3. Research envisaged and plan of work

3.1. Hypothesis

The development of bimetallic nanoparticles with chitosan derivatives encapsulated within a polyvinyl alcohol-chitosan nanofiber matrix will demonstrate enhanced antimicrobial efficacy against MDR pathogens through synergistic nanoparticle interactions and anti-efflux pump activity, while simultaneously accelerating wound healing via modulation of key signaling pathways and tissue regeneration markers. This combinatorial approach is hypothesized to overcome antimicrobial resistance limitations through three mechanisms:

- **Dual nanoparticle action:** The bimetallic Au-Ag system will exhibit broader-spectrum antimicrobial activity against MDR *E. coli., P. aeruginosa, S. aureus*, and *C. albicans* compared to conventional antibiotics through membrane disruption and reactive oxygen species generation.
- **Resistance mitigation:** TMC-functionalized nanoparticles will inhibit microbial efflux pumps (confirmed through acrA and mexB gene downregulation), reducing antibiotic clearance from bacterial cells.
- **Healing potentiation:** The nanofiber matrix will sustain nanoparticle release at wound sites, activating Wnt/β-catenin and TGF-β signaling pathways to promote re-epithelization (verified through E-cadherin/VEGF upregulation) while maintaining oxygen saturation >92% during healing (quantified via photoacoustic imaging).

This hypothesis predicts a ≥40% improvement in wound closure rates versus standard antimicrobial dressings in murine models, with complete microbial clearance achieved at nanoparticle concentrations ≤50 μg/mL (below cytotoxic thresholds). The structural integration within nanofibers is expected to reduce systemic nanoparticle exposure by 70-80% compared to topical solutions, addressing toxicity concerns of metallic nanoparticles.

3.2. Aims and objectives

The objective of the present work was to develop bimetallic nanoparticles of gold and silver capped with chitosan derivatives for antimicrobial efficacy and wound healing appliactions Present project has been divided under the following major specific aims:

- To develop bimetallic nanoparticles of gold and silver capped with chitosan derivatives for antimicrobial efficacy and wound healing appliactions.
- To characterize antimicrobial efficacy, physicochemical properties and *in vitro* properties of bimetallic nanoparticles
- To fabricate bimetallic nanoparticles loaded nanofibers against microbial infected wounds.
- To characterize physicochemical properties and *in vitro* properties of bimetallic nanoparticles loaded nanofibers.
- *In vivo* wound healing activity and histopathology study of microbial infected wounds.
- Live imaging using IVIS and ultrasound/photoacoustic imaging of microbial infected wounds.

3.3. Plan of work

I. Preparation and optimization* of gold and silver nanoparticles capped with trimethyl chitosan (Au-Ag-TMC-NPs) loaded nanofibers

II. Preparation and optimization* of gold and silver nanoparticles capped with carboxymethyl chitosan and antimicrobial peptide (G-S-CMC-Pep-NPs) loaded nanofibers and ultra-small magnesium nanoclusters (USM).

**Optimization of the process variables was based on particle size and zeta potential*

1. *In vitro* characterization

I. Particle size, PDI and surface charge

II. Shape and surface morphology

III. Elemental analysis

IV. *In vitro* drug release profile

V. Hemolysis study

2. Antimicrobial activity

I. MIC, MBC and MFC

II. Bacterial susceptibility

III. Bacterial viability test

IV. Antibiofilm activity

V. TEM microscopy study of test microbes

VI. Effect on the mRNA expression of biofilm-inducing efflux pump

3. Wound healing study

I. Wound closure

II. Histopathology

III. Western blotting

IV. *In vivo* imaging

V. Photoacoustic/Ultrasound imaging