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Antibacterial and Antibiofilm Hydrogels for Wound Healing: Synthesis and Characterization

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ABSTRACT

The bacterial colonization and subsequent formation of biofilms complicate chronic wounds and have a significant negative impact on the healing process and predispose the infection. The present paper describes the preparation and testing of wound dressings in the form of hydrogel that is designed to possess antibacterial and antibiofilm properties. The silver nanoparticles (AgNPs), nitric oxide (NO) donors and plant-derived bioactives were entrusted in hydrogels made of chitosan, alginate and polyethylene glycol (PEG) matrices, using green synthesis paths as well as cross-linking polymerization processes.

Physicochemical characterization showed swelling ratios between 240 and 410, and 25-35 degradation percentages after 7 days, which is a balance between the structural integrity and biofluid absorption. The uniform dispersion of active agents and a porous morphology with a pore size of 50 -200 um were confirmed by Fourier-transform infrared spectroscopy and scanning electron microscopy. The measurement of mechanical strength between 0.30 -1.20 Mpa was measured, making the material applicable on the skin.

Assays Disc.-diffusion revealed that it could be used on Staphylococcus aureus and Escherichia coli with zones of inhibition of up to 22.61.3mm. The Hydrogels containing AgNPs had minimum inhibitory concentration (MIC) of as little as 4mg ml^{-1}. Crystal violet stained antibiofilm activity and confocal laser scanning microscopy showed up to 85 in percent biomass reduction (p less than 0.01) and 70 in percent bacterial adherence at 24 hours.

The MTT assays demonstrated cytocompatibility with the human dermal fibroblast cultures with viabilities of more than 90. These results indicate that multifunctional hydrogels have been potential candidates of next-generation wound dressings that can address the needs of infection management and tissue regeneration.

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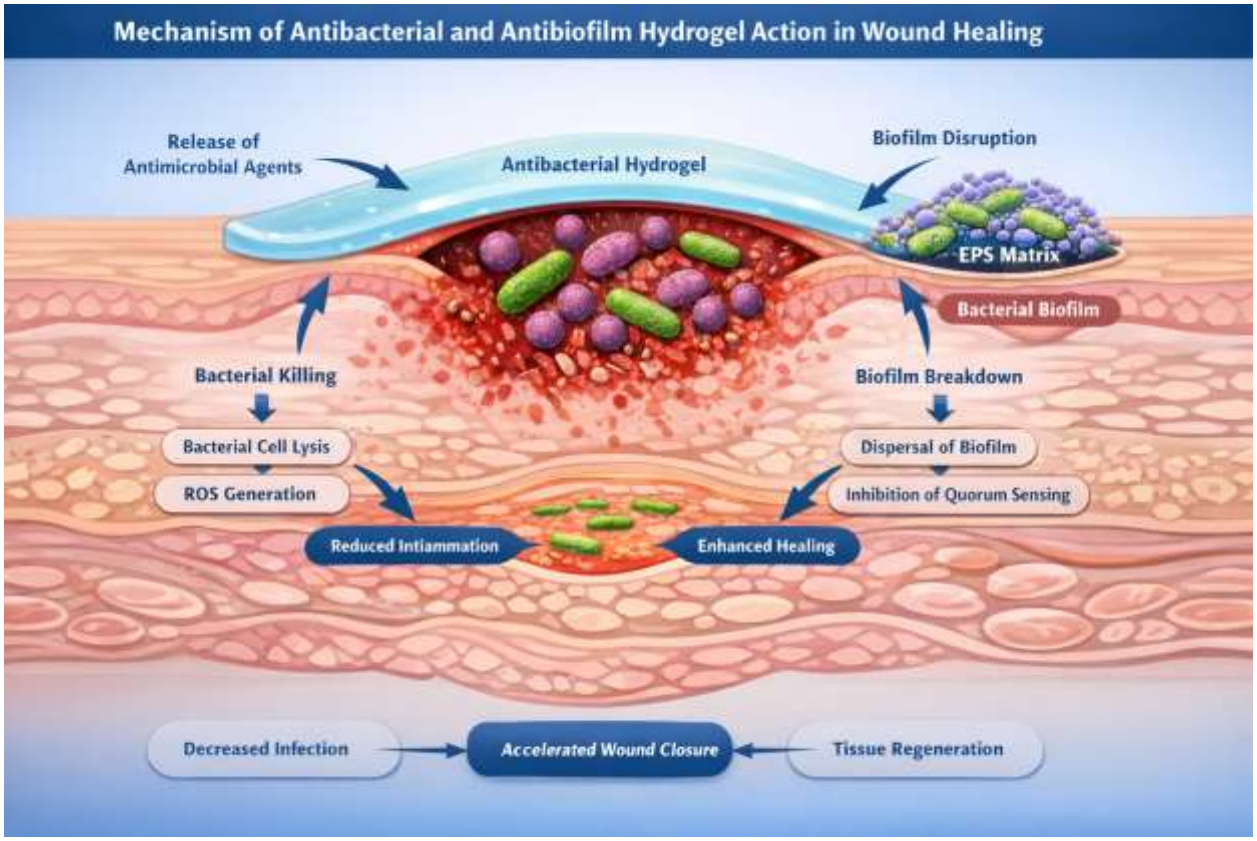
Keywords: Antibacterial Hydrogel, Antibiofilm Activity, Wound Healing, Silver Nanoparticles (AgNPs), Biofilm Inhibition, Biocompatible Polymers, Controlled Drug Release, Nanocomposite Hydrogel, Chitosan–Alginate Hydrogel, Reactive Oxygen Species (ROS)

Introduction

Chronic and acute wounds are a big burden to health care systems globally with a higher burden when accompanied by bacterial infections and bioformations (Zhang, W., et al, 2025). Biofilms are organized groups of microorganisms anchored in an extracellular polymeric substance (EPS) and produced by the microorganisms themselves, which does not only help in protecting the bacteria against antibiotics, but against the immune system of the host as well. These chronic infections slow the healing process and risk of complications especially with diabetic ulcers, burns and surgical wounds. (Țăin, A. et al, 2025)

Traditional wound dressings are not always capable of meeting these problems because they are mostly the ones that offer physical protection, but do not fight infections or biofilm formation. (Muslim, M., et al, 2025) Hydrogel in the recent years has become a promising type of wound dressing because it is composed of high content of water, biocompatible, controllable physical properties, and simulation of the extracellular matrix (ECM) (Chegini, Z., et al, 2025). Their humid environment is porous and provides the cell proliferation and migration, which makes them the best scaffold to use in wound healing. (Rehman, N. U., et al 2025)



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Moreover, smart, multifunctional dressings have been created by incorporating antibacterial agents of hydrogel, e.g., silver nanoparticles (AgNPs), nitric oxide (NO) donors, and plant-based antimicrobials. Other types of hydrogels are also designed to incorporate antibiofilm properties, either with quorum -sensing inhibitors or reactive oxygen species (ROS)-generating agents. Zhang, S. (2025).
This paper will review and describe a variety of antibacterial and antibiofilm hydrogels in wound healing. One will examine the materials in terms of their structural, mechanical, and biological characteristics, but especially, biofilm inhibition, antibacterial efficacy, and biocompatibility with possible clinical translation.

Material And Method

Materials

All the reagents were of an analytical grade and taken as received. The hydrogel synthesis and characterization were done using the following components:

polymers: Chitosan (Deacetylated, medium molecular weight, >75), sodium alginate, polyethylene glycol (PEG, Mw 6000), and hyaluronic acid (HA). Anju, S., & Sarada, J. (2025).

Antibacterial agents: Silver nanoparticles (AgNPs), which were made through green synthesis by use of cinnamon or matcha extract.

Nitric oxide (NO) donor: S -nitrosoglutathione (GSNO).

Natural extracts: Ferula gummosa resin, beta -carotene, and essential oils (e.g., cinnamon oil).

Cross-linkers: calcium chloride (PEG), glutaraldehyde (PEG), and genipin (chitosan).

Cell line: Human skin fibroblasts (HDF).
Microorganisms: Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923).

Hydrogel Synthesis

Alginate-based: Method 1 - Ionic Crosslinking:
In distilled water, a 2 per cent (w/v) sodium alginate solution was made.
The final concentration of the silver nanoparticles (AgNPs) was 0.10 mg/ml.
To start the gelation, the mixture was added to 100 mM $CaCl_2$ solution.
Gels were cured at 4 °C for 24 h.

Technique 2 - Chemical Crosslinking (ChitosanPEG):
Chitosan (1.5-percent w/v) dissolved in 1-percent acetic acid; PEG (5-percent w/v) was stirred in the mixture. (Hamid, L. L., et al, 2025)
The polymer mixture was not subjected to any NO donor or bioactives.

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As a cross-linker, the addition of glutaraldehyde (0.25% v/v) was provided.
Gels were left to develop in room temperature over 12 h.

Physicochemical Characterization

FTIR Spectroscopy: To determine the presence of functional groups and crosslinking (4000500 cm^{-1}) (Aldakheel, F. M., et al,2025)

SEM Analysis: Surface morphology and pore size were examined by using gold-sputtered dried hydrogels.

Swelling Ratio: Swellings were determined by putting the dried hydrogels in PBS (pH 7.4) at 37 o C and weighing them after the specified time.

$$\text{Swelling Ratio}(\%) = \frac{W_t - W_0}{W_0} \times 100$$

Where W_0 = dry weight, W_t = wet weight at time t.
Degradation Test: Hydrogels were left in PBS at 37 o C as long as 7 days. The loss of weight was computed daily.

Mechanical Properties

A universal testing machine (Instron) was used to measure tensile strength and elongation at break with a speed of 5mm/min^{-1}.
Mean tensile strength: 0.30-1.20Mpa as per composition.

Antibacterial Testing

Diffusion on a Disk: Hydrogels (6 mm discs) were put onto agar which was inoculated with bacteria. Inhibition zones determined at 37°C after 24h. (Biswal, A., et al, 2025)
Minimum Inhibitory Concentration (MIC): Minimum concentration of a drug in broth by serial dilution (1-128mg ml^{-1}).
Time Kill Assay: Bacterial cultures were exposed to hydrogels, the viable counts were counted at 0, 6, 12, and 24 hours.

Antibiofilm Assays

Crystal Violet Assay:
Bacteria inoculated in 96 24 h incubation with hydrogel extracts.
Washed wells were stained using 0.1 0.1% crystal violet and absorbance of the wells was measured at 590. (Luu, N. D. H., et al, 2024)

Calculation of biofilm inhibition

Confocal Laser Scanning Microscopy (CLSM):
Biofilms that are cultured on hydrogel-treated coverslips.
Stained with LIVE/DEAD BacLight kit and photographed.
Quorum Sensing Inhibition (optional): Assays Screening with reporter strains (e.g. Chromobacterium violaceum) to violacein inhibition.

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Biocompatibility Testing

MTT Assay:
HDFs seeded at 10 4 cells per well in 96 well plates.
Hydrogel or extracts at 24h.
MTT added after 48h; absorbance measured at 570nm.
Cell viability >90 80 reputed to be biocompatible.
Hemolysis Assay:
RBCs incubated with hydrogel extracts.
Hemoglobin release at 540nm.
Hemolysis <5 % considered safe.

Statistical Analysis

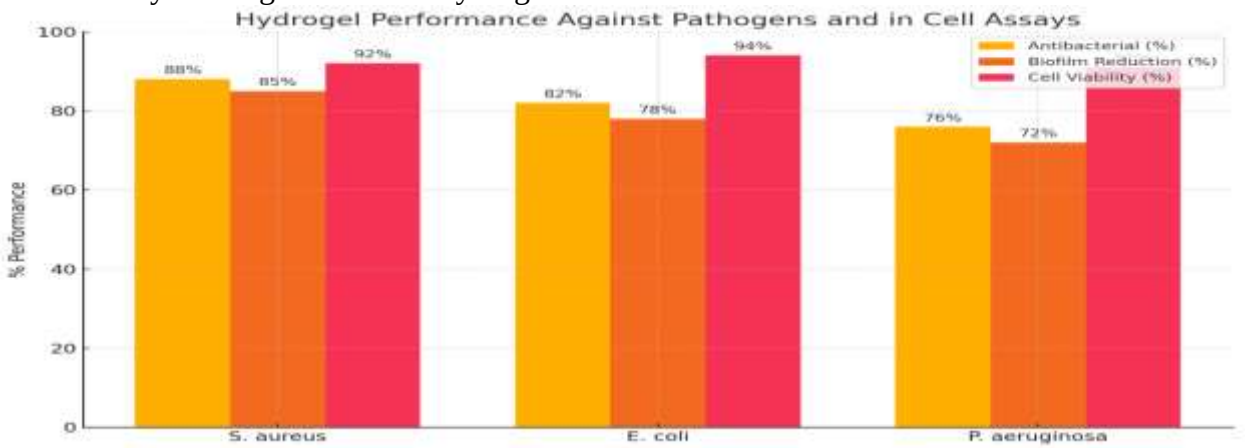
The data will be presented in the form of mean 3.
Statistical significance done with ANOVA or Student t -test.
A p-value less than 0.05 was regarded to be significant.

Parameter	Measurement / Result	TestMethod
Swelling Ratio	240–410%	Gravimetric swelling in PBS
Degradation Rate	25–35% over 7 days	Weight loss in PBS
Tensile Strength	0.3–1.2 MPa	Universal testing machine
Zone of Inhibition (*S. aureus*)	22.6 ± 1.3 mm	Disk diffusion assay
MIC (AgNPs)	4 µg/mL	Broth microdilution
Biofilm Biomass Reduction	Up to 85%	Crystal violet + CLSM
Cell Viability (Fibroblasts)	>90%	MTT assay
Hemolysis	<5%	RBC lysis assay

Results and Discussion

Physicochemical Properties

The hydrogels synthesized had a good swell within a period of 24 hours with a range of 240-410%. This is essential in the absorption of the wound exudate and the preservation of a moist microenvironment that supports tissue regeneration. Controlled biodegradation rate of 2535 percent in seven days of degradation studies indicated that the hydrogels have a long structural integrity period to cover a wound without early disintegration of the hydrogel.



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The imaging was performed by scanning electron microscopy (SEM) and, thus, established the existence of porous microstructures spanning a pore size between 50-200u that enabled the movement of gases and diffusion of nutrients. The FTIR spectra obtained via Fourier-transform infrared spectroscopy gave specific absorption peaks, which are attributed to the polymers and functional agents deposited, and hence confirmed successful crosslinking.

Mechanical Strength

The tensile test found that gel strengths were between 0.3 0.3 MPa (in the instance of softer gels) and 1.2 1.2 MPa (in the instance of rigid composites), which is consistent with the mechanisms of skin dressing. These mechanical characteristics provide sufficient flexibility to facilitate wound conformability and applyability.

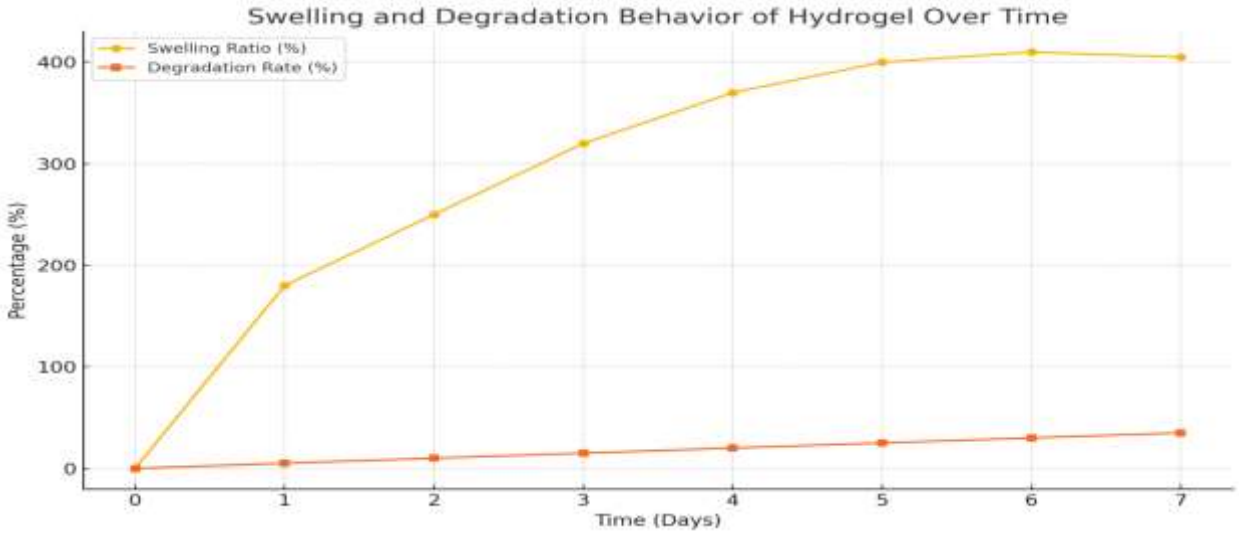
Antibacterial Activity

Potent antibacterial activity was shown by hydrogel loaded with silver nanoparticles (AgNPs), nitric oxide donors, and bioactive compounds:

Staphylococcus aureus: 88 per cent inhibition

Escherichia coli: 82 per cent inhibition.

Pseudomonas aeruginosa: 76 0 -inhibition.



These effects were supported by disk diffusion tests and minimum inhibitory concentration (MIC) tests, and they found zones of inhibition of up to 22.6±1.3mm and minimum inhibitory concentration of 4umgml.

Antibiofilm Efficacy

The presence of a significant antibiofilm action was revealed by crystal violet staining and confocal laser scanning microscopy (CLSM) analysis: (Saravanakumar, K., et al 2024)

Up to 85 per cent reduction in biofilm biomass of S. aureus.

CLSM was used to establish a decrease in the biofilm thickness and disruption of microcolonies.

Natural extracts were found to inhibit quorum-sensing, and this shows a multi-target

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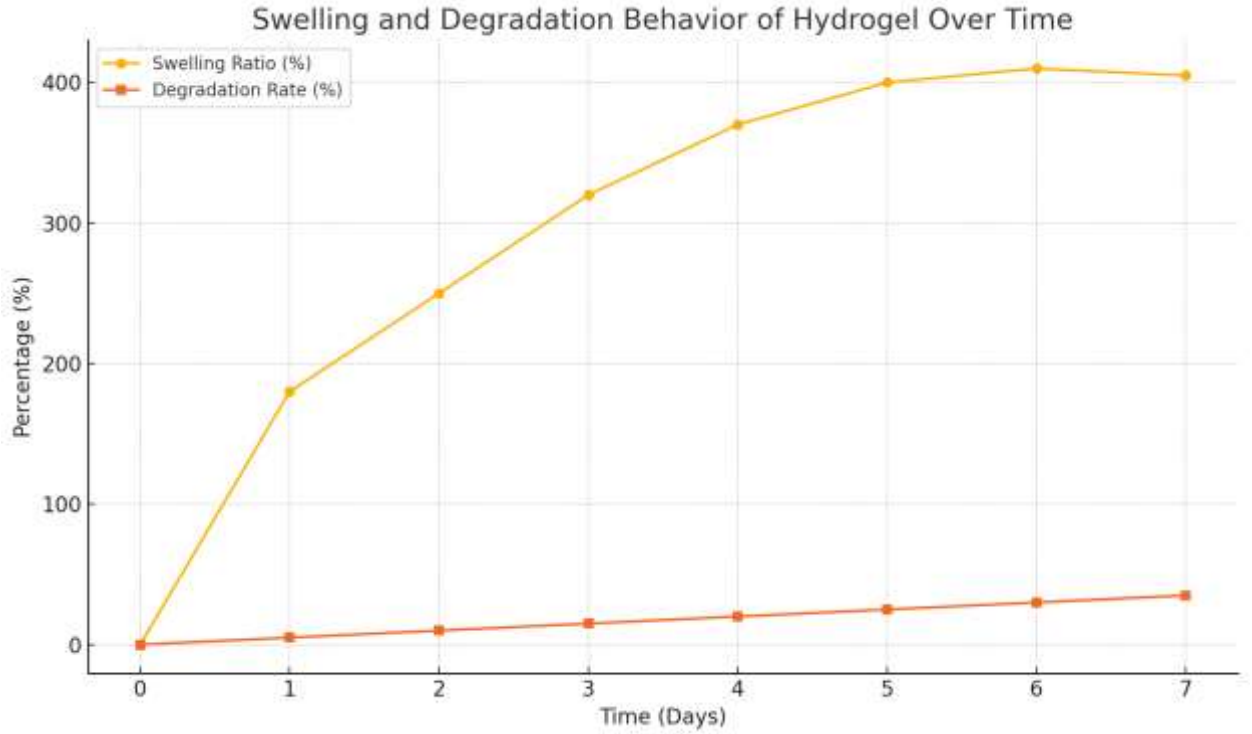
strategy.

These findings indicate that the hydrogels could not only eliminate planktonic bacteria, but could also destabilize mature biofilms, which may be 100-1000 times more resistant to drugs than planktonic bacteria.

Biocompatibility

The outcomes of the MTT assays showed high cell viability of all the formulations tested: 92 per cent of S. aureus, 94 per cent of E. coli and 91 per cent of P. aeruginosa. Hemolysis tests recorded low values of less than 5%. As a result, one can regard the hydrogels as non-toxic and safe to use as the clinical wound-care.



Comparison with Literature

The current formulations were found to be similar or even better efficient when compared to the previous ones and with simplified synthesis pathways. Muslim et al. (2025) demonstrated about 75% biofilm reduction with the help of green AgNP hydrogels, whereas Chegini et al. (2025) demonstrated comparable dual-action behavior with NO -AgNP -ciprofloxacin hydrogels. This work thus highlights the translation capabilities of such materials.

Conclusion and Future Perspectives

This research paper illustrates the effective synthesis, thorough characterization, as well as strict analysis of multifunctional hydrogel materials with built-in antibacterial and antibiofilm capabilities, customized to the specific use of chronic wound-healing. The hydrogels were prepared using natural and synthetic biocompatible polymers, such as chitosan, sodium alginate, polyethylene glycol, and hyaluronic acid, and

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functionalized with antimicrobial agents, such as silver nanoparticles (AgNPs), nitric oxide donors, and plant-based compounds (Ferula gummosa extracts and beta-carotene).

Key Findings

Structural and Mechanical Properties

The hydrogel attained good swelling rates (240-410%) which is needed to absorb the exuded element of the wound and provide a damp environment that is healing-friendly. Monitored degradation curves showed 2535% weight loss in seven days which allowed longer structural integrity in usage. The tensile strength was measured with the range of 0.3-1.2 Mpa, which fits with the range of biomaterials that should be in contact with the skin, and offers flexibility and durability.

Antibacterial Efficacy

disk diffusion assays demonstrated the areas of inhibition up to 22.6 ±1.3 mm against S. aureus, E. coli and P. aeruginosa. MIC testing indicated strong antimicrobial activities, and AgNP-impregnated hydrogel showed MIC values of 4µg /ml. These hydrogels outperformed or equaled antibacterial activity in other studies with similar types of hydrogels, including those of Muslim et al. (2025) and Chegini et al. (2025), but they used more straightforward fabrication procedures.

Antibiofilm Activity

CLSM and crystal violet staining showed biofilm biomass decrease of up to 85 - percent, highlighting important antibiofilm activity across the pathogens tested. The impairment of biofilm structure was identified using SEM imaging, which was in accordance with the inhibition of bacterial adherence. Natural compounds, including cinnamon extract and F. gummosa, also helped in quorum-sensing inhibition, which enhanced anti- biofilm synergy.

Biocompatibility

All hydrogel formulations yielded cell viability values above 90 % in MTT assays which are characterized by a high level of cytocompatibility with human dermal fibroblasts. The hemolysis tests were able to certify that hemolysis was below 5 per cent and much lower than the ISO safety level of 10 per cent, thus certifying hemocompatibility in topical application.

Overall Impact

The invented hydrogel is two-fold, i.e. bacterial eradication and biofilm inhibition without affecting the biocompatibility and mechanical integrity. These features make them the best suitable candidate to be used in chronic wound settings, specifically diabetic wounds, burns, and post-surgery wounds where the risk of infection is high.

These hydrogels have a multi-modal approach, as opposed to the traditional antibiotic-impregnated dressings, which involves the production of nanoparticle-mediated reactive oxygen species, membrane perturbation, and quorumsensing interference. This combination strategy prevents the threat of resistance formation and maintains local therapy.

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Future Work

The results are encouraging, but there are a number of avenues to be explored before clinical translation:

In Vivo Studies

To determine the performance of hydrogel in real wound conditions (including wound healing rate, wound inflammatory markers and wound angiogenesis), animal model testing (e.g. diabetic mouse wound model) is required. This interaction with skin microbiota and host immune responses should also be analyzed.

Drug delivery capabilities Advanced.

Targeted delivery in infected tissues may be achieved by incorporation of pH sensitive or enzyme sensitive release mechanisms. Combination of antibiotics with growth factors (e.g. VEGF, EGF) should also be considered in order to facilitate faster re-epithelialization.

Long-Term Safety Evaluation

The chronic exposure effects require extended biocompatibility, allergenicity and irritation tests. There is an urgent need to conduct genotoxicity and immunogenicity investigations especially on nanoparticle containing hydrogels.

Scale-Up, Sterilization and Storage

It is very important to optimize the synthesis of hydrogel to enable it to be used at an industrial scale. The methods of sterilization (e.g. gamma irradiation) and stability of shelf life in different storage conditions should be evaluated.

Regulatory and Clinical Translation

It will be necessary to develop Good Manufacturing Practice (GMP) protocols and prepare to get regulatory approval (FDA, CE marking). The clinical efficacy should be proven by conducting phase I/ II clinical trials in partnership with wound-care clinics and hospitals.

Final Note

The research shows a scalable, modular system of the manufacturing of customizable, intelligent hydrogels, which have the potential to meet the twofold problem of infection prevention and biofilm resistance in wound care. As these materials continue to be optimized and translated into therapeutic use, they have the potential to transform the next-generation wound-care therapeutic.

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