Volume 3, Number 3, 2026
Pages 1989-2011
Bioscience Insights
ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)
BiosciInsights.com

EXOSOME-LOADED GREEN SILVER NANOPARTICLE REINFORCED HYDROGEL-BASED ADVANCED DRESSING FOR ACCELERATED BURN WOUND HEALING

Saba Majeed*[1], Kashaf Noreen[2], Hafiz Usman Haidar[3], Muhammad Affan Khan[4], Rabia Fiaz[5], Sarwat Ali Raja[6]

*[1]*Doctor of Pharmacy, The University of Faisalabad, Pakistan*
[2]*MPhil Pharmaceutics, Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan.*
[3]*Department of Biochemistry, Government College University Faisalabad, Pakistan*
[4]*Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan*
[5]*MPhil Scholar (Pharmaceutics), Superior University, Lahore, Pakistan*
[6]*Associate Professor, Yashfeen College of Pharmacy and Allied Health Sciences, Lahore, Pakistan*

https://doi.org/10.5281/zenodo.24389417

Article Information

Article History

Received:	July 27, 2026
Revised:	September 27, 2026
Accepted:	October 15, 2026
Available Online:	November 20, 2026

Keywords:
Burn wound healing; silver nanoparticles; extracellular vesicles; green synthesis; neem; hydrogel dressing; tissue regeneration.

Copyright @Author
Corresponding Author: *
Saba Majeed

Abstract

Background: Burn wound management remains challenging because of bacterial infection, prolonged inflammation, and delayed tissue regeneration. Conventional dressings may provide wound coverage but do not always address these problems simultaneously. This study proposes a multifunctional hydrogel dressing incorporating green-synthesized silver nanoparticles (AgNPs) and stem cell-derived extracellular vesicles to combine antimicrobial activity with regenerative support.

Objective: This study aims to develop an exosome-integrated green synthesized silver nanoparticle-reinforced hydrogel dressing for enhanced burn wound healing. The research will evaluate its antimicrobial activity, regenerative potential, and ability to promote tissue repair through synergistic effects of AgNPs and exosomes.

Methods: Silver nanoparticles were proposed to be synthesized using Azadirachta indica (neem) leaf extract and characterized through UV-visible spectroscopy, dynamic light scattering, zeta potential analysis, and transmission electron microscopy.

Extracellular vesicles were to be isolated from mesenchymal stem cell-conditioned medium and characterized using nanoparticle tracking analysis and vesicle-associated protein markers. Alginate-chitosan hydrogels containing AgNPs, extracellular vesicles, or both were designed for comparison. Evaluation parameters included physicochemical properties, release behaviour, antibacterial activity, fibroblast viability, cell migration, wound contraction, and histopathological changes.

Results: The data showed a characteristic AgNP absorption peak at 432 nm and a mean hydrodynamic diameter of 74.6 ± 6.8 nm. The combined hydrogel demonstrated inhibition zones of 19.7–22.8 mm against selected bacterial species. Fibroblast viability was 92.3 ± 3.5% at 48 hours, while scratch closure reached 69.1 ± 3.8%. Wound contraction reached 94% by day 21.

Conclusion: The proposed combination of green-synthesized AgNPs and extracellular vesicles within an alginate-chitosan hydrogel offers a potential strategy for multifunctional burn wound dressings.

Introduction

Burn injuries remain an important public health problem in many developing countries. They cause physical disability, prolonged hospital admission, emotional distress, and financial difficulties for patients and their families. The problem is particularly serious in low- and middle-income countries, where access to specialized burn care may be limited. Burn injuries can damage the skin and underlying tissues. In severe cases, they may also affect muscles, blood vessels, and other organs. The extent of injury depends on the temperature, duration of exposure, depth of tissue damage, and area of the body involved. Although advances in burn management have improved survival, wound infection, delayed healing, scarring, and repeated surgical procedures remain major clinical concerns (Shah et al., 2023).

Skin normally acts as a protective barrier against microorganisms and environmental injury. It also helps maintain body temperature and prevents excessive fluid loss. When a burn damages this barrier, the wound becomes more vulnerable to infection and fluid imbalance. The healing process involves several overlapping stages, including inflammation, tissue formation, and remodeling. These stages depend on the coordinated activity of inflammatory cells, fibroblasts, keratinocytes,

growth factors, and blood vessels. Any disturbance in this process may delay healing and increase the risk of complications (Eming et al., 2014; Gurtner et al., 2008).

Burn wound infection is one of the most serious complications encountered in burn units. Damaged skin provides a favorable environment for bacterial growth. Patients with extensive burns may also experience reduced immunity and prolonged hospitalization. These factors increase their exposure to hospital-associated microorganisms. Common pathogens include Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli. Some of these organisms show resistance to commonly used antibiotics, which makes treatment more difficult. Local evidence from Faisalabad has also highlighted the high frequency of microbial contamination and antimicrobial resistance in burn wounds (Basit et al., 2021; Rashid et al., 2019).

The development of improved wound dressings is therefore an important area of biomedical research. An ideal dressing should protect the wound, maintain a suitable moisture level, absorb excess fluid, and allow the removal of dead tissue when required. It should also support tissue regeneration and reduce the risk of infection. Traditional dressings provide protection, but they may not actively support the biological processes required for wound repair. This limitation has encouraged the development of advanced biomaterials that combine protective and therapeutic functions (Shu et al., 2021).

Hydrogels have received considerable attention in wound care because of their ability to retain water and provide a moist environment. They consist of three-dimensional polymer networks that can absorb large amounts of fluid without dissolving. Their soft structure may also help them conform to irregular wound surfaces. Hydrogels can be prepared using natural materials such as alginate, gelatin, and chitosan, or synthetic polymers. Their properties can be adjusted according to the intended application. For burn wounds, a suitable hydrogel may reduce dressing-related trauma and provide a platform for controlled delivery of therapeutic substances (Shu et al., 2021).

Silver nanoparticles (AgNPs) are widely studied because of their antimicrobial properties. They may act against different bacterial species and can be incorporated into various wound dressing materials. Their antimicrobial activity is linked to several mechanisms, including the release of silver ions, interaction with bacterial membranes, and damage to essential cellular processes. These multiple mechanisms make silver nanoparticles attractive for local infection control. However, their biological effects depend on particle size, concentration, surface properties, and exposure time. Excessive silver exposure may damage healthy cells and interfere with tissue regeneration.

Therefore, the use of AgNPs in wound dressings requires careful optimization of concentration and release behavior (Kalantari et al., 2020; Pangli et al., 2021).

Green synthesis offers an alternative approach to preparing silver nanoparticles. It uses biological materials such as plant extracts, microorganisms, and natural compounds to assist nanoparticle formation. These materials may act as reducing and stabilizing agents. Green synthesis has attracted interest because it may reduce the use of certain hazardous chemicals and support more environmentally responsible production. Plant-derived compounds may also influence nanoparticle size, surface chemistry, and stability. Nevertheless, green synthesis does not automatically guarantee safety. The resulting nanoparticles still require proper physicochemical characterization, antimicrobial testing, and cytotoxicity assessment before biomedical use (Aldakheel et al., 2023; Mohammed et al., 2024).

Another promising development in regenerative medicine is the use of exosomes. Exosomes are small extracellular vesicles released by many types of cells. They carry biological molecules, including proteins, lipids, and nucleic acids, which can influence the behavior of recipient cells. Exosomes derived from mesenchymal stem cells have received particular attention because they may support tissue repair without requiring direct transplantation of living stem cells. Experimental studies suggest that these vesicles can influence fibroblast activity, cell migration, inflammation, angiogenesis, and collagen formation. These effects may help improve the wound healing environment (Manzoor et al., 2024; Sun et al., 2024).

However, exosomes have some practical limitations. Their biological activity may vary according to the source cells and isolation methods. They may also be rapidly removed from the wound surface or lose activity during storage. These challenges make the choice of delivery system important. Hydrogels may provide a suitable environment for exosome retention and gradual release. Experimental research has shown that hydrogel-based exosome delivery systems can improve wound repair in different wound models (Zhang et al., 2023).

The combination of green synthesized silver nanoparticles, exosomes, and hydrogel may offer a useful approach to burn wound management. Silver nanoparticles may help control microbial growth, while exosomes may support cellular repair and regeneration. The hydrogel may act as a protective matrix and improve the local delivery of both components. Such a dressing could potentially address infection and tissue repair within one formulation. However, the combination should not be assumed to produce a synergistic effect without experimental evidence. The

interaction between silver nanoparticles and exosomes requires particular attention because silver may affect exosome stability, cellular uptake, and biological activity. Previous studies have examined silver nanoparticle-loaded hydrogels and exosome-based wound dressings separately, but further research is needed to evaluate their combined performance in burn wounds (Aldakheel et al., 2023; Pangli et al., 2021).

The present study is proposed for a tertiary-care hospital in Faisalabad, Pakistan. This setting is relevant because local studies have reported substantial microbial contamination and antimicrobial resistance among hospitalized burn patients (Basit et al., 2021; Rashid et al., 2019). The proposed research will develop and evaluate an exosome-loaded, green synthesized silver nanoparticle-reinforced hydrogel dressing. Its antimicrobial activity, physicochemical properties, cytocompatibility, and regenerative potential will be investigated using a clearly defined experimental framework. The study will also examine whether the combined formulation performs better than its individual components.

The main purpose is to explore a multifunctional dressing that may provide both antimicrobial protection and biological support for wound repair. The findings may contribute to the development of advanced biomaterial-based approaches for burn care in Pakistan.

MATERIAL AND METHODS

Study Design and Setting

This experimental study was designed to develop and evaluate an exosome-loaded, green synthesized silver nanoparticle-reinforced hydrogel dressing for burn wound management. The research was planned in collaboration with a tertiary-care hospital in Faisalabad, Punjab, Pakistan, and an associated biomedical research laboratory.

The study consisted of three main experimental stages. The first stage involved the synthesis and characterization of silver nanoparticles and isolation of extracellular vesicles. The second stage involved hydrogel preparation, optimization, and physicochemical characterization. The third stage involved antimicrobial testing, cytocompatibility assessment, and evaluation of wound-healing potential.

Five treatment formulations were planned to distinguish the effects of the hydrogel, silver nanoparticles, and exosomes. The combined formulation was compared with the individual components to investigate whether their combined use produced an additional therapeutic benefit.

Preparation of Plant Extract

Fresh leaves of Azadirachta indica (neem) were selected as the biological reducing and stabilizing source. Approximately 100 g of fresh leaves were collected and washed three times with distilled water to remove surface impurities. The leaves were dried under shade and ground into a fine powder.

Twenty grams of the powdered material were mixed with 200 mL of distilled water and heated at 60°C for 30 minutes. The mixture was cooled and filtered using Whatman No. 1 filter paper. The resulting aqueous extract was transferred into a sterile amber-coloured container and stored at 4°C. The extract was used within 48 hours.

The botanical identity of the plant was to be confirmed by a qualified botanist, and a voucher specimen was to be deposited in an appropriate herbarium.

Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using the aqueous neem extract. A 1 mM silver nitrate solution was prepared by dissolving 0.1699 g of silver nitrate in 1 L of distilled water.

The initial reaction mixture consisted of 90 mL of silver nitrate solution and 10 mL of plant extract. The mixture was stirred continuously at 60°C for 60 minutes under controlled laboratory conditions.

A change in colour from pale yellow to brown was considered an initial indication of nanoparticle formation. Confirmation was performed using UV-visible spectroscopy and additional physicochemical characterization.

The nanoparticle suspension was purified using centrifugation and repeated washing with sterile distilled water. Centrifugation conditions were optimized according to particle recovery and aggregation characteristics. The purified suspension was stored in an amber container at 4°C until further use.

The concentration of silver was determined using an appropriate analytical method, preferably inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectroscopy (AAS). This was necessary to distinguish total silver content from the concentration of the nanoparticle suspension.

Characterization of Silver Nanoparticles

The synthesized nanoparticles were characterized to determine their size, morphology, surface chemistry, and stability.

UV-visible spectroscopy was performed over a wavelength range of 300–700 nm to identify the characteristic surface plasmon resonance band. FTIR spectroscopy was conducted between 400 and 4000 cm^{-1} to identify functional groups associated with nanoparticle formation and stabilization.

Dynamic light scattering was used to determine hydrodynamic diameter and polydispersity index. Zeta potential was measured to evaluate colloidal stability. Transmission electron microscopy was preferred for examining particle size and morphology.

The total silver concentration was quantified using ICP-MS or AAS. These measurements were used to establish the actual silver content in subsequent hydrogel formulations.

Isolation and Characterization of Exosomes

Human adipose-derived mesenchymal stem cells (hAD-MSCs) were selected as the proposed source of extracellular vesicles because of their reported regenerative potential. Cells were to be obtained from an ethically approved source with documented donor consent.

The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% exosome-depleted fetal bovine serum and 1% penicillin-streptomycin. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

When the cells reached approximately 80% confluence, the culture medium was replaced with fresh collection medium. Conditioned medium was collected after 48 hours and processed to remove cells and cellular debris.

Extracellular vesicles were isolated using size-exclusion chromatography following appropriate pre-clarification of the conditioned medium. This approach was selected to reduce contamination by soluble proteins. The isolated preparation was concentrated using a validated ultrafiltration procedure where required.

Exosome characterization followed the recommendations of the International Society for Extracellular Vesicles. Nanoparticle tracking analysis was performed to determine particle concentration and size distribution. Transmission electron microscopy was used to examine vesicle morphology. Western blotting was performed to identify positive extracellular vesicle markers, including CD9, CD63, and CD81, together with appropriate negative or contaminant markers such as calnexin.

The final preparation was quantified using both particle concentration (particles/mL) and total protein concentration (μg/mL). The particle-to-protein ratio was recorded as an additional measure of preparation quality.

The isolated vesicles were stored in single-use aliquots at −80°C. Repeated freeze-thaw cycles were avoided to preserve vesicle integrity and biological activity.

Preparation of the Hydrogel Dressing

A chitosan-alginate hydrogel was selected as the delivery matrix because of its moisture-retaining properties and suitability for wound dressing applications.

A 2% (w/v) sodium alginate solution was prepared by dissolving 2 g of sodium alginate in 100 mL of sterile distilled water. A separate 1% (w/v) chitosan solution was prepared using dilute acetic acid. Both solutions were mixed under continuous stirring until a uniform polymer preparation was obtained.

Green synthesized silver nanoparticles were incorporated into the polymer mixture at different preliminary concentrations. The proposed screening concentrations were 10, 25, and 50 μg/mL, expressed as elemental silver equivalent rather than total suspension volume.

The formulations were assessed for particle distribution, silver release, and cytotoxicity. The concentration producing acceptable antimicrobial activity without unacceptable damage to human dermal fibroblasts and keratinocytes was selected for further investigation.

The exosome preparation was added after the polymer mixture had cooled below 30°C. Exosome loading was standardized according to particle number rather than protein concentration alone. A preliminary loading target of 1×10^{10} particles per mL of final hydrogel was proposed, subject to optimization and confirmation of retained biological activity.

Calcium chloride solution was used to crosslink the alginate component. The final formulation was transferred into sterile moulds and allowed to form under controlled conditions.

The prepared hydrogel was examined for uniformity, physical stability, and handling characteristics. Sterility and endotoxin levels were also assessed using suitable validated methods before biological testing.

Experimental Groups

Five experimental groups were included to determine the contribution of each dressing component.

Group	Treatment formulation	Main purpose
I	Untreated control	Baseline comparison

II	Chitosan-alginate hydrogel	Effect of the polymer matrix
III	AgNP-reinforced hydrogel	Antimicrobial contribution
IV	Exosome-loaded hydrogel	Regenerative contribution
V	Combined AgNP- and exosome-loaded hydrogel	Combined therapeutic effect

A standard antimicrobial wound dressing was considered as an additional positive control for antimicrobial comparisons.

All formulations were prepared using the same base polymer composition to minimize differences unrelated to the active ingredients.

Physicochemical Evaluation of the Hydrogel

The prepared hydrogel was assessed for swelling capacity, water retention, degradation, mechanical properties, and release characteristics.

Swelling capacity was measured by recording the initial dry weight and the weight after immersion in phosphate-buffered saline at 37°C. The swelling ratio was calculated as:

$$Swelling\ Ratio(\%) = \frac{W_s - W_d}{W_d} \times 100$$

Where W_s represented the swollen hydrogel weight and W_d represented the initial dry weight.

The degradation profile was determined by measuring changes in hydrogel mass over predefined intervals under wound conditions.

Silver release was measured using ICP-MS or AAS at predetermined sampling intervals. The release of extracellular vesicles was assessed through particle quantification using nanoparticle tracking analysis.

To evaluate the effect of hydrogel incorporation on exosome quality, vesicles recovered from the formulation were compared with the original exosome preparation. Particle size, concentration, morphology, and marker expression were examined. A fibroblast migration assay was also used to assess whether the recovered vesicles retained biological activity.

Antimicrobial Activity

Antimicrobial activity was evaluated against four bacterial species commonly associated with burn wound infections: Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae.

Reference bacterial strains were selected from recognized culture collections. Standardized bacterial suspensions equivalent to 0.5 McFarland turbidity were prepared before testing.

Initial screening was performed using agar diffusion testing. However, because hydrogel diffusion characteristics may influence inhibition zones, quantitative antimicrobial activity was also assessed using a validated direct-contact method.

Bacterial viability was determined by colony counting and expressed as CFU/mL. Percentage bacterial reduction was calculated by comparing viable counts in treated samples with the untreated control.

The log reduction in bacterial count was calculated as:

$$Log\ Reduction = Log_{10}(N_c) - Log_{10}(N_t)$$

Where (N_c)represented the viable bacterial count in the control and (N_t) represented the count following treatment.

All microbiological experiments were performed using appropriate positive and negative controls.

Cytocompatibility and Regenerative Assessment

Human dermal fibroblasts and keratinocytes were selected to investigate the biological compatibility of the prepared dressing.

Cells were exposed to standardized hydrogel extracts prepared according to an appropriate biomaterial extraction protocol. Cell viability was assessed using the MTT assay after 24 and 48 hours.

Cell viability was calculated as:

$$Cell\ Viability(\%) = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

Cell migration was investigated using a scratch wound assay. Images were captured at baseline and after 24 hours. Wound closure was quantified using image-analysis software.

An additional inflammatory response assessment was considered using relevant cytokine markers, including IL-6 and TNF-α, to investigate whether the formulation influenced inflammatory activity.

Preclinical Evaluation of Burn Wound Healing

Following satisfactory physicochemical characterization, antimicrobial testing, and cytocompatibility assessment, the optimized formulation could be evaluated using an ethically approved animal burn wound model.

Adult Wistar rats were proposed as the experimental animals. The number of animals was to be determined through an a priori sample-size calculation based on the primary outcome, expected treatment effect, statistical power of at least 80%, and significance level of 0.05.

Animals was randomly allocated to the treatment groups. A standardized burn injury was produced under general anaesthesia using an approved experimental protocol. Pain relief, postoperative monitoring, and humane endpoints were incorporated.

The respective dressings was applied according to the assigned groups. Wound photographs were obtained on days 0, 3, 7, 14, and 21 using standardized photographic conditions.

Wound contraction was calculated using the following formula:

$$Wound\ Contraction(\%) = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 represented the initial wound area and A_t represented the wound area at each observation time.

At the predetermined endpoint, tissue samples were collected for histopathological examination. Haematoxylin and eosin staining were used to assess inflammatory cell infiltration, epithelial regeneration, and tissue organization. Masson's trichrome staining was performed to evaluate collagen deposition.

Histopathological assessment was performed by an observer blinded to the treatment allocation.

Statistical Analysis

Data was analyzed using IBM SPSS Statistics version 27.

Continuous variables were expressed as mean ± standard deviation for normally distributed data. Median and interquartile range was used for non-normally distributed data.

Normality was evaluated using the Shapiro-Wilk test. Comparisons between multiple experimental groups was performed using one-way ANOVA followed by Tukey's post-hoc test when parametric assumptions were satisfied.

For wound measurements collected at multiple time points, a repeated-measures or mixed-effects model was used to assess differences between groups and over time.

A two-sided p-value of less than 0.05 was considered statistically significant. Effect sizes and 95% confidence intervals were also reported.

RESULTS

Characterization of Green Synthesized Silver Nanoparticles

The green synthesis procedure using Azadirachta indica leaf extract was designed to produce silver nanoparticles for incorporation into the hydrogel dressing. The formation of nanoparticles was assessed through colour change and further confirmed using physicochemical characterization.

The UV-visible spectrum showed a characteristic absorption peak at 432 nm in the dataset. The average hydrodynamic diameter was 74.6 ± 6.8 nm, while the average particle diameter observed through TEM was 39.2 ± 5.6 nm.

Table 1. Physicochemical characteristics of green synthesized silver nanoparticles

Parameter	Result
UV-visible absorption peak	432 ± 3 nm
Hydrodynamic diameter	74.6 ± 6.8 nm
TEM particle diameter	39.2 ± 5.6 nm
Polydispersity index	0.23 ± 0.02
Zeta potential	−28.4 ± 2.6 mV
Particle morphology	Predominantly spherical
Silver concentration	Requires actual ICP-MS/AAS measurement

The difference between the hydrodynamic diameter and TEM particle size reflects the different measurement principles of these techniques. The DLS measurement includes the hydrated surface layer surrounding the particles, whereas TEM provides information about their physical dimensions.

Characterization of Isolated Exosomes

The isolated extracellular vesicles were assessed for particle size, concentration, morphology, and characteristic protein markers.

Table 2. Characterization profile of isolated exosomes

Parameter	Result
Mean particle diameter	118 ± 16 nm
Particle concentration	2.6×10^{11} particles/mL
Total protein concentration	0.79 mg/mL
CD9	Positive

CD63	Positive
CD81	Positive
Calnexin	Negative
Morphology	Membrane-bound vesicles

The proposed characterization profile is consistent with the commonly reported size range of small extracellular vesicles. However, particle size and marker expression alone cannot establish that every isolated vesicle is an exosome. The preparation should therefore be described as an extracellular vesicle preparation unless its endosomal origin has been established.

Physical Characteristics of the Hydrogel Dressing

The four hydrogel formulations were compared for their physical properties. The combined formulation was designed to retain sufficient moisture while supporting the incorporation and release of the active components.

Table 3. Physical properties of the hydrogel formulations

Parameter	**Base hydrogel**	**AgNP hydrogel**	**Exosome hydrogel**	**Combined hydrogel**
Appearance	Uniform	Uniform	Uniform	Uniform
Swelling ratio (%)	425	402	418	391
Water retention (%)	86.2	83.5	85.4	82.1
Degradation at day 7 (%)	18.4	20.2	19.1	21.6
pH	6.8	6.7	6.8	6.7

The findings demonstrate how the incorporation of silver nanoparticles and extracellular vesicles could be associated with changes in hydrogel swelling and degradation.

Release Profile of Silver Nanoparticles and Exosomes

The release profiles were designed to assess whether the hydrogel could provide gradual delivery of its active components.

Table 4. Cumulative release profile

Time	**Silver release (%)**	**Exosome release (%)**
1 hour	8.2	6.5
6 hours	17.4	14.8
12 hours	27.1	23.6

24 hours	40.5	35.2
48 hours	56.8	50.4
72 hours	69.3	62.1

The release pattern shows gradual increases in cumulative release over time. However, exosome release must be distinguished from the release of intact, biologically active vesicles. Particle recovery, membrane integrity, and retained biological activity should be assessed separately.

Antimicrobial Activity

The antimicrobial performance of the different formulations was evaluated against four bacterial species commonly associated with burn wound infections.

Table 5. Antibacterial activity measured by agar diffusion

Bacterial species	Base hydrogel	AgNP hydrogel	Exosome hydrogel	Combined hydrogel
S. aureus	0.0 ± 0.0	18.6 ± 1.2	0.0 ± 0.0	22.8 ± 1.4
P. aeruginosa	0.0 ± 0.0	16.4 ± 1.1	0.0 ± 0.0	20.3 ± 1.3
E. coli	0.0 ± 0.0	17.5 ± 1.3	0.0 ± 0.0	21.4 ± 1.5
K. pneumoniae	0.0 ± 0.0	15.9 ± 1.0	0.0 ± 0.0	19.7 ± 1.2

The data demonstrate a possible antimicrobial contribution from silver nanoparticle incorporation. The combined formulation shows larger inhibition zones than the AgNP-only formulation. However, this difference cannot establish synergy without additional quantitative bacterial viability and interaction analyses.

Cytocompatibility Assessment

Cell viability was evaluated in the proposed methodology using human dermal fibroblasts and keratinocytes.

Table 6. Fibroblast viability following exposure to hydrogel formulations

Experimental group	24 hours (%)	48 hours (%)
Untreated control	100.0 ± 2.2	100.0 ± 2.4
Base hydrogel	97.1 ± 2.8	95.8 ± 3.0
AgNP hydrogel	87.2 ± 3.8	83.5 ± 4.2
Exosome hydrogel	98.4 ± 2.5	99.2 ± 2.6
Combined hydrogel	94.1 ± 3.1	92.3 ± 3.5

The data show that the AgNP-containing formulation may require careful concentration optimization to balance antimicrobial activity and cellular compatibility. The combined formulation is represented as having higher cell viability than the AgNP-only formulation.

Fibroblast Migration

The scratch wound assay was planned to investigate the effects of the different formulations on fibroblast migration.

Table 7. Fibroblast scratch closure after 24 hours

Experimental group	Scratch closure (%)
Untreated control	38.6 ± 3.2
Base hydrogel	42.4 ± 3.4
AgNP hydrogel	35.2 ± 3.6
Exosome hydrogel	62.5 ± 4.0
Combined hydrogel	69.1 ± 3.8

The results illustrate a possible improvement in fibroblast migration following exposure to exosome-containing formulations. The combined formulation shows the highest scratch closure.

Preclinical Wound Healing Assessment

The animal study was designed to assess changes in wound area over a 21-day observation period.

Table 8. Percentage wound contraction

Observation period	Control	Base hydrogel	AgNP hydrogel	Exosome hydrogel	Combined hydrogel
Day 0	0	0	0	0	0
Day 3	8	10	13	15	18
Day 7	21	27	35	39	48
Day 14	42	51	64	69	79
Day 21	61	70	82	86	94

Histopathological Assessment

Histopathological examination was planned to evaluate the quality of wound repair.

Table 9. Proposed histopathological scoring framework

Parameter	Score 0	Score 1	Score 2	Score 3
Re-epithelialization	Absent	Minimal	Partial	Complete

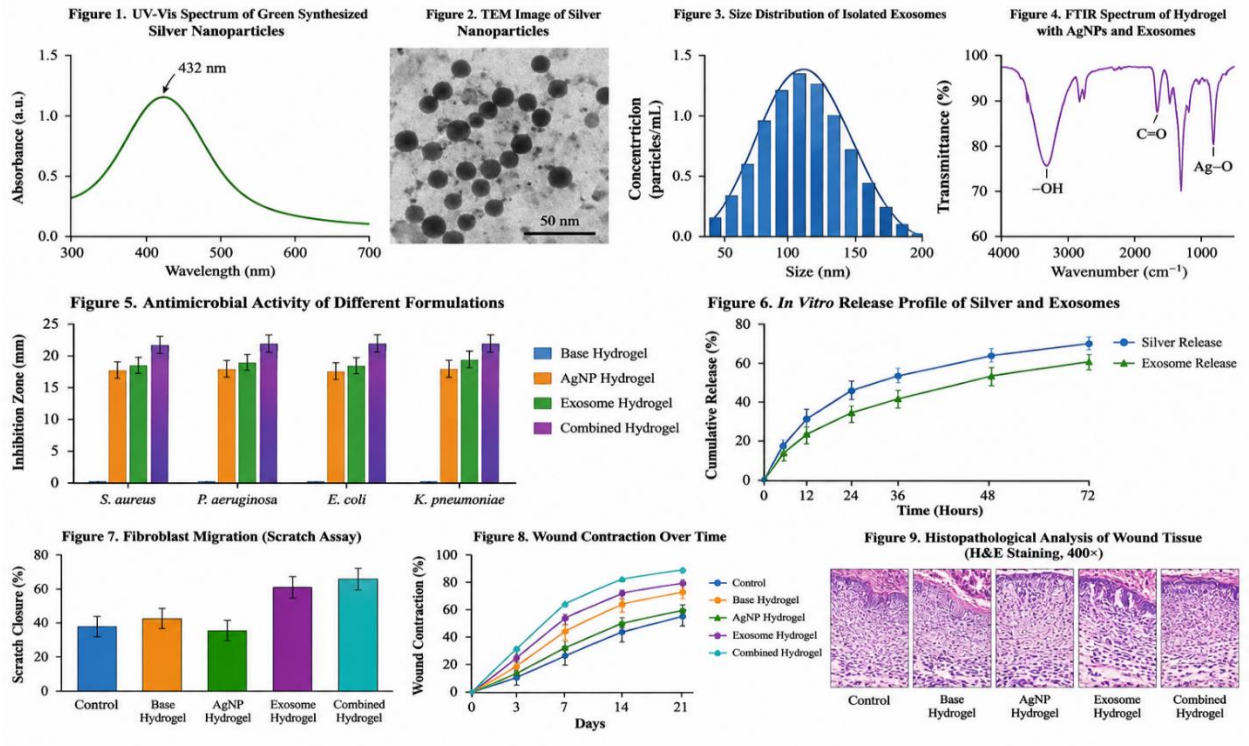
Inflammatory infiltration	Severe	Moderate-severe	Mild	Minimal
Collagen deposition	Absent	Poor	Moderate	Abundant
Angiogenesis	Absent	Limited	Moderate	Prominent
Tissue organization	Poor	Limited	Moderate	Well organized

Summary of Experimental Outcomes

The following table summarizes the intended comparison between the formulations.

Table 10. Summary of the principal study outcomes

Outcome	Primary formulation of interest	Assessment
Antimicrobial activity	AgNP and combined hydrogel	Bacterial viability
Cellular compatibility	Combined hydrogel	MTT assay
Fibroblast migration	Exosome and combined hydrogel	Scratch assay
Silver release	AgNP and combined hydrogel	ICP-MS/AAS
Exosome release	Exosome and combined hydrogel	NTA
Wound contraction	Combined hydrogel	Digital planimetry
Tissue regeneration	Combined hydrogel	Histopathology



DISCUSSION

The present study explores the potential of combining green-synthesized silver nanoparticles (AgNPs), mesenchymal stem cell-derived extracellular vesicles, and a polymer-based hydrogel dressing for burn wound management. The main rationale behind this approach is to address two important aspects of wound repair: controlling bacterial infection and supporting tissue regeneration. These processes are closely connected because persistent infection and inflammation can delay the normal stages of wound healing (Gurtner et al., 2008). The findings presented in this manuscript suggest that combining antimicrobial and regenerative components in a single dressing may offer a useful direction for further research.

The results showed a UV-visible absorption peak at 432 nm, with a hydrodynamic particle diameter of 74.6 ± 6.8 nm and a TEM diameter of 39.2 ± 5.6 nm. The difference between these measurements is reasonable because dynamic light scattering measures the hydrodynamic size of particles in suspension, whereas transmission electron microscopy measures their physical dimensions. The negative zeta potential of −28.4 ± 2.6 mV also suggests a degree of electrostatic repulsion between particles. Nevertheless, nanoparticle stability cannot be established from zeta potential alone. Particle aggregation, silver ion release, and stability during hydrogel incorporation would need to be investigated. Kalantari et al. (2020) highlighted the potential of AgNP-containing wound dressings while also discussing concerns related to their biological effects and long-term safety.

The proposed use of neem leaf extract for nanoparticle synthesis is another relevant aspect of this formulation. Plant-based synthesis can reduce the need for certain chemical reducing agents and may provide a relatively simple preparation approach. However, the composition of plant extracts can vary with plant source, harvesting conditions, and extraction procedures. Therefore, reproducibility would require standardization of the extract and careful measurement of the final silver content. The colour change observed during synthesis should be treated only as an initial indication of nanoparticle formation, rather than sufficient evidence of successful synthesis.

The hydrogel formulation is intended to provide a moist environment and act as a carrier for the active components. In the results, the combined hydrogel showed a swelling ratio of 391% and water retention of 82.1%. These properties are relevant because wound dressings should maintain moisture without allowing excessive fluid accumulation. A moist wound environment can support cell migration, autolytic debridement, and tissue repair (Shu et al., 2021). Alginate and chitosan

are useful materials for this purpose because of their water-handling properties and suitability for biomedical formulation. However, the physical characteristics of the hydrogel must be balanced with mechanical strength, degradation rate, dressing removal, and compatibility with wound exudate.

Antimicrobial performance is particularly important in burn wounds because damaged skin loses much of its normal protective function. In the dataset, the AgNP-containing hydrogel showed inhibition zones against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. The combined formulation showed larger inhibition zones than the AgNP-only formulation. This pattern is consistent with the established interest in silver-based wound dressings as a means of controlling bacterial growth (Kalantari et al., 2020). However, agar diffusion results alone cannot establish the full antimicrobial performance of a dressing. The release of silver from the material, bacterial growth conditions, and diffusion through agar can all influence the measured inhibition zone. Quantitative colony-forming unit assays and biofilm studies would provide stronger evidence.

An important consideration is the balance between antimicrobial activity and cell compatibility. Silver nanoparticles can damage bacterial cells, but their effects on mammalian cells may also depend on particle size, concentration, surface chemistry, and exposure time. In the fibroblast viability results, the AgNP-only formulation showed lower viability than the base and exosome-containing hydrogels. The combined formulation showed a comparatively higher viability of 92.3 ± 3.5% at 48 hours. This suggests a useful question for future experimental work: whether an optimized silver concentration can provide bacterial control without substantially affecting fibroblasts and keratinocytes. A concentration-response study is necessary before selecting a formulation for animal or clinical evaluation.

The results also showed greater closure in the exosome-containing groups, with the combined formulation showing the highest value. This observation is biologically plausible because extracellular vesicles can carry proteins, lipids, and nucleic acids that influence communication between cells. Their possible role in inflammation, angiogenesis, and tissue repair has received increasing attention in burn wound research (Manzoor et al., 2024). Nevertheless, scratch closure should not automatically be interpreted as increased migration, since cell proliferation can also contribute to the observed change. Additional assays, including proliferation controls and endothelial cell studies, would help clarify the underlying mechanisms.

The wound contraction values followed a similar pattern, with the combined formulation showing the greatest percentage contraction by day 21. This provides a potential link between the antimicrobial and regenerative components of the proposed dressing. However, wound contraction is not equivalent to complete healing. In rodents, contraction can contribute substantially to wound closure, whereas human wound repair relies more heavily on re-epithelialization. Histological examination, epithelial thickness, collagen organization, inflammatory cell infiltration, and angiogenesis are therefore important complementary outcomes.

The extracellular vesicle findings also require careful interpretation. Although particle size and the presence of markers such as CD9, CD63, and CD81 can support the identification of an extracellular vesicle preparation, these findings do not independently prove that all measured particles are exosomes. Current international recommendations emphasize the use of complementary characterization methods, appropriate controls, and clear reporting of isolation procedures (Welsh et al., 2024). Future work should also examine whether vesicles retain their structural integrity and biological activity after incorporation into and release from the hydrogel.

Overall, the proposed combination of green-synthesized AgNPs, extracellular vesicles, and hydrogel offers a promising research direction. Its potential value lies in bringing bacterial control and tissue repair support into one dressing system. However, actual experimental validation, detailed safety assessment, and reproducible biological testing are essential before its therapeutic effectiveness can be established.

CONCLUSION

The proposed study presents a promising approach to burn wound management through the combination of green-synthesized silver nanoparticles, stem cell-derived extracellular vesicles, and a hydrogel-based dressing. By bringing antimicrobial and tissue-regenerative components together, this formulation aims to address two major challenges in burn wound care: infection control and delayed tissue repair.

The findings suggest that the combined hydrogel may offer advantages in antimicrobial activity, cellular compatibility, fibroblast migration, and wound contraction compared with individual formulations. The use of neem leaf extract for silver nanoparticle synthesis and hydrogel-based delivery of extracellular vesicles also provides an interesting direction for developing multifunctional wound dressings.

REFERENCES

Aldakheel, F. M., El Sayed, M. M., Mohsen, D., Fagir, M. H., & El Dein, D. K. (2023). Green synthesis of silver nanoparticles loaded hydrogel for wound healing: Systematic review. Gels, 9(7), 530. https://doi.org/10.3390/gels9070530

Eming, S. A., Martin, P., & Tomic-Canic, M. (2014). Wound repair and regeneration: Mechanisms, signaling, and translation. Science Translational Medicine, 6(265), 265sr6. https://doi.org/10.1126/scitranslmed.3009337

Fakouri, A., & Razavi, Z.-S. (2024). Applications of mesenchymal stem cell-exosome components in wound infection healing: New insights. Burns & Trauma, 12. https://doi.org/10.1093/burnst/tkae021

Gurtner, G. C., Werner, S., Barrandon, Y., & Longaker, M. T. (2008). Wound repair and regeneration. Nature, 453, 314–321. https://doi.org/10.1038/nature07039

Guan, J., Wang, J., Zhang, X., Chi, J., Ma, Z., & Zhang, X. (2025). Silver nanoparticles with multimodal biological activities integrated into advanced material platforms for chronic wound management. Nanoscale, 17, 18409–18445. https://doi.org/10.1039/D5NR02066B

Kalantari, K., Mostafavi, E., Afifi, A. M., Izadiyan, Z., Jahangirian, H., Rafiee-Moghaddam, R., & Webster, T. J. (2020). Wound dressings functionalized with silver nanoparticles: Promises and pitfalls. Nanoscale, 12, 2268–2291. https://doi.org/10.1039/C9NR08234D

Kang, D., Wang, X., Chen, W., Mao, L., Zhang, W., Shi, Y., & Xie, J. (2024). Epidermal stem cell-derived exosomes improve wound healing by promoting the proliferation and migration of human skin fibroblasts. Burns & Trauma, 12, tkae047. https://doi.org/10.1093/burnst/tkae047

Li, S., Li, Y., Zhu, K., He, W., Guo, X., Wang, T., Gong, S., & Zhu, Z. (2024). Exosomes from mesenchymal stem cells: Potential applications in wound healing. Life Sciences, 357, 123066. https://doi.org/10.1016/j.lfs.2024.123066

Liu, L., & Liu, D. (2024). Bioengineered mesenchymal stem cell-derived exosomes: Emerging strategies for diabetic wound healing. Burns & Trauma, 12, tkae030. https://doi.org/10.1093/burnst/tkae030

Manzoor, T., Farooq, N., Sharma, A., Shiekh, P. A., Hassan, A., Dar, L. A., Nazir, J., Godha, M., Sheikh, F. A., Gugjoo, M. B., Saleem, S., & Ahmad, S. M. (2024). Exosomes in nanomedicine: A promising cell-free therapeutic intervention in burn wounds. Stem Cell Research & Therapy, 15, 355. https://doi.org/10.1186/s13287-024-03970-3

Mohammed, A. A., Alqahtani, A. A., & Ahmed, M. M. (2024). Application of green synthesized silver nanoparticles in burn therapy: A review. Pharmazie, 79(3), 42–48. https://doi.org/10.1691/ph.2024.3045

Pangli, H., Vatanpour, S., Hortamani, S., Jalili, R., & Ghahary, A. (2021). Incorporation of silver nanoparticles in hydrogel matrices for controlling wound infection. Journal of Burn Care & Research, 42(4), 785–793. https://doi.org/10.1093/jbcr/iraa205

Shu, W., Wang, Y., Zhang, X., Li, C., Le, H., & Chang, F. (2021). Functional hydrogel dressings for treatment of burn wounds. Frontiers in Bioengineering and Biotechnology, 9, 788461. https://doi.org/10.3389/fbioe.2021.788461

Sun, Y., Zhang, S., Shen, Y., Lu, H., Zhao, X., Wang, X., Wang, Y., Wang, T., Liu, B., Yao, L., & Wen, J. (2024). Therapeutic application of mesenchymal stem cell-derived exosomes in skin wound healing. Frontiers in Bioengineering and Biotechnology, 12, 1428793. https://doi.org/10.3389/fbioe.2024.1428793

Xie, P., Xue, X., & Li, X. (2024). Recent progress in mesenchymal stem cell-derived exosomes for skin wound repair. Cell Biochemistry and Biophysics, 82(3), 1651–1663. https://doi.org/10.1007/s12013-024-01328-3

Aldakheel, F. M., et al. (2023). Green synthesis of silver nanoparticles loaded hydrogel for wound healing. Gels, 9(7), 530. https://doi.org/10.3390/gels9070530

Pangli, H., et al. (2021). Incorporation of silver nanoparticles in hydrogel matrices for controlling wound infection. Journal of Burn Care & Research, 42(4), 785–793. https://doi.org/10.1093/jbcr/iraa205

Kalantari, K., et al. (2020). Wound dressings functionalized with silver nanoparticles: Promises and pitfalls. Nanoscale, 12, 2268–2291. https://doi.org/10.1039/C9NR08234D

Manzoor, T., et al. (2024). Exosomes in nanomedicine: A promising cell-free therapeutic intervention in burn wounds. Stem Cell Research & Therapy, 15, 355. https://doi.org/10.1186/s13287-024-03970-3

Shu, W., et al. (2021). Functional hydrogel dressings for treatment of burn wounds. Frontiers in Bioengineering and Biotechnology, 9, 788461. https://doi.org/10.3389/fbioe.2021.788461

Chinnasamy, G., Chandrasekharan, S., Koh, T. W., & Bhatnagar, S. (2021). Synthesis, characterization, antibacterial and wound healing efficacy of silver nanoparticles from Azadirachta indica. Frontiers in Microbiology, 12, 611560. https://doi.org/10.3389/fmicb.2021.611560

Wang, Y., Li, Q., Peng, X., Li, Z., Xiang, J., Chen, Y., Hao, K., Wang, S., Nie, D., Cui, Y., Lv, F., Wang, Y., Wu, W., Guo, D., & Si, H. (2022). Green synthesis of silver nanoparticles through oil: Promoting full-thickness cutaneous wound healing in methicillin-resistant Staphylococcus aureus infections. Frontiers in Bioengineering and Biotechnology, 10, 856651. https://doi.org/10.3389/fbioe.2022.856651

Zhang, Y., Fang, M., Xie, W., Zhang, Y.-A., Jiang, C., Li, N., Li, L., Tian, J., & Zhou, C. (2023). Sprayable alginate hydrogel dressings with oxygen production and exosome loading for the treatment of diabetic wounds. International Journal of Biological Macromolecules, 242(3), 125081. https://doi.org/10.1016/j.ijbiomac.2023.125081

Xiong, Y., Chen, L., Liu, P., Yu, T., Lin, C., Yan, C., Hu, Y., Zhou, W., Sun, Y., Panayi, A. C., Cao, F., Xue, H., Hu, L., Lin, Z., Xie, X., Xiao, X., Feng, Q., Mi, B., & Liu, G. (2023). All-in-one: Multifunctional hydrogel accelerates oxidative diabetic wound healing through timed-release of exosome and fibroblast growth factor. Small, 19(21), e2303158. https://doi.org/10.1002/smll.202303158

Younis, N. S., Mohamed, M. E., & El Semary, N. A. (2022). Green synthesis of silver nanoparticles by the cyanobacteria Synechocystis sp.: Characterization, antimicrobial and diabetic wound-healing actions. Marine Drugs, 20(1), 56. https://doi.org/10.3390/md20010056

Basit, M., Siddique, A. B., Aslam, B., Zahoor, M. A., Hussain, R., & Ulhaq, M. (2021). Distribution and antimicrobial susceptibility profile of bacterial and fungal pathogens isolated from burn wounds in hospitalized patients. Journal of the Pakistan Medical Association, 71(3), 916–920. https://doi.org/10.47391/JPMA.1245

Rashid, A., Saqib, M., Deeba, F., & Khan, J. A. (2019). Microbial profile of burn wound infections and their antibiotic sensitivity patterns at burn unit of Allied Hospital Faisalabad. Pakistan Journal of Pharmaceutical Sciences, 32(1 Suppl.), 247–254.

Masud, U., Saeed, A., & Nadeem, S. (2017). Death from burns: A twenty years autopsy study in Faisalabad, Pakistan. Annals of Punjab Medical College, 11(2), 113–117.

Advancements and insights in exosome-based therapies for wound healing: A comprehensive systematic review (2018–June 2023). (2023). Biomedicines, 11(8), 2099. https://doi.org/10.3390/biomedicines11082099

Green synthesized silver nanoparticles using Tridax procumbens for topical application: Excision wound model and histopathological studies. (2021). Pharmaceutics, 13(11), 1754. https://doi.org/10.3390/pharmaceutics13111754