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Formulation of *Aloe vera* and Forest Honey Hydrogel Dressing for Diabetic Ulcer Healing

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ABSTRACT

Hydrogel dressings are widely used for chronic wound management because they maintain a moist microenvironment, accommodate exudate, and support tissue repair. This study formulated a chitosan-based forest honey–*Aloe vera* hydrogel dressing and evaluated its physicochemical characteristics and wound-healing performance in an alloxan-induced hyperglycaemic excisional wound model in male rats (*Rattus norvegicus*). Four formulations were prepared by varying the proportions of forest honey and *Aloe vera*: F1 (20% honey), F2 (20% *Aloe vera*), F3 (15% honey + 5% *Aloe vera*), and F4 (5% honey + 15% *Aloe vera*). Physicochemical evaluation included organoleptic characteristics and homogeneity, pH, spreadability, adhesion time, and swelling capacity. All formulations produced homogeneous, translucent hydrogels without visible phase separation. The pH values were mildly acidic (4.85–5.40), spreadability ranged from 5.80 to 6.50 cm, adhesion time ranged from 8.1 to 14.2 min, and swelling capacity ranged from 190% to 230%. In vivo, complete macroscopic wound closure occurred by Day 10 for F2 and the positive control, followed by Day 11 for F4, Day 12 for F3, and Day 13 for F1; the untreated negative control did not reach closure by Day 14. Overall, the *Aloe vera*–dominant hydrogel (F2) showed the fastest closure kinetics in this dataset.

Keywords :
Aloe vera; Forest honey; Chitosan hydrogel dressing; Diabetic wound model; Alloxan; *Rattus norvegicus*

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1. Introduction

Diabetes mellitus remains a major global health burden, with a continuously expanding population at risk of chronic complications, including diabetes-related foot ulcers and other chronic wounds that are clinically recalcitrant and economically costly [1]. Among these complications, diabetic foot ulcers are particularly consequential because they are frequently complicated by infection, ischemia, and neuropathy, and they contribute substantially to hospitalisation, lower-limb amputation, and recurrence after apparent closure [2]. Contemporary guidance therefore emphasises a structured, evidence-based approach to wound bed preparation and adjunctive therapies to

accelerate healing and prevent deterioration, while maintaining strict attention to infection control and tissue viability [3].

From a biological perspective, diabetic ulcers commonly exhibit prolonged inflammation, impaired angiogenesis, dysregulated extracellular matrix remodelling, and delayed re-epithelialisation, which collectively undermine orderly tissue repair. These constraints create a rational need for wound dressings that can stabilise the local microenvironment, maintain moisture balance, absorb exudate, minimise microbial bioburden, and, where possible, deliver bioactive agents in a controlled manner. In this context, hydrogel dressings have received sustained attention because their hydrated, three-dimensional polymeric networks can emulate aspects of soft tissue, support autolytic debridement through moisture retention, and function as matrices for tunable diffusion- or swelling-mediated release of therapeutic constituents [4].

Natural bioactive materials, including *Aloe vera* and honey, are frequently investigated as functional additives within hydrogel platforms because they offer complementary mechanisms relevant to chronic wound management, such as modulation of inflammation, antioxidant activity, and antimicrobial effects. Clinical and translational evidence has also suggested that honey-based dressings can improve wound-related outcomes in chronic wounds, although effect sizes vary across settings and comparators, supporting the need for formulation-specific evaluation and rigorous testing [5]. Similarly, composite systems incorporating *Aloe* polysaccharides, honey, and synthetic polymers (e.g., poly(vinyl alcohol), PVA) have demonstrated favourable physicochemical properties and biologically relevant performance in wound models, reinforcing the plausibility of synergistic functionality when these components are integrated into a single hydrogel dressing [6].

Based on this rationale, the present study is positioned to develop and evaluate a hydrogel dressing formulated from *Aloe vera* and forest honey, with the intent to optimise physicochemical characteristics and to support diabetic ulcer healing through a moist, protective microenvironment and bioactive contribution from natural constituents. This formulation-driven approach is expected to be particularly relevant for settings where accessible, biologically active materials can be incorporated into a robust polymer network to yield a practical and scalable wound dressing candidate.

2. Methods

Research Design

This study was an experimental laboratory investigation conducted in three sequential phases: (1) formulation of a biodegradable chitosan-based hydrogel dressing incorporating *Aloe vera* gel and forest honey, (2) physicochemical evaluation of the prepared hydrogel formulations, and (3) *in vivo* assessment of wound-healing performance using an alloxan-induced hyperglycaemic rat excisional wound model [7], [9],[10].

Materials

Fresh *Aloe vera* leaves were used as the source of *Aloe vera* gel. Gorontalo forest honey (commonly associated with *Apis dorsata*) was used as the natural bioactive ingredient. Chitosan was used as the biodegradable polymer matrix for hydrogel preparation. All materials and reagents were of pharmaceutical/analytical grade.

Formulation Design and Preparation

Four hydrogel formulations (F1–F4) were prepared by varying the proportions of *Aloe vera* gel and forest honey while maintaining the chitosan-based hydrogel base

constant across formulations [7]. Total composition was adjusted to 100% (w/w). The formulation scheme is shown in **Table 1**.

Table 1. Composition of *Aloe vera*–Forest Honey Hydrogel Formulations (w/w%)

Ingredient	Function	F1	F2	F3	F4
Forest honey	Bioactive component	20	0	15	5
Aloe vera gel	Bioactive component	0	20	5	15
Chitosan-based hydrogel base	Matrix/vehicle (constant)	80	80	80	80
Total		100	100	100	100

Aloe vera gel was obtained by washing fresh leaves, removing the outer rind, collecting the inner gel, and homogenising it to obtain a uniform gel phase. A chitosan-based hydrogel base was prepared by hydrating/dissolving chitosan in an acidic aqueous medium under continuous stirring until a homogeneous gel matrix was formed [7]. After base formation, forest honey and *Aloe vera* gel were incorporated gradually according to **Table 1** under continuous mixing until uniform. The hydrogels were allowed to equilibrate to minimise entrapped air prior to testing and *in vivo* application.

Physicochemical Evaluation

Organoleptic properties and homogeneity. Appearance (clarity/colour), odour, and consistency were assessed visually. Homogeneity was evaluated by spreading a small amount on a clean glass surface to confirm the absence of coarse particles and phase separation.

pH measurement. pH was determined using a calibrated pH meter at room temperature. The same measurement procedure was applied for all formulations to ensure comparability.

Spreadability (dispersion). Spreadability was assessed using a plate-based method for semisolid preparations. A fixed amount of hydrogel was placed between two plates under a standardised load for a defined time, and the spread diameter was recorded in centimetres (cm) [8].

Adhesion time. Adhesion time was evaluated by placing the hydrogel between two glass surfaces, applying a defined compressive load for a defined contact period, and recording the time required for detachment/separation. Adhesion was expressed as detachment time (minutes).

Swelling ratio. Swelling capacity was determined gravimetrically. A pre-weighed hydrogel specimen (W_0) was immersed in an aqueous medium for a defined duration, excess surface liquid was gently removed, and the swollen specimen was reweighed (W_t). Swelling (%) was calculated as:

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

In vivo Evaluation of Wound Healing

Animals. Eighteen male albino rats (*Rattus norvegicus*) weighing 180–250 g were acclimatised prior to experimentation and maintained under standard husbandry conditions [10].

Induction of hyperglycaemia. Hyperglycaemia was induced using alloxan to simulate diabetic conditions. Hyperglycaemia was confirmed by measuring blood glucose before wound creation using the study's predefined threshold [9].

Excisional wound model. After confirmation of hyperglycaemia, a standardised full-thickness excisional wound (approximately 1 cm diameter) was created under anaesthesia using aseptic technique [10].

Grouping and treatments. Animals were allocated into six groups (n = 3 rats/group), as described in **Table 2**. Treatments were applied topically **once daily** in an amount sufficient to cover the wound bed uniformly.

Table 2. Animal Groups and Treatments (n = 3 per group)

Group	Designation	Treatment
G1	Negative control (untreated)	No topical treatment
G2	Positive control	Commercial Cavidagel hydrogel dressing
G3	F1	Hydrogel: 0% *Aloe vera* + 20% forest honey
G4	F2	Hydrogel: 20% *Aloe vera* + 0% forest honey
G5	F3	Hydrogel: 5% *Aloe vera* + 15% forest honey
G6	F4	Hydrogel: 15% *Aloe vera* + 5% forest honey

Clinical outcome parameters. Wound healing was evaluated using (i) wound closure rate (%) as the primary indicator of healing progression, (ii) total healing time (days) defined as the day of complete macroscopic wound closure, and (iii) qualitative assessment of exudate and inflammatory signs (redness and swelling) [10].

Data Analysis

Data are presented as mean ± SD. Comparisons among formulations (F1–F4) were performed using one-way ANOVA with appropriate post hoc testing; if assumptions were not met, Kruskal–Wallis was applied. Longitudinal wound measurements were analysed using a repeated-measures approach (mixed-effects model) with group and time as fixed effects. A two-sided $p < 0.05$ was considered statistically significant.

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee (Komisi Etik Penelitian Kesehatan), Universitas Negeri Gorontalo (Protocol No. 229/UN47.B7/KE/2025; November 12, 2025). All experimental procedures were conducted in accordance with institutional animal welfare requirements.

3. Results and Discussion

Visual characteristics and formulation acceptability

Across formulations, the predicted organoleptic pattern is driven primarily by the relative contribution of forest honey and *Aloe vera* gel. Formulations containing higher honey fractions (F1 and F3) are expected to exhibit a deeper yellow–amber colour and a more recognisable honey odour, whereas formulations enriched with *Aloe vera* gel (F2 and F4) tend to appear clearer and less aromatic, reflecting the comparatively translucent nature of *Aloe vera* gel and the absence or lower level of honey-derived chromophores. This directionality is consistent with prior hydrogel characterisation studies in which increasing *Aloe vera* content modifies visual appearance and rheological perception, particularly when incorporated into polymeric matrices intended for topical application [12], [13].

Table 3. Organoleptic characteristics and homogeneity of F1–F4

Parameter	F1 (0% *Aloe vera*; 20% honey)	F2 (20% *Aloe vera*; 0% honey)	F3 (5% *Aloe vera*; 15% honey)	F4 (15% *Aloe vera*; 5% honey)
Appearance/Colour	Amber to dark yellow, translucent	Clear to pale-yellow, translucent	Yellow-amber, translucent	Pale yellow, translucent
Odour	Distinct honey odour	Mild herbal/neutral odour	Honey odour (moderate)	Slight honey odour
Consistency	Gel-like, moderately viscous	Gel-like, relatively more viscous	Gel-like, moderate viscosity	Gel-like, moderately viscous
Homogeneity	Homogeneous; no visible particles	Homogeneous; no visible particles	Homogeneous; no visible particles	Homogeneous; no visible particles
Phase separation (24 h, visual)	Not observed	Not observed	Not observed	Not observed

From a formulation-performance standpoint, the most critical acceptability criterion at this stage is homogeneity without phase separation, because macroscopic separation often indicates inadequate compatibility or insufficient network integrity, which can compromise dose uniformity and reproducible topical coverage. The absence of visible phase separation (as predicted here across F1–F4) is mechanistically plausible for a chitosan-based hydrogel system, where polymer network formation and hydrogen-bonding interactions can stabilise dispersed bioactive components and maintain a coherent gel structure, supporting its suitability as a wound-dressing platform [4], [7]. Additionally, literature on *Aloe vera* composite hydrogels—particularly those developed for antibacterial and wound-related applications—commonly emphasises that stable, homogeneous matrices are a prerequisite before claiming functional performance, because macroscopic instability confounds both physicochemical readouts and biological outcomes [14].

Physicochemical characterisation of F1–F4 and functional implications

The pH profile provides an initial indicator of topical compatibility and matrix stability, particularly in chitosan-based hydrogels that are commonly formed in mildly acidic media and may be further acidified by honey-derived constituents. From a hydrogel-design perspective, a stable pH is also informative because marked pH drift can reflect component incompatibility or network instability, which can ultimately compromise consistency and performance [4], [7]. The predicted pH differences in Table 4 follow a plausible compositional logic: higher honey fractions tend to shift pH toward a more acidic range, whereas higher *Aloe vera* fractions yield comparatively less acidic formulations. This directionality is consistent with published physicochemical characterisation of *Aloe vera*-based hydrogels showing that bioactive loading can modulate fundamental formulation properties, including pH behaviour and perceived stability [12], [13].

Table 4. Physicochemical characteristics of hydrogel formulations

Parameter	F1 (0% *Aloe vera*; 20% honey)	F2 (20% *Aloe vera*; 0% honey)	F3 (5% *Aloe vera*; 15% honey)	F4 (15% *Aloe vera*; 5% honey)	p-value	Post hoc (Tukey)
pH	4.85 ± 0.08^{a}	5.40 ± 0.10^{c}	5.02 ± 0.07^{b}	5.22 ± 0.09^{bc}	<0.001	a≠b≠c
Spreadability (cm)	5.80 ± 0.25^{a}	6.50 ± 0.30^{c}	6.20 ± 0.28^{bc}	6.05 ± 0.22^{b}	0.002	a<b<c
Adhesion time (min)	14.2 ± 1.1^{c}	8.1 ± 0.9^{a}	12.0 ± 1.0^{b}	10.3 ± 0.8^{b}	<0.001	a<b<c
Swelling (%)	200 ± 15^{a}	190 ± 14^{a}	230 ± 18^{b}	210 ± 16^{ab}	0.010	b>a

Notes: Values are mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences (one-way ANOVA followed by Tukey's test, $p < 0.05$)

Spreadability has immediate practical relevance because it determines ease of application and the ability to form a uniform coating over the wound bed. Methodologically, plate-based spreadability testing is widely used in semisolid dosage forms to capture differences in flow and application performance across formulations [8]. The predicted spreadability trends can be rationalised as reflecting compositional modulation of the gel's viscoelastic response, in which both honey and *Aloe vera* can alter the effective water fraction and intermolecular interactions within the polymer network. In wound-dressing applications, adequate spreadability should be balanced against retention characteristics to prevent rapid displacement after application [7].

Adhesion time represents the ability of the hydrogel to remain in contact with the wound surface, which is directly linked to sustained bioactive exposure and the stability of the applied layer during the dosing interval. A plausible pattern is that honey-enriched formulations exhibit greater tackiness and cohesion, whereas *Aloe vera*-dominant systems display moderate adhesion depending on network density and water content. This interpretation aligns with the broader rationale for designing hydrogel dressings in which polymer–bioactive interactions and network architecture jointly determine adhesion and functional residence time [4], [7]. Reports on honey–*Aloe* composite hydrogels also commonly emphasise enhanced contact and retention as a practical motivation for combining these bioactives within a single matrix [6], [18], [19].

Swelling ratio is a key functional attribute for chronic wounds because it reflects fluid-uptake capacity and the ability to maintain a moist microenvironment while managing exudate. Within hydrogel science, swelling is governed by the effective crosslink density, network hydrophilicity, and the balance between water uptake and structural integrity [4]. The predicted swelling behaviour in Table 4 can be interpreted as a network-level consequence of *Aloe vera* polysaccharides and the hygroscopic nature of honey, both of which can increase water affinity and free volume within the gel. Comparable studies on *Aloe vera* hydrogels and honey-containing hydrogel dressings frequently link such physicochemical shifts to improved dressing functionality through exudate control and maintenance of a favourable healing milieu [12], [13], [18], [19]. Nevertheless, excessive swelling may compromise mechanical stability and effective adhesion, so the final interpretation should explicitly consider the trade-off among swelling, adhesion, and spreadability when selecting the most rational formulation for subsequent efficacy claims [4], [7].

Validation of the hyperglycaemic wound model

As shown in **Table 5**, all experimental groups exhibited elevated blood glucose levels following alloxan induction, supporting successful establishment of a

hyperglycaemic condition prior to excisional wound creation, consistent with the expected diabetogenic profile of alloxan in rodent models [9]. The absence of a statistically detectable difference in baseline glucose across groups (one-way ANOVA, $p = 0.88$) indicates that animals entered the treatment phase with comparable glycaemic severity, thereby reducing the likelihood that subsequent between-group differences in wound closure kinetics and total healing time are attributable to baseline metabolic imbalance rather than treatment-related effects; such baseline comparability is also aligned with good reporting practice in animal experiments [11].

Table 5. Blood glucose levels after alloxan induction (mg/dL; mean ± SD)

Group	Designation	Blood glucose (mg/dL), mean ± SD
G1	Negative control (untreated)	312 ± 28
G2	Positive control (Cavidagel)	305 ± 24
G3	F1	318 ± 31
G4	F2	299 ± 22
G5	F3	321 ± 26
G6	F4	309 ± 27

Note: Baseline comparison across all groups: one-way ANOVA, $p = 0.88$ ($n = 3$ rats/group)

Wound closure kinetics under different treatments

Table 6 and **Figure 1** depict the longitudinal wound-length profiles over 14 days in the alloxan-induced hyperglycaemic excisional wound model. The untreated negative control (G1) showed negligible macroscopic improvement throughout follow-up, with wound length remaining close to the initial value and indicating persistent impairment of closure under hyperglycaemic conditions, which is consistent with the delayed-healing phenotype typically expected in diabetic wound settings [2], [10]. In contrast, all treated groups demonstrated a clear time-dependent decline in wound length, with trajectory separation becoming evident within the early observation window and widening thereafter, suggesting that topical hydrogel coverage—particularly when incorporating natural bioactives—may meaningfully modulate closure dynamics relative to the untreated condition [10].

Table 6. Wound length trajectories across groups (Day 1–14)

Day	G2 Positive control (Cavidagel)	G1 Negative control (untreated)	G3 F1	G4 F2	G5 F3	G6 F4
1	10.00	10.00	10.00	10.00	10.00	10.00
2	8.13	9.56	9.06	9.30	9.33	9.33
3	7.33	9.26	8.30	7.46	8.66	8.46
4	6.93	9.10	7.03	5.73	8.00	8.00
5	6.00	8.96	6.60	4.40	7.23	6.93
6	5.13	9.13	6.06	3.76	6.53	5.70
7	4.46	8.86	5.53	2.23	5.76	4.83
8	2.60	8.80	4.30	1.30	4.33	2.86
9	0.86	8.73	3.13	0.43	3.00	1.00
10	0.00	8.66	2.43	0.00	2.00	0.43
11	0.00	8.80	1.66	0.00	0.76	0.00
12	0.00	8.66	1.10	0.00	0.00	0.00
13	0.00	9.13	0.00	0.00	0.00	0.00
14	0.00	9.23	0.00	0.00	0.00	0.00

Among treated groups, the commercial hydrogel comparator (G2; Cavidagel) exhibited rapid reduction in wound length and achieved complete macroscopic closure by Day 10. A closely comparable kinetic pattern was observed for the *Aloe vera*-enriched formulation without honey (G4/F2), which approached near-complete closure by Day 9 and reached complete closure by Day 10. The remaining formulations achieved complete closure at later time points, with G6/F4 reaching complete closure by Day 11, G5/F3 by Day 12, and G3/F1 by Day 13. Taken together, these trajectories indicate that the *Aloe vera*-dominant formulation produced the earliest closure among the experimental hydrogels and performed comparably to the commercial comparator under the conditions tested, whereas mixed honey–*Aloe vera* compositions retained a measurable benefit over the untreated group but converged more gradually toward full closure.

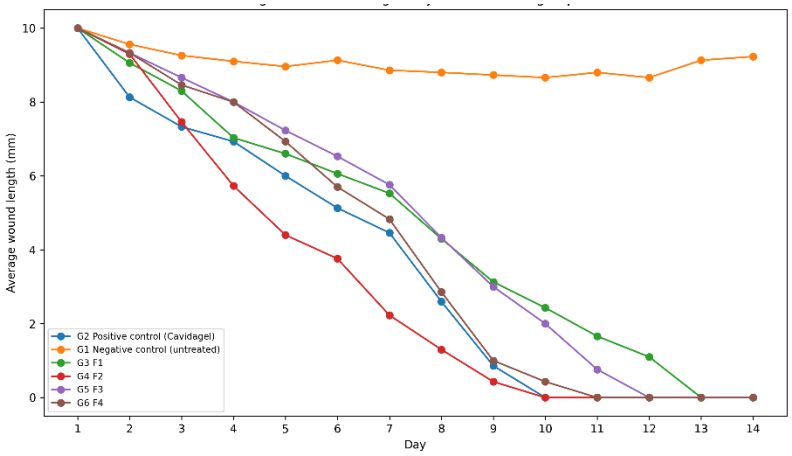

Figure 1. Wound-length trajectories over 14 days in an alloxan-induced hyperglycaemic excisional wound model.

Biologically, the observed trajectory patterns are plausible when interpreted through the functional roles of hydrogel dressings and the known bioactivity landscape of honey and *Aloe vera*. Honey dressings have been associated with improved healing outcomes in chronic wound contexts, with proposed contributions including antimicrobial activity and modulation of inflammatory burden, although effect estimates can vary across wound types and study designs [5]. In parallel, *Aloe vera*-containing hydrogels have been reported to support wound repair in preclinical models, including outcomes consistent with enhanced re-epithelialisation and tissue restoration, providing a coherent rationale for the accelerated closure observed in the *Aloe vera*-enriched formulation [12], [20]. Moreover, hydrogel platforms combining honey and *Aloe vera* have been described as capable of accelerating wound closure compared with controls in related systems, supporting the conceptual justification for the improved kinetics seen in the combined formulations relative to untreated animals [18], [20]. Nonetheless, given the clinical heterogeneity and recurrence risk characteristic of diabetic foot ulcers and the emphasis of contemporary

guidelines on evidence-based adjunct selection, the present findings should be interpreted as indicating adjunctive potential within a preclinical framework rather than as a direct proxy for clinical superiority in human diabetic ulcers [2], [3].

Total healing time and clinical inflammatory/exudative signs

The total healing-time patterns in Table 7 are internally consistent with the trajectory separation shown in Table 6 and Figure 1. The untreated hyperglycaemic group (G1) failed to reach complete closure by Day 14, reinforcing the impaired healing phenotype expected in diabetic wound settings and underscoring the clinical relevance of evaluating adjunctive wound dressings under hyperglycaemia [2], [10]. In contrast, both the commercial comparator (G2; Cavidagel) and the *Aloe vera*–enriched hydrogel without honey (G4/F2) achieved complete closure by Day 10, indicating the earliest macroscopic healing endpoint among the tested conditions. The mixed-composition hydrogels reached closure sequentially thereafter (G6/F4 by Day 11, G5/F3 by Day 12, and G3/F1 by Day 13), suggesting that compositional differences altered the time to complete closure even though all bioactive-loaded hydrogels improved healing relative to the untreated control.

Table 7. Total healing time (days) derived from the first day of complete macroscopic closure

Group	Designation	Total healing time (days)*
G1	Negative control (untreated)	Not achieved by Day 14
G2	Positive control (Cavidagel)	10
G3	F1 (20% honey; 0% *Aloe vera*)	13
G4	F2 (0% honey; 20% *Aloe vera*)	10
G5	F3 (15% honey; 5% *Aloe vera*)	12
G6	F4 (5% honey; 15% *Aloe vera*)	11

With respect to inflammatory and exudative signs, the available dataset supports macroscopic inference rather than quantitative scoring. Conceptually, earlier closure typically corresponds to earlier termination of the open wound-bed phase, which is the period most strongly associated with visible exudation and inflammatory features such as erythema and swelling. This interpretation is coherent with broader evidence that honey-based dressings can support improved wound outcomes—often discussed through antimicrobial and inflammation-modulating plausibility—while acknowledging that the magnitude and certainty of benefit vary by wound context and comparator [5]. Reviews of honey-containing hydrogels similarly emphasise that moisture balance and exudate handling are central functional attributes of hydrogel dressings, linking physicochemical capacity (e.g., swelling/absorbency) to clinically meaningful management of chronic wound microenvironments [19]. Likewise, *Aloe vera* hydrogel studies in full-thickness wound models commonly report improved wound outcomes consistent with supportive tissue repair trajectories, providing a rational biological context for the comparatively earlier

closure observed in the *Aloe vera*–dominant formulation in the present dataset [20].

Table 8. Clinical inflammatory/exudative signs

Group	Macroscopic status within follow-up	Structured interpretation of inflammatory/exudative signs
G1	Wound remained open through Day 14	Because complete closure was not reached, resolution of exudate/erythema/swelling within the observation window cannot be inferred.
G2	Closed by Day 10	Complete closure by Day 10 implies earlier resolution of wound-bed exudation and visible inflammatory features at the macroscopic level.
G3	Closed by Day 13	Later closure suggests a more prolonged phase of open wound-bed exposure compared with G2 and G4.
G4	Closed by Day 10	Closure kinetics comparable to G2 implies earlier macroscopic resolution of open-wound inflammatory features.
G5	Closed by Day 12	Intermediate healing time suggests an intermediate duration of the inflammatory/exudative phase relative to the faster groups.
G6	Closed by Day 11	Closure earlier than G3/G5 but later than G2/G4 suggests a correspondingly intermediate resolution window.

Integrated interpretation and practical relevance

Synthesising the findings across Sections 3.1–3.5, the overall performance profile indicates that the 20% *Aloe vera*–enriched hydrogel (F2/G4) represents the most rational candidate formulation under the present experimental conditions, because it combined favourable physicochemical functionality with the earliest *in vivo* macroscopic closure endpoint that was comparable to the commercial hydrogel comparator (complete closure by Day 10). From a formulation-science standpoint, selection of an "optimal" hydrogel dressing should not be based on a single parameter, but rather on a balanced trade-off among spreadability (uniform wound coverage), adhesion (residence time/contact), and swelling capacity (exudate handling and maintenance of a moist microenvironment). These functions are fundamentally governed by hydrogel network architecture and polymer–bioactive interactions, which collectively determine the dressing's ability to remain in place while providing controlled hydration and supporting the wound-bed milieu [4]. Within this framework, the chitosan-based matrix provides a coherent platform for topical wound dressing development due to its established compatibility with hydrogel dressing design principles and its suitability as a biodegradable carrier for bioactive incorporation [7].

The comparative pattern between single-bioactive and combined-bioactive formulations merits a nuanced interpretation. Although honey-containing groups displayed improved kinetics relative to the untreated

condition, the fastest closure was observed in the *Aloe vera*–dominant formulation in this dataset. This does not negate the therapeutic plausibility of honey, but rather suggests that, within the current matrix and composition range, the network-level balance among viscosity/adhesion and swelling-driven moisture management may have favoured the *Aloe vera*–enriched system. Reports of honey–*Aloe vera* hydrogels in the literature frequently describe accelerated closure and functional wound-dressing performance, indicating that combined systems can be advantageous when the matrix architecture and component ratios are optimised to avoid compromising spreadability or over-increasing tackiness and diffusion resistance [18], [20]. Similarly, reviews of honey-containing hydrogels emphasise that outcome variability is influenced by wound context, comparator choice, and formulation design, reinforcing that "bioactive addition" is not inherently sufficient; rather, it must be embedded within a matrix that sustains an appropriate microenvironment and delivery profile [19]. Consequently, the present data support a practical interpretation: F2 is the most rational candidate among the tested formulations, while the honey–*Aloe vera* combinations remain mechanistically credible and may warrant further optimisation of ratio and network properties.

From a clinical relevance perspective, these findings should be positioned as adjunctive preclinical evidence rather than a direct proxy for superiority in human diabetic foot ulcers. Diabetic foot ulcers are characterised by chronicity and high recurrence rates, and guideline-based care emphasises structured selection of adjuncts in the context of standard wound-bed preparation and evidence strength [2], [3]. Accordingly, the practical implication is that a chitosan-based hydrogel incorporating *Aloe vera* (and potentially honey, after optimisation) may represent a plausible topical dressing candidate for diabetic-wound settings, but translation requires cautious framing and additional confirmatory endpoints.

Limitations of the study

Several limitations constrain the strength and generalisability of the present interpretation. First, the animal cohort was small (n = 3 per group), which limits statistical power and increases uncertainty around effect estimates; moreover, some longitudinal outcomes were available only as group means without animal-level variability, restricting robust inference on between-group differences and precluding fully transparent mixed-effects modelling outputs in the present narrative. Second, outcomes were predominantly macroscopic (wound length/closure timing) without histological confirmation (e.g., re-epithelialisation thickness, granulation tissue quality, collagen deposition) or biochemical biomarkers of inflammation and microbial burden, thereby limiting mechanistic attribution to honey or *Aloe vera*–specific pathways. Third, the hyperglycaemic model was validated via glucose levels, but the broader metabolic context (e.g., weight change, glycaemic trajectories during follow-up) was not reported, which may influence wound dynamics. Finally, ARRIVE-aligned reporting elements such as allocation concealment, blinding of outcome

assessors, and predefined exclusion criteria were not fully documented, which can affect reproducibility and risk-of-bias appraisal in animal research.

4. Conclusion

The findings of this study demonstrate that male albino rats treated with the bioactive hydrogel formulations, including those containing Gorontalo forest honey and *Aloe vera*, exhibited faster wound closure than the untreated negative control. Among the tested formulations, Formula 2 (20% *Aloe vera*) showed the most rapid reduction in wound length and reached complete macroscopic closure by Day 10, indicating that *Aloe vera* may contribute meaningfully to accelerated healing within the chitosan-based hydrogel matrix, plausibly through supportive antimicrobial and anti-inflammatory effects. Overall, incorporation of the bioactive ingredients produced a marked improvement in wound-length reduction compared with the untreated group, while the negative control did not achieve complete closure within the 14-day observation period, underscoring the potential of *Aloe vera*– and honey-containing hydrogels as adjunctive wound-dressing candidates for diabetic wound care.

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Conflict of Interest:

The author declares that there are no conflicts of interest in this research.

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