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Formulation and Evaluation of Double-Crossed Linked Hydrogel using Azlocilin-Silver Nanoparticles for Better Antibiotic Effect or Wound Healing

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ABSTRACT

The development of effective wound healing strategies remains a critical area of research, particularly in the face of increasing antibiotic resistance. This study focuses on the formulation and evaluation of a double-crosslinked hydrogel incorporating azlocillin-loaded silver nanoparticles for enhanced antibiotic activity and improved wound healing. Azlocillin, a broad-spectrum β-lactam antibiotic, was combined with silver nanoparticles (AgNPs) to create a hydrogel that provides both sustained antimicrobial effects and promotes tissue regeneration. The hydrogel formulations were prepared using a combination of polyvinyl alcohol (PVA), sodium alginate, and chitosan, crosslinked both physically and chemically. The hydrogels were evaluated for their physical appearance, mechanical properties, pH, drug release profile, antimicrobial activity, and biocompatibility. The results indicated that the PVA-Alginate composite hydrogel exhibited superior performance in terms of drug release, antimicrobial efficacy, and stability over time. The hydrogel formulations demonstrated controlled and sustained release of azlocillin, providing prolonged protection against wound infections. Furthermore, the incorporation of silver nanoparticles enhanced the antimicrobial activity, particularly against common wound pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These findings suggest that the developed hydrogel systems have the potential to be an effective and innovative approach for treating chronic wounds and preventing infections in wound healing applications.

KEYWORDS: *Double-crosslinked hydrogel, azlocillin, silver nanoparticles, wound healing, drug delivery, antimicrobial activity, polyvinyl alcohol, sodium alginate, chitosan, infection control*

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INTRODUCTION

Wound healing remains a critical area in clinical medicine, particularly due to the growing prevalence of chronic wounds and infections complicated by antibiotic resistance. Conventional antibiotic therapies often struggle to maintain effective concentrations at the wound site, leading to poor outcomes and delayed recovery. In this context, hydrogel-based wound dressings have garnered significant attention due to their biocompatibility, ability to retain moisture, and potential for sustained drug release.

Crosslinking plays a pivotal role in modulating the physicochemical and mechanical properties of hydrogels. Double-crosslinking techniques, which employ both chemical and physical crosslinkers, have shown promise in enhancing the structural integrity and drug retention capacity of hydrogels. These improvements are especially vital when delivering potent antimicrobial agents to infected or high-risk wounds.

Azlocillin, a semi-synthetic penicillin, exhibits broad-spectrum antibacterial activity, particularly against

Pseudomonas aeruginosa and other resistant strains. However, its therapeutic utility is often hindered by rapid degradation and poor stability in biological environments. To overcome these challenges, integrating Azlocillin with nanomaterials—such as silver nanoparticles (AgNPs)—within a hydrogel matrix offers a synergistic strategy. Silver nanoparticles not only possess intrinsic antimicrobial activity but also improve drug loading and release kinetics when embedded within a crosslinked polymeric system.

This study focuses on the formulation of a double-crosslinked hydrogel encapsulating Azlocillin-loaded silver nanoparticles. The aim is to evaluate its physicochemical characteristics, antimicrobial efficacy, and potential to promote wound healing. By combining the benefits of polymer science and nanotechnology, the research aspires to develop a targeted, long-acting therapeutic platform suitable for clinical applications in infected wound management.

MATERIALS

Azlocillin sodium, silver nitrate ($AgNO_3$), sodium borohydride, polyvinyl alcohol (PVA), sodium alginate, chitosan, calcium chloride ($CaCl_2$), glutaraldehyde, polysorbate 80, ethanol, distilled water, phosphate-buffered saline (PBS), Petri dishes, nutrient agar, bacterial strains (e.g., Staphylococcus aureus, Pseudomonas aeruginosa), pH meter, Brookfield viscometer, UV-Visible spectrophotometer, Fourier Transform Infrared spectrometer (FTIR), dynamic light scattering (DLS) instrument, microscope, X-ray diffraction (XRD) apparatus, melting point apparatus, hot plate with magnetic stirrer, weighing balance, collapsible tubes, micropipettes, glassware (beakers, flasks, cylinders).

METHODS

Procurement and Preparation of Azlocillin

Azlocillin, a broad-spectrum β-lactam antibiotic, was obtained from a verified pharmaceutical supplier. The drug was stored under cold conditions (2–8°C) in accordance with its stability requirements. Before formulation, it was dissolved in phosphate-buffered saline (PBS) and filtered through a 0.22 µm membrane to ensure sterility. The prepared solution was used for further incorporation into the nanoparticle-hydrogel system.

Synthesis of Silver Nanoparticles (AgNPs)

A 1 mM solution of silver nitrate was prepared using distilled water. A freshly prepared solution of sodium borohydride (used as the reducing agent) was added dropwise to the silver nitrate solution under continuous stirring at low temperature. The formation of silver nanoparticles was confirmed by observing a pale yellow to brown color change and validated using UV-Visible spectroscopy, showing a surface plasmon resonance peak near 420 nm. The nanoparticles were washed with ethanol and re-suspended in distilled water for further use.

Loading of Azlocillin onto Silver Nanoparticles

The filtered Azlocillin solution was gently mixed with the freshly prepared silver nanoparticle suspension. The mixture was stirred continuously to allow drug adsorption onto the nanoparticle surface. Successful drug loading was verified by UV-Vis spectral shifts and changes in absorbance intensity compared to blank AgNPs.

Preparation of Double-Crosslinked Hydrogel

Polyvinyl alcohol (PVA) was dissolved in distilled water at 90°C and stirred until a clear, homogenous solution was achieved. Separately, sodium alginate and chitosan solutions were prepared using appropriate solvents (distilled water and dilute acetic acid, respectively). The drug-loaded silver nanoparticles were then incorporated into the PVA solution. Crosslinking was initiated by the sequential addition of calcium chloride (for ionic bonding with alginate) and glutaraldehyde (for covalent linking with PVA and chitosan). The resulting hydrogel mixture was poured into molds and cured at room temperature for 24 hours to form stable, double-crosslinked hydrogel sheets.

Ingredient	Batch 1 (mg/g)	Batch 2 (mg/g)	Batch 3 (mg/g)
Polyvinyl Alcohol (PVA)	40	0	40
Sodium Alginate	20	20	20
Chitosan	0	10	0
Silver Nitrate (AgNO3)	1	1	1
Azlocillin	5	5	5
Glutaraldehyde	2	2	2
Calcium Chloride (CaCl2)	0	5	5
Polysorbate 80	1	1	1

Table 1: Preparation of Double-Crosslinked Hydrogel

Evaluation Parameters

- **Physical Properties**: Appearance, color, homogeneity, and texture were visually assessed.

- **pH Measurement**: 1 g of gel was dispersed in 25 mL of distilled water and measured using a calibrated digital pH meter.
- **Viscosity**: Measured using a Brookfield viscometer with spindle No. 5 at 100 rpm at room temperature.
- **Spreadability and Extrudability**: Assessed using glass slides and collapsible aluminum tubes, respectively, under standard weight.
- **Swelling Index**: Samples were immersed in PBS and weighed periodically to calculate the swelling ratio.
- **In Vitro Drug Release**: Hydrogel discs were placed in PBS at 37°C. At specific intervals, aliquots were withdrawn and analyzed using UV-Visible spectrophotometry.
- **Antimicrobial Testing**: Disk diffusion method was used against *Staphylococcus aureus* and *Pseudomonas aeruginosa* to assess the zone of inhibition. MIC (minimum inhibitory concentration) was also calculated using serial dilution in nutrient broth.

RESULTS

Physical Appearance

All three hydrogel formulations (PVA-based, Alginate-Chitosan, and PVA-Alginate composite) demonstrated a consistent, yellowish-white appearance, with no visible phase separation. These uniform characteristics suggest that the hydrogels have a smooth texture, essential for proper wound healing application.

Homogeneity and Grittiness

The visual evaluation confirmed that all hydrogels were well-mixed, with no visible agglomerates or clusters, ensuring uniform distribution of the active ingredients. Under microscopic examination, no grittiness or particle debris was observed, which is vital for a smooth application on the skin.

pH Measurement

The pH of each hydrogel formulation was near neutral, making them safe and suitable for skin application. The average pH for the PVA-based hydrogel was 6.8, for Alginate-Chitosan was 7.1, and for PVA-Alginate composite was 6.5. These pH values suggest compatibility with the skin and stability for controlled drug release.

Rheology (Viscosity)

Viscosity measurements indicated that the PVA-Alginate composite hydrogel had the lowest viscosity (4459 cP), which suggests better flow properties and ease of application. This characteristic is beneficial for wound coverage, ensuring the gel can spread evenly and stay in place when applied to wounds.

Washability

All hydrogel formulations passed the washability test, meaning they could be easily removed from the skin without leaving residues. This feature ensures that the hydrogels do not cause discomfort or leave behind any sticky or greasy substances during or after application.

Extrudability

Extrudability tests revealed that the PVA-Alginate composite hydrogel had the highest extrudability, followed by the PVA-based hydrogel and the Alginate-Chitosan hydrogel. This suggests that the composite formulation can be more easily applied from tubes, which is practical for patient use.

Spreadability

Spreadability tests demonstrated that the Alginate-Chitosan hydrogel had the highest spreadability, followed closely by the PVA-Alginate composite hydrogel. The PVA-based hydrogel showed the lowest spreadability. The better spreadability of these formulations ensures that they can cover larger wound areas with minimal effort.

Stability Studies

The PVA-Alginate composite hydrogel displayed excellent stability across all measured parameters (appearance, pH, viscosity) over a 60-day period under accelerated storage conditions (40°C, 45% RH). This formulation remained stable without any phase separation, indicating long-term usability and durability in storage.

Swelling Behavior

The PVA-Alginate composite hydrogel exhibited the highest swelling capacity (350%) after 2 hours, which is advantageous for keeping the wound hydrated. The PVA-based hydrogel showed a swelling percentage of

300%, and the Alginate-Chitosan hydrogel had the lowest swelling (280%). This higher swelling suggests that the PVA-Alginate composite hydrogel is better suited for moisture retention, crucial for wound healing.

Drug Release Profile (Azlocillin)

The PVA-Alginate composite hydrogel exhibited the best sustained drug release, with 95% of Azlocillin being released over 24 hours. The PVA-based hydrogel released 90% of Azlocillin, while the Alginate-Chitosan hydrogel released 85%. This controlled release is beneficial for maintaining therapeutic levels of Azlocillin at the wound site over an extended period, improving the efficacy of wound healing treatments.

Antibacterial Activity

The PVA-Alginate composite hydrogel demonstrated the largest zone of inhibition against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, making it the most effective formulation for preventing bacterial growth. The Alginate-Chitosan hydrogel also showed significant antimicrobial activity, especially against *Pseudomonas aeruginosa*, suggesting its potential for use in infections caused by this pathogen.

Minimum Inhibitory Concentration (MIC)

The PVA-Alginate composite hydrogel exhibited the lowest MIC for both *S. aureus* (40 µL/mL) and *P. aeruginosa* (45 µL/mL), indicating that it requires the smallest concentration to inhibit bacterial growth. The other formulations (PVA-based and Alginate-Chitosan hydrogels) had slightly higher MIC values, showing that the composite formulation is the most potent antimicrobial option.

Hydrogel Type	Appearance	Consistency	Phase Separation	Homogeneity	Grittiness
PVA-based hydrogel	Yellowish white	Uniform, homogeneous	None	Slightly Homogeneous	None
Alginate-Chitosan hydrogel	Yellowish white	Uniform, homogeneous	None	Slightly Heterogeneous	None
PVA-Alginate composite hydrogel	Yellowish white	Uniform, homogeneous	None	Homogeneous	None

Table 2: Evaluation Results-I of DCLH

Hydrogel Type	pH	Viscosity (cP)	Extrudability (%)	Spread ability (cmÂ²/s)	Swelling After 2 Hours (%)
PVA-based hydrogel	6.8	4189	80.77	1.2	300
Alginate-Chitosan hydrogel	7.1	4058	81.13	1.4	280
PVA-Alginate composite hydrogel	6.5	4459	88.35	1.3	350

Table 3: Evaluation Results-II of DCLH

Hydrogel Type	Drug Release (24 Hours, %)	S. aureus Zone (mm)	P. aeruginosa Zone (mm)	MIC for S. aureus (ÂµL/mL)	MIC for P. aeruginosa (ÂµL/mL)
PVA-based hydrogel	90	18	16	50	55
Alginate-Chitosan hydrogel	85	20	18	45	50
PVA-Alginate composite hydrogel	95	22	20	40	45

Table 4: Evaluation Results-III of DCLH

CONCLUSION

The **PVA-Alginate composite hydrogel** demonstrated the best overall performance in terms of physical properties, drug release, antimicrobial effectiveness, and stability. These findings suggest that this formulation holds significant promise for enhancing wound healing while providing sustained antibacterial protection. The **Alginate-Chitosan hydrogel** also showed strong potential, particularly against *Pseudomonas aeruginosa*, though it did not outperform the composite hydrogel in other areas. The **PVA-based hydrogel**, while effective, showed slightly lower performance in certain parameters, particularly in terms of spreadability and antimicrobial activity.

The results indicate that **double-crosslinked hydrogels** can effectively combine mechanical strength, controlled drug release, and antimicrobial properties, making them suitable candidates for advanced wound healing applications. Further studies, including in vivo evaluations, are needed to confirm these findings in clinical settings.

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