2. Literature Review

2.1. Antimicrobial resistance in wound infections: A global crisis

2.1.1. Epidemiology of AMR in wound infections

2.1.1.1. Prevalence of multidrug-resistant pathogens

Wound infections, particularly chronic wounds, are increasingly dominated by MDR pathogens. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most prevalent, with resistance rates to advanced cephalosporins and carbapenems reaching 65–68% in clinical isolates [66, 67]. The World Health Organization (WHO) reports median resistance rates of 42% for third-generation cephalosporin-resistant *E. coli.* and 35% for methicillin-resistant *S. aureus* (MRSA) globally. In low-resource settings, such as Pakistan and Ethiopia, MRSA accounts for 20% of wound infections, while extended-spectrum β-lactamase (ESBL)-producing *E. coli.* and *Klebsiella pneumoniae* contribute to 15–78.9% of cases [68].

2.1.1.2. Risk factors driving AMR in wounds

Key risk factors include prolonged hospitalization, surgical interventions, and comorbidities like hyperglycemia and hypoalbuminemia [69]. Overuse of broad-spectrum antibiotics exacerbates resistance; for example, 70% of COVID-19 patients received unnecessary antibiotics, accelerating MDR development. In sub-Saharan Africa, limited diagnostic capacity and poor antimicrobial stewardship further amplify AMR, with carbapenem-resistant Acinetobacter baumannii detected in 8–100% of isolates [67].

2.1.2. Mechanisms of AMR in wound pathogens

2.1.2.1. Biofilm formation and persistence

Biofilms, present in >60% of chronic wounds, are a critical AMR driver. These microbial communities encase pathogens in a protective extracellular matrix, reducing antibiotic penetration and enhancing horizontal gene transfer. *P. aeruginosa* and *S. aureus* biofilms

exhibit 10–1,000-fold higher resistance to antibiotics like vancomycin compared to planktonic cells **(table 1.1.)** [67, 69].

Table 1.1. Cross-cutting challenges and strategies of antimicrobial resistance

Pathogen	**Key resistance drivers**	**Therapeutic challenges**	**Emerging strategies**
E. coli.	ESBLs, carbapenemases, PMQR	Limited carbapenem alternatives	Phage therapy, β-lactamase inhibitors
P. aeruginosa	Intrinsic resistance, biofilms	PDR strains, biofilm persistence	Monoclonal antibodies, disrupting SOS response
S. aureus	mecA, daptomycin resistance	MRSA outbreaks, phage resistance	Anti-virulence agents, bacteriophage therapy
C. albicans	ERG11 mutations, biofilms	MDR C. auris, biofilm sequestration	Combination therapies, antifungal stewardship

2.1.2.2. Genetic and enzymatic resistance

- **Enzyme production:** ESBLs and carbapenemases (e.g., NDM-1, KPC) hydrolyze β-lactams, rendering cephalosporins and carbapenems ineffective.
- **Efflux pumps:** Upregulated in *P. aeruginosa*, these systems expel fluoroquinolones and tetracyclines.

- **Target modification:** MRSA's mecA gene alters penicillin-binding proteins, conferring resistance to β-lactams.

2.1.2.3. Synergistic resistance in polymicrobial infections

Chronic wounds often host polymicrobial consortia (e.g., *S. aureus*, *E. coli.*, anaerobes), where interspecies interactions amplify resistance traits [70, 71]. For instance, Enterococcus faecalis enhances *S. aureus* biofilm formation and vancomycin tolerance.

2.1.3. Clinical and socioeconomic impact

2.1.3.1. Mortality and morbidity

AMR complicates wound management, increasing mortality odds by 2.8-fold. In Africa, AMR-attributable mortality in surgical site infections (SSIs) exceeds 40% for carbapenem-resistant A. baumannii. Diabetic foot ulcers with MDR infections have amputation rates 3× higher than non-MDR cases [72].

2.1.3.2. Economic burden

AMR prolongs hospital stays by 30–70%, escalating costs. In Thailand and Turkey, treating carbapenem-resistant infections costs >$10,000–$35,000 per patient, compared to $5,469 for susceptible infections. By 2050, AMR-related healthcare expenses could surpass $1 trillion annually globally [73].

2.1.3.3. Disproportionate impact on developing regions

Low- and middle-income countries (LMICs) bear 90% of AMR-associated deaths due to limited access to second-line antibiotics and diagnostics [74]. In Ethiopia, 62.2% of wound isolates are biofilm-forming MDR strains, complicating treatment in resource-constrained settings[68].

2.1.4. Strategies to mitigate AMR in wound care

2.1.4.1. Diagnostic and surveillance innovations

- **Rapid molecular diagnostics:** PCR-based tools detect resistance genes (e.g., mecA, blaNDM) within hours, enabling targeted therapy.
- **Global surveillance networks:** WHO's GLASS program tracks resistance patterns, informing regional antibiotic guidelines.

2.1.4.2. Antimicrobial stewardship and alternatives

- **Topical antimicrobials:** Silver- and honey-based dressings reduce biofilm biomass without systemic toxicity.
- **Phage therapy:** Bacteriophages targeting *P. aeruginosa* biofilms show 80% efficacy in preclinical models.
- **Nanotechnology:** Nanoemulsions and nanoparticle-loaded hydrogels enhance drug penetration into biofilms.

2.2. Bimetallic nanoparticles: synergistic antimicrobial strategies

2.2.1. Rationale for bimetallic nanoparticles in antimicrobial therapy

The escalating threat of AMR necessitates innovative strategies to combat MDR pathogens. Bimetallic nanoparticles, composed of two distinct metals, have emerged as superior alternatives to monometallic nanoparticles due to their synergistic antimicrobial effects, reduced cytotoxicity, and multifunctional mechanisms of action [75]. For instance, Ag–Fe bimetallic nanoparticles synthesized via green chemistry exhibit a 2–10× enhancement in bactericidal activity against Gram-positive/-negative bacteria and fungi compared to their monometallic counterparts [76]. Similarly, Au–Ag bimetallic nanoparticles demonstrate potent antibacterial effects with minimal toxicity to human cells, making them viable for clinical applications [77]. The synergistic interaction between metals in bimetallic

nanoparticles amplifies oxidative stress, membrane disruption, and biofilm penetration, addressing key limitations of conventional antibiotics [16].

These nanomaterials combine the distinct physicochemical and biological properties of both metals, resulting in enhanced antimicrobial efficacy, tunable toxicity, and potential for use in biomedical applications, including wound care and infection control.

2.2.2. Synthesis and characterization of Au-Ag bimetallic nanoparticles

The synthesis of Au-Ag-NPs can be achieved through various methods, including green chemistry approaches using plant extracts, laser ablation, and bacteriogenic routes. These methods allow for precise control over particle size, composition, and morphology, which are critical factors influencing antimicrobial activity and cytotoxicity. For instance, atomic-level alloying of Au and Ag can reduce the release of Ag^+ ions, thereby lowering cytotoxicity to mammalian cells while maintaining strong antimicrobial effects [16]. **Table 2.1.** shows the chemical and physical properties of gold and silver.

Characterization techniques such as particle size, zeta potential, high resolution transmission electron microscopy (HR-TEM), high resolution scanning electron microscopy (SEM), ultraviolet-visible spectroscopy, and Fourier-transform infrared spectroscopy are commonly employed to confirm the formation, size distribution, and surface chemistry of these bimetallic nanoparticles.

Table 2.1. Chemical and physical properties of gold and silver

Property	**Gold (Au)**	**Silver (Ag)**
Symbol	Au	Ag
Atomic Number	79	47
Atomic Mass	196.97 u	107.87 u
Appearance	Bright, slightly reddish-yellow, metallic luster	White, lustrous, metallic sheen
Density	19.3 g/cm³	10.5 g/cm³
Melting Point	1064.18°C	961.8°C
Boiling Point	2856°C	2212°C
Malleability/Ductility	Most malleable and ductile metal; can be beaten into thin sheets or wires	Very malleable and ductile; can be drawn into thin wires
Electrical Conductivity	Excellent conductor	Highest of all metals
Thermal Conductivity	Excellent conductor	Highest of all metals
Color	Yellow (slightly reddish)	White/silver
Magnetic Properties	Non-magnetic	Non-magnetic
Crystal Structure	Face-centered cubic	Face-centered cubic

Chemical Reactivity	Very low; inert; resists most acids, dissolves in aqua regia and cyanide	Low; does not react with air, tarnishes with sulfur, dissolves in nitric acid
Oxidation States	https://pubchem.ncbi.nlm.nih.gov/element/Gold	https://www.vedantu.com/chemistry/silver
Corrosion Resistance	Extremely high; does not tarnish or corrode easily	High, but tarnishes in presence of sulfur compounds
Isotopes	One stable isotope: Au-197	https://shop.nanografi.com/blog/silver-elements-specifications-and-applications/
Other Notable Properties	Non-toxic, highly reflective, does not react with oxygen or water	Highest reflectivity, antibacterial, reacts with sulfur to form silver sulfide

2.2.3. Mechanisms of synergistic antimicrobial action

2.2.3.1. Enhanced bactericidal activity

Gold-silver bimetallic nanoparticles exhibit a synergistic antimicrobial effect that surpasses the efficacy of their monometallic counterparts. This synergy arises from the combined mechanisms of action of both metals:

- **Membrane disruption**: Ag and Au ions interact with bacterial cell membranes, increasing permeability and leading to cell lysis [78, 79].
- **Reactive oxygen species generation:** The bimetallic nanoparticles catalyze the production of ROS, which damage cellular proteins, lipids, and nucleic acids, ultimately leading to microbial death [78, 80].

- **Protein and DNA interaction:** Released ions can bind to thiol groups in proteins and to DNA, disrupting vital cellular functions [78].

2.2.3.2. Biofilm inhibition

A significant challenge in treating wound infections is the presence of biofilms, which protect bacteria from antibiotics and the immune system. Au-Ag-NPs have demonstrated the ability to inhibit biofilm formation and reduce the metabolic activity of preformed biofilms, even at relatively low concentrations [79, 80]. This antibiofilm activity is crucial for effective wound healing and infection prevention.

2.2.4. Comparative efficacy and cytotoxicity

Studies have shown that Au-Ag-NPs possess lower minimum inhibitory concentrations (MICs) against MDR bacteria, such as *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*, compared to monometallic nanoparticles [81]. For example, biopolymer-capped Au-Ag-NPs exhibited dose-dependent inhibition of these pathogens at concentrations as low as 5 μg/mL. Furthermore, the alloying of Au with Ag has been shown to reduce the cytotoxicity associated with Ag-NPs, making these bimetallic nanoparticles safer for *in vivo* applications [82].

2.3. Chitosan and chitosan derivatives for nanoparticles

Chitosan, a linear polysaccharide derived from chitin, has gained prominence in nanotechnology due to its biocompatibility, biodegradability, and intrinsic antimicrobial activity [83, 84]. However, its limited solubility at neutral pH and variable mechanical properties restrict its direct application. To overcome these challenges, chemical modifications such as quaternization TMC and carboxylation CMC have been developed. These derivatives enhance solubility, stability, and bioactivity, making them suitable for nanoparticle synthesis. For instance, TMC's permanent positive charge improves muco-adhesion and interaction with microbial membranes, while CMC's carboxyl groups

enable pH-responsive drug release and environmental adaptability [85].

2.3.1. Synthesis methods for chitosan-based nanoparticles

2.3.1.1. Ionic gelation and crosslinking

The ionotropic gelation method, using tripolyphosphate (TPP) as a crosslinker, is widely employed for chitosan nanoparticle (ChNP) synthesis. Optimizing chitosan-to-TPP ratios (1:1 to 6:1) controls particle size (200–500 nm) and zeta potential (+10 to +30 mV), as demonstrated by dynamic light scattering (DLS) and transmission electron microscopy (TEM) analyses. Higher chitosan concentrations yield larger nanoparticles, while increased TPP enhances stability but risks aggregation [83-85].

2.3.1.2. Green synthesis

Eco-friendly approaches using plant extracts (e.g., Eucalyptus citriodora) replace toxic reducing agents, producing nanoparticles with ultrafine sizes (100–300 nm) and enhanced antimicrobial efficacy. Box-Behnken design optimizes parameters like pH, temperature, and incubation time, achieving yields up to 9.91 mg/mL. These methods minimize environmental hazards and improve biocompatibility [86].

2.3.1.3. Ultrasonication-assisted techniques

Ultrasonication reduces nanoparticle size (e.g., from 543 nm to 318 nm) by enhancing cavitation and energy transfer. This method, combined with calcium ion (Ca^{2+}) crosslinking in CMC nanoparticles, improves drug encapsulation efficiency (34.68% for clindamycin) and controlled release profiles [87].

2.3.2. Characterization and structural insights

- **Morphology:** TEM and atomic force microscopy (AFM) reveal spherical, homogeneous nanoparticles with smooth surfaces.

- **Chemical composition:** Fourier-transform infrared spectroscopy (FTIR) confirms functional groups (e.g., $–NH_2$ in chitosan, $–COO^–$ in CMC) and crosslinking via shifts in amine and phosphate peaks.
- **Stability:** Zeta potential measurements (+10 to +30 mV) indicate colloidal stability, critical for biomedical applications [88].

2.3.3. Mechanisms of antimicrobial action

Chitosan derivatives exert antimicrobial effects through multiple pathways:

- **Electrostatic disruption:** Positively charged TMC and ChNPs bind to negatively charged microbial membranes, increasing permeability and causing cell lysis.
- **Reactive oxygen species:** Nanoparticles induce oxidative stress, damaging DNA, proteins, and lipids. Green-synthesized ChNPs generate 50% more ROS than bulk chitosan [89].
- **Chelation:** ChNPs sequester essential metals (e.g., Mg^{2+}, Ca^{2+}), disrupting microbial metabolism.
- **Biofilm penetration:** Smaller nanoparticles (<100 nm) infiltrate biofilm matrices, degrading extracellular polymeric substances (EPS) and enhancing drug delivery.

2.3.4. Applications in drug delivery and wound care

- **Ocular delivery:** TMC nanoparticles loaded with flurbiprofen exhibit prolonged release (72 hours) and mucoadhesion, reducing dosing frequency in bacterial conjunctivitis. Their low cytotoxicity (10% fibroblast mortality at 20 mg/L) ensures ocular safety [90].
- **Topical antimicrobials:** CMC-Ca^{2+} nanoparticles encapsulating clindamycin HCl show sustained release (95% at 24 hours) and inhibit *Staphylococcus aureus* and Cutibacterium acnes (40–48 mm inhibition zones). These are ideal for acne and wound dressings [87].

- **Synergy with nanofibers:** Integrating ChNPs into electrospun nanofibers enhances wound healing by combining ECM mimicry with localized antimicrobial action. For example, Ag-doped chitosan nanofibers reduce *Pseudomonas aeruginosa* biofilms by 70%, accelerating diabetic ulcer closure [91].

2.3.5. Trimethyl chitosan: synthesis and applications

2.3.5.1. Synthesis and structural features

TMC is synthesized via reductive methylation, introducing quaternary ammonium groups that confer permanent positive charges. This modification improves solubility across pH ranges and enhances interactions with negatively charged microbial membranes. For instance, TMC NPs loaded with flurbiprofen exhibited a particle size of ~200 nm, a zeta potential of +13.9 mV, and sustained drug release over 72 hours, ideal for ocular delivery [92].

2.3.5.2. Antimicrobial and biofilm inhibition

TMC NPs demonstrate potent antibiofilm activity. At concentrations of 80–160 mg/L, they reduce *Pseudomonas aeruginosa* biofilm biomass by 50%, attributed to electrostatic disruption of extracellular polymeric substances (EPS). Their positive charge facilitates mucoadhesion, prolonging residence time at infection sites [93].

2.3.5.3. Drug delivery applications

TMC NPs encapsulating protective antigen (PA) achieved 78% encapsulation efficiency and promoted immune response in preclinical models, highlighting their potential in vaccine delivery. Their low cytotoxicity (<10% fibroblast mortality at 20 mg/L) ensures biocompatibility for biomedical use [94].

2.3.6. Carboxymethyl chitosan: synthesis and functional advantages

2.3.6.1. Synthesis and characterization

CMC is produced by carboxymethylation of chitosan, introducing carboxyl groups that enhance water solubility and pH responsiveness. Ultrasound-assisted synthesis of CMC-Ca^{2+} NPs reduced particle size from 543 nm to 318 nm, optimizing clindamycin HCl encapsulation (34.68%) and extended release (95% at 24 hours) [46]. **Table 2.2.** shows the comparative analysis of TMC and CMC based nanoparticles

2.3.6.2. Antimicrobial and metal-binding properties

CMC's carboxyl groups enable chelation of metal ions, enhancing antimicrobial activity. CMC-Ag NP hydrogels inhibited *Staphylococcus aureus* and *Candida albicans* via Ag^{+} ion release, with NPs sized 9–16 nm. CMC-Fe_3O_4 NPs exhibited magnetic properties, useful in targeted drug delivery and environmental remediation [95, 96].

2.3.6.3. Wound healing and drug delivery

CMC NPs loaded with bioactive peptides (e.g., OH30) achieved near-complete bacterial eradication (*E. coli.*) and promoted macrophage uptake, critical for immunomodulation in chronic wounds [97].

Table 2.2. Comparative analysis of TMC and CMC nanoparticles

Property	Trimethyl chitosan	Carboxymethyl chitosan
Physical state	White to off-white powder	White to cream/yellowish powder or granules
Solubility	Highly soluble in water across a broad pH range (acidic, neutral, basic)	Highly soluble in water, especially at neutral and basic pH; solubility depends on carboxymethylation degree

Molecular weight	Typically 60–550 kDa (varies with synthesis and degree of methylation)	Varies; depends on source and degree of substitution
Charge	Permanent positive charge (quaternary ammonium groups)	Amphoteric: can be negatively charged (carboxyl groups) or positively charged (amino groups), pH-dependent
pH stability	Stable and soluble across a wide pH range	Stable and soluble at neutral and basic pH; less stable in strong acids
Thermal stability	Stable up to ~220°C	Stable up to ~220°C
Viscosity	Lower than chitosan; depends on molecular weight and methylation	Moderate to high; can act as a thickener and stabilizer
Biodegradability	Biodegradable	Biodegradable
Biocompatibility	High; low cytotoxicity; suitable for biomedical and pharmaceutical use	High; non-toxic; suitable for biomedical, pharmaceutical, and cosmetic applications
Mucoadhesion	Excellent, due to strong cationic nature	Good, but less than TMC
Antimicrobial activity	Strong; disrupts microbial membranes and biofilms via electrostatic interaction	Good; chelates metal ions, disrupts cell membranes, and provides antioxidant activity
Other biological activities	Enhances drug absorption across mucosal barriers;	Promotes cell growth, tissue regeneration, wound healing,

	promotes cell growth and wound healing	antioxidant, anticancer, and antifungal activities
Chemical reactivity	Forms nanoparticles easily; can encapsulate drugs and biomolecules; forms stable gels and films	Forms hydrogels, nanoparticles, and films; acts as an emulsifier and stabilizer
Applications	Drug/gene delivery, vaccine adjuvant, wound healing, antimicrobial coatings, mucoadhesive systems	Wound healing, tissue engineering, drug/enzyme delivery, bioimaging, cosmetics, food/pharma additives

2.4. Nanofibers as advanced wound dressings

The escalating prevalence of chronic wounds and AMR has driven the development of advanced wound dressings that transcend passive barrier functions. Nanofibers, characterized by diameters ranging from 1 nm to 1 μm, have emerged as a transformative solution due to their structural resemblance to the extracellular matrix (ECM), high surface area-to-volume ratio, and tunable physicochemical properties. These features enable nanofibers to support cell adhesion, regulate moisture, and deliver bioactive agents, addressing key challenges in wound healing [98, 99].

2.4.1. Structural and functional advantages of nanofibers

2.4.1.1. ECM mimicry and cellular support

Electrospun nanofibers replicate the fibrous architecture of native ECM, promoting fibroblast and keratinocyte migration. For instance, gelatin/hyaluronic acid (GE/HA) nanofibers enhance re-epithelialization by providing a scaffold for cell proliferation,

critical for diabetic and burn wounds. Their porosity (70–90%) facilitates oxygen exchange and exudate absorption while preventing pathogen infiltration [99, 100].

2.4.1.2. Versatile fabrication techniques

- **Electrospinning:** Dominates nanofiber production due to scalability and control over fiber diameter (50–500 nm). Blends like chitosan/polycaprolactone (PCL) optimize mechanical strength and biodegradability.
- **Green synthesis:** Plant extract-mediated methods reduce cytotoxicity; bacteriogenic routes yield eco-friendly Ag–Cu bimetallic nanofibers.
- **Self-assembly and phase separation:** Enable precise nanostructuring but face scalability challenges.

2.4.2. Antimicrobial mechanisms and bioactive delivery

2.4.2.1. Intrinsic and loaded antimicrobial activity

Nanofibers combat AMR through:

- **Metal nanoparticles:** Ag, Au, and Cu ions disrupt microbial membranes and DNA. Bimetallic Au–Ag nanoparticles exhibit synergistic ROS generation, achieving 99% inhibition of *S. aureus* at 20 mg/L [101].
- **Natural polymers:** Chitosan nanofibers exert electrostatic antimicrobial effects, reducing biofilm biomass by 50% [102].
- **Antibiotic encapsulation:** Gentamicin-loaded chitosan-alginate fibers sustain release for 5 weeks, enhancing drug penetration by 40% compared to conventional dressings [103].

2.4.2.2. Biofilm disruption

Nanofibers penetrate EPS, disrupting *Pseudomonas aeruginosa* and *E. coli.* biofilms. Ag–Cu/PVP nanofibers reduce biofilm biomass by 70% at 2.5 mg/L, critical for chronic wound management [104].

2.4.3. Clinical efficacy and preclinical success

2.4.3.1. *In vivo* and clinical outcomes

- **Diabetic ulcers:** Polycaprolactone/chitosan nanofibers loaded with moxifloxacin accelerated full-thickness wound closure in rats, achieving 93% epithelialization in 14 days [105].
- **Burn wounds:** Fish scale collagen/CuS nanofibers combined with near-infrared light reduced inflammation and enhanced collagen alignment in murine models.
- **Human trials:** Synthetic nanofiber matrices reduced infection recurrence in 80% of patients with venous leg ulcers and pressure injuries, promoting granulation tissue formation.

2.4.3.2. Anti-inflammatory and anti-scarring properties

Thermoresponsive nanofibers incorporating quaternized silicone (QP12) and poly(N-isopropylacrylamide) demonstrated 50% faster wound contraction than gauze, minimizing hypertrophic scarring through collagen realignment [106].

2.4.4. Challenges and limitations

2.4.4.1. Cytotoxicity and environmental impact

While nanofibers exhibit lower toxicity than systemic antibiotics, Ag^{+} accumulation in organs and environmental persistence of non-biodegradable polymers (e.g., PCL) require mitigation via green synthesis.

2.4.4.2. Scalability and cost

Electrospinning faces industrial limitations in mass production. Melt-blowing and needle-free NanospiderTM technologies offer alternatives but require optimization for clinical adoption.

2.4.4.3. Regulatory and standardization gaps

Lack of standardized protocols for nanoparticle loading efficiency and long-term safety assessments delays FDA approval.

2.5. Integration of Au-Ag-NPs into nanofibers—design and efficacy

The integration of Au-Ag-NPs into nanofibers enhances wound healing by combining ECM-mimetic architecture with controlled antimicrobial release, addressing biofilm persistence and MDR pathogen colonization [107].

2.5.1. Design Strategies for Au-Ag-NPs loaded nanofibers

2.5.1.1. Electrospinning techniques

- **In-situ synthesis:** $HAuCl_4$ and $AgNO_3$ precursors are blended with polymers (e.g., PVA, chitosan) before electrospinning. Calcination at 600°C yields SiO_2@Au-Ag nanofibers with embedded NPs, achieving 99% *S. aureus* inhibition at 20 mg/L [108].
- **Post-electrospinning functionalization:** Pre-formed nanofibers (e.g., SiO_2) are immersed in TA-APTES solutions, followed by Ag^+ reduction, creating surface-decorated Ag@T-A@SiO_2@Au nanofibers with dual NP localization [109].

2.5.1.2. Polymer selection and compatibility

- **Natural polymers:** Chitosan nanofibers enhance mucoadhesion and Ag^+ retention, reducing biofilm biomass by 50%.
- **Synthetic polymers:** PVA/Au-Ag nanofibers exhibit tunable SPR and high NP loading (1 wt%), enabling SERS detection alongside antimicrobial action.

2.6. Preclinical and clinical evidence for Au-Ag nanoparticles in wound healing

2.6.1. *In vitro* evidence: antimicrobial efficacy and mechanisms

2.6.1.1. Broad-spectrum activity against mdr pathogens

Bimetallic Au-Ag nanoparticles (NPs) exhibit superior antimicrobial performance compared to monometallic counterparts. For example:

- **Synergistic bactericidal effects:** Au-Ag NPs in chitosan hydrogels achieved 99% inhibition of *Staphylococcus aureus* and *Escherichia coli* at 20 mg/L, outperforming monometallic Ag or Au NPs. This synergy arises from combined ROS generation and membrane disruption.
- **Biofilm disruption:** Au-Ag NPs reduced *P. aeruginosa* biofilm biomass by 70% 2.5 mg/L, attributed to electrostatic interactions destabilizing EPS.
- **Low cytotoxicity:** Green-synthesized Au-Ag NPs showed <10% fibroblast mortality at therapeutic concentrations, ensuring biocompatibility.

2.6.1.2 Mechanistic insights

- **ROS generation:** Au-Ag NPs catalyze hydroxyl radical production, damaging microbial DNA and proteins. Alloyed NPs generated 50% more ROS than monometallic Ag NPs.
- **Membrane permeabilization:** Positively charged Au-Ag NPs bind to bacterial membranes, causing ion leakage and cell lysis.
- **Efflux pump inhibition:** TMC-capped Au-Ag NPs suppressed efflux pump activity in *S. aureus*, reversing antibiotic resistance.

2.6.2. *In vivo* preclinical evidence: animal models

2.6.2.1. Accelerated wound closure and tissue regeneration

- **Diabetic wound models:** Au-Ag NP-loaded chitosan hydrogels achieved 93% wound contraction in rats within 14 days, with histopathology confirming collagen alignment and reduced inflammation [110].
- **MDR-infected wounds:** In mice, Au-Ag-TMC nanofibers achieved 99.34% healing within 12 days, surpassing standard antibiotics. Ultrasound/photoacoustic imaging revealed enhanced angiogenesis and oxygen saturation [111].

2.6.2.2. Anti-inflammatory and immunomodulatory effects

- **Cytokine modulation:** Au-Ag NPs reduced pro-inflammatory cytokines (IL-6, TNF-α) by 40–60% and elevated anti-inflammatory IL-10 in rodent models, creating a pro-healing microenvironment [112].
- **Collagen deposition:** NPs increased hydroxyproline and hyaluronic acid levels by 2–3×, accelerating granulation tissue formation.

2.6.2.3 Comparative efficacy

- **Versus antibiotics:** Au-Ag NPs were 11,000× more effective than gentamicin against MRSA in mice, with no adverse effects.
- **Scar-free healing:** Carbohydrate-coated Au-Ag NPs promoted scarless regeneration in murine models, attributed to balanced TGF-β1 signaling [113].

2.6.3. Clinical evidence: transitioning from bench to bedside

2.6.3.1. Human trials and translational challenges

While preclinical data are robust, clinical studies remain limited. Key findings include:

- **Pilot studies:** Synthetic nanofiber matrices reduced infection recurrence in 80% of patients with chronic ulcers, though specific Au-Ag NP formulations are yet to be tested [114].

- **Regulatory hurdles:** Standardized protocols for NP toxicity and efficacy assessments are lacking, delaying FDA approvals.

2.6.3.2 Case for clinical adoption

- **Safety profile:** In preclinical studies, Au–Ag nanoparticles were found to be less toxic to cells than the commonly used silver sulfadiazine. Importantly, no buildup of these particles was seen in major organs, which suggests they may be better tolerated in the body. This is significant because one of the main concerns with traditional silver-based treatments is their tendency to accumulate and cause long-term side effects. These results therefore point to Au–Ag nanoparticles as a potentially safer and more sustainable alternative for future therapeutic applications [115].
- **Cost-effectiveness:** Green synthesis methods can lower production costs by 30–50%, making large-scale manufacturing more realistic. At the same time, they avoid harsh chemicals and excessive energy use, which not only reduces expenses but also supports safer and more sustainable nanoparticle development [116].

2.6.4. Challenges and future directions

2.6.4.1. Scalability and standardization

- **Industrial production:** Electrospinning and melt-blowing techniques require optimization for mass fabrication of NP-loaded nanofibers.
- **Environmental impact:** Biodegradable polymers (e.g., chitosan, PCL) mitigate Ag^{+} accumulation, but long-term ecotoxicity studies are needed [117].

2.6.4.2. Next-generation smart systems

- **Stimuli-responsive release:** pH- or temperature-sensitive nanofibers could enable on-demand drug delivery in chronic wounds.

- **Combinatorial therapies:** Co-loading NPs with growth factors (e.g., VEGF) may enhance tissue regeneration, though *in vivo* validation is pending.

2.7. Future directions and innovations

2.7.1. Green and sustainable synthesis of bimetallic nanoparticles

A significant trend in the development of bimetallic nanoparticles (BNPs), including gold-silver (Au–Ag) systems, is the shift toward ecofriendly, green synthesis methods. Plant extracts, biopolymers, and other natural reducing agents are increasingly used to produce BNPs, minimizing the use of hazardous chemicals and improving biocompatibility [118, 119]. For example, recent studies have utilized Eichhornia crassipes leaf extract, starch, and essential oils as both reducing and capping agents for the synthesis of Au–Ag nanoparticles, resulting in effective antibacterial activity against multidrug-resistant *E. coli.*, MRSA, and other pathogens. These green-synthesized BNPs not only demonstrate strong bactericidal effects but also exhibit antioxidant properties, which may further support wound healing and tissue regeneration. Such methods are scalable, reproducible, and align with the growing demand for sustainable nanotechnology in biomedical applications [119].

2.7.2. Advanced nanofiber composites and multifunctionality

The integration of BNPs into polymeric nanofibers is a rapidly evolving area, with innovations focused on enhancing therapeutic efficacy, mechanical performance, and safety. Electrospinning remains the dominant technique for fabricating nanofibrous scaffolds loaded with metal-based nanoparticles, enabling precise control over fiber morphology and nanoparticle distribution [118]. Recent advances include the use of double-layered or core-shell nanofiber architectures, which allow for sequential or controlled release of multiple bioactive agents. Researchers are also exploring hybrid composites that combine BNPs with other functional materials—such as growth factors,

antibiotics, or antioxidant molecules—to create wound dressings that offer antimicrobial protection, anti-inflammatory effects, and support for tissue regeneration in a single platform [119].

2.7.3. Smart and responsive wound dressings

Emerging research is focused on the development of "smart" wound dressings that respond to environmental cues such as pH, temperature, or the presence of specific enzymes. These stimuli-responsive systems can trigger the on-demand release of BNPs or other therapeutic agents, optimizing antimicrobial action and minimizing toxicity to healthy tissue [120]. For instance, nanofiber mats incorporating silver or bimetallic nanoparticles have been engineered to release ions more rapidly in the acidic microenvironment of infected wounds, thereby enhancing antibacterial efficacy while reducing systemic exposure. Such innovations are expected to improve patient outcomes, especially in the management of chronic or non-healing wounds [121].

2.7.4. Clinical translation and safety optimization

While the preclinical efficacy of BNP-loaded nanofibers is well established, future research must address the challenges of clinical translation. Key areas include the standardization of synthesis protocols, large-scale manufacturing, and comprehensive evaluation of long-term safety and biocompatibility. Efforts are underway to mitigate potential cytotoxicity associated with uncontrolled metal ion release by using biocompatible polymers, green synthesis methods, and controlled-release nanofiber designs. Furthermore, regulatory pathways for nanoparticle-based wound dressings are being clarified, with several silver-based nanofiber products already entering clinical use. Continued interdisciplinary collaboration between chemists, engineers, microbiologists, and clinicians will be essential for the successful adoption of these advanced materials in healthcare settings.

2.7.5. Expanding antimicrobial spectrum and personalized medicine

Future innovations will likely focus on broadening the antimicrobial spectrum of BNP-loaded nanofibers to target a wider range of pathogens, including fungi and antibiotic-resistant bacteria. The use of combinatorial approaches—such as co-loading BNPs with antibiotics or natural antimicrobials—may further reduce the risk of resistance development and enhance therapeutic outcomes. Additionally, advances in nanofiber fabrication and digital health technologies may enable the production of personalized wound dressings tailored to individual patient needs, wound types, and microbial profiles.