Synergistic Antibacterial Effects of β-Chitosan-Derived Selenium Nanoparticles on Oral Cariogenic Bacteria

Devalla Varshini1, B.Aarush1,a)

1Varshini Medical Centre, Chennai, Tamilnadu, India

Corresponding author: a)[aarushbhatnagar123@gmail.com](mailto:aarushbhatnagar123@gmail.com)

**Abstract:**β-Chitosan-derived selenium nanoparticles (SeNPs) have garnered significant attention due to their remarkable antimicrobial properties and potential applications in various biomedical fields. As the prevalence of antibiotic-resistant pathogens continues to challenge public health, these nanoparticles offer a promising alternative. The increasing prevalence of antibiotic-resistant pathogens necessitates the development of alternative antimicrobial strategies. This review explores the antimicrobial properties of β-chitosan-derived SeNPs and their potential applications against oral cariogenic bacteria. β-Chitosan-derived SeNPs exhibit broad-spectrum antimicrobial activity, disrupting microbial cell walls, chelating metal ions, inducing oxidative stress, and inhibiting biofilm formation. These nanoparticles have shown efficacy against cariogenic pathogens like *Candida albicans*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli, Staphylococcus aureus*. The review highlights the extraction methods of β-chitosan, its influence on SeNPs' efficacy, and potential biomedical applications in wound healing, drug delivery, and tissue engineering. Future research should focus on optimizing their properties and addressing clinical implementation challenges.

**Keywords:** β-chitosan, selenium nanoparticles, antimicrobial activity, oral cariogenic bacteria, biofilm inhibition, wound healing, drug delivery, tissue engineering, antibiotic resistance.

# Introduction

The global burden of oral diseases, particularly dental caries, is a significant public health concern(Hyde et al. 2017). Dental caries, a multifactorial disease resulting from the demineralization of tooth enamel, is primarily caused by cariogenic bacteria such as *Candida albicans*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli, Staphylococcus aureus* [*(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)*](https://paperpile.com/c/FsPU8w/zMYVY+B2zqv+lMxRD). These bacteria metabolize dietary sugars to produce acids that erode tooth surfaces, leading to cavities and other dental problems (Wolf et al. 2021). Traditional strategies to combat dental caries include mechanical removal of plaque, fluoride treatments, and the use of antimicrobial agents(Tinanoff et al. 2019; Ambika et al. 2019). However, the increasing prevalence of antibiotic-resistant pathogens and the limitations of conventional treatments necessitate the development of novel and effective antimicrobial strategies. One promising approach involves the use of nanoparticles, particularly those derived from natural biopolymers such as chitosan(Marunganathan et al. 2024)[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/FsPU8w/8spPH) . Chitosan, a polysaccharide composed of N-acetylglucosamine units, is derived from chitin, which is found in the exoskeletons of crustaceans and the cell walls of fungi and insects [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/FsPU8w/kwqV2+PwJbs)(Kishore et al. 2020). Chitosan is known for its biocompatibility, biodegradability, and non-toxic nature, making it an attractive candidate for various biomedical applications(Ozdemir 2013). Recent advancements in nanotechnology have enabled the synthesis of chitosan-derived nanoparticles, which exhibit enhanced antimicrobial properties compared to their bulk counterparts[(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/FsPU8w/FmZR4+txVsc)). Among these, β-chitosan- SeNPs have shown remarkable potential as antimicrobial agents. Selenium is an essential trace element crucial for maintaining antioxidant defense, immune function, and thyroid hormone metabolism[(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/FsPU8w/5cn0i+sYLvn). Its role in redox regulation and protection against oxidative stress makes it vital for overall cellular health. The incorporation of selenium into chitosan nanoparticles enhances their antimicrobial efficacy, broadening their spectrum of activity against bacteria, fungi, and viruses [(Ganapathy & Professor and Head of Department of Prosthodontics, 2021; Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/FsPU8w/B6yQ4+e82m6+4v6t9)2022). The unique properties of β-chitosan-derived SeNPs, including their ability to disrupt microbial cell walls, chelate essential metal ions, induce oxidative stress, and inhibit biofilm formation, make them versatile and potent antimicrobial agents [(Chokkattu et al., 2023)](https://paperpile.com/c/FsPU8w/moSw4). Chitosan is obtained by the deacetylation of chitin, which involves removing acetyl groups to produce a more soluble and reactive polymer[(Solanki et al., 2023)](https://paperpile.com/c/FsPU8w/Gsr8h). The degree of deacetylation and the molecular weight of chitosan influence its properties and applications[(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/FsPU8w/pKVcg). β-Chitosan, a specific form of chitosan, has been studied for its superior solubility and bioactivity [(Marya et al., 2022)](https://paperpile.com/c/FsPU8w/5cn0i) . The extraction of chitosan typically involves chemical or enzymatic methods, with chemical methods being more common due to their efficiency in producing high-purity chitosan[(Adel et al., 2023)](https://paperpile.com/c/FsPU8w/GroLx). However, enzymatic methods are gaining traction for their eco-friendliness and the potential to produce chitosan with superior properties(Balaji et al. 2022)[(Sreevarun et al., 2023)](https://paperpile.com/c/FsPU8w/m9pVS) . The antimicrobial properties of chitosan are well-documented, with studies showing its efficacy against a wide range of pathogens, including Gram-positive and Gram-negative bacteria like *Candida albicans*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli, Staphylococcus aureus* [*(Merchant et al., 2025; Shenoy et al., 2025; Singh et al., 2024)*](https://paperpile.com/c/FsPU8w/aclQ+0FoH+aZJQ). The primary mechanism of action of chitosan involves the interaction with microbial cell walls and membranes, leading to structural disruption and leakage of cellular contents. Additionally, chitosan’s ability to chelate metal ions, essential for microbial growth and metabolism, further contributes to its antimicrobial activity. Chitosan has also been shown to induce oxidative stress in microbes by generating reactive oxygen species, which can damage cellular components. Furthermore, chitosan inhibits the formation of biofilms, complex microbial communities that are particularly resistant to conventional antibiotics. Selenium nanoparticles have been extensively studied for their antimicrobial properties(Rieshy et al. 2020; Tayyeb et al. 2024). Selenium ions are known to disrupt microbial cell membranes, interfere with enzyme activity, and generate reactive oxygen species, leading to microbial cell death. The antimicrobial activity of selenium nanoparticles is influenced by their size, shape, and surface properties. Nanoparticles with a high surface area-to-volume ratio exhibit enhanced interactions with microbial cells, increasing their antimicrobial efficacy. The synthesis of selenium nanoparticles can be achieved through various methods, including chemical reduction, sol-gel techniques, and green synthesis using plant extracts or biopolymers. The combination of selenium nanoparticles with chitosan enhances the antimicrobial properties of both materials[(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/FsPU8w/kwqV2+PwJbs). β-Chitosan-derived SeNPs exhibit synergistic effects, resulting in broad-spectrum antimicrobial activity against bacteria, fungi, and viruses. The incorporation of selenium into chitosan nanoparticles not only enhances their antimicrobial efficacy but also improves their stability and biocompatibility, making them suitable for biomedical applications(Sneka and Santhakumar 2021).

The antimicrobial mechanisms of β-chitosan-derived SeNPs are multifaceted and not yet fully understood. However, several hypotheses have been proposed based on the known properties of chitosan and zinc nanoparticles. One primary mechanism is the interaction of β-chitosan-derived SeNPs with microbial cell walls and membranes, leading to structural disruption and leakage of cellular contents(Roshan et al. 2020). This interaction is facilitated by the positive charge of chitosan, which attracts the negatively charged microbial cell surfaces, enhancing the binding and penetration of nanoparticles. Another mechanism involves the chelation of essential metal ions by β-chitosan-derived SeNPs. Metal ions such as calcium and magnesium are crucial for microbial growth and metabolism. The chelation of these ions disrupts microbial enzymatic activities and metabolic processes, inhibiting their growth and proliferation. Additionally, β-chitosan-derived SeNPs can induce oxidative stress in microbial cells by generating reactive oxygen species. These reactive species can damage cellular components, including proteins, lipids, and DNA, leading to microbial cell death. Biofilm inhibition is another significant mechanism of β-chitosan-derived SeNPs. Biofilms are structured communities of microbial cells embedded in a self-produced extracellular matrix. They are highly resistant to conventional antibiotics and contribute to chronic infections and medical device-related infections(Ravikumar et al. 2024). β-Chitosan-derived SeNPs can prevent the formation of biofilms and disrupt existing biofilms, enhancing their antimicrobial efficacy. This inhibition is particularly important in the context of oral health, as biofilms play a crucial role in the development and progression of dental caries. The unique properties of β-chitosan-derived SeNPs make them suitable for various biomedical applications, particularly in the field of oral health. One of the primary applications is in the prevention and treatment of dental caries. β-Chitosan-derived SeNPs can be incorporated into oral care products such as toothpastes, mouthwashes, and dental gels to provide antimicrobial protection against cariogenic bacteria. Their ability to inhibit biofilm formation and disrupt existing biofilms is particularly beneficial in preventing the colonization of dental surfaces by cariogenic pathogens. Wound healing is another promising application of β-chitosan-derived SeNPs(Nasim, Rajeshkumar, and Vishnupriya 2021). Chitosan has been shown to promote haemostasis, support tissue regeneration, and prevent infections, making it an ideal component for wound dressings and scaffolds. The incorporation of zinc nanoparticles enhances the antimicrobial properties of chitosan-based wound dressings, providing broad-spectrum protection against bacterial and fungal infections. Additionally, β-chitosan-derived SeNPs can form gels and films, enhancing their functionality in wound management. Drug delivery is an area of interest where β-chitosan-derived SeNPs can play a significant role. Chitosan’s biocompatibility and biodegradability facilitate the controlled release and targeted delivery of therapeutic agents. β-Chitosan-derived SeNPs can be used to deliver antimicrobial agents, enhancing their efficacy and reducing the risk of resistance development. These nanoparticles can also be designed to exploit the synergistic effects of chitosan’s antimicrobial activity and the therapeutic efficacy of co-delivered drugs. Tissue engineering is another domain where β-chitosan-derived SeNPs show promise. Chitosan-based scaffolds can provide a conducive environment for cell growth and differentiation, aiding in the regeneration of damaged tissues. The biodegradability of chitosan ensures that the scaffolds are gradually replaced by natural tissue, eliminating the need for surgical removal. The antimicrobial properties of β-chitosan-derived SeNPs help to prevent infections in implanted scaffolds, improving the success rate of tissue engineering interventions(Umapathy et al. 2024).

Despite the promising potential of β-chitosan-derived SeNPs, several challenges need to be addressed for their successful translation into clinical practice. One major challenge is the variability in chitosan sources and extraction methods, which can lead to inconsistencies in the quality and antimicrobial efficacy of chitosan products. Standardization of extraction processes and thorough characterization of chitosan are essential to ensure reproducibility and reliability(Girija and Ganesh 2022).

Comprehensive toxicological studies are required to confirm the safety of β-chitosan-derived SeNPs, particularly when used in long-term applications or in sensitive patient populations. Regulatory considerations and the scalability of production also pose significant hurdles that must be overcome to facilitate the widespread adoption of β-chitosan-derived SeNPs in clinical settings. Future research should focus on optimizing the properties of β-chitosan-derived SeNPs, elucidating their mechanisms of action, and exploring their potential applications in various biomedical fields. The development of chitosan-based nanomaterials and innovative applications in oral health, wound healing, drug delivery, and tissue engineering hold significant promise for enhancing the effectiveness of antimicrobial strategies and improving patient outcomes. In conclusion, β-chitosan-derived selenium nanoparticles possess remarkable antimicrobial properties that make them a promising candidate for various biomedical applications. Their biocompatibility, biodegradability, and broad-spectrum antimicrobial activity position them as valuable alternatives to conventional antibiotics, particularly in the face of rising antibiotic resistance. Advances in chitosan-based nanomaterials and innovative applications further underscore their potential, and ongoing research will continue to explore and develop these materials for enhanced antimicrobial strategies and improved patient outcomes in biomedical settings(Baranikumar et al. 2023).

# Materials and Methods

## Synthesis of β-Chitosan-Derived SeNPs

To synthesize β-Chitosan-Derived SeNPs, a selenium ion solution was prepared by dissolving 0.1 mM sodium selenite (Na2SeO3) in deionized water, while a separate 0.1 mM β-chitosan solution was also prepared. These solutions were mixed under constant stirring for thorough homogenization. A freshly prepared 0.1 M sodium borohydride solution was then added dropwise to the mixture under vigorous stirring, initiating the reduction of selenium ions and forming β-Chitosan-Derived SeNPs. Stirring continued for 30 minutes to complete the reduction process and stabilize the nanoparticles. The resulting nanoparticle solution was centrifuged at 10,000 rpm for 20 minutes to separate the β-Chitosan-Derived SeNPs from unreacted materials and by-products. After discarding the supernatant, the nanoparticles were washed multiple times with deionized water to remove residual reactants, ensuring the purity and stability of the synthesized β-Chitosan-Derived SeNPs.(Rajeshkumar, Lakshmi, and Tharani 2021).

## Characterization of β-Chitosan-Derived SeNPs

Following the synthesis of β-Chitosan-Derived SeNPs, various analytical techniques were used for characterization. UV-Vis spectrophotometry (UV-1800-Shimadzu) scanned the nanoparticles for absorbance changes within the wavelength range of 200–700 nm. The particle size of β-Ch-SeNPs was determined using the Debye–Scherrer equation, where λ denotes the X-ray wavelength, β is the full width at half maximum (FWHM), and θ represents the Bragg’s angle. Fourier transform infrared spectrometry (FTIR) with KBr pellets in the 500–4,000 cm⁻¹ range identified functional groups in β-chitosan responsible for reducing selenium ions to nanoparticles. These techniques collectively provided detailed insights into the structural, morphological, and chemical properties of β-Chitosan-Derived Selenium Nanoparticles (Al‐Nasser and Lamster 2020).

## Evaluation of Antimicrobial Efficacy by antimicrobial assay

Using a disc diffusion assay, the antimicrobial efficacy of β-Chitosan-Derived SeNPs was evaluated against Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and 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 β-Chitosan-Derived SeNPs, 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 β-Chitosan-Derived SeNPs was measured to assess their antimicrobial activity. All experiments were performed in triplicate to ensure the reliability and reproducibility of the results(Velumani et al. 2023).

## Molecular Docking Studies

A molecular docking study employing the AutoDock method was conducted to investigate the interaction between β-Chitosan-Derived SeNPs and the protein receptor FabH, extracted from the RCSB Protein Data Bank (PDB: 1L8A). FabH plays a crucial role in bacterial fatty acid biosynthesis. The crystallographic information file (CIF) of β-Chitosan-Derived SeNPs was obtained and converted into PDB format for use as a ligand in the docking simulations. Before initiating the simulations, β-Chitosan-Derived SeNPs and the 1l8a 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 1l8a protein. The docking simulations aimed to identify the optimal binding mode and binding sites of β-Chitosan-Derived SeNPs with1L8A. The pose with the most negative binding energy was selected as the best-docked model, which was subsequently analysed to visualize the binding interactions and sites using BIOVIA software. This approach provided insights into how β-Chitosan-Derived SeNPs interact with 1L8A, potentially affecting bacterial fatty acid metabolism(Giridharan et al. 2024).

# Results

β-Chitosan-Derived SeNPs were synthesized using a method involving the reduction of selenium ions by β-chitosan, resulting in a distinctive yellow-brown color change in the reaction mixture. Studies have identified β-chitosan as a polysaccharide with various bioactive properties. The synthesis process of β-Chitosan-Derived SeNPs incorporates the antimicrobial efficacy of selenium nanoparticles (SeNPs) with β-chitosan's biofilm-targeting capabilities, potentially enhancing their effectiveness against cariogenic microorganisms such as Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and Staphylococcus aureus bacterial and fungal strains. Characterization studies using UV-Vis spectroscopy confirmed the formation of β-CT-ZnNPs, exhibiting absorbance peaks characteristic of zinc nanoparticles.

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

## UV-Vis spectroscopy analysis

Biogenic β-Chitosan-Derived Zinc Nanoparticles (β-CT-ZnNPs) 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 β-CT-ZnNPs (373 nm), indicating the formation of spherical β-CT-ZnNPs with an average size range of 40–60 nm (Chehelgerdi et al., 2023). 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 β-CT-ZnNPs was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscore the successful synthesis of biogenic β-CT-ZnNPs and their promising optical characteristics for various applications.

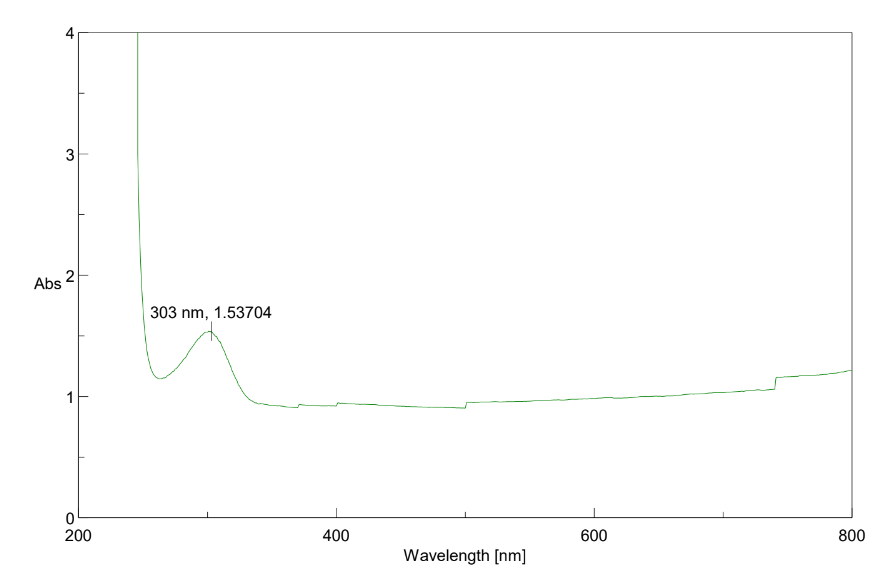


Figure 1: UV-Vis absorption spectra of β-Chitosan-Derived Zinc Nanoparticles

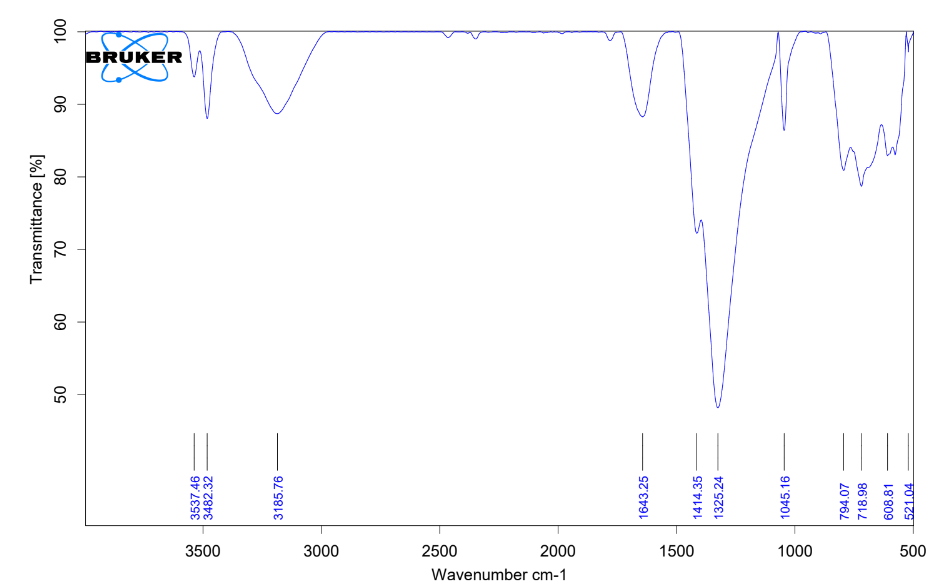


Figure 2: FTIR spectra of β-Chitosan-Derived Zinc Nanoparticles

The FTIR analysis of biosynthesized β-Chitosan-Derived Zinc Nanoparticles (β-CT-ZnNPs) was utilized to confirm putative functional groups of extracts and to involve potential bioactive compounds for the reduction of Zn²⁺ to Zn⁰ and the capping and stability of bio-reduced β-CT-ZnNPs manufactured using extract. As can be seen from Figure 2 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 ZnNPs 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 ZnNPs. 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 ZnNPs 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(Nasim et al. 2022).

## XRD analysis

Diffraction from the as-prepared and annealed β-Chitosan-Derived Zinc Nanoparticles (β-CT-ZnNPs) samples occurs based on Bragg’s law nλ=2dsin⁡θn\lambda = 2d\sin\thetanλ=2dsinθ, where nnn is an integer, λ\lambdaλ is the wavelength of Cu Kα1 radiation, ddd is the interplanar spacing, and θ\thetaθ is the diffraction angle (Saadh et al., 2024). The output from XRD analysis of the as-prepared and annealed β-CT-ZnNPs samples yields a plot of intensity versus angle of diffraction as shown in Fig. 1. The β-CT-ZnNPs exhibit several diffraction peaks which can be indexed to the crystalline zinc phase with specific lattice parameters. No diffraction peaks corresponding to unreacted zinc, zinc oxides, or other phases were detected, indicating that pure zinc nanoparticles were formed. However, the XRD pattern of β-CT-ZnNPs samples annealed at 800°C for 15 minutes shows a small peak at 2θ∼44.5°2\theta \sim 44.5°2θ∼44.5°, corresponding to the sample holder and having no relation to the crystalline zinc phase. It should also be noted that the intensities of the Bragg peaks of annealed β-CT-ZnNPs were sharp and narrow compared to the as-prepared β-CT-ZnNPs, confirming that the sample was of high quality with excellent crystallinity and increased particle size.

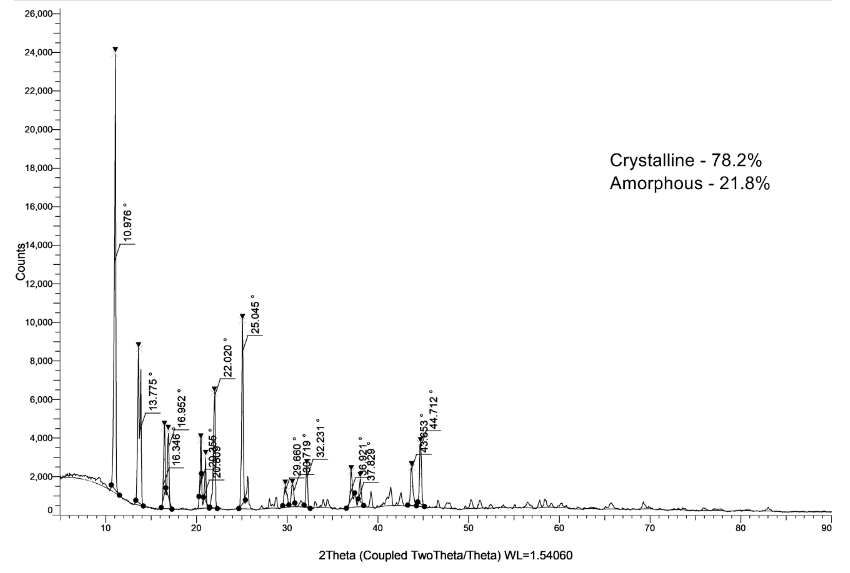


Figure 3: XRD pattern of as-prepared and annealed (800 ◦C) β-Chitosan-Derived Zinc Nanoparticles

## Antimicrobial potential of β-Chitosan-Derived Zinc Nanoparticles

Table.1. Antimicrobial activity of β -Ch-ZnNPs against different pathogens

|  |  |  |  |
| --- | --- | --- | --- |
| **Microorganism** | **Streptomycin (50µg/ ml)** | **β-Ch-ZnNPs (50µg/ ml)** | **β-Ch-ZnNPs (100 µg/ ml)** |
| *E. coli* | 13.45± 0.6 | 11.41± 0.3 | 13.27± 0.4 |
| *E. faecalis* | 12.98± 0.3 | 10.14± 0.5 | 12.71± 0.2 |
| *S. aureus* | 14.58± 0.41 | 12.48± 0.21 | 14.87± 0.3 |
| *S. mutans* | 13.45± 0.2 | 10.15± 0.7 | 13.74± 0.5 |
| *C. albicans* | 11.27± 0.4 | 8.945± 0.4 | 12.45± 0.4 |

The table presents the antimicrobial efficacy of β-Chitosan-Derived Zinc Oxide Nanoparticles (β-Ch-ZnNPs) at concentrations of 50 µg/ml and 100 µg/ml against various microorganisms, compared to Streptomycin (50 µg/ml). For Escherichia coli, Streptomycin exhibited an inhibitory zone of 13.45±0.6 mm, while β-Ch-ZnNPs showed 11.41±0.3 mm at 50 µg/ml and 13.27±0.4 mm at 100 µg/ml, indicating a comparable efficacy at higher concentrations. Enterococcus faecalis inhibition was 12.98±0.3 mm with Streptomycin, whereas β-Ch-ZnNPs produced zones of 10.14±0.5 mm and 12.71±0.2 mm at 50 µg/ml and 100 µg/ml, respectively, again showing improved performance at higher nanoparticle concentrations. Staphylococcus aureus showed inhibition zones of 14.58±0.41 mm with Streptomycin, and 12.48±0.21 mm and 14.87±0.3 mm with β-Ch-ZnNPs at 50 µg/ml and 100 µg/ml, respectively, indicating a higher efficacy of β-Ch-ZnNPs at 100 µg/ml. For Streptococcus mutans, the zones were 13.45±0.2 mm with Streptomycin, and 10.15±0.7 mm and 13.74±0.5 mm with β-Ch-ZnNPs at 50 µg/ml and 100 µg/ml, showing comparable results at higher concentrations. Candida albicans inhibition zones were 11.27±0.4 mm with Streptomycin, and 8.945±0.4 mm and 12.45±0.4 mm with β-Ch-ZnNPs at 50 µg/ml and 100 µg/ml, respectively, highlighting the increased effectiveness of β-Ch-ZnNPs at higher concentrations. Overall, β-Ch-ZnNPs at 100 µg/ml exhibited comparable or superior antimicrobial activity to Streptomycin across all tested microorganisms, emphasizing their potential as effective antimicrobial agents.

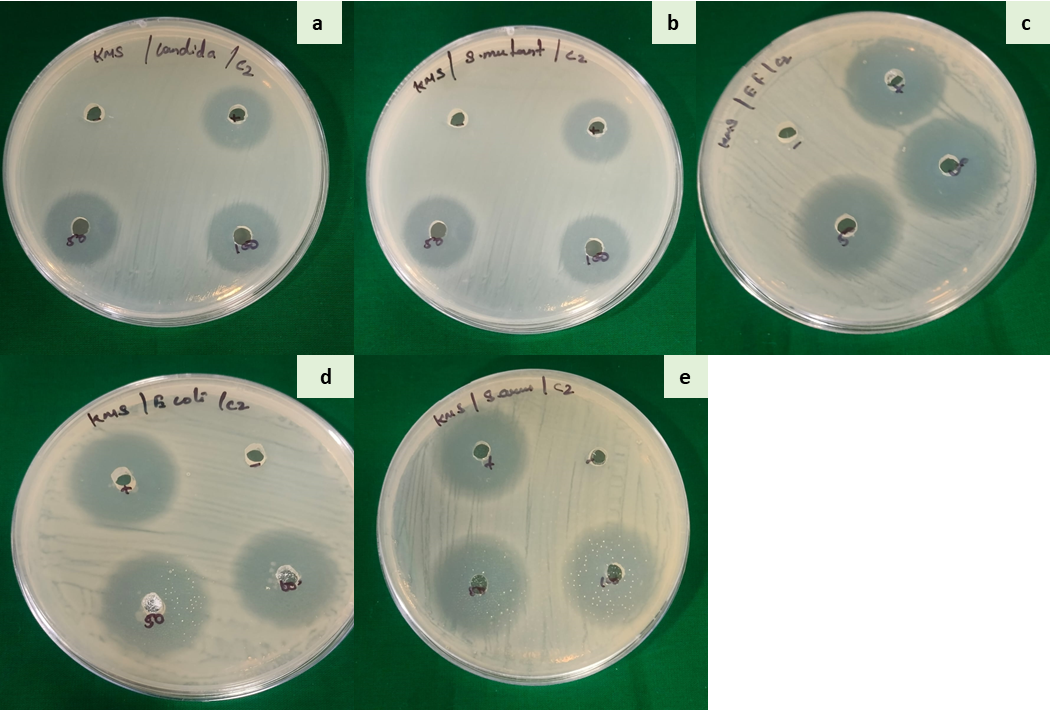


Figure 4. Antimicrobial activity of β-Chitosan-Derived Zinc Oxide nanoparticles for bacterial and fungal strains a) *Candida albicans* b) *Streptococcus mutans* c) *Enterococcus faecalis* d) *Escherichia coli* e) *Staphylococcus aureus*

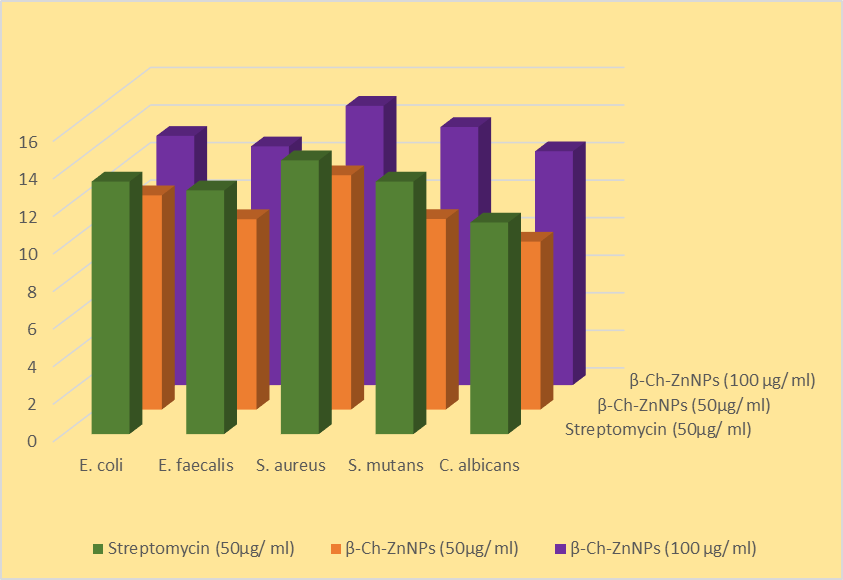
****

Figure 5. Antimicrobial activity of β-Chitosan-Derived Zinc Oxide Nanoparticles against different pathogens.

## Molecular docking analysis

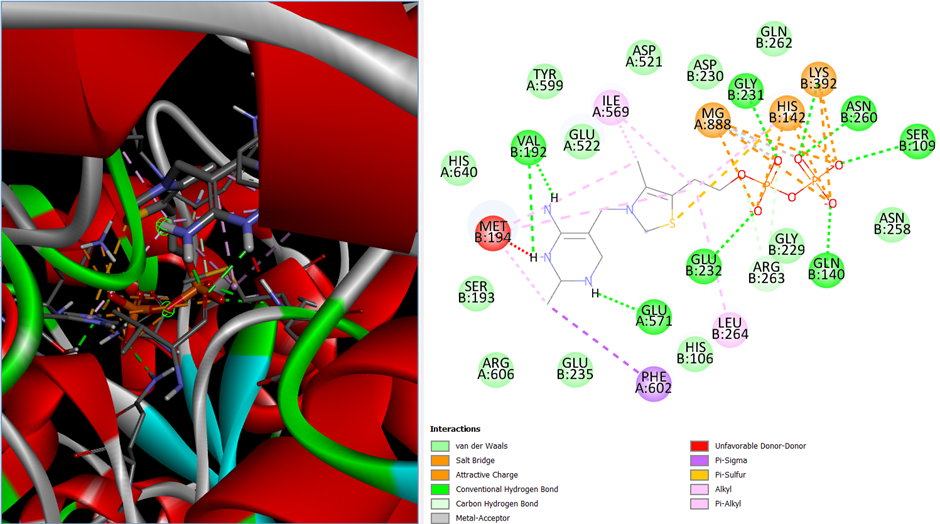


Figure 5: Molecular docking study of receptor, ligand (β-Chitosan) best docking pose and various β-Chitosan-Derived Zinc Nanoparticles interactions with amino acids contribute to cavity formation

A catalytic tunnel composed of various protiens are found in the active site of 1L8A (PDB:1L8A). 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 β-Chitosan-Derived Zinc Nanoparticle receptor are conserved across Gram-positive and Gram-negative bacteria, making 1L8A protein a promising therapeutic target for the development of innovative and broad-spectrum antimicrobial drugs as selective and nontoxic 1L8A inhibitors. To predict the in vitro efficiency of β-CT-ZnNPs, the ligand 1L8A model was used to perform a molecular docking study. Docking of β-CT-ZnNPs into a modelled receptor named 1L8A was done to investigate proper nanoparticle orientation within the receptor and obtain useful information for the active mechanism, including non-covalent interactions between the active site of the receptor and β-CT-ZnNPs, which could lead to the development of new drugs for further biological research(Anbarasu et al. 2024).

# Discussion

β-Chitosan-derived zinc nanoparticles (β-CT-ZnNPs) represent a cutting-edge advancement in the fight against antimicrobial resistance, particularly relevant in the context of oral health. The increasing prevalence of antibiotic-resistant pathogens necessitates innovative approaches, and β-CT-ZnNPs offer a promising alternative due to their potent antimicrobial properties and diverse biomedical applications. Dental caries, primarily caused by cariogenic bacteria like Streptococcus mutans and Lactobacillus species, is a significant public health issue(Wright 2018). These bacteria metabolize dietary sugars into acids, eroding tooth enamel and leading to cavities. Traditional strategies such as mechanical plaque removal, fluoride treatments, and conventional antimicrobial agents are becoming less effective due to the rise of antibiotic-resistant strains. This scenario underscores the need for new antimicrobial strategies, where β-CT-ZnNPs come into play. β-Chitosan, derived from chitin through deacetylation, is known for its biocompatibility, biodegradability, and non-toxic nature(Selwitz, Ismail, and Pitts 2007). Its superior solubility and bioactivity compared to regular chitosan make it an excellent candidate for biomedical applications. When combined with zinc, a trace element with inherent antimicrobial properties, the resulting nanoparticles exhibit enhanced efficacy. β-CT-ZnNPs disrupt microbial cell walls, chelate essential metal ions, induce oxidative stress, and inhibit biofilm formation. These mechanisms collectively broaden their spectrum of activity, making them effective against bacteria, fungi, and viruses. The extraction and synthesis of β-CT-ZnNPs involve either chemical or enzymatic methods. Chemical methods, although efficient, pose environmental concerns, while enzymatic methods offer an eco-friendlier alternative, potentially yielding chitosan with superior properties. The degree of deacetylation and molecular weight of chitosan significantly influence the properties and efficacy of the synthesized nanoparticles(Quock 2015).

In vitro studies have demonstrated the efficacy of β-CT-ZnNPs against oral pathogens. These nanoparticles have shown substantial antimicrobial activity against Streptococcus mutans and Lactobacillus species, key players in dental caries. By disrupting biofilms and microbial cell walls, β-CT-ZnNPs prevent the formation and progression of cavities, offering a significant advantage over traditional treatments. Beyond oral health, β-CT-ZnNPs hold potential in various biomedical fields. Their antimicrobial properties make them suitable for wound healing, where they can prevent infections and promote faster recovery. In drug delivery, their biocompatibility and ability to target specific sites enhance therapeutic outcomes. Furthermore, in tissue engineering, β-CT-ZnNPs can be used to create scaffolds that support cell growth and tissue regeneration while preventing microbial contamination. Despite these promising applications, challenges remain. The optimization of β-CT-ZnNP properties for specific applications, scale-up production, and ensuring consistent quality are critical areas for future research. Additionally, clinical trials are necessary to validate their safety and efficacy in real-world settings. In conclusion, β-Chitosan-derived zinc nanoparticles represent a versatile and potent antimicrobial strategy with significant potential to address the challenges posed by antibiotic-resistant pathogens. Their broad-spectrum activity, coupled with biocompatibility and diverse applications, positions them as a promising tool in modern medicine and dentistry. Further research and development could pave the way for their widespread clinical adoption, offering a sustainable and effective solution to current and emerging health threats(Bogale et al. 2021; Khalid et al. 2024).

# Conclusion

β-Chitosan-derived zinc nanoparticles (β-CT-ZnNPs) offer a promising solution to combat antibiotic-resistant pathogens, particularly in dental health. Their broad-spectrum antimicrobial activity, biocompatibility, and potential applications in wound healing, drug delivery, and tissue engineering make them versatile and effective. These nanoparticles disrupt microbial cell walls, chelate metal ions, induce oxidative stress, and inhibit biofilm formation. While promising, further research is needed to optimize their properties and address clinical implementation challenges. β-CT-ZnNPs represent a significant advancement in developing innovative, sustainable antimicrobial strategies for public health.

# References

1. Al‐Nasser, Lubna, and Ira B. Lamster. 2020. “Prevention and Management of Periodontal Diseases and Dental Caries in the Older Adults.” *Periodontology 2000* 84 (1): 69–83.
2. Ambika, Subramanian, Yesaiyan Manojkumar, Sankaralingam Arunachalam, Balakrishnan Gowdhami, Kishore Kumar Meenakshi Sundaram, Rajadurai Vijay Solomon, Ponnambalam Venuvanalingam, Mohammad Abdulkader Akbarsha, and Muthuraman Sundararaman. 2019. “Biomolecular Interaction, Anti-Cancer and Anti-Angiogenic Properties of Cobalt (III) Schiff Base Complexes.” *Scientific Reports* 9 (1): 2721.
3. Anbarasu, Murugan, Viswanathan Vinitha, Ponmudi Priya, Taniya Mary Martin, Lavanya Prathap, Meenakshi Sundaram Kishore Kumar, Mohammed Rafi Shaik, Ajay Guru, and Vajiravelu Sivamurugan. 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.
4. Balaji, A. P., Srinivasan Bhuvaneswari, Leon Stephan Raj, Giridharan Bupesh, Kishore Kumar Meenakshisundaram, and Konda Mani Saravanan. 2022. “A Review on the Potential Species of the Zingiberaceae Family with Anti-Viral Efficacy towards Enveloped Viruses.” *J Pure Appl Microbiol* 16 (2): 796–813.
5. Baranikumar, Dhanushree, Meenakshi Sundaram Kishore Kumar, Venkataramanan Natarajan, and Lavanya Prathap. 2023. “Activation of Nuclear Factor Kappa B (NF-KB) Signaling Pathway Through Exercise-Induced Simulated Dopamine Against Colon Cancer Cell Lines.” *Cureus* 15 (10).
6. Bogale, Birke, Fasikawit Engida, Charlotte Hanlon, Martin J. Prince, and Jennifer E. Gallagher. 2021. “Dental Caries Experience and Associated Factors in Adults: A Cross-Sectional Community Survey within Ethiopia.” *BMC Public Health* 21: 1–12.
7. Giridharan, Bupesh, Amutha Chinnaiah, Konda Mani Saravanan, Sudharsan Parthasarathy, Kishore Kumar Meenakshi Sundaram, Siva Vijayakumar Tharumasivam, Pranay Punj Pankaj, Archunan Govindaraju, Dayalan Haripriya, and Uttam Kumar Sahoo. 2024. “Characterization of Novel Antimicrobial Peptides from the Epidermis of Clarias Batrachus Catfish.” *International Journal of Peptide Research and Therapeutics* 30 (2): 11.
8. Girija, A. S. Smiline, and Pitchaipillai Sankar Ganesh. 2022. “Functional Biomes beyond the Bacteriome in the Oral Ecosystem.” *Japanese Dental Science Review* 58: 217–26.
9. Hyde, Susan, Veronique Dupuis, Boipelo P. Mariri, and Sophie Dartevelle. 2017. “Prevention of Tooth Loss and Dental Pain for Reducing the Global Burden of Oral Diseases.” *International Dental Journal* 67: 19–25.
10. Khalid, Jabir Padathpeedika, Taniya Mary Martin, Lavanya Prathap, Milind Abhimanyu Nisargandha, Nisha Boopathy, and Meenakshi Sundaram Kishore Kumar. 2024. “Exploring Tumor-Promoting Qualities of Cancer-Associated Fibroblasts and Innovative Drug Discovery Strategies With Emphasis on Thymoquinone.” *Cureus* 16 (2).
11. Kishore, S. O. G., A. J. Priya, L. Narayanan, S. R. Kumar, and G. Devi. 2020. “Controlling of Oral Pathogens Using Turmeric and Tulsi Herbal Formulation Mediated Copper Nanoparticles.” *Plant Cell Biotechnol Mol Biol*, 33–37.
12. Marunganathan, Vanitha, Meenakshi Sundaram Kishore Kumar, Zulhisyam Abdul Kari, Jayant Giri, Mohammed Rafi Shaik, Baji Shaik, and Ajay Guru. 2024. “Marine-Derived κ-Carrageenan-Coated Zinc Oxide Nanoparticles for Targeted Drug Delivery and Apoptosis Induction in Oral Cancer.” *Molecular Biology Reports* 51 (1): 89.
13. Nasim, Iffat, Zohra Jabin, S. Rajesh Kumar, and V. Vishnupriya. 2022. “Green Synthesis of Calcium Hydroxide-Coated Silver Nanoparticles Using Andrographis Paniculata and Ocimum Sanctum Linn. Leaf Extracts: An Antimicrobial and Cytotoxic Activity.” *Journal of Conservative Dentistry and Endodontics* 25 (4): 369–74.
14. Nasim, Iffat, S. Rajeshkumar, and V. Vishnupriya. 2021. “Green Synthesis of Reduced Graphene Oxide Nanoparticles, Its Characterization and Antimicrobial Properties against Common Oral Pathogens.” *Int J Dentistry Oral Sci* 8 (2): 1670–75.
15. Ozdemir, Dogan. 2013. “Dental Caries: The Most Common Disease Worldwide and Preventive Strategies.” *International Journal of Biology* 5 (4): 55.
16. Quock, Ryan L. 2015. “Dental Caries: A Current Understanding and Implications.” *Journal of Nature and Science* 1 (1): 27.
17. Rajeshkumar, S., T. Lakshmi, and M. Tharani. 2021. “Green Synthesis of Copper Nanoparticles Synthesized Using Black Tea and Its Antibacterial Activity against Oral Pathogens.” *Int J Dent Oral Sci* 8 (9): 4156–59.
18. Ravikumar, O. V., Vanitha Marunganathan, Meenakshi Sundaram Kishore Kumar, Magesh Mohan, Mohammed Rafi Shaik, Baji Shaik, Ajay Guru, and Khairiyah Mat. 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): 352.
19. Rieshy, V., Jothi Priya, Lakshminarayanan Arivarasu, S. Rajesh Kumar, and Gayatri Devi. 2020. “Enhanced Antimicrobial Activity of Herbal Formulation Mediated Copper Nanoparticles against Clinical Pathogens.” *Plant Cell Biotechnology and Molecular Biology* 21 (53–54): 52–56.
20. Roshan, A., A. Jothipriya, Lakshminarayanan Arivarasu, Rajesh Kumar, and Gayatri Devi. 2020. “Antifungal Activity of Tulsi and Turmeric Assisted Copper Nano Particle.” *Plant Cell Biotechnology and Molecular Biology* 21 (27–28): 9–13.
21. Selwitz, Robert H., Amid I. Ismail, and Nigel B. Pitts. 2007. “Dental Caries.” *The Lancet* 369 (9555): 51–59.
22. Senthil, Renganthan, Kishore Kumar Meenakshi Sundaram, Giridharan Bupesh, Singaravelu Usha, and Konda Mani Saravanan. 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.
23. Sneka, Sneka, and Preetha Santhakumar. 2021. “Antibacterial Activity of Selenium Nanoparticles Extracted from Capparis Decidua against Escherichia Coli and Lactobacillus Species.” *Research Journal of Pharmacy and Technology* 14 (8): 4452–54.
24. Tayyeb, Jehad Zuhair, Madhu Priya, Ajay Guru, Meenakshi Sundaram Kishore Kumar, Jayant Giri, Akash Garg, Rutvi Agrawal, Khairiyah Binti Mat, and Jesu Arockiaraj. 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.
25. Tinanoff, Norman, Ramon J. Baez, Carolina Diaz Guillory, Kevin J. Donly, Carlos Alberto Feldens, Colman McGrath, Prathip Phantumvanit, Nigel B. Pitts, W. Kim Seow, and Nikolai Sharkov. 2019. “Early Childhood Caries Epidemiology, Aetiology, Risk Assessment, Societal Burden, Management, Education, and Policy: Global Perspective.” *International Journal of Paediatric Dentistry* 29 (3): 238–48.
26. Umapathy, Suganiya, Ieshita Pan, Praveen Kumar Issac, Meenakshi Sundaram Kishore Kumar, Jayant Giri, Ajay Guru, and Jesu Arockiaraj. 2024. “Selenium Nanoparticles as Neuroprotective Agents: Insights into Molecular Mechanisms for Parkinson’s Disease Treatment.” *Molecular Neurobiology*, 1–28.
27. Velumani, Kadhirmathiyan, Abirami Arasu, Praveen Kumar Issac, Meenakshi Sundaram Kishore Kumar, Ajay Guru, and Jesu Arockiaraj. 2023. “Advancements of Fish-Derived Peptides for Mucormycosis: A Novel Strategy to Treat Diabetic Compilation.” *Molecular Biology Reports* 50 (12): 10485–507.
28. Wolf, Thomas Gerhard, Maria Grazia Cagetti, Julian-Marcus Fisher, Gerhard Konrad Seeberger, and Guglielmo Campus. 2021. “Non-Communicable Diseases and Oral Health: An Overview.” *Frontiers in Oral Health* 2: 725460.
29. Wright, John Timothy. 2018. “The Burden and Management of Dental Caries in Older Children.” *Pediatric Clinics* 65 (5): 955–63.
30. [Adel, S. M., El-Harouni, N., & Vaid, N. R. (2023). White Spot lesions: State of the art biomaterials and workflows used in prevention, progression and treatment. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/FsPU8w/GroLx)[10.1053/j.sodo.2023.01.002](http://dx.doi.org/10.1053/j.sodo.2023.01.002)
31. [Aparna, J., Maiti, S., & Jessy, P. (2021). Polyether ether ketone - As an alternative biomaterial for Metal Richmond crown-3-dimensional finite element analysis. *Journal of Conservative Dentistry : JCD*, *24*(6), 553–557.](http://paperpile.com/b/FsPU8w/zMYVY)
32. Chehelgerdi M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P. & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Molecular cancer, 22(1), 169.
33. [Chokkattu, J. J., Mary, D. J., Shanmugam, R., & Neeharika, S. (2022). Embryonic Toxicology Evaluation of Ginger- and Clove-mediated Titanium Oxide Nanoparticles-based Dental Varnish with Zebrafish. *The Journal of Contemporary Dental Practice*, *23*(11), 1157–1162.](http://paperpile.com/b/FsPU8w/kwqV2)
34. [Chokkattu, J. J., Neeharika, S., & Rameshkrishnan, M. (2023). Applications of Nanomaterials in Dentistry: A Review. *Journal of International Society of Preventive & Community Dentistry*, *13*(1), 32–41.](http://paperpile.com/b/FsPU8w/moSw4)
35. [Ganapathy, D., & Professor and Head of Department of Prosthodontics, (2021). Awareness of hazards caused by long-term usage of polyethylene terephthalate (PET) bottles. *International Journal of Dentistry and Oral Science*, 2976–2980.](http://paperpile.com/b/FsPU8w/4v6t9)
36. [Jain, R. K., & Verma, P. (2022). Visual assessment of extent of White Spot lesions in subjects treated with fixed orthodontic appliances: A retrospective study. *World Journal of Dentistry*, *13*(3), 245–249.](http://paperpile.com/b/FsPU8w/sYLvn)
37. [Laghari, I. A., Pandey, A. K., Samykano, M., Aljafari, B., Kadirgama, K., Sharma, K., & Tyagi, V. V. (2023). Thermal energy harvesting of highly conductive graphene-enhanced paraffin phase change material. *Journal of Thermal Analysis and Calorimetry*, *148*(18), 9391–9402.](http://paperpile.com/b/FsPU8w/txVsc)
38. [Marya, A., Venugopal, A., Karobari, M. I., & Rokaya, D. (2022). White Spot lesions: A serious but often ignored complication of orthodontic treatment. *The Open Dentistry Journal*, *16*(1). https://doi.org/](http://paperpile.com/b/FsPU8w/5cn0i)[10.2174/18742106-v16-e2202230](http://dx.doi.org/10.2174/18742106-v16-e2202230)
39. [Merchant, A., Ganapathy, D. M., & Maiti, S. (2022). Effectiveness of local and topical anesthesia during gingival retraction. *Brazilian Dental Science*, *25*(1), e2591.](http://paperpile.com/b/FsPU8w/e82m6)
40. [Merchant, A., Pandurangan, K. K., Shenoy, A. D., Nallaswamy, D., & Singh, P. N. (2025). Comparison of marginal fit between milled and three-dimensional printed polymethylmethacrylate prostheses for single crowns, anterior bridges, and pier abutment bridges: An in vitro study. *Journal of Indian Prosthodontic Society*, *25*(1), 67–73.](http://paperpile.com/b/FsPU8w/aclQ)
41. [Muthuswamy Pandian, S., Subramanian, A. K., Ravikumar, P. A., & Adel, S. M. (2022). Biomaterial testing in contemporary orthodontics: Scope, protocol and testing apparatus. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/FsPU8w/8spPH)[10.1053/j.sodo.2022.12.011](http://dx.doi.org/10.1053/j.sodo.2022.12.011)
42. [Pandiyan, I., Sri, S. D., Indiran, M. A., Rathinavelu, P. K., Prabakar, J., & Rajeshkumar, S. (2022). Antioxidant, anti-inflammatory activity of -mediated selenium nanoparticles: An study. *Journal of Conservative Dentistry : JCD*, *25*(3), 241–245.](http://paperpile.com/b/FsPU8w/B6yQ4)
43. [Poornima, P., Krithikadatta, J., Ponraj, R. R., Velmurugan, N., & Kishen, A. (2021). Biofilm formation following chitosan-based varnish or chlorhexidine-fluoride varnish application in patients undergoing fixed orthodontic treatment: a double blinded randomised controlled trial. *BMC Oral Health*, *21*(1), 465.](http://paperpile.com/b/FsPU8w/lMxRD)
44. [Ramakrishnan, M., Shanmugam, R., Neeharika, S., Selvaraj, S., Chokkattu, J. J., & Thangavelu, L. (2023). Anti-inflammatory potential of a mouthwash formulated using clove and ginger mediated by zinc oxide nanoparticles: An in vitro study. *World Journal of Dentistry*, *14*(5), 394–401.](http://paperpile.com/b/FsPU8w/FmZR4)
45. [Ramamurthy, S., Thiagarajan, K., Varghese, S., Kumar, R., Karthick, B. P., Varadarajan, S., & Balaji, T. M. (2022). Assessing the in vitro antioxidant and anti-inflammatory activity of Moringa oleifera crude extract. *The Journal of Contemporary Dental Practice*, *23*(4), 437–442.](http://paperpile.com/b/FsPU8w/PwJbs)
46. Saadh, M. J., Rasulova, I., Almoyad, M. A. A., Kiasari, B. A., Ali, R. T., Rasheed, T. & Ciongradi, C. I. (2024). Recent progress and the emerging role of lncRNAs in cancer drug resistance; focusing on signaling pathways. Pathology-Research and Practice, 253, 154999.
47. [Shenoy, A., Maiti, S., Nallaswamy, D., & Srinivasan, M. (2025). A double-blind randomized crossover trial comparing the esthetic outcomes of CAD-CAM provisional restorations fabricated using CBCT and IOS acquisition methods. *Journal of Dentistry*, *153*, 105545.](http://paperpile.com/b/FsPU8w/0FoH)
48. [Singh, P., Shenoy, A., Nallaswamy, D., & Maiti, S. (2024). Comparative Evaluation of Microbial Adhesion on Provisional Crowns Fabricated With Milled Polymethyl Methacrylate (PMMA) and Conventional Acrylic Resin: A Prospective Clinical Trial. *Cureus*, *16*(7), e64469.](http://paperpile.com/b/FsPU8w/aZJQ)
49. [Solanki, L. A., Dinesh, S. P. S., Jain, R. K., & Balasubramaniam, A. (2023). Effects of titanium oxide coating on the antimicrobial properties, surface characteristics, and cytotoxicity of orthodontic brackets - A systematic review and meta analysis of in-vitro studies. *Journal of Oral Biology and Craniofacial Research*, *13*(5), 553–562.](http://paperpile.com/b/FsPU8w/Gsr8h)
50. [Sreevarun, M., Ajay, R., Suganya, G., Rakshagan, V., Bhanuchander, V., & Suma, K. (2023). Formulation, Configuration, and Physical Properties of Dental Composite Resin Containing a Novel 2π + 2π Photodimerized Crosslinker - Cinnamyl Methacrylate: An Research. *The Journal of Contemporary Dental Practice*, *24*(6), 364–371.](http://paperpile.com/b/FsPU8w/m9pVS)
51. [Subramanian, A., & Harikrishnan, S. (2023). 3D printing in orthodontics: A narrative review. *Journal of International Oral Health: JIOH*, *15*(1), 15.](http://paperpile.com/b/FsPU8w/pKVcg)
52. [Verma, P., & Muthuswamy Pandian, S. (2021). Bionic effects of nano hydroxyapatite dentifrice on demineralised surface of enamel post orthodontic debonding: in-vivo split mouth study. *Progress in Orthodontics*, *22*(1), 39.](http://paperpile.com/b/FsPU8w/B2zqv)