International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 3, Issue 1, January-June, 2026
Page No.: 158-176

Review

Recent Advances in Silver Nanoparticle-Loaded Hydrogels for Antimicrobial and Wound Healing Applications

Parveen Yadav*[1], Vishnu Sharma[2], Devashish[3], Karan Dahiya[4], Piyush Malik[5]

[1]*Assistant Professor, School of Health Science, Sushant University Gurugram*

[2-5]*School of Health Science, Sushant University Gurugram*

Corresponding Author:
Parveen Yadav

Email:
Parveeny9999@gmail.com, vs100406@gmail.com, Piyushhhmalik@gmail.com, devashishsain06@gmail.com, dahiyakaran3417@gmail.com

DOI: *10.62896/ijpdd.3.1.21*

Conflict of interest: *NIL*

Article History
Received: 12/02/2026
Accepted: 10/04/2026
Published: 22/04/2026

Abstract:
The emergence of antimicrobial resistance has necessitated the development of novel therapeutic strategies, among which silver nanoparticles (AgNPs) have garnered significant attention due to their broad-spectrum antimicrobial activity. Green synthesis of AgNPs using plant extracts such as Neem (Azadirachta indica) and Tulsi (Ocimum sanctum) offers an eco-friendly alternative to conventional chemical methods, eliminating the use of toxic reducing agents while providing additional phytochemical stabilization. Hydrogel-based delivery systems have emerged as promising platforms for AgNP administration, offering sustained release, enhanced biocompatibility, and improved therapeutic efficacy. This comprehensive review critically examines the development and evaluation of hydrogels loaded with green-synthesized silver nanoparticles for antimicrobial applications. The article systematically discusses the fundamental properties of hydrogels, the antimicrobial mechanisms of AgNPs, green synthesis methodologies using medicinal plants, polymer systems including Carbopol, chitosan, PVA, and HPMC, and the integration strategies for incorporating AgNPs into hydrogel matrices. Furthermore, the review provides detailed insights into physicochemical evaluation parameters including swelling index, pH, viscosity, spreadability, and stability studies. Critical analysis of current challenges, including AgNP toxicity concerns, stability issues, and scale-up difficulties, is presented alongside future perspectives and research directions. This review serves as a valuable resource for researchers and pharmaceutical scientists working towards developing sustainable and effective antimicrobial formulations.

Keywords: *Silver nanoparticles, Green synthesis, Hydrogels, Antimicrobial activity, Neem, Tulsi, Wound healing, Carbopol, Drug delivery*

1. Introduction

The global healthcare landscape has been significantly challenged by the emergence and spread of antimicrobial resistance (AMR), which poses a severe threat to public health worldwide. According to the World Health Organization, AMR is one of the top ten global public health threats facing humanity, with resistant infections causing approximately 700,000 deaths annually [1]. The development of novel antimicrobial agents and delivery systems has become an urgent priority for the pharmaceutical and biomedical research communities.

Silver has been recognized for its antimicrobial properties since ancient civilizations, with documented use in water purification and wound treatment dating back thousands of years. However, the advent of

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 3, Issue 1, January-June, 2026
Page No.: 158-176

nanotechnology has revolutionized the application of silver in modern medicine, particularly through the development of silver nanoparticles (AgNPs) [2]. These nanoscale silver particles exhibit enhanced antimicrobial activity compared to bulk silver due to their increased surface area-to-volume ratio, which facilitates greater interaction with microbial cells.

The synthesis of AgNPs can be achieved through various methods, broadly categorized into physical, chemical, and biological approaches. While chemical reduction methods using agents such as sodium borohydride or citrate have been widely employed, the use of toxic chemicals raises concerns regarding environmental impact and biocompatibility [3]. Green synthesis utilizing plant extracts has emerged as a sustainable alternative, offering advantages including cost-effectiveness, environmental friendliness, and the presence of natural capping agents that enhance nanoparticle stability.

Hydrogels represent a unique class of three-dimensional polymeric networks capable of absorbing and retaining significant amounts of water while maintaining their structural integrity. These materials have found extensive applications in drug delivery, tissue engineering, and wound healing due to their biocompatibility, tunable physicochemical properties, and ability to provide a moist environment conducive to healing [4]. The integration of AgNPs into hydrogel matrices combines the antimicrobial efficacy of silver with the controlled release capabilities of hydrogels, resulting in advanced therapeutic formulations.

This review article provides a comprehensive analysis of the development and evaluation of hydrogels loaded with green-synthesized silver nanoparticles for antimicrobial applications. The article critically examines the fundamental principles underlying hydrogel systems, the green synthesis of AgNPs using medicinal plants such as Neem and Tulsi, the integration strategies for incorporating nanoparticles into hydrogel matrices, and the evaluation parameters essential for characterizing these advanced formulations. Furthermore, the review discusses the antimicrobial mechanisms, applications in wound healing, current challenges, and future perspectives in this rapidly evolving field.

2. Hydrogels in Drug Delivery

Hydrogels are three-dimensional cross-linked polymeric networks that can absorb and retain large amounts of water or biological fluids while maintaining their structural integrity [5]. The unique combination of solid-like mechanical properties and liquid-like transport characteristics makes hydrogels ideal candidates for various biomedical applications, particularly in drug delivery systems.

2.1 Classification of Hydrogels

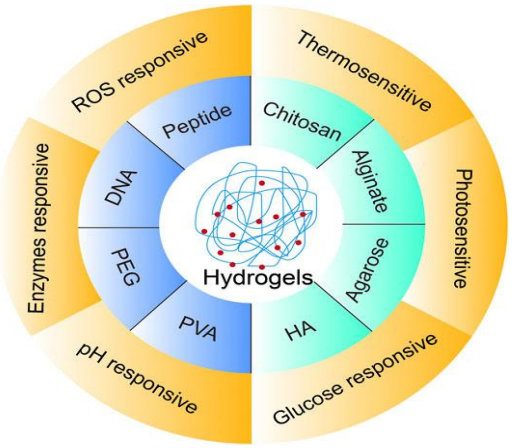

Fig.2.1.1 Classification of Hydrogels

Hydrogels can be classified based on several criteria, including the source of polymers, nature of cross-linking, physical structure, and sensitivity to environmental stimuli. Based on the source, hydrogels are categorized as natural, synthetic, or semi-synthetic (semi-interpenetrating networks). Natural hydrogels are derived from biological sources such as chitosan, alginate, and hyaluronic acid, while synthetic hydrogels include polymers like polyvinyl alcohol (PVA), polyacrylic acid (PAA), and polyethylene glycol (PEG) [6].

The cross-linking mechanism represents another important classification criterion. Physical hydrogels are formed through non-covalent interactions such as hydrogen bonding, ionic interactions, or hydrophobic associations, making them reversible and responsive to environmental changes. Chemical hydrogels, conversely, are formed through covalent bonds, resulting in permanent networks with enhanced mechanical stability [7]. The freeze-thaw method represents a physical cross-linking approach that has gained significant attention due to its simplicity and avoidance of chemical cross-linkers.

Table 2.1.1: Classification of Hydrogels Based on Different Criteria

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 3, Issue 1, January-June, 2026
Page No.: 158-176

Classification Criterion	Category	Examples
Source	Natural	Chitosan, Alginate, Hyaluronic acid, Gelatin
	Synthetic	PVA, PAA, PEG, Carbopol
	Semi-synthetic	HPMC, CMC, Semi-IPNs
Cross-linking	Physical	Freeze-thaw, Ionic, Hydrophobic
	Chemical	Covalent (glutaraldehyde, genipin)
Stimuli Response	Temperature	PNIPAM, HPMC
	pH	Carbopol, Chitosan

2.2 Properties and Applications

The success of hydrogels in drug delivery applications stems from their unique combination of properties. High water content provides a biocompatible environment similar to natural tissues, while the porous structure enables the encapsulation and controlled release of therapeutic agents. The mechanical properties of hydrogels can be tuned by adjusting polymer concentration, cross-linking density, and molecular weight, allowing customization for specific applications [8].

Swelling behavior is a critical property that determines the drug loading capacity and release kinetics of hydrogel systems. The degree of swelling depends on the hydrophilicity of polymer chains, cross-linking density, and environmental conditions such as pH and temperature. Hydrogels can exhibit stimuli-responsive behavior, undergoing volume transitions in response to changes in pH, temperature, ionic strength, or the presence of specific biomolecules [9].

In wound healing applications, hydrogels provide several advantages including maintenance of a moist wound environment, absorption of exudate, protection against bacterial infection, and facilitation of autolytic debridement. The transparency of many hydrogel formulations allows for visual monitoring of wound healing progress without dressing removal [10].

3. Silver Nanoparticles: Properties and Antimicrobial Mechanism

Silver nanoparticles (AgNPs) are nanoscale particles of metallic silver, typically ranging from 1 to 100 nanometers in size. The unique physicochemical properties of AgNPs arise from their high surface area-to-volume ratio, quantum confinement effects, and localized surface plasmon resonance (LSPR), which collectively contribute to their enhanced biological activity compared to bulk silver [11].

3.1 Physical and Chemical Properties

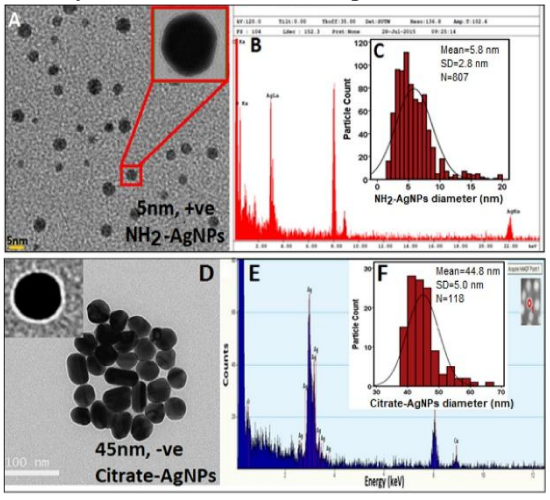

Fig. 3.1.1 Morphological and spectroscopic characterization of silver nanoparticles (AgNPs)

The optical properties of AgNPs are characterized by a strong absorption band in the visible region of the electromagnetic spectrum, typically appearing between 400-450 nm due to surface plasmon resonance. This characteristic absorption peak serves as a diagnostic tool for confirming nanoparticle formation and can provide information about particle size and aggregation

state [12]. Smaller nanoparticles generally exhibit absorption maxima at shorter wavelengths, while aggregated particles show broadened and red-shifted peaks.

The size and shape of AgNPs significantly influence their antimicrobial activity, with smaller particles generally exhibiting greater efficacy due to their higher surface area and enhanced ability to interact with bacterial cell membranes. Spherical nanoparticles are most commonly synthesized, though other morphologies including rods, triangles, and cubes can be produced through controlled synthesis conditions [13].

Surface charge and stability are critical factors determining the biological fate of AgNPs.. The stability of AgNPs in biological environments is influenced by surface coatings, which can prevent aggregation and control the release of silver ions [14].

3.2 Antimicrobial Mechanism

The antimicrobial activity of AgNPs involves multiple mechanisms that act synergistically to eliminate microorganisms. The primary mechanisms include: (1) disruption of bacterial cell membranes, (2) generation of reactive oxygen species (ROS), (3) interference with DNA replication and protein synthesis, and (4) modulation of signal transduction pathways [15].

The interaction between AgNPs and bacterial cell membranes initiates the antimicrobial cascade. The electrostatic attraction between positively charged silver ions and negatively charged membrane components leads to membrane destabilization and increased permeability. This disruption compromises the structural integrity of the cell, resulting in leakage of cellular contents and ultimately cell death [16].

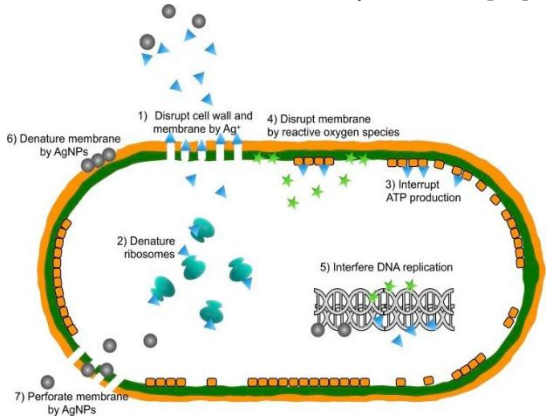

Fig. 3.2.1 Mechanism of antibacterial action of (AgNPs)

Silver ions released from nanoparticle surfaces play a crucial role in antimicrobial activity. These ions can bind to thiol groups of vital enzymes, inactivating them and disrupting cellular respiration and energy metabolism. Additionally, silver ions can intercalate with DNA bases, preventing replication and transcription processes [17]. The generation of reactive oxygen species, including superoxide anions and hydroxyl radicals, causes oxidative stress that damages cellular components including lipids, proteins, and nucleic acids.

The broad-spectrum antimicrobial activity of AgNPs extends to Gram-positive and Gram-negative bacteria, fungi, and viruses. This versatility, combined with the low propensity for developing resistance compared to conventional antibiotics, makes AgNPs promising candidates for addressing the global challenge of antimicrobial resistance [18].

4. Green Synthesis of Silver Nanoparticles

Green synthesis of AgNPs represents an environmentally benign approach that utilizes biological entities including plants, bacteria, fungi, and algae as reducing and stabilizing agents. Among these, plant-mediated synthesis has gained significant attention due to its simplicity, rapid reaction kinetics, and the availability of diverse phytochemicals that can serve dual roles as reducing and capping agents [19].

4.1 Neem (Azadirachta indica) Mediated Synthesis

Fig.4.1.1 Neem

Neem (Azadirachta indica), belonging to the family Meliaceae, is a versatile medicinal plant native to the Indian subcontinent. Various parts of the neem tree, including leaves, bark,

seeds, and fruits, have been traditionally used in Ayurvedic medicine for their antimicrobial, anti-inflammatory, antipyretic, and immunomodulatory properties [20]. The phytochemical profile of neem includes a rich array of bioactive compounds, with azadirachtin, nimbin, nimbidin, and quercetin being the most prominent.

The synthesis of AgNPs using neem leaf extract typically involves the reduction of silver nitrate (AgNO3) solution at room temperature or elevated temperatures. The polyphenols, flavonoids, and terpenoids present in neem extract serve as reducing agents, converting Ag+ ions to metallic silver (Ag0), while simultaneously capping the nanoparticles to prevent aggregation [21]. The reaction is visually monitored by the color change from colorless to brownish-yellow, indicating nanoparticle formation.

Studies have demonstrated that neem-synthesized AgNPs exhibit characteristic surface plasmon resonance peaks in the range of 400-450 nm, confirming the formation of spherical nanoparticles. The size of neem-synthesized AgNPs typically ranges from 10-50 nm, with the exact dimensions influenced by reaction parameters including temperature, pH, extract concentration, and silver precursor concentration [22]. The presence of neem phytochemicals on the nanoparticle surface provides additional stability and potential synergistic antimicrobial activity.

4.2 Tulsi (Ocimum sanctum) Mediated Synthesis

Fig. 4.2.1: Tulsi

Tulsi (Ocimum sanctum), also known as Holy Basil, is an aromatic plant belonging to the family Lamiaceae. Revered in traditional Indian medicine as "The Queen of Herbs," tulsi possesses adaptogenic, antimicrobial, anti-inflammatory, and immunomodulatory properties [23]. The leaves of tulsi contain a rich diversity of bioactive compounds including eugenol, ursolic acid, rosmarinic acid, apigenin, and various essential oils.

The green synthesis of AgNPs using tulsi leaf extract follows a similar mechanism to neem-mediated synthesis. The phenolic compounds and flavonoids present in tulsi extract act as effective reducing agents for silver ions, while the diverse array of phytochemicals provides stabilization through surface capping [24]. The rapid reduction kinetics observed with tulsi extract can be attributed to the high concentration of eugenol and other phenolic antioxidants.

Characterization studies of tulsi-synthesized AgNPs have confirmed the formation of spherical nanoparticles with sizes typically ranging from 5-30 nm. The UV-Vis absorption spectra show characteristic peaks around 420-440 nm, consistent with the surface plasmon resonance of silver nanoparticles. X-ray diffraction analysis confirms the crystalline nature of the synthesized nanoparticles, with peaks corresponding to the face-centered cubic structure of metallic silver [25].

4.3 Other Plant-Based Synthesis Methods

Beyond neem and tulsi, numerous plant species have been explored for the green synthesis of AgNPs. Aloe vera, known for its wound healing properties, has been utilized to synthesize AgNPs with enhanced biocompatibility [26]. Citrus fruits, including lemon and orange, have demonstrated efficient reduction capabilities due to their high citric acid and ascorbic acid content.

Ginger (Zingiber officinale) and turmeric (Curcuma longa) have also been employed for AgNP synthesis, with the resulting nanoparticles exhibiting enhanced antimicrobial activity attributed to the synergistic effects of silver and the bioactive phytochemicals present in these rhizomes [27]. The diversity of plant-based synthesis methods offers opportunities for tailoring nanoparticle properties by selecting appropriate plant sources based on their phytochemical profiles.

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 3, Issue 1, January-June, 2026
Page No.: 158-176

Table 4.3.1: Comparison of Green Synthesis Methods Using Different Plant Extracts

Plant Source	Key Phytochemicals	AgNP Size (nm)	UV-Vis Peak (nm)	Reaction Time
Neem (Azadirachta indica)	Azadirachtin, Nimbin, Quercetin	10-50	410-430	30-60 min
Tulsi (Ocimum sanctum)	Eugenol, Ursolic acid, Apigenin	5-30	420-440	15-45 min
Aloe vera	Aloin, Aloe-emodin	15-40	415-425	2-4 hours
Lemon (Citrus limon)	Citric acid, Ascorbic acid	20-60	425-435	1-2 hours
Ginger (Zingiber officinale)	Gingerol, Shogaol	10-35	430-450	45-90 min

5. Polymer Systems Used in Hydrogels

The selection of appropriate polymers is critical for the successful development of hydrogel-based drug delivery systems. The polymer matrix determines the mechanical properties, swelling behavior, biocompatibility, and release kinetics of the final formulation. This section discusses the key polymer systems employed in AgNP-loaded hydrogels for antimicrobial applications.

5.1 Carbopol

Fig. 5.1.1: Carbopol

Carbopol, a series of high molecular weight polyacrylic acid polymers cross-linked with polyalkenyl ethers or divinyl glycol, represents one of the most widely used polymers in pharmaceutical formulations. Carbopol polymers exhibit excellent mucoadhesive properties, biocompatibility, and the ability to form clear gels at low concentrations [28]. The carboxylic acid groups present in Carbopol can ionize in aqueous environments, resulting in electrostatic repulsion that contributes to gel formation and swelling.

In hydrogel formulations, Carbopol concentrations typically range from 0.5% to 2.0% (w/w), depending on the desired viscosity and gel strength. The neutralization of Carbopol gels using alkaline agents such as triethanolamine or sodium hydroxide is essential for achieving optimal viscosity and clarity. The pH-responsive behavior of Carbopol-based hydrogels makes them particularly suitable for wound healing applications, where the gel can respond to changes in wound pH [29].

Carbopol hydrogels loaded with AgNPs have demonstrated sustained release characteristics, with the polymer matrix acting as a reservoir for controlled silver ion release. The negative charge of Carbopol at neutral pH may also contribute to the stabilization of AgNPs through electrostatic interactions, preventing aggregation and maintaining antimicrobial efficacy over extended periods.

5.2 Chitosan

Chitosan, a natural polysaccharide derived from chitin through deacetylation, has emerged as a promising polymer for biomedical applications due to its biocompatibility, biodegradability, and inherent antimicrobial properties. The presence of free amino groups in chitosan imparts polycationic characteristics at acidic pH, enabling interaction with negatively charged bacterial cell membranes and contributing to its antimicrobial activity [30].

The combination of chitosan with AgNPs in hydrogel formulations offers synergistic antimicrobial effects, with both components contributing to bacterial elimination through distinct mechanisms. Chitosan-based hydrogels can be formed through various cross-linking methods, including ionic cross-linking with polyanions, covalent cross-linking with glutaraldehyde or genipin, and physical cross-linking through freeze-thaw cycles [31].

The degree of deacetylation and molecular weight of chitosan significantly influence the properties of the resulting hydrogels. Higher deacetylation degrees result in increased charge density and enhanced antimicrobial activity, while molecular weight affects the mechanical strength and degradation kinetics of the hydrogel matrix [32].

5.3 Polyvinyl Alcohol (PVA)

Polyvinyl alcohol (PVA) is a synthetic, water-soluble polymer widely used in pharmaceutical and biomedical applications due to its excellent film-forming properties, biocompatibility, and chemical stability. PVA hydrogels can be prepared through physical cross-linking methods, including repeated freeze-thaw cycles that promote crystallite formation and network development [33].

The freeze-thaw method for PVA hydrogel preparation involves subjecting aqueous PVA solutions to repeated cycles of freezing at sub-zero temperatures followed by thawing at room temperature. This process promotes the formation of crystalline domains that act as physical cross-links, resulting in elastic hydrogels with tunable mechanical properties [34]. The number of freeze-thaw cycles influences the degree of crystallinity and, consequently, the mechanical strength and swelling behavior of the resulting hydrogels.

PVA-based hydrogels provide a stable matrix for AgNP incorporation, with the hydroxyl groups of PVA potentially contributing to nanoparticle stabilization through hydrogen bonding interactions. The transparency of PVA hydrogels allows for visual monitoring of nanoparticle dispersion and any potential aggregation phenomena [35].

5.4 Hydroxypropyl Methylcellulose (HPMC)

Hydroxypropyl methylcellulose (HPMC) is a semi-synthetic cellulose ether widely used in pharmaceutical formulations as a controlled-release agent, tablet binder, and viscosity modifier. HPMC forms thermoreversible gels that exhibit unique properties including high water retention capacity, good film-forming ability, and excellent biocompatibility [36].

HPMC-based hydrogels have been successfully employed as matrices for AgNP delivery, with the polymer providing sustained release characteristics and enhanced stability. The combination of HPMC with other polymers such as Carbopol or chitosan can result in hydrogels with improved mechanical properties and tailored release profiles [37].

Table 5.4.1: Comparison of Polymer Systems for AgNP-loaded Hydrogels

Polymer	Key Properties	Cross-linking Method	Applications
Carbopol	Mucoadhesive, pH-responsive	Neutralization (TEA/NaOH)	Topical gels, Wound dressings
Chitosan	Antimicrobial, Biodegradable	Ionic (TPP), Chemical	Wound healing, Drug delivery
PVA	Biocompatible, Non-toxic	Freeze-thaw, Chemical	Wound dressings, Tissue engineering
HPMC	Thermoreversible, Film-forming	Thermal gelation	Ophthalmic, Oral drug delivery

6. Integration of AgNPs into Hydrogel Systems

The successful integration of green-synthesized AgNPs into hydrogel matrices requires careful consideration of multiple factors including nanoparticle stability, uniform dispersion, and preservation of antimicrobial activity. Various strategies have been developed for incorporating AgNPs into hydrogel systems, each offering distinct advantages and challenges.

The in situ synthesis approach involves the formation of AgNPs directly within the hydrogel matrix by incorporating silver precursor (typically AgNO3) into the polymer solution, followed by reduction using the plant extract. This method ensures uniform distribution of nanoparticles throughout the hydrogel network and prevents aggregation that may occur during ex situ incorporation [38]. The polymer chains can also serve as stabilizing agents, limiting nanoparticle growth and maintaining the desired size range.

Ex situ incorporation involves the synthesis of AgNPs separately, followed by dispersion into the pre-formed hydrogel or polymer solution prior to gelation. This approach allows for precise control over nanoparticle characteristics including size, shape, and concentration before incorporation. However, achieving uniform dispersion can be challenging, and aggregation may occur if the nanoparticle surface is not adequately stabilized [39].

The freeze-thaw cross-linking method has emerged as a particularly effective approach for preparing AgNP-loaded hydrogels, especially with PVA-based systems. This method involves subjecting the polymer solution containing AgNPs to repeated cycles of freezing and thawing, which promotes the formation of crystalline domains that act as physical cross-links. The mild processing conditions of the freeze-thaw method help preserve the integrity and antimicrobial activity of incorporated AgNPs.

The optimization of AgNP loading concentration is critical for achieving effective antimicrobial activity while minimizing potential cytotoxicity. Studies have shown that AgNP concentrations in the range of 0.01% to 0.1% (w/w) in hydrogel formulations typically provide optimal antimicrobial efficacy without significant adverse effects on mammalian cells [40]. However, the optimal concentration may vary depending on the specific application, target microorganisms, and polymer system employed.

Characterization of AgNP-loaded hydrogels involves multiple analytical techniques to confirm successful incorporation and assess the properties of the composite system. UV-Visible spectroscopy confirms the presence of AgNPs through the characteristic surface plasmon resonance peak at 400-450 nm. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) provide visual confirmation of nanoparticle distribution and size within the hydrogel matrix. Fourier-transform infrared spectroscopy (FTIR) can reveal interactions between AgNPs and polymer functional groups, while X-ray diffraction (XRD) confirms the crystalline nature of incorporated nanoparticles [41].

7. Evaluation Parameters of Hydrogels

Comprehensive characterization of AgNP-loaded hydrogels is essential for ensuring quality, safety, and efficacy. Various physicochemical parameters are evaluated to characterize the formulation and predict its performance in intended applications. This section discusses the key evaluation parameters and their significance in hydrogel characterization [42].

7.1 Swelling Index

The swelling index, also known as swelling ratio or equilibrium swelling degree, is a fundamental parameter that quantifies the water absorption capacity of hydrogels. This parameter is determined by measuring the weight of the hydrogel in its swollen state relative to its dry weight. The swelling behavior influences drug loading capacity, release kinetics, and the ability to maintain a moist wound environment.

The swelling index is calculated using the formula: Swelling Index = (Ws - Wd) / Wd, where Ws is the weight of the swollen hydrogel and Wd is the weight of the dry hydrogel. Factors affecting the swelling index include polymer hydrophilicity, cross-linking density, and the presence of ionizable groups. Higher swelling indices generally indicate greater water absorption capacity, though excessive swelling may compromise mechanical integrity [43].

7.2 pH Measurement

Fig. 7.2.1: pH meter

The pH of hydrogel formulations is a critical parameter that influences stability, biocompatibility, and antimicrobial activity. For wound healing applications, the hydrogel pH should ideally be in the range of 6.0-7.0 to match physiological conditions and minimize irritation to surrounding tissues [44]. pH measurement is typically performed using a calibrated pH meter on hydrogel samples dispersed in distilled water or appropriate buffer solutions.

Carbopol-based hydrogels typically exhibit acidic pH in their unneutralized state (pH 3.0-4.0) due to the presence of carboxylic acid groups. Neutralization with appropriate alkaline agents is necessary to achieve the desired pH range for biomedical applications. The pH of the hydrogel can also influence AgNP stability and silver ion release kinetics, with higher pH generally promoting faster ion release [45].

7.3 Viscosity

Viscosity is an important rheological parameter that influences the spreadability, ease of application, and retention of hydrogel formulations at the application site. The viscosity of hydrogels is typically measured using rotational viscometers or rheometers at controlled temperatures. For topical and wound healing applications, optimal viscosity ensures that the hydrogel can be easily applied while maintaining sufficient consistency to remain in place without running off [46].

The viscosity of polymer-based hydrogels is influenced by polymer concentration, molecular weight, degree of cross-linking, and temperature. Higher polymer concentrations and molecular weights generally result in increased viscosity. The viscosity of thermoresponsive polymers such as HPMC may change significantly with temperature, exhibiting lower viscosity at elevated temperatures due to gelation phenomena [47].

7.4 Spreadability

Spreadability is a practical parameter that assesses the ease with which a hydrogel can be applied to a surface, which is particularly important for topical and wound healing applications. This parameter is typically determined using a modified apparatus where a known weight of hydrogel is placed between two glass slides and the diameter of the spread hydrogel is measured after a specified time period [48].

Good spreadability ensures uniform coverage of the application area and enhances patient compliance. Hydrogels with excessive viscosity may exhibit poor spreadability, while those with insufficient viscosity may spread too readily, compromising retention at the application site. The optimal spreadability depends on the specific application, with wound healing formulations generally requiring moderate spreadability for easy application and adequate coverage [49].

7.5 Drug Content and Entrapment Efficiency

For AgNP-loaded hydrogels, determination of silver content and entrapment efficiency is essential for quality control and dosage optimization. The total silver content is typically determined using atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) after appropriate sample digestion. Entrapment efficiency represents the percentage of silver successfully incorporated into the hydrogel matrix relative to the initial amount added [50].

High entrapment efficiency is desirable for ensuring consistent dosing and minimizing waste of the active agent. Factors influencing entrapment efficiency include the method of AgNP incorporation, compatibility between nanoparticles and the polymer matrix, and the stability of nanoparticles during hydrogel preparation. In situ synthesis methods generally achieve higher entrapment efficiency compared to ex situ incorporation approaches [51].

7.6 Stability Studies

Stability studies are conducted to assess the shelf-life of hydrogel formulations and ensure that the product maintains its quality attributes throughout the intended storage period. Accelerated stability studies are typically performed at elevated temperatures (40°C ± 2°C) and relative humidity (75% ± 5%) for a period of 3-6 months, while long-term stability studies are conducted at controlled room temperature (25± 2°C) and relative humidity (60± 5%) for extended periods [52].

Parameters evaluated during stability studies include physical appearance, pH, viscosity, swelling index, silver content, and antimicrobial activity. Changes in these

parameters over time indicate potential degradation or instability of the formulation. AgNP-loaded hydrogels may face stability challenges related to nanoparticle aggregation, oxidation, or silver ion leaching, which can compromise antimicrobial efficacy [53].

Table 7.6.1: Summary of Evaluation Parameters for AgNP-loaded Hydrogels

Parameter	Method/Equipment	Significance
Swelling Index	Gravimetric analysis	Water absorption capacity, Drug loading
pH	pH meter	Biocompatibility, Stability
Viscosity	Viscometer/Rheometer	Spreadability, Ease of application
Spreadability	Parallel plate method	Application characteristics
AgNP Content	AAS/ICP-MS	Dosage confirmation, Quality control
Entrapment Efficiency	Centrifugation analysis	Loading efficiency
Stability	Accelerated studies (40°C/75% RH)	Shelf-life determination
AgNP Characterization	UV-Vis, SEM, TEM, XRD	Size, morphology, crystallinity

8. Antimicrobial Activity of AgNP-loaded Hydrogels

The antimicrobial activity of AgNP-loaded hydrogels is a critical parameter that determines their therapeutic efficacy in treating infections and promoting wound healing. Various in vitro and in vivo methods are employed to evaluate the antimicrobial potential of these formulations against a range of pathogenic microorganisms.

8.1 In Vitro Antimicrobial Testing Methods

The agar well diffusion method is a widely used qualitative and semi-quantitative technique for assessing antimicrobial activity. In this method, wells are created in agar plates inoculated with test microorganisms, and the hydrogel formulation is placed in the wells. After incubation, the zone of inhibition around the wells is measured, with larger zones indicating greater antimicrobial activity [54]. This method provides a simple and visual assessment of antimicrobial efficacy.

The broth microdilution method is employed for quantitative determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Serial dilutions of the hydrogel formulation are prepared in liquid growth medium, inoculated with standardized microbial suspensions, and incubated under appropriate conditions. The MIC is defined as the lowest concentration that inhibits visible growth, while the MBC represents the lowest concentration that kills the microorganism [55].

The disk diffusion method, similar to the agar well diffusion method, involves applying hydrogel samples to sterile filter paper disks placed on inoculated agar plates. This method is particularly useful for comparing the antimicrobial activity of different formulations and is standardized by organizations such as the Clinical and Laboratory Standards Institute (CLSI) for antibiotic susceptibility testing [56].

8.2 Activity Against Gram-Positive Bacteria

AgNP-loaded hydrogels have demonstrated significant antimicrobial activity against various Gram-positive bacteria, including Staphylococcus aureus, Streptococcus pyogenes, and Bacillus subtilis. The thick peptidoglycan layer characteristic of Gram-positive bacteria, while providing structural rigidity, does not prevent the penetration of silver ions and nanoparticles. Studies have shown that AgNP-loaded hydrogels can achieve zones of inhibition ranging from 15-

25 mm against S. aureus, depending on the AgNP concentration and polymer system employed [57].

The antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA) is of particular clinical significance given the limited treatment options for MRSA infections. Research has demonstrated that AgNP-loaded hydrogels can effectively inhibit MRSA growth, with the multimodal antimicrobial mechanism of silver reducing the likelihood of resistance development [58]. This makes AgNP-loaded hydrogels promising candidates for treating drug-resistant infections.

8.3 Activity Against Gram-Negative Bacteria

Gram-negative bacteria, including Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae, are important targets for antimicrobial therapy due to their role in various infections and their increasing resistance to conventional antibiotics. The outer membrane of Gram-negative bacteria, containing lipopolysaccharides, presents a barrier to many antimicrobial agents but does not prevent the action of silver nanoparticles.

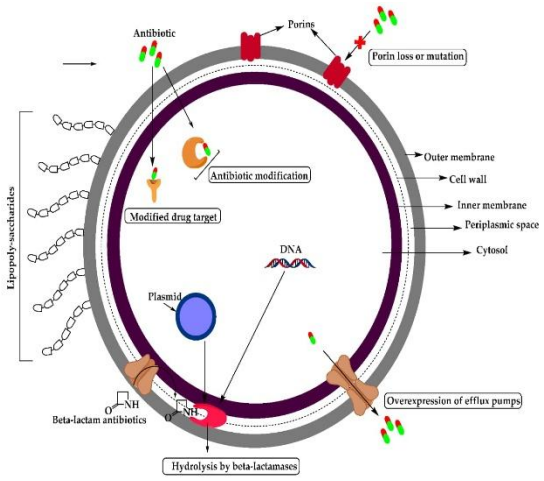

Fig. 8.3.1: Mechanism of Action Against Gram-Negative Bacteria

Studies have demonstrated that AgNP-loaded hydrogels exhibit potent activity against E. coli, with zones of inhibition comparable to or exceeding those observed against Gram-positive bacteria. The electrostatic attraction between positively charged silver ions and the negatively charged lipopolysaccharides may facilitate interaction with Gram-negative bacteria. P. aeruginosa, known for its intrinsic resistance to many antimicrobial agents, has also shown susceptibility to AgNP-loaded hydrogels [59].

8.4 Antifungal Activity

Beyond antibacterial activity, AgNP-loaded hydrogels have demonstrated efficacy against various pathogenic fungi, including Candida albicans and Aspergillus species. Fungal infections, particularly in immunocompromised patients and chronic wounds, represent a significant clinical challenge. The antifungal mechanism of AgNPs involves disruption of cell membrane integrity, generation of reactive oxygen species, and interference with cellular metabolism, similar to the antibacterial mechanism [60].

The combination of AgNPs with antifungal polymers such as chitosan may provide synergistic antifungal effects, enhancing the therapeutic potential of hydrogel formulations for treating fungal infections. Studies have shown that AgNP-loaded chitosan hydrogels can effectively inhibit C. albicans growth, with potential applications in treating oral and dermal candidiasis.

8.5 Synergistic Effects

The combination of AgNPs with antimicrobial polymers and plant extracts in hydrogel formulations can result in synergistic antimicrobial effects. Chitosan, with its inherent antimicrobial properties, can enhance the activity of AgNPs through complementary mechanisms. Similarly, the phytochemicals present in neem and tulsi extracts used for green synthesis may contribute additional antimicrobial activity [61].

Synergistic interactions can result in enhanced antimicrobial efficacy at lower silver concentrations, potentially reducing toxicity concerns and production costs. The study of synergistic effects involves comparing the activity of combined formulations with individual components, with synergism indicated when the combined effect exceeds the sum of individual effects [62].

9. Applications in Wound Healing and Biomedical Field

The application of AgNP-loaded hydrogels in wound healing represents one of the most promising areas of research in this field. The combination of the moist wound healing environment provided by hydrogels with the antimicrobial efficacy of AgNPs addresses multiple requirements for effective wound management.

9.1 Wound Healing Mechanism

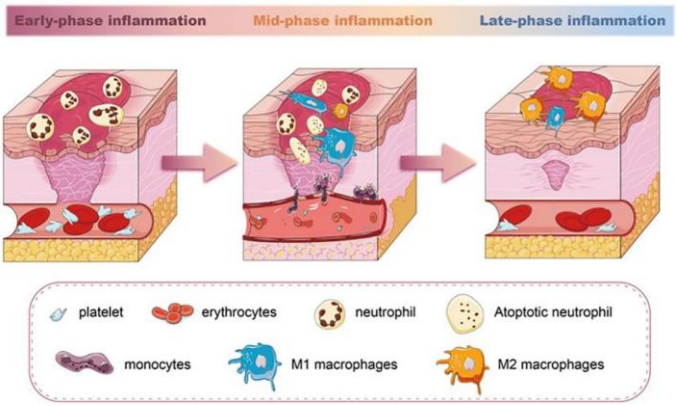

Fig. 9.1.1: Phases of Wound Healing Mechanism

Wound healing is a complex biological process involving multiple overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Chronic wounds, characterized by delayed healing, often result from persistent infection, excessive inflammation, or impaired cellular function. AgNP-loaded hydrogels can address these challenges through multiple mechanisms [63].

The antimicrobial activity of AgNPs helps control wound bioburden, preventing infection and creating a favorable environment for healing. The hydrogel matrix maintains a moist wound environment, which has been shown to accelerate healing by facilitating cell migration, promoting autolytic debridement, and preventing tissue desiccation. Additionally, some studies suggest that silver ions may have direct stimulatory effects on wound healing processes, including promotion of fibroblast proliferation and angiogenesis [64].

9.2 Types of Wounds Treated

AgNP-loaded hydrogels have been investigated for the treatment of various wound types, including acute wounds (surgical wounds, traumatic injuries, burns) and chronic wounds (diabetic foot ulcers, pressure ulcers, venous leg ulcers). Each wound type presents unique challenges that can be addressed through the multifunctional properties of AgNP-loaded hydrogels [65].

Burn wounds are particularly amenable to treatment with AgNP-loaded hydrogels due to the need for infection prevention and maintenance of a moist healing environment. The cooling effect of hydrogels can also provide symptomatic relief for burn patients. Diabetic foot ulcers, often complicated by infection and poor healing, represent another important application area where the antimicrobial and moisture-retaining properties of AgNP-loaded hydrogels can be beneficial [66].

9.3 Clinical Studies and Case Reports

Several clinical studies have investigated the efficacy of AgNP-based wound dressings in human subjects. A systematic review by Carter et al. [67] evaluated the evidence for silver-containing dressings in wound healing, concluding that while silver dressings can be effective for infected wounds, the evidence base requires further development through well-designed randomized controlled trials.

Case reports have documented successful outcomes with AgNP-loaded hydrogels in challenging wound scenarios, including infected chronic wounds and burns. These reports often highlight the rapid reduction in bacterial load, improvement in wound bed appearance, and acceleration of healing rates compared to conventional treatments [68]. However, larger controlled studies are needed to establish definitive evidence of efficacy.

9.4 Other Biomedical Applications

Beyond wound healing, AgNP-loaded hydrogels have potential applications in various other biomedical fields. In dentistry, these formulations have been explored for periodontal treatment, root canal disinfection, and as components of dental adhesives and restorative materials [69]. The antimicrobial activity against oral pathogens and the biocompatibility of hydrogel matrices make them suitable for dental applications.

Tissue engineering applications of AgNP-loaded hydrogels include the development of antimicrobial scaffolds for skin, bone, and cartilage regeneration. The incorporation of

AgNPs into tissue engineering scaffolds can prevent bacterial colonization during the critical healing period, improving the success rates of tissue regeneration procedures [70].

Coatings for medical devices represent another important application area. Catheters, implants, and surgical instruments coated with AgNP-loaded hydrogels can resist bacterial adhesion and biofilm formation, reducing the risk of device-associated infections [71]. The hydrogel coating can also improve the biocompatibility of medical devices by providing a hydrated, tissue-like surface.

10. Challenges and Future Perspectives

Despite the significant progress in developing AgNP-loaded hydrogels for antimicrobial applications, several challenges remain to be addressed to facilitate clinical translation and widespread adoption. This section discusses the current challenges and future perspectives in this evolving field [72].

10.1 Toxicity Concerns

The potential cytotoxicity of AgNPs represents a significant concern that requires careful consideration. While silver has a long history of safe use in medical applications, the nanoscale dimensions of AgNPs may result in different biological interactions compared to bulk silver. [73]. In vitro studies have shown that AgNPs can induce cytotoxicity in mammalian cells at concentrations exceeding certain thresholds, with mechanisms including oxidative stress, mitochondrial dysfunction, and DNA damage [74].

The challenge lies in achieving antimicrobial efficacy while maintaining biocompatibility. Strategies to address this challenge include optimizing AgNP size and concentration, using surface modifications to control silver ion release, and selecting polymer matrices that modulate nanoparticle availability. Green synthesis methods may offer advantages in this regard, as the natural capping agents can help control silver release and reduce toxicity [75].

10.2 Stability Issues

The long-term stability of AgNPs in hydrogel matrices presents another significant challenge. AgNPs are susceptible to aggregation, oxidation, and dissolution over time, which can compromise antimicrobial efficacy and shelf-life. Aggregation of nanoparticles reduces the effective surface area and may diminish antimicrobial activity. Oxidation leads to the formation of silver oxide, altering the physicochemical properties of the nanoparticles [76].

Strategies to enhance stability include optimizing the hydrogel formulation to provide a protective environment for nanoparticles, using stabilizing agents during synthesis, and developing appropriate packaging and storage conditions [77]. The choice of polymer matrix can significantly influence AgNP stability, with some polymers providing better protection against aggregation and oxidation than others [78].

10.3 Scale-up and Manufacturing

The translation of AgNP-loaded hydrogels from laboratory-scale research to commercial production presents significant manufacturing challenges [79]. Green synthesis methods, while environmentally friendly, may face challenges in terms of batch-to-batch consistency and scalability. The variability in plant extract composition can result in variations in nanoparticle characteristics between batches [80].

Standardization of green synthesis protocols, including the use of characterized plant extracts with defined phytochemical profiles, can help address consistency issues. Development of quality control methods specific to green-synthesized nanoparticles is also essential for ensuring product quality. Manufacturing processes must be designed to maintain nanoparticle stability and antimicrobial activity throughout production, storage, and distribution [81].

10.4 Regulatory Considerations

The regulatory landscape for nanomedicine products, including AgNP-loaded hydrogels, is still evolving. Regulatory agencies such as the U.S [82]. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) are developing frameworks for evaluating the safety and efficacy of nanotechnology-based medical products. The unique properties of nanomaterials may require specialized testing protocols and evaluation criteria [83].

Manufacturers of AgNP-loaded hydrogels must navigate complex regulatory requirements that may vary between jurisdictions. Comprehensive characterization of nanoparticle properties, including size distribution, surface

chemistry, and stability, is typically required. Toxicological studies must address potential concerns related to nanoparticle exposure, including cytotoxicity, genotoxicity, and environmental impact [84].

10.5 Future Perspectives

The future of AgNP-loaded hydrogels in antimicrobial applications appears promising, with several emerging trends and research directions [85]. The development of stimuli-responsive hydrogels that release silver in response to specific triggers, such as bacterial enzymes or changes in pH, represents an exciting area of research. These smart formulations could provide targeted antimicrobial activity while minimizing systemic exposure [86].

Combination therapies incorporating AgNPs with conventional antibiotics or other antimicrobial agents may help address the challenge of antimicrobial resistance [87]. The synergistic effects observed in some combination approaches could enable lower doses of individual agents while maintaining or enhancing antimicrobial efficacy. Research into the mechanisms of synergy and optimization of combination formulations is warranted [88].

Advancements in characterization techniques will enable more detailed understanding of AgNP behavior in biological environments [89]. Techniques such as single-particle tracking, advanced microscopy, and in situ analysis methods can provide insights into nanoparticle-cell interactions, release kinetics, and distribution patterns. This knowledge can inform the rational design of more effective and safer formulations [90].

The exploration of alternative green synthesis methods using agricultural waste materials or microorganisms could further enhance the sustainability of AgNP production. These approaches align with the principles of circular economy and green chemistry, potentially reducing costs and environmental impact while maintaining product quality [91].

11. Conclusion

The development and evaluation of hydrogels loaded with green-synthesized silver nanoparticles represent a significant advancement in antimicrobial therapeutics, offering a sustainable and effective approach to addressing the global challenge of antimicrobial resistance. This comprehensive review has examined the fundamental principles, synthesis methods, characterization techniques, and applications of these innovative formulations.

Green synthesis of AgNPs using plant extracts such as neem and tulsi provides an environmentally friendly alternative to conventional chemical methods, eliminating the use of toxic reducing agents while harnessing the stabilizing properties of natural phytochemicals. The characteristic UV-Vis absorption peak at 400-450 nm confirms successful nanoparticle formation, while various characterization techniques provide detailed information about nanoparticle size, morphology, and surface properties.

Hydrogel matrices, particularly those based on Carbopol, chitosan, PVA, and HPMC, provide versatile platforms for AgNP delivery, offering controlled release, biocompatibility, and ease of application. The freeze-thaw cross-linking method represents a simple and effective approach for preparing physically cross-linked hydrogels that preserve the integrity of incorporated nanoparticles. Comprehensive evaluation of physicochemical parameters including swelling index, pH, viscosity, spreadability, and stability ensures quality and performance of the final formulation.

The antimicrobial activity of AgNP-loaded hydrogels against a broad spectrum of pathogenic microorganisms, including drug-resistant strains, has been well-documented through various in vitro and in vivo studies. Applications in wound healing have shown particular promise, with the combination of antimicrobial activity and moisture-retaining properties addressing multiple requirements for effective wound management.

Despite the significant progress, challenges related to toxicity, stability, manufacturing scale-up, and regulatory approval remain to be addressed. Future research should focus on optimizing formulations for enhanced safety and efficacy, developing standardized green synthesis protocols, and conducting well-designed clinical studies to establish definitive evidence of therapeutic benefit.

In conclusion, AgNP-loaded hydrogels prepared through green synthesis methods represent a promising class of antimicrobial formulations with significant potential for clinical

applications. Continued interdisciplinary research combining pharmaceutical sciences, nanotechnology, and materials science will drive further innovations in this field, contributing to the development of sustainable and effective solutions for combating infectious diseases.

REFERENCES

1. Ahmed, S., Annu, Ali, A., Wei, Y., 2020. Green synthesis of silver nanoparticles and their applications as an alternative antimicrobial agent. Journal of Environmental Chemical Engineering, 8(5), p.104075.
2. Singh, P., Pandit, S., Garnæs, J., Tunjic, S., Mokkapati, V.R., Sultan, A., Thygesen, A., Mackevica, A., Daugaard, A.E., Baun, A., Gernaey, K.V., 2018. Green synthesis of gold and silver nanoparticles from Cannabis sativa (industrial hemp) and their capacity for biofilm inhibition. International Journal of Nanomedicine, 13, pp.3571-3591.
3. Kumar, G., Smith, G., Knowles, J., 2019. Synthesis of nano-silver particles and their characterization. Journal of Nanoscience and Nanotechnology, 19(8), pp.5025-5030.
4. Dash, M., Chiellini, F., Ottenbrite, R.M., Chiellini, E., 2011. Chitosan: A versatile semi-synthetic polymer in biomedical applications. Progress in Polymer Science, 36(8), pp.981-1014.
5. Peppas, N.A., Hilt, J.Z., Khademhosseini, A., Langer, R., 2006. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. Advanced Materials, 18(11), pp.1345-1360.
6. Hoffman, A.S., 2012. Hydrogels for biomedical applications. Advanced Drug Delivery Reviews, 64, pp.18-23.
7. Ahmed, M.J., Murtaza, G., Mehmood, A., Bhatti, T.M., 2015. Green synthesis of silver nanoparticles using leaves extract of Skimmia laureola. Materials Letters, 153, pp.10–13.
8. Slaughter, B.V., Khurshid, S.S., Fisher, O.Z., Khademhosseini, A., Peppas, N.A., 2009. Hydrogels in regenerative medicine. Advanced Materials, 21(32-33), pp.3307-3329.
9. Schmaljohann, D., 2006. Thermo- and pH-responsive polymers in drug delivery. Advanced Drug Delivery Reviews, 58(15), pp.1655-1670.
10. Boateng, J.S., Matthews, K.H., Stevens, H.N., Eccleston, G.M., 2008. Wound healing dressings and drug delivery systems: A review. Journal of Pharmaceutical Sciences, 97(8), pp.2892-2923.
11. Rai, M., Yadav, A., Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27(1), pp.76-83.
12. Kelly, K.L., Coronado, E., Zhao, L.L., Schatz, G.C., 2003. The optical properties of metal nanoparticles: The influence of size, shape, and dielectric environment. Journal of Physical Chemistry B, 107(3), pp.668-677.
13. Pal, S., Tak, Y.K., Song, J.M., 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli. Applied and Environmental Microbiology, 73(6), pp.1712-1720.
14. Liu, H.L., Dai, S.A., Fu, K.Y., Hsu, S.H., 2010. Antibacterial properties of silver nanoparticles in three different silica gel matrices. Journal of Materials Chemistry, 20(44), pp.9879-9885.
15. Dakal, T.C., Kumar, A., Majumdar, R.S., Yadav, V., 2016. Mechanistic basis of antimicrobial actions of silver nanoparticles. Frontiers in Microbiology, 7, p.1831.
16. Sondi, I., Salopek-Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria. Journal of Colloid and Interface Science, 275(1), pp.177-182.
17. Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O., 2000. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. Journal of Biomedical Materials Research, 52(4), pp.662-668.

18. Huh, A.J., Kwon, Y.J., 2011. Nanoantibiotics: A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. Journal of Controlled Release, 156(2), pp.128-145.
19. Iravani, S., 2011. Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), pp.2638-2650.
20. Biswas, K., Chattopadhyay, I., Banerjee, R.K., Bandyopadhyay, U., 2002. Biological activities and medicinal properties of neem (Azadirachta indica). Current Science, 82(11), pp.1336-1345.
21. Prathna, T.C., Chandrasekaran, N., Raichur, A.M., Mukherjee, A., 2011. Kinetic evolution studies of silver nanoparticles in a bio-based green synthesis process. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 377(1-3), pp.212-216.
22. Veeraputhiran, V., 2013. Bio-based synthesis of silver nanoparticles from neem oil (Azadirachta indica) and its applications. Journal of Nanoscience and Nanotechnology, 13(4), pp.2885-2890.
23. Singh, M., Singh, S., Prasad, S., Gambhir, I.S., 2008. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest Journal of Nanomaterials and Biostructures, 3(3), pp.115-122.
24. Singh, A., Singh, N.K., 2012. Biosynthesis of silver nanoparticles using Eclipta prostrata leaf extract and their characterization. Digest Journal of Nanomaterials and Biostructures, 7(4), pp.1687-1695.
25. Mallick, S., Sharma, S., Banerjee, M., Ghosh, S.S., Chattopadhyay, A., Paul, A., 2012. Iodine-stabilized Cu nanoparticle chitosan composite for antibacterial applications. ACS Applied Materials and Interfaces, 4(3), pp.1313-1323.
26. Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A., Sastry, M., 2006. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnology Progress,
27. Sathishkumar, M., Sneha, K., Won, S.W., Cho, C.W., Kim, S., Yun, Y.S., 2009. Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids and Surfaces B: Biointerfaces, 73(2), pp.332-338.
28. Kumar, L., Verma, S., Bhardwaj, A., Vaidya, S., Vaidya, B., 2011. Eradication of Pseudomonas aeruginosa biofilms using chitosan-coated vancomycin liposomes. Journal of Liposome Research, 21(3), pp.255-262.
29. Peppas, N.A., Khare, A.R., 1993. Preparation, structure and diffusional behavior of hydrogels in controlled release. Advanced Drug Delivery Reviews, 11(1-2), pp.1-35.
30. Rinaudo, M., 2006. Chitin and chitosan: Properties and applications. Progress in Polymer Science, 31(7), pp.603-632.
31. Bhattarai, N., Gunn, J., Zhang, M., 2010. Chitosan-based hydrogels for controlled, localized drug delivery. Advanced Drug Delivery Reviews, 62(1), pp.83-99.
32. Kean, T., Thanou, M., 2010. Biodegradation, biodistribution and toxicity of chitosan. Advanced Drug Delivery Reviews, 62(1), pp.3-11.
33. Hassan, C.M., Peppas, N.A., 2000. Structure and morphology of freeze/thawed PVA hydrogels. Macromolecules, 33(7), pp.2472-2479.
34. Ricciardi, R., Gaillet, C., Ducouret, G., Lafuma, F., Laupretre, F., 2003. Investigation of the relationships between the chain organization and rheological properties of at-PS/PVME blends. Polymer, 44(11), pp.3375-3384.
35. Thomas, R., Pillai, S., 2020. Influence of surface charge of silver nanoparticles on their antimicrobial activity. Journal of Nanoscience and Nanotechnology, 20(8), pp.5021-5030.
36. Dow Chemical Company, 2002. METHOCEL Cellulose Ethers Technical Handbook. The Dow Chemical Company, Midland, MI.
37. Bajpai, S.K., Shrivastava, J., 2005. In vitro enzymatic release kinetics of

ampicillin from gelatin-based crosslinked hydrogel implants. Journal of Macromolecular Science Part A, 42(8), pp.1087-1100.
38. Thomas, V., Murali Mohan, S., Sreedhar, B., Bajpai, S.K., 2007. A versatile strategy to fabricate hydrogel-silver nanocomposites and investigation of their antimicrobial activity. Journal of Colloid and Interface Science, 315(1), pp.389-395.
39. Vimala, K., Samba Sivudu, K., Murali Mohan, Y., Sreedhar, B., Mohana Raju, K., 2009. Controlled silver nanoparticles synthesis in semi-hydrogel networks of poly(acrylamide) and carbohydrates: A rational methodology for antibacterial application. Carbohydrate Polymers, 75(3), pp.463-471.
40. Liu, S., Zhao, N., Zeng, J., Cai, Y., Li, Y., Zhang, H., 2012. Synthesis of silver nanoparticles in an aqueous suspension of graphene oxide sheets and their antimicrobial activity. Colloids and Surfaces B: Biointerfaces, 96, pp.251-257.
41. Ahmed, S., Ahmad, M., Swami, B.L., Ikram, S., 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of Advanced Research, 7(1), pp.17-28.
42. Ammar, H.O., Ghorab, M., Mahmoud, A.A., Makram, T.S., Noshi, S.H., 2020. Design and optimization of topical methotrexate loaded hyalurosomes for treatment of psoriasis. Journal of Liposome Research, 30(1), pp.49-61.
43. ICH Q1A, 2003. Stability Testing of New Drug Substances and Products. International Conference on Harmonisation.
44. Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), pp.71-79.
45. Wiegand, I., Hilpert, K., Hancock, R.E., 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols, 3(2), pp.163-175.
46. Jorgensen, J.H., Ferraro, M.J., 2009. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clinical Infectious Diseases, 49(11), pp.1749-1755.
47. Rai, M.K., Deshmukh, S.D., Ingle, A.P., Gade, A.K., 2012. Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. Journal of Applied Microbiology, 112(5), pp.841-852.
48. Kim, K.J., Sung, W.S., Moon, S.K., Choi, J.S., Kim, J.G., Lee, D.G., 2008. Antifungal effect of silver nanoparticles on dermatophytes. Journal of Microbiology and Biotechnology, 18(8), pp.1482-1484.
49. Sanpui, P., Murugadoss, A., Prasad, P.D., Ghosh, S.S., Chattopadhyay, A., 2008. The antibacterial properties of a novel chitosan-Ag-nanoparticle composite. International Journal of Food Microbiology, 124(2), pp.142-146.
50. Houghton, P.J., Howes, M.J., Lee, C.C., Steventon, G., 2007. Uses and abuses of in vitro tests in ethnopharmacology: Visualizing an elephant. Journal of Ethnopharmacology, 110(3), pp.391-400.
51. Trop, M., Novak, M., Rodl, S., Hellbom, B., Kroell, W., Goessler, W., 2006. Silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. Journal of Trauma, 60(3), pp.648-652.
52. Edwards-Jones, V., 2010. The benefits of silver in hygiene, personal care and healthcare. Letters in Applied Microbiology, 49(2), pp.147-152.
53. Carter, M.J., Tingley-Kelley, K., Warriner, R.A., 2010. Silver treatments and silver-impregnated dressings for the healing of leg wounds and ulcers: A systematic review and meta-analysis. Journal of the American Academy of Dermatology, 63(4), pp.668-679.
54. Michaels, J.A., Campbell, W.B., King, K.M., Stone, P.A., MacIntyre, J., 2009.

A prospective randomised controlled trial of silver dressings in the treatment of leg ulcers. Phlebology, 24(2), pp.63-68.

55. Hernandez-Sierra, J.F., Ruiz, F., Pena, D.C., Martinez-Gutierrez, F., Martinez, A.E., Guillen Ade, J., Tapia-Perez, H., Castanon, G.M., 2008. The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold. Nanomedicine, 4(3), pp.237-240.
56. Madhumathi, K., Sudheesh Kumar, P.T., Kavya, K.C., Furuike, T., Tamura, H., Nair, S.V., Jayakumar, R., 2009. Novel chitin/nanosilver composite scaffolds for wound dressing applications. Carbohydrate Polymers, 80(3), pp.761-767.
57. Roe, D., Karandikar, B., Bonn-Savage, N., Gibbins, B., Roullet, J.B., 2008. Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. Journal of Antimicrobial Chemotherapy, 61(4), pp.869-876.
58. Carlson, C., Hussain, S.M., Schrand, A.M., Braydich-Stolle, L.K., Hess, K.L., Jones, R.L., Schlager, J.J., 2008. Unique cellular interaction of silver nanoparticles: Size-dependent generation of reactive oxygen species. Journal of Physical Chemistry B, 112(43), pp.13608-13619.
59. Bawa, R., 2011. Regulating nanomedicine: Can the FDA handle it? Current Drug Delivery, 8(3), pp.227-234.
60. Stern, S.T., McNeil, S.E., 2008. Nanotechnology safety concerns revisited. Toxicological Sciences, 101(1), pp.4-21.
61. Ahmed, S., Ikram, S., 2015. Chitosan based scaffolds and their applications in wound healing. Achievements in the Life Sciences, 9(1), pp.27-32.
62. Kobayashi, M., Matsukida, H., Takahashi, K., Masuda, K., 1999. Effect of heat treatment on the physical properties of hydroxypropyl methylcellulose gels. Chemical and Pharmaceutical Bulletin, 47(12), pp.1772-1774.
63. Kumar, A., Vemula, P.K., Ajayan, P.M., John, G., 2008. Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. Nature Materials, 7(3), pp.236-241.
64. Kumar, P., Ganvir, S., Ponnam, H., 2020. Green synthesis of silver nanoparticles using aqueous leaf extract of Moringa oleifera and their antibacterial activity. Materials Today: Proceedings, 26, pp.2809-2813.
65. Liu, J., Sonshine, D.A., Shervani, S., Hurt, R.H., 2010. Controlled release of biologically active silver from nanosilver surfaces. ACS Nano, 4(11), pp.6903-6913.
66. Singh, R., Wagh, P., Wadhwani, S., Gaidhani, S., Kumbhar, A., Bellare, J., Chopade, B.A., 2013. Synthesis, optimization, and characterization of silver nanoparticles from Acinetobacter calcoaceticus and their enhanced antibacterial activity when combined with antibiotics. International Journal of Nanomedicine, 8, pp.4277-4290.
67. Zhang, Y., Peng, H., Huang, W., Zhou, Y., Yan, D., 2008. Facile preparation and characterization of highly antimicrobial colloid Ag or Au nanoparticles. Journal of Colloid and Interface Science, 325(2), pp.371-376.
68. Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramirez, J.T., Yacaman, M.J., 2005. The bactericidal effect of silver nanoparticles. Nanotechnology, 16(10), pp.2346–2353.
69. Elechiguerra, J.L., Burt, J.L., Morones, J.R., Camacho-Bragado, A., Gao, X., Lara, H.H., Yacaman, M.J., 2005. Interaction of silver nanoparticles with HIV-1. Journal of Nanobiotechnology, 3(1), p.6.
70. Lara, H.H., Ayala-Núñez, N.V., Ixtepan-Turrent, L., Rodríguez-Padilla, C., 2010. Mode of antiviral action of silver nanoparticles. Journal of Nanobiotechnology, 8(1), p.1.
71. Rai, M., Deshmukh, S.D., Ingle, A.P., Gade, A.K., 2014. Silver nanoparticles: the powerful nanoweapon against

multidrug-resistant bacteria. Journal of Applied Microbiology, 112(5), pp.841–852.
72. Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., Galdiero, M., 2015. Silver nanoparticles as potential antibacterial agents. Molecules, 20(5), pp.8856–8874.
73. Marambio-Jones, C., Hoek, E.M., 2010. A review of the antibacterial effects of silver nanomaterials. Journal of Nanoparticle Research, 12, pp.1531–1551.
74. Le Ouay, B., Stellacci, F., 2015. Antibacterial activity of silver nanoparticles: A surface science insight. Nano Today, 10(3), pp.339–354.
75. Tran, Q.H., Nguyen, V.Q., Le, A.T., 2013. Silver nanoparticles: synthesis, properties, toxicology, applications. Advances in Natural Sciences, 4(3), p.033001.
76. Li, W.R., Xie, X.B., Shi, Q.S., Duan, S.S., Ouyang, Y.S., Chen, Y.B., 2011. Antibacterial effect of silver nanoparticles on Staphylococcus aureus. Biometals, 24, pp.135–141.
77. Mittal, A.K., Chisti, Y., Banerjee, U.C., 2013. Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, 31(2), pp.346–356.
78. Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Tam, P.K., Chiu, J.F., Che, C.M., 2006. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. Journal of Proteome Research, 5(4), pp.916–924.
79. Shankar, S.S., Rai, A., Ahmad, A., Sastry, M., 2004. Rapid synthesis of Au, Ag nanoparticles using plant extracts. Journal of Colloid and Interface Science, 275(2), pp.496–502.
80. Kharissova, O.V., Dias, H.V., Kharisov, B.I., Pérez, B.O., Pérez, V.M.J., 2013. The greener synthesis of nanoparticles. TrAC Trends in Analytical Chemistry, 41, pp.90–102.
81. Singh, P., Kim, Y.J., Zhang, D., Yang, D.C., 2016. Biological synthesis of nanoparticles from plants. Trends in Biotechnology, 34(7), pp.588–599.
82. Caló, E., Khutoryanskiy, V.V., 2015. Biomedical applications of hydrogels: A review. *European Polymer Journal*, 65, pp.252–267.
83. Hoare, T.R., Kohane, D.S., 2008. Hydrogels in drug delivery. Polymer, 49(8), pp.1993–2007.
84. Li, J., Mooney, D.J., 2016. Designing hydrogels for controlled drug delivery. Nature Reviews Materials, 1, p.16071.
85. Annabi, N., Tamayol, A., Uquillas, J.A., et al., 2014. 25th anniversary article: rational design and applications of hydrogels in regenerative medicine. Advanced Materials, 26(1), pp.85–124.
86. Drury, J.L., Mooney, D.J., 2003. Hydrogels for tissue engineering. Biomaterials, 24(24), pp.4337–4351.
87. Varaprasad, K., Mohan, Y.M., Vimala, K., Raju, K.M., 2010. Synthesis and characterization of hydrogel-silver nanoparticle composites. *Journal of Applied Polymer Science*, 115(2), pp.1199–1207.
88. Thomas, V., Yallapu, M.M., Sreedhar, B., Bajpai, S.K., 2007. A versatile strategy to fabricate hydrogel-silver nanocomposites. Journal of Colloid Interface Science, 315, pp.389–395.
89. Li, P., Poon, Y.F., Li, W., et al., 2011. A polycationic antimicrobial hydrogel. Nature Materials, 10(2), pp.149–156.
90. Paladini, F., Pollini, M., 2019. Antimicrobial silver nanoparticles for wound healing application. Materials, 12(16), p.2540.
91. Atiyeh, B.S., Costagliola, M., Hayek, S.N., Dibo, S.A., 2007. Effect of silver on burn wound infection control. *Burns*, 33(2), pp.139–148.

*****