In Situ Synthesis and Characterization of a Strontium Nitrate-Impregnated Starch-Collagen Hydrogel for Antimicrobial Applications

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Abstract

Background: The development of effective antimicrobial biomaterials remains a critical challenge in healthcare applications, requiring materials that can provide sustained therapeutic action while maintaining biocompatibility.

Objective: To develop and characterize a novel hybrid hydrogel system incorporating strontium nitrate for antimicrobial drug delivery applications

Methods: A hybrid hydrogel was synthesized using natural polymers (starch, collagen, gelatin) and synthetic polymers (polyvinyl alcohol [PVA], polyethylene glycol [PEG]). Strontium nitrate ($Sr(NO_3)_2$) was incorporated and reduced in situ within the hydrogel matrix using glutaraldehyde crosslinking. The hydrogel was characterized using UV-visible spectroscopy, scanning electron microscopy (SEM), swelling studies in simulated tear fluid, antimicrobial assays against Staphylococcus aureus and Pseudomonas aeruginosa, and strontium ion release profiling over 24 hours.

Results: The hydrogel demonstrated a porous microstructure suitable for fluid absorption. The system exhibited high swelling capacity (>1200% of dry weight) in simulated tear fluid, reaching equilibrium within 6 hours. Antimicrobial testing showed significant activity with inhibition zones of 18 mm against S. aureus and 15 mm against P. aeruginosa. Strontium ion release exhibited an initial burst (30% within 4 hours) followed by sustained release, achieving 85% cumulative release over 24 hours.

Conclusions: The developed starch-collagen-strontium nitrate hybrid hydrogel demonstrates promising characteristics for antimicrobial applications, combining high fluid absorption, broad-spectrum antimicrobial activity, and controlled strontium ion release. These properties position it as a viable candidate for advanced therapeutic platforms in infection management.

Keywords: Hydrogel, strontium nitrate, antimicrobial, drug delivery, starch, collagen, controlled release

Introduction

The increasing prevalence of antimicrobial resistance has created an urgent need for novel therapeutic approaches in infection management [1]. Hydrogels have emerged as promising vehicles for drug delivery due to their high water content, biocompatibility, and ability to provide sustained drug release [2]. The combination of natural and synthetic polymers in hybrid hydrogel systems offers unique advantages, including enhanced mechanical properties, controlled degradation, and tunable release characteristics [3].

Natural polymers such as starch and collagen provide excellent biocompatibility and biodegradability, making them ideal candidates for biomedical applications [4]. Starch, derived from various plant sources, is abundant, cost-effective, and exhibits excellent gelling properties [5]. Collagen, being a major component of the extracellular matrix, provides exceptional biocompatibility and can enhance tissue integration [6].

The incorporation of synthetic polymers like polyvinyl alcohol (PVA) and polyethylene glycol (PEG) can enhance the mechanical properties and stability of natural polymer-based hydrogels [7]. PVA is particularly valuable for its excellent film-forming properties and biocompatibility, while PEG serves as an effective crosslinking agent and can facilitate controlled drug release [8].

Strontium compounds have gained attention for their antimicrobial properties and potential therapeutic applications [9]. Strontium ions have been shown to exhibit antimicrobial activity against various bacterial strains while demonstrating good biocompatibility [10]. The controlled release of strontium ions from hydrogel matrices offers advantages in terms of sustained antimicrobial action and reduced systemic toxicity [11].

The objective of this study was to develop and comprehensively characterize a novel hybrid hydrogel system combining natural polymers (starch and collagen) with synthetic polymers (PVA and PEG) for the controlled release of strontium nitrate. We hypothesized that this hybrid system would demonstrate superior antimicrobial activity while maintaining the biocompatibility required for therapeutic applications

Materials and Methods

Materials

Potato starch (pharmaceutical grade), bovine collagen type I, gelatin (bloom strength 250), polyvinyl alcohol (PVA, Mw 89,000-98,000, 99% hydrolyzed), polyethylene glycol (PEG 4000), strontium nitrate ($Sr(NO_3)_2$, ≥99%), glutaraldehyde (25% aqueous solution), and acetic acid (glacial, ≥99.7%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used as received without further purification. Deionized water (resistivity >18 MΩ·cm) was used throughout the study.

Preparation of Polymer Stock Solutions

All polymer solutions were prepared under sterile conditions:

1% Gelatin Solution: 0.1 g of gelatin was dispersed in 10 mL of distilled water, allowed to hydrate for 30 minutes at room temperature, then heated to 40°C with continuous stirring for 1 hour until complete dissolution.

10% PVA Solution: 10 g of PVA was gradually added to 100 mL of deionized water and heated to 40-50°C with vigorous stirring for 1-3 hours until a clear, homogeneous solution was obtained.

10% PEG Solution: 5 g of PEG 4000 was dissolved in 50 mL of deionized water at room temperature with gentle stirring for 30 minutes.

1% Starch Solution: 0.5 g of potato starch was dispersed in 50 mL of deionized water and heated to 48°C with continuous stirring for 1-3 hours to achieve complete gelatinization.

1% Collagen Solution: 0.1 g of bovine collagen type I was dissolved in 10 mL of 2% (v/v) acetic acid solution with gentle stirring for 24 hours at 4°C.

Synthesis of Strontium-Impregnated Hydrogel

The hybrid hydrogel was synthesized using a multi-step process:

Base Hydrogel Preparation: Equal volumes of 1% starch, 1% collagen, and 10% PVA solutions were combined in a 1:1:1 ratio and mixed thoroughly using a magnetic stirrer for 30 minutes at room temperature.

Strontium Incorporation: A 0.1 M strontium nitrate solution was slowly added to the polymer blend under continuous stirring. The mixture was stirred for an additional 15 minutes.

Stabilization: A small amount of 10% PEG solution was added as a stabilizer. The solution was stirred for 45 minutes.

Crosslinking: Crosslinking was achieved by dropwise addition of 2.5% glutaraldehyde solution over a period of 10 minutes with continuous stirring. The final mixture was stirred for an additional 30 minutes.

Gel Formation and Processing: The resulting gel was cast into sterile molds and allowed to cure at room temperature for 24 hours. The gels were then lyophilized using a freeze-dryer for 48 hours.

Control Hydrogel: A control hydrogel was prepared following the identical protocol but omitting the strontium nitrate addition step.

Characterization Methods

UV-Visible Spectroscopy

The incorporation of strontium nitrate was analyzed using UV-Vis spectroscopy (UV-2600, Shimadzu, Japan). Hydrogel samples were dissolved in deionized water (1 mg/mL) and analyzed over a wavelength range of 200-400 nm.

Scanning Electron Microscopy (SEM)

The morphological characteristics and internal structure of lyophilized hydrogel samples were examined using scanning electron microscopy (JSM-7100F, JEOL, Japan). Samples were mounted on aluminum stubs, sputter-coated with gold (10 nm thickness), and examined at accelerating voltages of 5-15 kV.

Swelling behavior was evaluated using simulated tear fluid (STF) prepared with the following composition: NaCl (6.78 g/L), $NaHCO_3$ (2.18 g/L), $CaCl_2 \cdot 2H_2O$ (0.084 g/L), and KCl (1.38 g/L), adjusted to pH 7.4 ± 0.1.

Dry hydrogel samples (20 ± 2 mg) were immersed in 10 mL of STF at 37°C ± 0.5°C. At predetermined time intervals, samples were carefully removed, excess surface fluid was blotted, and the swollen weight was recorded. The swelling ratio (SR) was calculated using:

$$SR (\%) = [(Ws - Wd) / Wd] \times 100 \quad ... (1)$$

Where Ws is the weight of the swollen hydrogel and Wd is the initial dry weight.

Antimicrobial Activity Assessment

Antimicrobial efficacy was evaluated using the disk diffusion method against Staphylococcus aureus (ATCC 25923) and Pseudomonas aeruginosa (ATCC 27853).

Bacterial strains were cultured in Mueller-Hinton broth at 37°C for 18-24 hours. The bacterial suspension was adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Mueller-Hinton agar plates were inoculated with 100 µL of bacterial suspension. Hydrogel discs (6 mm diameter) were aseptically placed on the inoculated plates. Plates were incubated at 37°C for 24 hours, and the diameter of inhibition zones was measured. Each experiment was performed in triplicate.

Strontium Ion Release Study

Strontium ion release kinetics were determined using atomic absorption spectroscopy (AAS, AA-7000, Shimadzu, Japan). Hydrogel samples (50 ± 5 mg) were immersed in 25 mL of phosphate-buffered saline (PBS, pH 7.4) at 37°C ± 0.5°C under gentle agitation.

At specified time points (1, 4, 8, 12, and 24 hours), 1 mL aliquots were withdrawn and replaced with fresh PBS. Samples were analyzed for strontium ion concentration using AAS. The cumulative release percentage was calculated using:

Cumulative Release (%) = (ΣQt / Q∞) × 100 ... (2)

Where Qt is the amount of strontium released at time t, and Q∞ is the total amount of strontium incorporated in the hydrogel.

Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test. Statistical significance was set at $p < 0.05$.

Results and Discussion

Hydrogel Formation and Visual Characteristics

The hybrid hydrogel was successfully synthesized through the combination of natural and synthetic polymers with strontium nitrate incorporation. The hydrogel blend turned a characteristic color upon the addition of glutaraldehyde and PEG, indicating successful crosslinking and strontium incorporation within the polymer matrix. The control hydrogel remained colorless throughout the synthesis process, confirming that any color change was specifically due to strontium nitrate incorporation and crosslinking reactions.

UV-Visible Spectroscopy Analysis

UV-Vis spectroscopy confirmed the successful incorporation of strontium nitrate within the hydrogel matrix. The strontium-loaded hydrogel showed characteristic absorption patterns in the UV region, distinct from the control hydrogel. The absorption profile indicated successful integration of strontium ions within the polymer network, providing a foundation for controlled release applications.

Morphological Characterization

SEM analysis revealed significant structural characteristics of both control and strontium-loaded hydrogels. The control hydrogel displayed a highly porous, interconnected three-dimensional network structure typical of freeze-dried polymer hydrogels. The pores ranged from 10-100 μm in diameter, providing extensive surface area for fluid absorption and drug release.

The strontium-loaded hydrogel maintained the porous structure while showing evidence of strontium incorporation throughout the polymer matrix. The porous architecture remained intact, suggesting that strontium incorporation did not significantly compromise the structural integrity of the hydrogel network. This porous structure is crucial for effective fluid absorption and controlled release of the incorporated therapeutic agent.

Swelling Behavior Analysis

Both control and strontium-loaded hydrogels demonstrated excellent swelling capacity in simulated tear fluid, reaching equilibrium within 6 hours. The hydrogel achieved a maximum swelling ratio of over 1200% of its dry weight, indicating exceptional fluid absorption capability.

The swelling kinetics followed a typical pattern with rapid initial swelling (0-2 hours) where approximately 70% of maximum swelling was achieved, followed by gradual equilibration (2-6 hours). This behavior is attributed to initial rapid water uptake by hydrophilic polymer chains, followed by network relaxation and equilibration.

The high swelling capacity demonstrates the hydrogel's ability to absorb significant amounts of fluid while maintaining structural integrity, making it suitable for various biomedical applications where fluid management and drug delivery are required.

Antimicrobial Activity Evaluation

The strontium-loaded hydrogel exhibited significant antimicrobial activity against both tested bacterial strains. Clear zones of inhibition were observed with diameters of approximately 18 mm against S. aureus and 15 mm against P. aeruginosa.

Table 1: Antimicrobial Activity Results

Sample	**S. aureus (mm)**	**P. aeruginosa (mm)**
Strontium-loaded hydrogel	18 ± 1.2	15 ± 1.1
Control hydrogel	No inhibition	No inhibition

The control hydrogel showed no antimicrobial activity, confirming that the observed effects were specifically due to the incorporated strontium nitrate. The slightly larger inhibition zone against S. aureus compared to P. aeruginosa suggests differential susceptibility of Gram-positive and Gram-negative bacteria to strontium ions.

The antimicrobial mechanism likely involves the release of strontium ions from the hydrogel matrix, which can interact with bacterial cell components and disrupt cellular processes. The sustained release of strontium ions ensures prolonged antimicrobial activity, which is beneficial for preventing reinfection and biofilm formation.

Strontium Ion Release Kinetics

The strontium ion release profile from the loaded hydrogel exhibited a biphasic behavior characteristic of drug-loaded hydrogel systems. An initial burst release was observed during the first 4 hours, with approximately 30% of the total strontium content released. This initial phase is attributed to the rapid dissolution and diffusion of surface-bound strontium through the hydrated polymer network.

Following the burst phase, a more gradual and sustained release occurred, with an additional 55% of strontium released over the subsequent 20 hours, achieving a cumulative release of 85% at 24 hours. This sustained release phase is attributed to the gradual dissolution and diffusion through the polymer matrix.

The controlled release profile is advantageous for therapeutic applications, providing immediate antimicrobial action through the initial burst release while maintaining sustained activity through prolonged release. This release pattern can help maintain therapeutic concentrations while minimizing potential side effects associated with high peak concentrations.

Clinical Relevance and Biocompatibility Considerations

The developed hydrogel system demonstrates several characteristics that make it suitable for antimicrobial applications. The high water content and swelling capacity ensure compatibility with biological environments, while the controlled release of strontium ions provides sustained antimicrobial activity.

The use of natural polymers (starch and collagen) as primary matrix components enhances biocompatibility and biodegradability. Both polymers have established safety profiles and have been used in various biomedical applications. The incorporation of synthetic polymers (PVA and PEG) enhances mechanical properties while maintaining biocompatibility.

Comparison with Existing Systems

Compared to conventional antimicrobial delivery systems, the developed hydrogel offers several advantages: sustained drug release, improved bioavailability through prolonged contact time, reduced dosing frequency, and potential for combination therapy. The hybrid polymer approach provides improved mechanical properties and controlled release characteristics compared to single-polymer systems.

The in situ incorporation method ensures uniform distribution of strontium throughout the hydrogel matrix, providing consistent release characteristics and antimicrobial activity. This approach offers advantages over post-loading methods in terms of drug distribution and stability.

Limitations and Future Directions

While the results are promising, several areas require further investigation. Comprehensive cytotoxicity evaluation and in vivo biocompatibility studies are necessary to establish the safety profile. Long-term stability studies are needed to determine shelf-life and storage conditions.

Future research directions include optimization of strontium loading to balance antimicrobial efficacy with biocompatibility, development of stimuli-responsive release mechanisms, and evaluation of the system against a broader range of pathogenic microorganisms.

Conclusions

This study successfully developed and characterized a novel strontium nitrate-impregnated starch-collagen hydrogel for antimicrobial applications. The key findings include:

Successful Synthesis: The hybrid hydrogel was successfully synthesized with effective incorporation of strontium nitrate within the polymer matrix.

Optimal Physical Properties: The hydrogel demonstrated excellent swelling capacity (>1200%) in simulated tear fluid, reaching equilibrium within 6 hours.

Broad-Spectrum Antimicrobial Activity: Significant antimicrobial efficacy was demonstrated against both Gram-positive (S. aureus) and Gram-negative (P. aeruginosa) bacteria, with inhibition zones of 18 mm and 15 mm, respectively.

Controlled Release Profile: The hydrogel exhibited a clinically advantageous biphasic release pattern with initial burst release (30% in 4 hours) followed by sustained release (85% cumulative release over 24 hours).

Biocompatible Design: The use of natural polymers as primary matrix components, combined with established biocompatible synthetic polymers, provides a foundation for safe biomedical applications.

The developed hydrogel system represents a significant advancement in antimicrobial drug delivery, combining the benefits of natural and synthetic polymers with the antimicrobial properties of strontium nitrate. The controlled release characteristics and high swelling capacity make it suitable for various antimicrobial therapy applications.

These findings support the potential clinical translation of this hydrogel system for antimicrobial therapy. However, comprehensive in vivo studies are necessary to fully establish its safety and efficacy profile before clinical implementation.

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All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement
Not applicable. This study did not involve human subjects or animal testing.

Informed Consent Statement
Not applicable.

Data Availability Statement
The data presented in this study are available on request from the corresponding author.

Conflicts of Interest
The authors declare no conflicts of interest.

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