macromolecules **108**: 1128-1139.

11) Divya Sri, E., et al. (2020). "Awareness on the role of health care workers in COVID-19-need of the hour." International Journal of Current Research and Review: 122-127.

12) Giridharan, B., et al. (2024). "Characterization of Novel Antimicrobial Peptides from the Epidermis of Clarias batrachus Catfish." International Journal of Peptide Research and Therapeutics **30**(2): 1-13.

13) Haque, M., et al. (2019). "Dental infection and resistance—global health consequences." Dentistry journal **7**(1): 22.

14) Izumi, F. (1989). "RIETAN: a software package for the Rietveld analysis and simulation of X-ray and neutron diffraction patterns." The Rigaku Journal **6**(1): 10-20.

15) Jalan, S., et al. (2020). "Awareness on self isolation to prevent COVID 19 infection among elderly people of Tamilnadu-a survey." International Journal of Current Research and Review: S160-S164.

16) Kovacs, C. J. (2019). Defining functional roles for the rhamnose-glucose polysaccharides of Streptococcus mutans, University of Rochester.

17) Krzyściak, W., et al. (2014). "The virulence of Streptococcus mutans and the ability to form biofilms." European Journal of Clinical Microbiology & Infectious Diseases **33**: 499-515.

18) Mantravadi, P. K., et al. (2019). "The quest for novel antimicrobial compounds: emerging trends in research, development, and technologies." Antibiotics **8**(1): 8.

19) Martinson, J. N. and S. T. Walk (2020). "Escherichia coli residency in the gut of healthy human adults." EcoSal plus **9**(1): 10.1128/ecosalplus. ESP-0003-2020.

20) Palaniappan, C. S., et al. (2021). "Knowledge And Awareness On The Association Between Physical Inactivity, Junk Food Consumption And Obesity Among Adolescent Population-A Survey Based Analysis." Int J Dentistry Oral Sci **8**(03): 1946-1951.

21) Peres, M. A., et al. (2019). "Oral diseases: a global public health challenge." The Lancet **394**(10194): 249-260.

22) Prathap, L. and S. Jayaraman (2022). "Identification of Endogenous Superoxide Dismutase as a Potential Inhibitor for Pi3k/Akt Signaling In Colorectal Cancer-A Molecular Docking Study." Journal of Pharmaceutical Negative Results: 1374-1379.

23) Raj, P. S. M., et al. (2024). "Anti-psychotic Nature of Antibiotics: Vancomycin and Omadacycline Combination Ameliorating Stress in a Zebrafish Model." Cureus **16**(3).

24) Ram, A. J., et al. (2020). "Overexpression of BASP1 indicates a poor prognosis in head and neck squamous cell carcinoma." Asian Pacific Journal of Cancer Prevention: APJCP **21**(11): 3435.

25) Ravikumar, O., et al. (2024). "Zinc oxide nanoparticles functionalized with cinnamic acid for targeting dental pathogens receptor and modulating apoptotic genes in human oral epidermal carcinoma KB cells." Molecular Biology Reports **51**(1): 1-16.

26) Rezaei, T., et al. (2023). "Factors associated with Streptococcus mutans pathogenicity in the oral cavity." Biointerface Res Appl Chem **13**(4): 368.

27) Roberts, A. P. and P. Mullany (2010). "Oral biofilms: a reservoir of transferable, bacterial, antimicrobial resistance." Expert review of anti-infective therapy **8**(12): 1441-1450.

28) Rodrigues, M. E., et al. (2019). "Candida spp./bacteria mixed biofilms." Journal of Fungi **6**(1): 5.

29) Sadeghinejad, L. (2016). Adaptation Mechanisms of Streptococcus mutans to Stresses Induced by Resin Composite Biodegradation By-products, University of Toronto (Canada).

30) Sasikumar, T., et al. (2020). "Awareness and initiatives taken by the residents of condominium in a metro city to prevent infection (Covid-19) spread-a survey." International Journal of Current Research and Review: S116-S121.

31) Senthil, R., et al. (2022). "Identification of oxazolo [4, 5-g] quinazolin-2 (1H)-one Derivatives as EGFR Inhibitors for Cancer Prevention." Asian Pacific Journal of Cancer Prevention: APJCP **23**(5): 1687.

32) Soro, A. S., et al. (2024). Dental caries. Molecular Medical Microbiology, Elsevier**:** 915-930.

33) Sundaram, K. K. M., et al. (2022). "Instrumentals behind embryo and cancer: a platform for prospective future in cancer research." AIMS Molecular Science **9**(1): 25-45.

34) Tayyeb, J. Z., et al. (2024). "Multifunctional curcumin mediated zinc oxide nanoparticle enhancing biofilm inhibition and targeting apoptotic specific pathway in oral squamous carcinoma cells." Molecular Biology Reports **51**(1): 423.

35) Velumani, K., et al. (2023). "Advancements of fish-derived peptides for mucormycosis: a novel strategy to treat diabetic compilation." Molecular Biology Reports **50**(12): 10485-10507.

36) Vijesh, A., et al. (2013). "Molecular docking studies of some new imidazole derivatives for antimicrobial properties." Arabian Journal of Chemistry **6**(2): 197-204.

37) Wang, S., et al. (2019). "Theaflavin-3, 3′-digallate suppresses biofilm formation, acid production, and acid tolerance in Streptococcus mutans by targeting virulence factors." Frontiers in Microbiology **10**: 464819.

38) Yuvaraj, A., et al. (2020). "Perception of general public regarding pros and cons of technological advancements during lockdown due to covid 19 pandemic-an online survey." International Journal of Current Research and Review: 227-233.