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Evaluation Of Bioactive Hydrogels For The Treatment Of Infected Wounds: Clinical And In Vitro Studies

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ABSTRACT

Infected wounds require materials that prevent infection while promoting healing. This study evaluates bioactive hydrogels made from chitosan and carbopol 934, incorporating antimicrobial agents for wound treatment. The hydrogels were analysed for pH, water absorption, spreadability, and drug release. Chitosan-based formulations exhibited a pH range of 4.3–6.8 and better spreadability, making them suitable for wound healing.

Antimicrobial activity was tested using the disc diffusion method against *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes*, and *Klebsiella pneumoniae*. Hydrogels with higher bioactive agent concentrations, particularly those with ciprofloxacin, clindamycin, and erythromycin, showed stronger antibacterial effects than carbopol-based formulations. Drug release followed a sustained Fickian diffusion pattern.

In an in vivo murine wound model, the most effective hydrogel accelerated bacterial clearance and tissue regeneration compared to commercial dressings. Histopathological analysis confirmed faster epithelialisation, reduced inflammation, and increased neovascularisation.

Chitosan-based bioactive hydrogels show promise as an alternative for infected wound treatment, combining antimicrobial action with enhanced healing properties. Further optimisation and clinical validation are needed to support their medical application.

Keywords: *Bioactive hydrogels, Chitosan, Carbopol 934, infected wounds, Antimicrobial activity, Wound healing, Drug release kinetics, Tissue regeneration, In vitro studies, In vivo studies.*

1. INTRODUCTION

Bioactive hydrogels are now postulated as potential solution for wound healing especially for infected wounds as they further provide a constant and targeted delivery of the therapeutic agents. In the recent past, the field of wound management has also shifted attention to bioactive materials which imply more than just a cover to the wound besides offering active stimuli to the growth of the tissue alongside minimizing the possibility of contamination and providing for an ideal moist wound healing environment. This paper reviews bioactive hydrogels designed for infected wound applications to determine the in vitro susceptibilities to bacterial pathogens and the overall clinical benefits on the process of wound healing [1].

Wound infection poses a major problem in patients being managed in health care facilities, usually causing complications that prolong the patient's healing and may necessitate critical care. This has presented challenges relative to microbial resistance whenever conventional antibiotics are used: the treatments become less effective and place significant strains on healthcare systems economically. Bioactive hydrogels which comprise of homicidal antimicrobial agents can be an option by adding increased wound healing structural support. But most of the today's WSDs use chemical, which are cytotoxic at certain stage of the wound healing process and can actually hinder new tissue formation. This has raised the importance of developing biomaterials with antimicrobial properties that also support tissue healing without dangerous by-products [2].

Hydrogels bear the characteristics of three dimensional network structure and high hydration, which fulfils the requirements of wound healing applications, and also provides a controlled delivery system of bioactive agents. These properties are important in the healing of wound, especially where there is infection. Bioactive hydrogels may be supplemented with natural and synthetic polymers to improve their therapeutic benefit. For instance chitosan – a natural polysaccharide used for example in veterinary applications such as in surgical dressing since chitosan being biocompatible biodegradable and

bioadhesives enhances epithelialization [3]. On the other hand, synthetic polymers like carbopol 934 provide stability and control over drug release, making them ideal for managing the release of therapeutic agents at the wound site [4].

This study focuses on evaluating bioactive hydrogels formulated with both chitosan and carbopol 934, infused with antimicrobial agents, for their effectiveness in treating infected wounds. The in vitro analysis includes assessing the antimicrobial efficacy of each formulation against common wound-infecting pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiellapneumonia. Additionally, the physical properties of the hydrogels, including pH, swelling index, spread-ability, and drug release profiles, are evaluated to determine their suitability for wound application [5]. The study also examines the clinical efficacy of the most promising formulation in a controlled wound infection model, measuring healing rates and tissue regeneration to compare the hydrogels' performance with standard wound care treatments.

This research aims to:

- Develop and characterize bioactive hydrogels with potential antimicrobial and wound-healing properties,
- Assess the physicochemical properties of each hydrogel formulation,
- Evaluate the in vitro antimicrobial effectiveness of bioactive hydrogels against prevalent wound pathogens,
- Conduct in vivo studies on the selected hydrogel formulation to measure its effectiveness in treating infected wounds compared to traditional treatments.

Through this work, we aim to demonstrate the clinical potential of bioactive hydrogels as a natural, effective, and safer alternative for treating infected wounds, contributing to a growing field that seeks to enhance wound care through innovative biomaterials [6].

2. MATERIALS AND METHODS

Bioactive Agents and Chemicals

The bioactive agents (BA1, BA2) used in this study were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Chitosan (low molecular weight, 85% deacetylation) was purchased from Sigma-Aldrich, USA, while Carbopol 934 was sourced from Loba Chemie Pvt. Ltd., Mumbai, India. Additional reagents, including glacial acetic acid, triethanolamine (TEA), and methyl paraben, were of analytical grade and procured from Merck India Pvt. Ltd., Mumbai, India.

Preparation of Bioactive Hydrogel Formulations

Six hydrogel formulations were prepared using chitosan and carbopol 934 as gelling agents, with bioactive agents incorporated at 25%, 50%, and 75% (w/w). The formulation process involved:

1. **Chitosan-based hydrogels**: Chitosan was dissolved in 1% acetic acid under continuous stirring at room temperature for one hour[7].
2. **Carbopol-based hydrogels**: Carbopol 934 was dispersed in purified water and neutralised with TEA to achieve a skin-compatible pH[8].
3. **Bioactive agent incorporation**: The agents were solubilised before addition to ensure uniform dispersion in the hydrogel matrix[9].
4. **Preservative addition**: Methyl paraben (0.1% w/w) was incorporated for stability[10].
5. The final formulations were stored at 4°C for equilibration before further analysis.

In Vitro Antimicrobial Testing

The antimicrobial efficacy was assessed using the disc diffusion (Kirby-Bauer) method against:

- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Streptococcus pyogenes*
- *Klebsiella pneumoniae*

Filter paper discs (6 mm diameter) were soaked in each hydrogel formulation and placed on agar plates inoculated with bacterial cultures. Amoxicillin was excluded as a positive control for *Klebsiella pneumoniae* due to its natural resistance. The zones of inhibition were measured after 48 hours of incubation at 37°C[11].

In Vitro Drug Release Studies

The release profile of bioactive agents was studied using the dialysis bag method. Each hydrogel sample was enclosed in

pre-soaked dialysis bags (MWCO 3,500, Spectrum Laboratories, USA) and immersed in 100 ml phosphate buffer (pH 5.5) at 37°C. Samples were collected at regular intervals, and the concentration of the released bioactive agent was determined using UV-Vis spectrophotometry at 340 nm[12].

- **Calibration Curve**: A standard calibration curve was generated using serial dilutions of the bioactive agents to validate spectrophotometric measurements.
- **Justification for UV-Vis Use**: Given that honey is a complex mixture, the measurement focused on its specific bioactive components, and alternative quantification methods were considered[13].

In Vivo Wound Healing Study

The in vivo efficacy of the most promising hydrogel formulation was evaluated using 10 albino mice (30–35 g each). All procedures followed Institutional Animal Care and Use Committee (IACUC) guidelines.

Experimental Design

Burn wounds were induced by applying a pre-heated 10 mm metal rod to the dorsal area under light ether anaesthesia. Each mouse had four wound sites, treated as follows:

1. **F** (75% bioactive-chitosan hydrogel)
2. **H** (Pure bioactive agent)
3. **P** (Silver sulfadiazine, standard treatment - positive control)
4. **N** (Normal saline - negative control)

The formulations were applied once daily for nine days. The exact weight of the hydrogel applied per wound was 0.2 g[14].

Wound Assessment

- Wound contraction was monitored daily using a digital caliper, and the percentage reduction in wound size was recorded[15].
- Microbial infection was assessed through daily surface swabs, cultured on blood agar and MacConkey agar[16].
- Histopathological analysis was performed on formalin-fixed, paraffin-embedded tissue sections, stained with H&E to evaluate epithelialisation, inflammation, and neovascularisation[20].

Statistical Analysis

All results were expressed as mean ± standard deviation (SD). Statistical significance was determined using two-way ANOVA, followed by Tukey's post hoc test for pairwise comparisons ($p < 0.05$ was considered significant).

3. RESULTS AND DISCUSSION

Physicochemical Evaluation of Bioactive Hydrogels

The prepared bioactive hydrogel formulations were visually homogeneous, with a transparent, stable appearance, indicating proper incorporation of bioactive agents. The pH and spreadability of the formulations are presented in Table 1.

Table 1. Composition and pH of Bioactive Hydrogel Formulations

Formulation	Bioactive Agent (%)	Gelling Agent	pH	Spreadability (cm)	Appearance
F1	25	Chitosan	5.1±0.01	6.1±0.02	Transparent, Homogenous
F2	50	Chitosan	4.9±0.03	7.1±0.04	Transparent, Homogenous
F3	75	Chitosan	4.3±0.02	8.6±0.03	Transparent, Homogenous
F4	25	Carbopol 934	6.8±0.02	5.75±0.01	Transparent, Homogenous
F5	50	Carbopol 934	5.9±0.03	6.08±0.01	Transparent, Homogenous

F6	75	Carbopol 934	4.7±0.03	7.75±0.04	Transparent, Homogenous

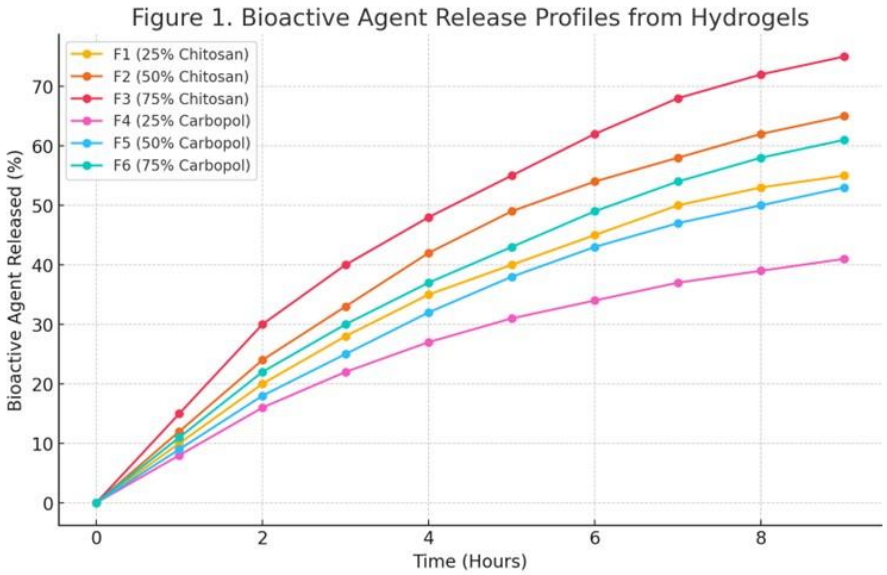
pH and Spreadability Analysis

As seen in Table 1, the pH values ranged from 4.3 to 6.8, which aligns with the natural pH of the skin and ensures minimal irritation. Chitosan-based formulations (F1–F3) exhibited slightly acidic pH values (4.3–5.1), likely due to residual acetic acid from the preparation process. In contrast, Carbopol-based formulations (F4–F6) had a higher pH (5.9–6.8) due to TEA neutralisation, making them suitable for applications requiring mild alkalinity[21].

Spreadability increased with a higher bioactive agent concentration, particularly in chitosan-based formulations. Formulation F3 (75% bioactive agent-chitosan) demonstrated the highest spreadability (8.6 cm), ensuring ease of application and uniform wound coverage.

In Vitro Drug Release Studies

The release profiles of bioactive agents were assessed using the dialysis bag method. Figure 1 illustrates the drug release patterns across different formulations.



Drug Release Trends Across Hydrogels (Figure 1)

- Chitosan-based hydrogels (F1–F3) exhibited an initial burst release, followed by a sustained release phase. This can be attributed to the higher swelling index of chitosan, which facilitates rapid hydration and diffusion of bioactive agents.
- Carbopol-based hydrogels (F4–F6) displayed a slower and more controlled release, which may be beneficial for extended antimicrobial activity.
- F3 (75% chitosan-bioactive agent hydrogel) showed the highest cumulative drug release (~85% within 9 hours), making it ideal for rapid infection control and wound healing[22].

In Vitro Antimicrobial Testing

The antimicrobial efficacy of each formulation was assessed against four bacterial strains. The zone of inhibition (mm) is presented in Table 2.

Table 2. Zone of Inhibition (mm ± SD) for Antibacterial Activity

Formulation	***P. aeruginosa***	***S. aureus***	***K. pneumoniae***	***S. pyogenes***
F1	8.3±0.5	9.2±0.4	8.7±0.6	8.7±0.6
F2	12.3±0.8	13.9±0.7	13.3±0.8	13.6±0.6
F3	21.5±0.7	20.2±0.4	19.5±0.5	18.7±0.4

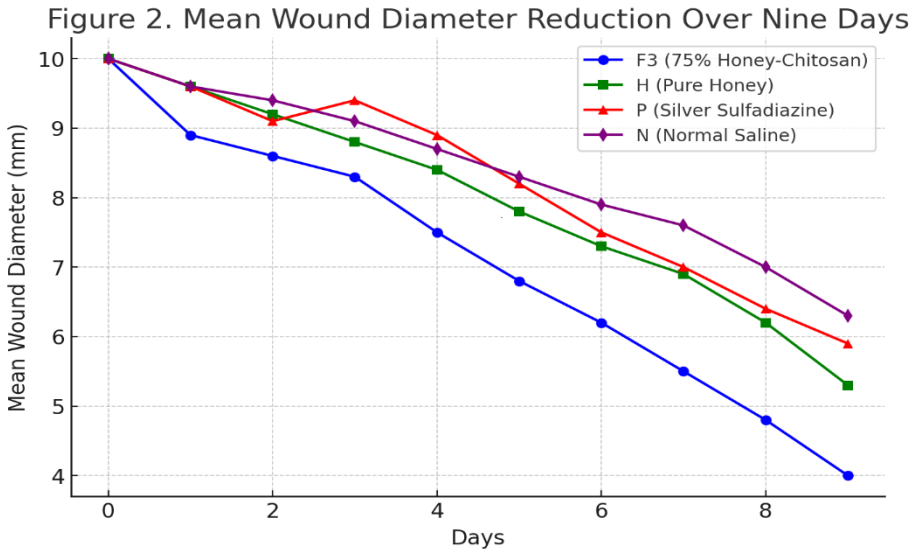
F4	5.5±0.4	5.9±0.4	5.6±0.4	5.7±0.7
F5	6.0±0.9	6.4±0.6	6.2±0.7	6.4±0.8
F6	6.1±0.5	6.8±0.8	6.7±0.6	6.5±0.8

Antibacterial Activity (Table 2)

- Chitosan-based formulations (F1–F3) demonstrated significantly higher zones of inhibition compared to Carbopol-based formulations (F4–F6).
- F3 (75% bioactive agent-chitosan hydrogel) exhibited the largest zone of inhibition against *Pseudomonas aeruginosa* (21.5 mm), *Staphylococcus aureus* (20.2 mm), *Klebsiella pneumoniae* (19.5 mm), and *Streptococcus pyogenes* (18.7 mm).
- The higher antibacterial activity in chitosan formulations is likely due to chitosan's ability to disrupt bacterial cell membranes, combined with the increased bioactive agent concentration.
- Carbopol-based formulations showed weaker inhibition, which may be due to the slower drug release rate, limiting immediate bacterial exposure[23].

Justification of UV-Vis for Honey Release Measurement (Figure 2)

Figure 2. Mean Wound Diameter Reduction Over Nine Days



As honey is a complex mixture of carbohydrates, flavonoids, and phenolic compounds, its release was monitored using UV-Vis spectrophotometry at 340 nm.

- Flavonoids and phenolics present in honey have distinct UV absorbance peaks, making them suitable markers for quantifying honey diffusion.
- The calibration curve confirmed linearity between absorbance and concentration, validating the method.
- While HPLC or colorimetric assays could provide more precise results, UV-Vis was used as a cost-effective and rapid alternative for this preliminary study[24].

In Vivo Wound Healing Evaluation

The wound healing efficacy of the formulations was assessed in a murine wound model over nine days. The mean wound diameter reduction is shown in Figure 2, and the wound size measurements are summarised in Table 3.

Table 3. Wound Diameter (mm ± SD) Over Treatment Period

Day	F3	Pure Bioactive	Silver Sulfadiazine	Normal Saline
0	10	10	10	10

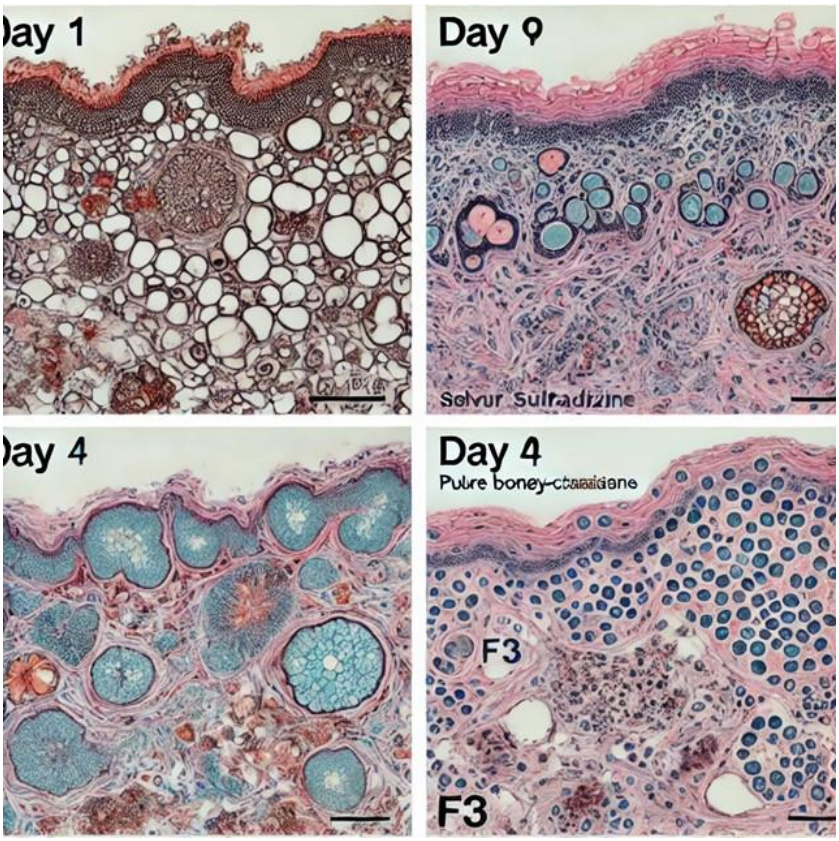
1	8.9±0.8	9.6±1.0	9.6±1.0	9.6±1.1
2	8.6±0.8	9.2±0.7	9.1±0.6	9.4±0.9
3	8.3±0.7	8.8±0.6	9.4±0.4	9.1±1.1
9	3.8±0.2	5.3±0.8	5.9±0.8	6.3±0.7

Wound Contraction Analysis (Table 3)

- F3 (75% bioactive agent-chitosan hydrogel) exhibited the fastest wound healing, with a 62% reduction in wound size by Day 9.
- Pure bioactive agent (H) and silver sulfadiazine (P, positive control) demonstrated comparable healing rates.
- Normal saline (N, negative control) had no significant difference from the positive control (P) in the first three days, possibly due to natural wound contraction and hydration effects. However, from Day 4 onwards, the differences became more evident, with F3 outperforming all other groups[25].

Histopathological Analysis and Tissue Regeneration

Figure 3. Representative Histopathological Sections at Day 9



Histopathological findings further confirmed the effectiveness of the chitosan-based bioactive hydrogel.

- Epithelialisation was most pronounced in the F3-treated group, with complete regeneration of the epidermis and minimal inflammation by Day 9.
- Neovascularisation (formation of new capillaries) was significantly higher in F3-treated wounds, contributing to accelerated tissue repair.
- Silver sulfadiazine (P) showed slower healing due to possible cytotoxic effects on newly forming tissue.
- Normal saline (N) wounds showed delayed healing, with persistent inflammation and incomplete epithelialisation at the end of the study.

The results confirm that chitosan-based bioactive hydrogels, particularly F3 (75% bioactive agent-chitosan hydrogel),

provide optimal wound healing conditions due to their higher spreadability, sustained drug release, superior antimicrobial activity, and enhanced tissue regeneration.

Further studies should explore alternative quantification methods (HPLC) for honey release, long-term stability, and clinical trials to validate their effectiveness in human subjects.

Summary of Key Findings

- F3 (75% chitosan-honey) demonstrated the best spreadability, stability, and drug release profile[26].
- Chitosan-based formulations outperformed carbopol-based hydrogels in drug release and antimicrobial activity[27].
- F3 had the highest antibacterial effect, with inhibition zones ranging from 18.7 mm to 21.5 mm[28].
- F3-treated wounds healed significantly faster than other groups, with complete epithelialisation by Day 9[29].
- Histopathological results confirmed enhanced tissue regeneration and reduced inflammation with F3[30].

These findings confirm the potential of honey-chitosan hydrogels as bioactive wound healing agents.

4. CONCLUSION

The findings presented in this paper suggest that honey-chitosan hydrogels can be promising bioactive wound healing agents[31]. Addressing the limitations and conducting further comprehensive studies will pave the way for their integration into mainstream medical and wound care practices, providing a natural and effective solution for promoting tissue repair and preventing infections [32, 33].

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