

Abstract

Introduction

Antimicrobial resistance (AMR) has been increasing at a horrifying rate, posing a significant public health challenge and a threat to global health and development. *Pseudomonas aeruginosa* (*P. aeruginosa*) a gram-negative strain and *Staphylococcus aureus* (*S. aureus*) gram positive bacterium can infect both localized and systemic regions in the human body thereby affecting population globally. Taken into serious consideration by World Health Organization (WHO) which is concerned about the emergence of antibiotic-resistant strains, has insisted that it imperative to urgently develop alternative therapeutic strategies, as these are also biofilm-forming bacteria identified from chronic wounds. According to the conclusions of the AMR review, a research study initiated by the UK Government, it is anticipated that by 2050, antimicrobial resistance will result in a significant rise in mortality, with an annual total of more than 10 million deaths (approx.). Biofilms having bacteria encased are shielded by the self-produced extracellular polymeric materials thereby rendering them resistive to sanitizers, ultraviolet rays, and standardized dose of antibiotics, consequently needing greater dosages of antibiotics for microbial eradication. Hence, antibiotic free bioengineered approaches against this disease, including those that can efficiently target biofilms, have become more important.

Chitosan, a natural polysaccharide derived from chitin, has garnered considerable attention as an antimicrobial agent due to its biocompatibility, biodegradability, and intrinsic antimicrobial activity, primarily attributed to its polycationic nature which enables interactions with negatively charged microbial cell membranes, leading to cell disruption and death. Its efficacy is further enhanced through chemical modifications such as N,N,N-trimethyl chitosan (TMC) and carboxymethyl chitosan (CMC), which improve solubility in physiological conditions and augment antimicrobial potency. TMC, with its permanent

positive charge across a broad pH range, exhibits superior membrane permeability and bioadhesiveness, facilitating enhanced interaction with microbial surfaces. CMC, on the other hand, combines chitosan's antimicrobial properties with improved water solubility and metal-chelating capacity, expanding its spectrum of activity. These modifications not only extend the functional range of chitosan in diverse biomedical and pharmaceutical applications but also address the limitations posed by its native form, such as poor solubility at neutral pH, thereby making chitosan and its derivatives promising candidates in the development of novel antimicrobial formulations.

Gold-silver bimetallic nanoparticles capped with chitosan polymer represent a synergistic and multifunctional platform for antimicrobial applications, combining the unique properties of both noble metals with the bioactive polymer matrix. Silver is well-known for its broad-spectrum antimicrobial activity, primarily through the release of Ag^+ ions that disrupt microbial membranes, generate reactive oxygen species (ROS), and interfere with DNA replication. Gold, though less inherently antimicrobial, enhances nanoparticle stability, bioavailability, and tunability of physicochemical properties such as size, shape, and surface charge. The bimetallic configuration often exhibits enhanced antimicrobial efficacy compared to their monometallic counterparts due to synergistic interactions at the nano-bio interface. Capping these nanoparticles with chitosan not only stabilizes the nanostructure but also introduces additional antimicrobial functionality through its polycationic nature, promoting strong electrostatic interactions with negatively charged microbial membranes. Furthermore, chitosan enhances biocompatibility, prevents aggregation, and facilitates targeted delivery, making gold-silver bimetallic nanoparticles capped with chitosan an advanced and efficient antimicrobial system with potential applications in wound healing, coatings, and infection control.

Further, nanoparticles-loaded nanofibers have emerged as a highly effective strategy in the development of advanced antimicrobial materials, particularly in the context of gold-silver bimetallic nanoparticles capped with chitosan. The integration of such nanoparticles into electrospun nanofibrous matrices offers a dual-functionality approach—leveraging the potent, broad-spectrum antimicrobial activity of the embedded nanoparticles alongside the structural and functional benefits of nanofibers. Nanofibers possess a high surface-area-to-volume ratio, porosity, and morphological similarity to the extracellular matrix, which facilitate sustained and localized release of nanoparticles, enhance contact with microbial cells, and improve wound healing potential. Furthermore, the incorporation of nanoparticles within chitosan-based or compatible polymeric nanofibers provides additional antimicrobial synergy and mechanical stability, while mitigating potential cytotoxicity associated with free nanoparticles. This system enables the controlled delivery of antimicrobial agents, prolongs their activity, and minimizes resistance development, making nanoparticles-loaded nanofibers a promising platform for applications in antimicrobial wound dressings, tissue engineering scaffolds, and protective textiles.

Moreover, in context of wound healing, nanofiber scaffolds loaded with chitosan-capped gold-silver bimetallic nanoparticles present a multifaceted therapeutic strategy that addresses both infection control and tissue regeneration. The antimicrobial efficacy of silver, potentiated by the stabilizing and biocompatible properties of gold, plays a critical role in preventing microbial colonization and biofilm formation at the wound site—factors that are often major impediments to effective healing. Chitosan not only enhances the antimicrobial effect through its polycationic interaction with microbial membranes but also contributes to hemostasis, cellular adhesion, and proliferation, thereby actively supporting the wound healing cascade. The nanofibrous architecture mimics the extracellular matrix, providing a conducive environment for fibroblast infiltration, angiogenesis, and collagen

deposition. Moreover, the sustained and localized release of nanoparticles from the nanofibers ensures prolonged antimicrobial protection while minimizing systemic toxicity. Collectively, this composite system accelerates wound closure, reduces inflammation, and promotes tissue remodeling, positioning it as a promising candidate for next-generation wound dressings in clinical settings.

Objectives

The objectives of the present research work include;

- To develop bimetallic nanoparticles of gold and silver capped with trimethyl chitosan and carboxy methyl chitosan for the potential treatment against antimicrobial resistant bacteria.
- To characterize physicochemical properties, antibacterial efficacy, *in vitro* characterization, and *in vivo* evaluation of bimetallic nanoparticles
- To fabricate bimetallic nanoparticles loaded nanofibers for wound dressing to microbial infected wounds.
- To characterize physicochemical properties, *in vitro* characterization, and *in vivo* evaluation of bimetallic nanoparticles loaded nanofibers.
- *In-vivo* wound healing study and Live imaging using IVIS and ultrasound/photoacoustic imaging of microbial infected wounds.

Methodology

We have developed chitosan capped gold-silver nanoparticles first time for the antimicrobial wound dressing and *in vivo* wound healing activity. The synthesis of gold-silver core-shell nanoparticles was carried out using a seeded growth synthesis approach, where Au nanoparticles will be utilised as the seed material. The chitosan capped gold-silver nanoparticles were encapsulated by electrospinning for the first time within a polyvinyl alcohol and chitosan-based nanofiber matrix for potential *in vivo* wound healing

applications and treating microbial resistant wounds with low or no adverse effects. Initially, developed chitosan capped gold-silver nanoparticles were characterized for particle size, zeta potential, poly dispersity index, surface morphology, and elemental analysis. Notably, chitosan capped gold-silver nanoparticles antimicrobial activity against MDR strains of *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans* were evaluated through *in vitro* experiments in comparison to the marketed broad-spectrum antibiotic. The chitosan capped gold-silver nanoparticles were evenly dispersed into nanofiber matrix by using the process of electrospinning. The nanofibers were then applied on MDR microbes-infected mice wound sites. The wound healing potency of nanofibers was assessed by the wound closures, reepithelization, wound marker protein expression levels and ultrasound/photoacoustic imaging. Likewise, the real time wound visualization, angiogenesis, vascularity, and wound oxygen saturation were also evaluated.

Summary and Conclusion

Conclusively, the present research embodies the development and characterization of bimetallic nanoparticles and nanofibers antimicrobial wound dressing and *in vivo* wound healing activity and imaging.

The research emphasises the versatility of chitosan with bimetallic nanoparticles and how it may be used as an agent for accelerated MDR microbial infected wound healing approach with no adverse effects over available marketed formulations. The synthesized wound dressing with chitosan capped gold-silver nanoparticles was found to have sustained release of therapeutic agents on wound sites with accelerated healing within 12 days. Furthermore, the real time wound visualization, angiogenesis, vascularity, and wound oxygen saturation by ultrasound/photoacoustic imaging system supports the improved oxygen saturation, increased vascularity and enhanced degree of wound closure. However, this study is limited

to the isolated MDR microbial strains from the local hospitals, and a single therapeutic delivery system was used, although in future the other possible delivery systems can be explored with the isolated MDR microbial strains from different sites globally. Overall, the chitosan capped gold-silver nanoparticles wound dressing can lay the foundation for the development of future antibacterial and antifungal agents with significant wound-healing potential.