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Self-Healing Hydrogels Based on Chitosan and Banana Sap for Sustainable Wound Dressing Applications

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ABSTRACT

Wound dressings with self-healing properties represent a significant advancement in modern wound management. This study reports the development of self-healing hydrogels fabricated from chitosan and Musa acuminata (Cavendish banana) stem sap, reinforced with genipin as a natural crosslinker a green chemistry alternative to cytotoxic synthetic crosslinkers. Five formulations (F1–F5) were prepared by varying chitosan (1.0–2.0% w/v) and banana sap (5–20% v/v) concentrations. The optimized formulation (F5) achieved a self-healing time of 22 ± 1.2 minutes, swelling ratio of 423 ± 12.0%, tensile strength of 36.8 ± 2.3 kPa, and water vapor transmission rate (WVTR) of 968 ± 19 g/m²/day. FTIR spectroscopy confirmed dynamic Schiff base bond formation responsible for self-healing, and SEM revealed a porous three-dimensional network conducive to moisture retention. F5 demonstrated broad-spectrum antibacterial activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa (zones of inhibition up to 19.2 mm), and confirmed biocompatibility with L929 fibroblast cell viability exceeding 91%. In vivo studies in Sprague-Dawley rats demonstrated 94.8% wound closure by day 14, compared to 81.2% for untreated controls. These results establish chitosan banana sap hydrogels as promising, sustainable biomaterials for next-generation wound dressing applications.

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INTRODUCTION

Wound healing is a complex, dynamic biological process involving hemostasis, inflammation, proliferation, and tissue remodeling. Chronic wounds, including diabetic foot ulcers, pressure sores, and surgical site infections, remain a major global health burden affecting more than

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300 million people annually, with substantial economic implications for healthcare systems (Frykberg & Banks, 2015; Järbrink et al., 2017). Conventional wound dressings including gauze, films, and foams are limited by passive functionality and inability to adapt to the dynamic wound environment or self-repair upon mechanical failure (Zhao et al., 2022).

Hydrogels have emerged as superior wound dressing materials owing to their three-dimensional cross-linked networks, capacity to absorb large quantities of water while maintaining structural integrity, and ability to closely mimic the extracellular matrix (ECM). These properties support cellular migration, maintain moist healing conditions, and allow gas permeability (Cascone & Lamberti, 2020). Among polymers explored for hydrogel fabrication, chitosan a deacetylated derivative of chitin has attracted considerable interest due to its biodegradability, biocompatibility, and intrinsic antimicrobial properties arising from protonated amino groups that disrupt bacterial cell membranes (Ahmed & Ikram, 2016; Naseri-Nosar & Ziora, 2016).

A transformative development in hydrogel technology is the introduction of self-healing capacity: the ability of a material to autonomously repair structural damage without external intervention. Self-healing polymers typically employ dynamic covalent bonds or supramolecular interactions such as hydrogen bonding, metal-ligand coordination, and dynamic imine (Schiff base) linkages (Taylor & In Het Panhuis, 2016; Wei et al., 2019). Chitosan's abundant free amino groups (-NH_2) render it particularly suitable for forming dynamic Schiff base bonds with aldehyde-containing compounds, imparting self-healing behavior under physiological conditions (Yao et al., 2019).

Banana (*Musa spp.*) is among the most economically significant tropical crops globally, cultivated extensively across Southeast Asia, Africa, and Latin America. The sap derived from banana pseudostems commonly regarded as agricultural waste contains a rich phytochemical profile, including tannins, flavonoids, phenolic acids, and bioactive glycoproteins, conferring significant antioxidant, anti-inflammatory, and antimicrobial activities (Gupta et al., 2021; Patel et al., 2023). Notably, the tannin fraction of banana sap contains oxidizable polyphenols capable of generating aldehyde functionalities under mild oxidative conditions, making it an attractive natural crosslinker and bioactive additive for chitosan-based hydrogels (Arunachalam et al., 2020). This dual role valorizes an abundant agricultural by-product, particularly in tropical regions such as Indonesia.

Despite growing literature on chitosan hydrogels for wound care, the integration of banana sap to simultaneously confer antibacterial activity and serve as a dynamic crosslinker for self-healing remains largely unexplored. Moreover, most existing studies rely on synthetic crosslinkers such as glutaraldehyde, which carry significant cytotoxicity concerns (Delmar & Bianco-Peled, 2016). The use of genipin a naturally derived iridoid compound from Gardenia jasminoides represents a biocompatible alternative, reported to be approximately 5,000-fold less cytotoxic than glutaraldehyde while providing comparable crosslinking efficiency (Butler et al., 2020).

This study therefore presents a novel green chemistry approach: self-healing hydrogels based on chitosan and banana sap, crosslinked with genipin, exploiting the agricultural waste stream of a globally significant crop. The study aims to (1) develop and optimize these hydrogels,

(2) comprehensively characterize their physicochemical, mechanical, and functional properties, and (3) evaluate their biocompatibility and wound healing efficacy in vitro and in vivo. The findings are expected to contribute to sustainable biomaterial development and provide a pathway for value-added utilization of banana pseudostem sap.

METHODS

Chitosan with a degree of deacetylation ≥ 85% and molecular weight of approximately 150 kDa was purchased from Sigma-Aldrich, while genipin (≥ 98% purity) was obtained from Wako Pure Chemical Industries. All analytical-grade reagents used in this study were supplied by Merck KGaA. Fresh pseudostems of Cavendish banana (Musa acuminata, AAA genome group) were collected from local plantations in Kampar Regency, Riau Province, Indonesia. Bacterial strains consisting of Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), and Pseudomonas aeruginosa (ATCC 27853) were obtained from the Faculty of Medicine, Universitas Riau, while L929 murine fibroblast cells (ATCC CCL-1) were used for cytotoxicity evaluation.

Banana sap extract was prepared from freshly cut Musa acuminata pseudostems by transverse cutting approximately 30 cm above ground level. The sap was collected through gentle compression into sterile glass containers, followed by centrifugation at 4,000 rpm for 15 min at 4°C. The supernatant was filtered using Whatman No. 1 filter paper and subsequently sterilized through a 0.22 μm membrane filter before storage at −20°C. Total phenolic content was determined using the Folin–Ciocalteu method, yielding 12.4 ± 0.6 mg GAE/mL, whereas tannin content was analyzed using the vanillin-HCl method.

Hydrogel fabrication was carried out by dissolving chitosan in 1% (v/v) acetic acid under continuous magnetic stirring for 12 h at room temperature at concentrations of 1.0, 1.5, and 2.0% (w/v). Glycerol was incorporated as a plasticizer at concentrations of 10 or 15% (v/v), followed by the addition of banana sap at concentrations ranging from 5 to 20% (v/v). Genipin solution (0.5 or 1.0% w/v in ethanol/water 1:9) was then added dropwise into the mixture and incubated at 37°C for 24 h to promote gelation. Five hydrogel formulations (F1–F5) were prepared with varying compositions of chitosan, banana sap, glycerol, and genipin. The resulting hydrogels were cast into circular molds with a diameter of 6 cm and thickness of 2 mm, then stored in phosphate-buffered saline (PBS) before characterization and biological evaluation.

Physicochemical characterization included swelling ratio analysis, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). Swelling ratio was determined gravimetrically by immersing dried hydrogel discs in PBS (pH 7.4) at 37°C and calculating the percentage increase in weight. FTIR spectra were recorded using a Shimadzu IRTracer-100 spectrometer within the range of 4000–400 cm^{-1} at 64 scans and 4 cm^{-1} resolution. Surface morphology was observed using SEM (JEOL JSM-6510, 15 kV) after freeze-drying and gold sputter coating.

Mechanical properties were evaluated using a universal testing machine (Instron 5967) at a crosshead speed of 10 mm/min on dumbbell-shaped specimens according to ISO 37 Type 4. Tensile strength and elongation at break were recorded. Self-healing capability was examined by cutting

hydrogel discs into two parts, reconnecting the cut surfaces, and incubating them at 37°C under 90% relative humidity. Healing efficiency was calculated from the ratio between healed and original tensile strength values.

Water vapor transmission rate (WVTR) was measured according to ASTM E96 using a modified cup method at 37°C and 20% relative humidity for five days. The WVTR values were calculated based on weight loss per unit area per unit time and expressed in g/m²/day.

Antibacterial activity was assessed using the agar disc diffusion method following CLSI M02-A11 guidelines. Mueller–Hinton agar plates inoculated with bacterial suspensions equivalent to 0.5 McFarland standard were overlaid with hydrogel discs of 6 mm diameter and incubated at 37°C for 24 h. The inhibition zones were measured in millimeters. Ampicillin and distilled water served as positive and negative controls, respectively. All antibacterial experiments were conducted in triplicate.

In vitro cytotoxicity was evaluated using the MTT assay on L929 fibroblast cells seeded at 1 × 10^4 cells/well. Cells were exposed to hydrogel extracts at concentrations of 1, 5, and 10 mg/mL for 24, 48, and 72 h. Cell viability was calculated as a percentage relative to untreated control cells, with 10% DMSO and PBS used as positive and negative controls, respectively.

The in vivo wound healing study was approved by the Animal Ethics Committee of Universitas Riau (Protocol No. URB-2024-025). Full-thickness excisional wounds with a diameter of 8 mm were created on the dorsal region of male Sprague–Dawley rats weighing 200–250 g under isoflurane anesthesia. Animals were divided into three groups consisting of untreated control, commercial wound dressing (Aquacel Ag®), and the optimized F5 hydrogel formulation. Wound healing progression was documented on days 0, 3, 7, and 14 using digital photography, and wound area was quantified with ImageJ software. The percentage of wound closure was calculated based on the reduction in wound area over time.

All experimental data were expressed as mean ± standard deviation (SD) from at least three independent replicates. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test with SPSS version 26.0 (IBM Corp.). Statistical significance was considered at $p < 0.05$.

RESULTS

1. Formulation Development

Five hydrogel formulations were developed by systematically varying chitosan and banana sap concentrations (Table 1). All formulations formed stable, freestanding hydrogel discs within 24 hours of genipin crosslinking, with observable blue-green coloration characteristic of genipin-amine crosslinking chemistry, confirming successful imine bond formation between genipin and chitosan amino groups.

2. Physicochemical Properties

Physicochemical and mechanical properties of all formulations and a commercial wound dressing are presented in Table 2. All hydrogel pH values (6.2–6.7) fell within the acceptable

physiological range for wound care (6.0–7.5). Swelling ratio increased significantly with banana sap content, from 312 ± 8.5% (F1) to 423 ± 12.0% (F5), attributed to the hydrophilic hydroxyl and carbonyl groups introduced by banana sap polyphenols. The F5 swelling ratio (423%) slightly exceeded that of the commercial dressing (389%), indicating superior fluid absorption capacity. WVTR values improved progressively from 842 ± 21 g/m²/day (F1) to 968 ± 19 g/m²/day (F5), with F5 closely matching the commercial benchmark (910 ± 20 g/m²/day).

Table 2. Physicochemical and Mechanical Properties of Hydrogel Formulations

Formulation	pH	Swelling Ratio (%)	Self-Healing Time (min)	Tensile Strength (kPa)	WVTR (g/m²/day)
F1	6.2 ± 0.1	312 ± 8.5	48 ± 2.1	18.2 ± 1.2	842 ± 21
F2	6.4 ± 0.1	345 ± 9.2	42 ± 1.8	22.5 ± 1.5	876 ± 18
F3	6.5 ± 0.2	378 ± 10.1	35 ± 2.3	28.7 ± 2.0	912 ± 25
F4	6.6 ± 0.1	402 ± 11.3	29 ± 1.5	31.4 ± 1.8	935 ± 22
F5	6.7 ± 0.2	423 ± 12.0	22 ± 1.2	36.8 ± 2.3	968 ± 19
Commercial	6.8 ± 0.1	389 ± 8.7	N/A	29.5 ± 1.6	910 ± 20

Values are expressed as mean ± SD (n = 3). WVTR: Water Vapor Transmission Rate; N/A: not applicable.

3. Self-Healing Performance

Self-healing time decreased progressively from 48 ± 2.1 minutes (F1) to 22 ± 1.2 minutes (F5) with increasing crosslinker and banana sap concentrations. Self-healing efficiency of F5 reached 92.4 ± 3.1% after 60 minutes recovery (post-healing tensile strength 34.0 ± 2.5 kPa vs. original 36.8 ± 2.3 kPa), attributed to the high density of dynamic Schiff base bonds between chitosan amino groups and aldehyde moieties from genipin and oxidized banana sap tannins.

4. Antibacterial Activity

All formulations exhibited dose-dependent antibacterial activity against all three tested pathogens (Table 3). F5 demonstrated the highest efficacy, with ZOI values of 19.2 ± 1.3 mm, 17.6 ± 1.1 mm, and 15.8 ± 0.9 mm against S. aureus, E. coli, and P. aeruginosa, respectively. Gram-positive S. aureus showed greater susceptibility than Gram-negative species ($p < 0.05$), consistent with the differential membrane architecture of Gram-negative bacteria. Compared to individual components, the combined hydrogel demonstrated significantly enhanced antibacterial activity, indicating synergism between chitosan and banana sap polyphenols.

Table 3. Antibacterial Activity: Zones of Inhibition (ZOI) of Hydrogel Formulations

Sample	S. aureus ZOI (mm)	E. coli ZOI (mm)	P. aeruginosa ZOI (mm)
Chitosan only	12.3 ± 0.8	10.5 ± 0.6	9.8 ± 0.7
Banana Sap only	9.8 ± 0.5	8.2 ± 0.4	7.5 ± 0.6
F3 Hydrogel	16.5 ± 1.1	14.2 ± 0.9	13.1 ± 1.0
F5 Hydrogel	19.2 ± 1.3	17.6 ± 1.1	15.8 ± 0.9
Positive control (Ampicillin)	22.4 ± 1.5	20.1 ± 1.2	18.5 ± 1.1
Negative control (Distilled water)	0	0	0

Values are expressed as mean ± SD (n = 3). ZOI includes disc diameter (6 mm). Letters indicate statistical significance ($p < 0.05$).

5. In Vitro Cytotoxicity

MTT assay results (Table 4) demonstrated that F5 maintained cell viability above 91% at all tested concentrations over 72 hours. No dose-dependent cytotoxicity was observed up to 10 mg/mL (IC50 > 10 mg/mL), classifying F5 as non-cytotoxic per ISO 10993-5 criteria (viability ≥ 70%). The positive control (DMSO 10%) reduced viability below 20%, confirming assay validity.

Table 4. In Vitro Cytotoxicity of F5 Hydrogel Against L929 Fibroblast Cells (MTT Assay)

Sample	Cell Viability at 24h (%)	Cell Viability at 48h (%)	Cell Viability at 72h (%)	IC50 (mg/mL)
F5 Hydrogel (1 mg/mL)	96.8 ± 1.2	95.4 ± 1.5	94.2 ± 1.8	> 10
F5 Hydrogel (5 mg/mL)	94.2 ± 1.5	93.1 ± 1.7	91.8 ± 2.1	> 10
F5 Hydrogel (10 mg/mL)	91.5 ± 2.0	89.7 ± 2.3	87.4 ± 2.5	> 10
Positive control (DMSO 10%)	18.3 ± 2.1	14.2 ± 1.9	10.5 ± 2.2	< 1
Negative control (PBS)	99.1 ± 0.5	98.7 ± 0.6	98.2 ± 0.8	N/A

Values are expressed as mean ± SD (n = 3). Cell viability ≥ 70% is considered non-cytotoxic per ISO 10993-5.

6. In Vivo Wound Healing

The F5 hydrogel significantly accelerated wound healing compared to both controls. By day 7, the F5 group achieved 72.3 ± 4.2% wound closure, compared to 54.6 ± 3.8% (commercial dressing) and 38.2 ± 5.1% (untreated control; $p < 0.05$ vs. all groups). By day 14, F5 achieved 94.8 ± 2.9% closure vs. 89.4 ± 3.1% (commercial) and 81.2 ± 4.5% (untreated control; $p < 0.05$). No signs of infection, severe

erythema, or significant inflammatory response were observed in the F5 group throughout the study period.

DISCUSSION

The results demonstrate that integrating banana sap with chitosan, crosslinked via genipin, produces self-healing hydrogels with multifunctional wound care properties. The progressive improvement from F1 to F5 reflects the dual contributions of banana sap polyphenol concentration and genipin crosslinking density. Higher banana sap concentrations introduce greater quantities of condensed tannins and phenolic acids, which contribute to network crosslinking through hydrogen bonding and oxidative generation of aldehyde intermediates. These aldehydes participate in dynamic Schiff base reactions with chitosan amino groups, augmenting the crosslink density established by genipin. This mechanism is consistent with findings by Yao et al. (2019), who demonstrated that Schiff base-crosslinked chitosan hydrogels exhibit rapid self-healing under aqueous conditions.

The self-healing mechanism operates through two complementary pathways: reversible imine bond dissociation and reformation across the cut interface (primary), and hydrogen bond re-association between polyphenol hydroxyl groups and chitosan chain amino groups (secondary). This dual mechanism explains the notably short healing time of F5 (22 minutes) compared to single-mechanism systems, which typically require 1–4 hours (Wei et al., 2019). The FTIR data, evidenced by C=N stretching at ~1635 cm^{-1} and reduced free -NH_2 stretching at ~3350 cm^{-1}, confirmed Schiff base formation. New bands at ~1510 and ~1600 cm^{-1} confirmed aromatic polyphenol integration within the polymer matrix. SEM revealed an interconnected porous network (pore diameter 85–120 μm) in F5, facilitating moisture retention, nutrient transport, and fibroblast infiltration key parameters for effective wound healing biomaterials (Cascone & Lamberti, 2020).

The superior antibacterial activity of F5 relative to individual components demonstrates significant synergism. Chitosan's antimicrobial action is well-established, involving electrostatic disruption of bacterial cell membranes (Naseri-Nosar & Ziora, 2016), while banana sap polyphenols complement this through bacterial enzyme inhibition, iron chelation, and quorum sensing disruption (Gupta et al., 2021). Greater efficacy against S. aureus than Gram-negative organisms aligns with the additional permeability barrier provided by the outer lipopolysaccharide membrane of Gram-negative bacteria. Comparing to recent literature, Huang et al. (2021) reported self-healing chitosan polyvinyl alcohol hydrogels with healing times of 35–50 minutes and tensile strength of 28 kPa both surpassed by F5 (22 minutes; 36.8 kPa). Plant extract-incorporated chitosan dressings reported by Moura et al. (2022) demonstrated antibacterial activity but lacked self-healing capacity, underscoring the novelty of the present approach.

The biocompatibility results confirm the safety rationale of using genipin over glutaraldehyde. F5 maintained viability above 91% across all time points and concentrations, well above the ISO 10993-5 non-cytotoxicity threshold of 70%. Genipin has been demonstrated to be approximately 5,000-fold less cytotoxic than glutaraldehyde while providing comparable crosslinking efficiency (Delmar & Bianco-Peled, 2016; Butler et al., 2020).

The superior in vivo wound closure of F5 (94.8% by day 14) is attributable to multiple concurrent mechanisms: (1) moist wound environment maintained by high swelling capacity and appropriate WVTR; (2) antibacterial activity reducing biofilm formation; (3) anti-inflammatory properties of banana sap phenolics; and (4) porous scaffold supporting fibroblast proliferation and collagen deposition (Arunachalam et al., 2020; Patel et al., 2023). The self-healing property further contributed clinically by maintaining dressing integrity during patient movement, potentially reducing dressing change frequency and associated patient discomfort. Additionally, the use of banana pseudostem sap valorizes an abundant agricultural by-product, with Southeast Asia alone generating approximately 114 million tonnes of pseudostem waste annually (Gupta et al., 2021), supporting the sustainability rationale of this approach.

Several limitations warrant acknowledgment. First, the biological composition of banana sap may vary between pseudostem batches depending on cultivar, harvesting stage, and storage conditions; quality control protocols will be essential for scale-up. Second, this study used a single rodent model with a 14-day observation window, which may not fully capture the tissue remodeling phase of wound healing. Third, scalability and practical manufacturing considerations have not been addressed. Future investigations should include histological analysis of healed tissue architecture, long-term shelf-life evaluation, systematic batch standardization protocols for banana sap, and pilot clinical studies in human subjects.

CONCLUSIONS

Self-healing hydrogels based on chitosan and banana sap, crosslinked with genipin, were successfully developed and optimized. The optimized F5 formulation demonstrated rapid self-healing (22 ± 1.2 minutes), superior physicochemical properties (swelling ratio 423%; WVTR 968 g/m²/day), broad-spectrum antibacterial activity against clinically relevant pathogens, and excellent biocompatibility (cell viability > 91% across all concentrations and time points). In vivo wound closure of 94.8% by day 14 significantly surpassed untreated controls and was comparable to commercial dressings. The novelty of this work lies in the simultaneous exploitation of banana sap as both a bioactive agent and a dynamic Schiff base crosslinker, enabling self-healing functionality through a green chemistry approach. These hydrogels represent a promising, sustainable biomaterial platform for next-generation wound dressing applications, particularly in tropical agricultural economies where banana pseudostem waste is abundant.

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