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Development of betaine–aloe vera–gelatin hydrogel formulation for the treatment of burn wounds

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Burn wounds present a significant clinical challenge, often leading to prolonged healing, infection, and substantial scarring. Traditional dressings frequently fall short in providing an optimal healing environment. Hydrogel-based wound dressings have gained significant attention in recent years due to their ability to provide a moist environment, facilitate cellular responses, and promote tissue regeneration. In this study we developed a novel composite hydrogel formulation composed of Betaine (B), Aloe vera (A), and Gelatin (G) termed BAG hydrogel to enhance burn wound healing through synergistic bioactivity and improved mechanical stability. The hydrogel was evaluated *in vivo* using a thermal burn wound model in rats to assess its therapeutic efficacy. Topical application of BAG hydrogel significantly accelerated wound closure, reduced wound area, and promoted faster skin regeneration compared to control and other groups BG and AG. Morphometric analysis confirmed enhanced epidermal thickness and re-epithelialization in BAG-treated wounds. Histopathological evaluation on days 7 and 21 post-injury demonstrated notable collagen deposition, reduced fibrosis, and improved dermal architecture. Among all treatment groups, BAG hydrogel showed the most consistent and significant wound healing profile, emphasizing its potential in skin repair. Overall, the BAG hydrogel formulation offers a promising and bioactive therapeutic approach for burn wound management with the potential to be further developed as a customized and clinically translatable dermal substitute.

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1. Introduction

Over the past decade hydrogels have emerged as a dynamic and transformative class of biomaterials within modern materials science and biomedical engineering.[1] Characterized by a three-dimensional network of hydrophilic polymer chains, hydrogels possess a remarkable ability to absorb and retain large quantities of water, closely mimicking the natural extracellular matrix (ECM) of biological tissues.[2] Their high-water content, soft and elastic nature, tunable mechanical properties, biocompatibility, and controlled biodegradability make them particularly attractive for diverse biomedical applications including drug delivery, tissue engineering, regenerative medicine, and wound healing.[3] Among these, wound dressing remains one of the most promising domains for hydrogel application due to the ability of hydrogels to maintain a moist wound environment, absorb exudates, allow gaseous exchange, and promote tissue regeneration.[4,5] In particular, burn wounds, which account for a significant global health burden, present a complex challenge due to the risk of infection, prolonged inflammation, and delayed re-epithelialization.[6] According to the World Health Organization, burns result in approximately 180 000 deaths annually, predominantly in low- and middle-income countries with millions more suffering from long-term disability.[7] Current treatment options, including synthetic dressings, antibiotics, and skin grafts, often fall short in terms of cost-effectiveness, bioactivity, and patient compliance.[8] In recent years, naturally derived hydrogels have gained significant attention due to their intrinsic biological properties, reduced toxicity, and multifunctionality.[9] Aloe vera (*Aloe barbadensis*) has been extensively utilized due to its broad pharmacological spectrum including wound healing, anti-inflammatory, antimicrobial, antioxidant, and immunomodulatory properties.[10–12] Aloe vera gel contains over 95% water along with a complex blend of bioactive compounds

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such as polysaccharides (acemannan), amino acids, enzymes, vitamins, and phenolic compounds.[13] These components contribute to its capacity to accelerate collagen synthesis, enhance fibroblast proliferation, modulate cytokine release, and mitigate oxidative stress, all of which are key events in the wound healing cascade.[14,15] However, its inherent limitations including low mechanical strength and poor structural integrity restrict its use as a standalone hydrogel for clinical applications. To overcome these drawbacks hydrogel systems based on composite matrices have been developed using structurally supportive biopolymers. Gelatin, a natural derivative of collagen, is widely employed due to its excellent biocompatibility, biodegradability, and its ability to form thermo-reversible gels.[16] It supports cellular adhesion, migration, and proliferation making it an ideal scaffold material. Despite these advantages, gelatin hydrogels also tend to lack stability and mechanical resilience under physiological conditions.[17] Betaine, a zwitterionic, naturally occurring molecule known for its osmoprotective and anti-inflammatory properties, has shown promise in biomedical research but remains underexplored in hydrogel systems for wound healing.[18] Betaine stabilizes proteins and cellular structures under stress, reduces oxidative stress, and modulates inflammatory responses.[19] Its incorporation into a hydrogel matrix could enhance the biological functionality of the dressing by improving the wound microenvironment and supporting tissue regeneration.[20] We report the development and evaluation of a novel composite hydrogel composed of betaine, aloe vera, and gelatin (BAG hydrogel) designed specifically for the treatment of burn injuries. To the best of our knowledge, this is the first study that integrates betaine into an aloe vera–gelatin matrix leveraging the combined properties of these three bioactive components to achieve enhanced wound healing performance. The formulation was prepared *via* a solvent-casting method yielding a hydrogel with favorable physicochemical and rheological characteristics including shear-thinning behavior, thermal responsiveness, and mechanical stability. Comprehensive characterization using Fourier transform infrared spectroscopy (ATR-FTIR), dynamic light Scattering (DLS), zeta potential, and rheological analysis confirmed the successful crosslinking and integration of the hydrogel components. The BAG hydrogel exhibited a highly porous structure, suitable viscoelastic properties, and stable particle distribution attributes essential for wound coverage and conformability. *In vivo* evaluation in a rat model of thermal burn injury demonstrated that the BAG hydrogel significantly accelerated wound contraction, reduced inflammatory response, and promoted tissue regeneration, as confirmed by macroscopic observation and histopathological analysis (H&E staining). The results showed enhanced re-epithelialization, fibroblast infiltration, and collagen deposition in the BAG-treated group compared to controls and aloe vera–gelatin-only formulations. This research presents a technologically novel and translationally relevant hydrogel dressing that offers synergistic bioactivity, biocompatibility, and ease of application, addressing the multifactorial challenges associated with burn wound healing (Fig. 1). The BAG hydrogel not only serves as a promising therapeutic platform for burn care but also opens avenues for the development of scalable, customizable, and cost-effective wound dressings, with potential extension to other chronic wound conditions such as diabetic ulcers and surgical wounds.

2. Experimental section

2.1. Materials

Gelatin powder (porcine, Type B; 120 g Bloom) was purchased from SRL Chemicals, India. Glutaraldehyde was purchased from TCI Chemicals, India. Spray-dried AV gel powder 200× (*Aloe barbadensis*) was received from Heilen Biopharm, Gandhinagar, India. Ultrapure water (12 MΩ cm), produced by a Millipore water purification system, was used for the preparation of aqueous solutions throughout all of the experimental work. All chemicals were of analytical grade and were used without further purification.

2.2. Synthesis of hydrogel

The hydrogel was synthesized using a solvent-casting method with gelatin, aloe vera, and betaine as key components. Initially, gelatin (5% and 10% w/v) was dispersed in distilled water and heated to 40–50 °C on a magnetic stirrer (200–300 rpm) until a clear homogeneous solution was obtained (~10–15 minutes) avoiding temperatures above 50 °C to prevent denaturation. Separately, aloe vera powder was dissolved in distilled water (10% w/v) and maintained at room temperature (25–30 °C). Betaine (5% w/v) was also dissolved in distilled water with gentle stirring at room temperature until fully solubilized (~5 minutes). The aloe vera solution was gradually added to the gelatin solution at 40 °C under continuous stirring followed by the addition of the betaine solution. The mixture was stirred until homogeneity was achieved. After cooling to room temperature, glycerol (5–10% v/v) was added as a plasticizer and stirred gently for 5–10 minutes. Finally, glutaraldehyde (0.06–0.1% v/v) was introduced as a crosslinking agent and the mixture was stirred at room temperature for 15–20 minutes to complete the crosslinking process. It was used at low concentration to ensure effective crosslinking while minimizing residual cytotoxicity. The resulting mixture was then cast and left undisturbed for 24–48 hours to allow complete drying and gel formation and was washed in DI water to remove excess crosslinker, if any (Fig. 2).[21–23]

2.3. Characterization

Comprehensive characterization of the formulated betaine–aloe vera–gelatin (BAG) hydrogels was carried out to assess their physicochemical and functional properties relevant to burn wound healing applications. Initially, the precursor solution of the hydrogel was analyzed to determine its zeta potential using dynamic light scattering (DLS) and electrophoretic light scattering (ELS), providing insight into the surface charge and colloidal stability of the formulation. Following gelation, the hydrogel's rheological and viscoelastic properties were sys-

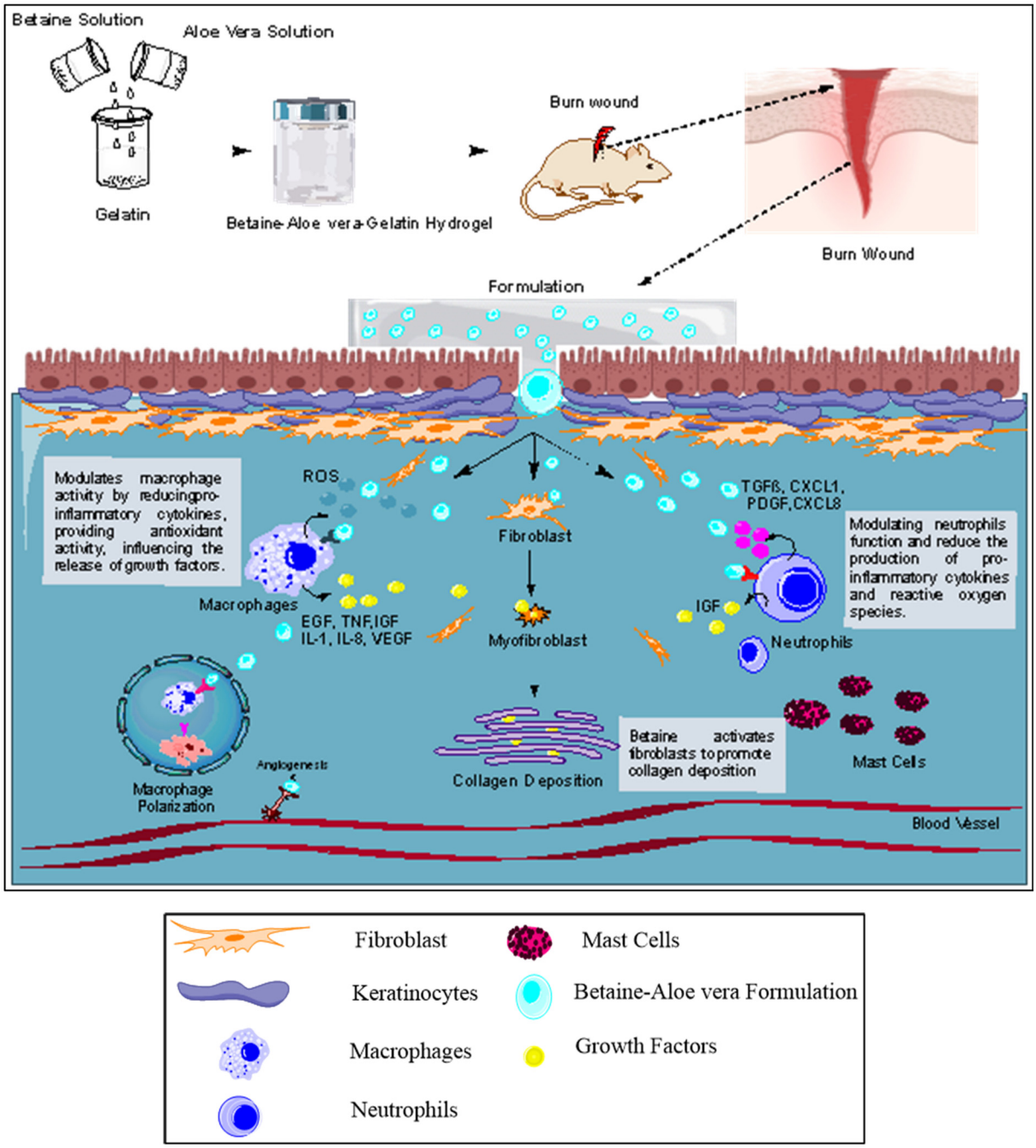

Fig. 1 Schematic representation of *in Vivo* burn wound healing.

tematically evaluated using a rotational rheometer under oscillatory mode. These characterization studies, including temperature ramp and frequency sweep tests, were conducted to understand the gelation behavior, thermal stability, and mechanical resilience of the BAG hydrogel under physiological conditions. To investigate the BAG hydrogel's chemical stability Fourier transform infrared spectroscopy (ATR-FTIR) was employed to monitor changes in functional groups. The thermal properties of the hydrogel were analyzed to determine its temperature ramp performance and overall stability, ensuring its suitability for application in burn wounds where temperature regulation and gel stability are vital. Collectively, these characterization techniques provided a detailed understanding of the BAG hydrogel's structural, mechanical, and bio-functional properties, supporting its potential as a novel wound dressing material.

2.4. *In vivo* burn wound model

A burn wound model was induced on the dorsal skin of rats as per the method described earlier. All groups were maintained

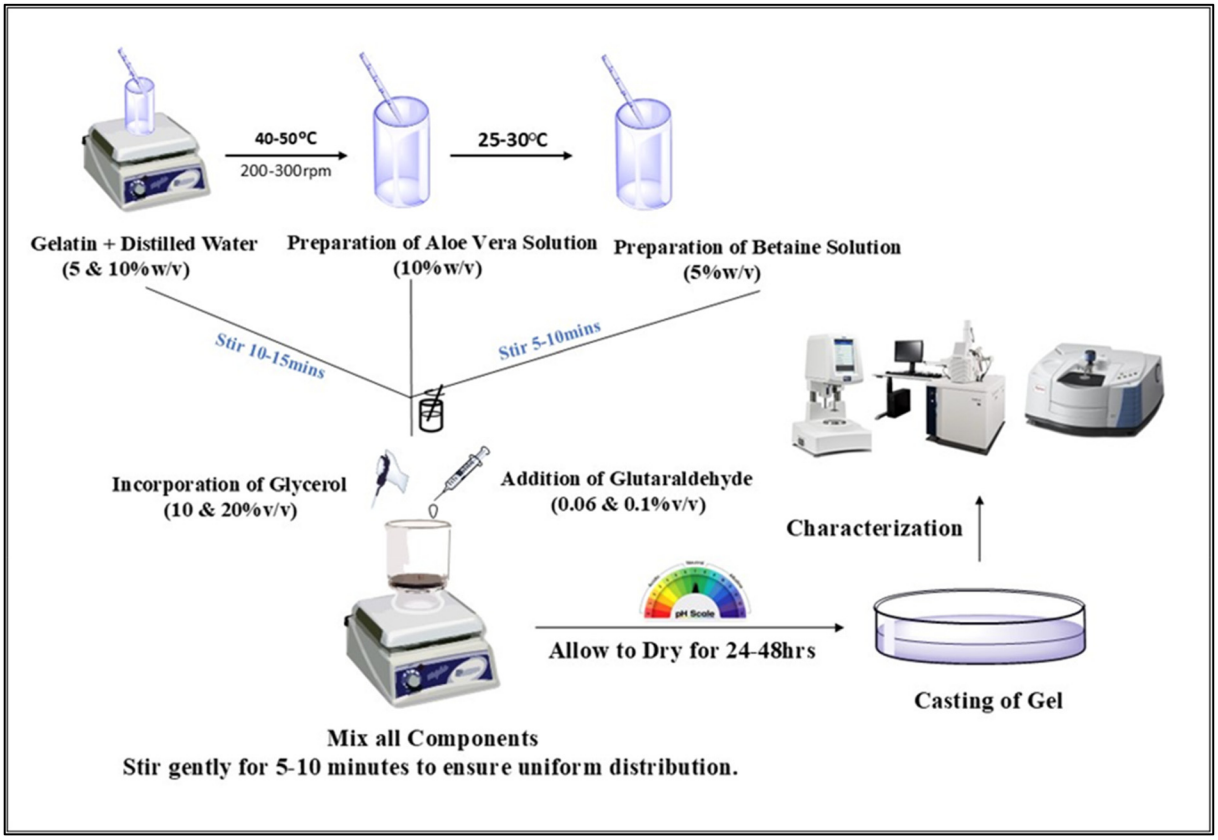

Fig. 2 Synthesis of the hydrogel.

under the same conditions, with free access to food and water before and after inducing the burn wound. The rats were anesthetized by administering ketamine (80 mg kg^{-1}, i.p.) (Troikaa Pharmaceuticals, Dehradun, Uttarakhand, India) and xylazine (20 mg kg^{-1}, i.p.) (India Immunologicals Ltd, Siddipet, Telangana, India). A grade-II burn wound was created by placing a solid aluminum bar (10 mm in diameter) previously heated in boiling water (100 °C) for 2–3 min in close contact with the animal's dorsal proximal area of skin for about 20 s. To prevent infection, 1% silver sulfadiazine ointment (Sun Pharmaceutical Industry Ltd, Surat, Gujarat, India) was applied once at the injury site five minutes after inducing the burn. Thereafter, the BAG hydrogel formulation was applied daily to the wound.[24]

2.5. *In vivo* wound healing examination

The *in vivo* wound healing efficacy of the formulated betaine–aloe vera–gelatin (BAG) hydrogel was systematically evaluated using a thermal burn wound model in Wistar rats. Standardized full-thickness burn injuries were induced under anesthesia by applying a pre-heated metal rod to the shaved dorsal skin of the animals. The rats were then randomly divided into three experimental groups: an untreated control group, a group treated with aloe vera–gelatin (AG) hydrogel, a group treated with betaine–gelatin (BG) hydrogel, and a group treated with the BAG hydrogel. Each treatment was applied topically once daily for 21 days, and the wounds were monitored at regular intervals. The extent of wound healing was assessed macroscopically by capturing digital images of the wound sites on days 0, 3, 7, 10 and 14 post-injury. Wound contraction was quantified by measuring the wound area and calculating the percentage of wound closure over time. To further substantiate the macroscopic findings, histopathological analysis was performed at the end of the treatment period. Excised wound tissues were fixed in formalin, embedded in paraffin, sectioned, and stained with haematoxylin and eosin (H&E). Microscopic examination revealed enhanced re-epithelialization, increased fibroblast proliferation, well-organized collagen deposition, and reduced inflammatory cell infiltration in the BAG hydrogel-treated wounds. The Institutional Animal Ethics Committee of the Indian Institute of Technology, Banaras Hindu University, Varanasi, approved all the experimental protocols under approval no. IIT(BHU)/IAEC/2025/I/056.

3. Results and discussion

3.1. Rheological analysis

3.1.1. Amplitude sweep test. The amplitude sweep test was performed to assess the viscoelastic behavior, structural integrity, and mechanical stability of various hydrogel formulations

by monitoring the variation of storage modulus (G') and loss modulus (G'') with increasing shear strain (Fig. 3). At low strain values all formulations exhibited a predominant elastic behavior ($G' > G''$) indicating the presence of a stable three-dimensional polymeric network capable of resisting deformation.[25] As the shear strain increased, both G' and G'' began to decrease marking the onset of the yield point and the end of the linear viscoelastic region. This decline signifies the progressive disruption of the internal gel structure and the transition from solid-like to liquid-like behavior. Among the formulations tested, sample SM8 exhibited the most favorable rheological profile. It demonstrated the highest G' values indicating that it could sustain a greater degree of deformation without structural collapse. The G' of SM8 remained significantly higher than G'' throughout the tested strain range and no crossover was observed suggesting that the formulation retained its elastic character even at higher strains. This behavior reflects a well-organized, highly entangled or densely crosslinked network structure that provides superior resistance to shear-induced breakdown. In contrast, other formulations such as SM6, LM6, and LM10 showed a sharp decline in G' at lower strain values indicating weaker network structures and lower mechanical strength. The corresponding LM8 formulation, while compositionally related to SM8, exhibited noticeably lower G' values and a narrower linear viscoelastic region suggesting that differences in formulation parameters including polymer concentration and crosslinking density significantly influenced mechanical performance. The superior rheological stability of SM8 makes it the most promising candidate for applications requiring robust mechanical properties, such as wound healing materials, injectable hydrogels or load-bearing biomedical scaffolds. These findings highlight the critical role of formulation composition in determining viscoelastic performance and underscore the need for careful optimization to enhance functional outcomes.

3.1.2. Frequency sweep test. The frequency sweep test was conducted to evaluate the viscoelastic behavior of the hydrogel formulations under varying oscillatory shear frequencies (0.1–10 rad s^{-1}) at constant strain within the linear viscoelastic region (Fig. 4). The variations in the storage modulus (G') and loss modulus (G'') were recorded as a function of angular frequency. Across all the tested samples, the storage modulus (G') consistently remained higher than the loss modulus (G'') throughout the entire frequency range, indicating a predominantly elastic or solid-like behavior characteristic of crosslinked hydrogel networks. This dominance of G' over G'' confirms the structural

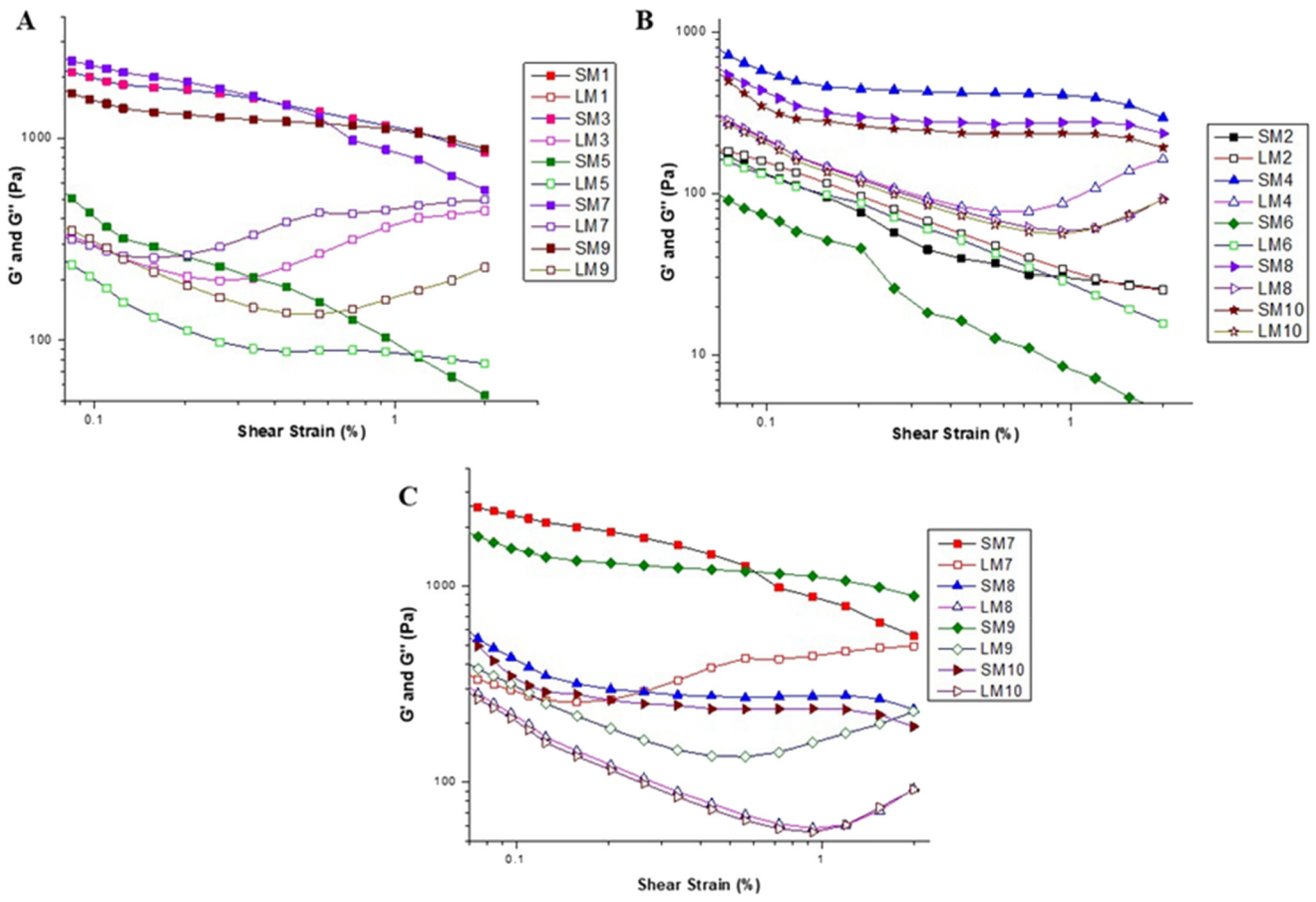

Fig. 3 Amplitude sweep of each hydrogel sample at a constant frequency of 1 rad s^{-1}. Different colored symbols represent hydrogel samples, with filled and unfilled symbols respectively denoting storage modulus (SM) and loss modulus (LM).

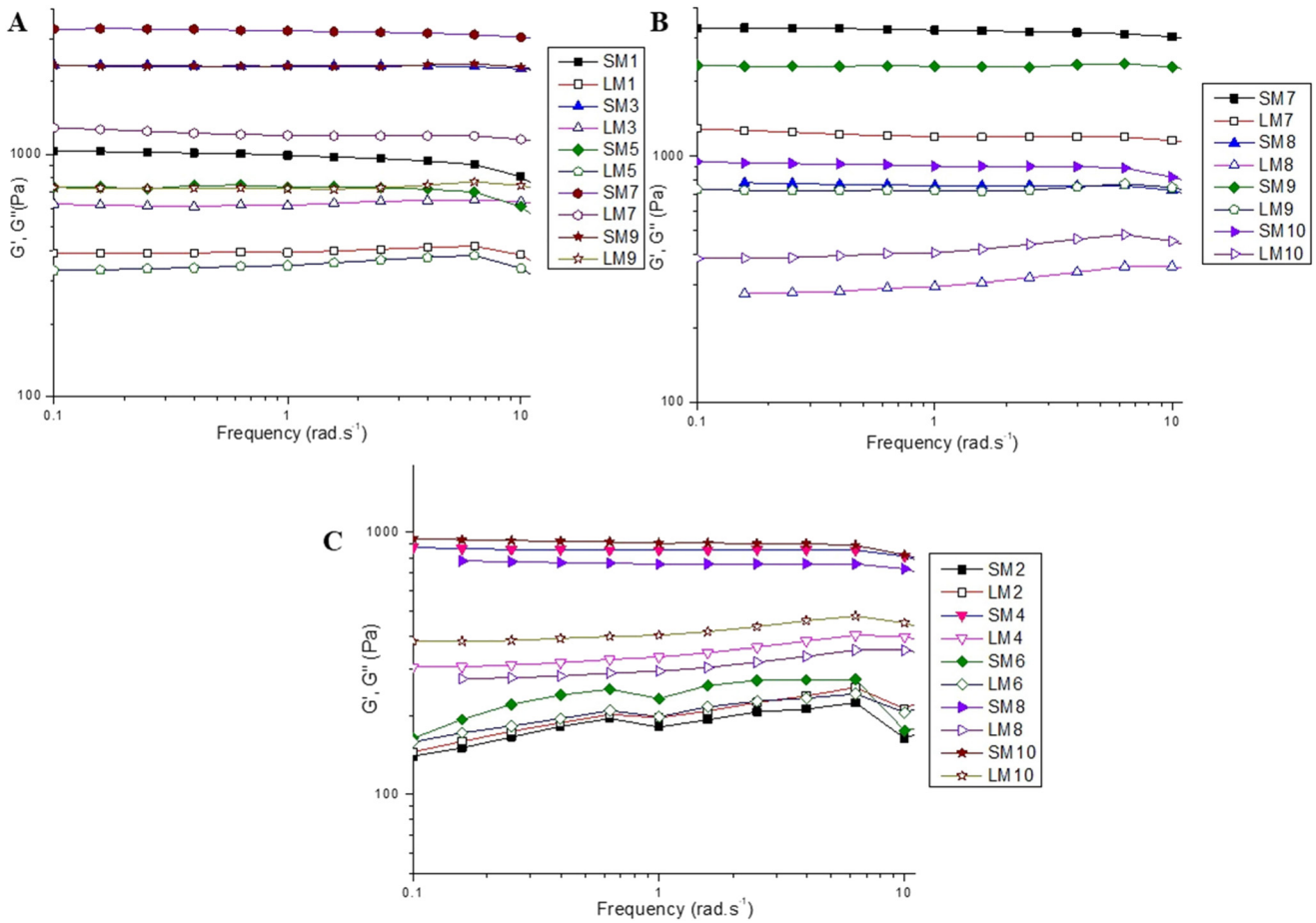

Fig. 4 Frequency sweep of each hydrogel sample at a constant shear strain of 1%. Different colored symbols represent hydrogel samples, with filled and unfilled symbols respectively denoting storage modulus (SM) and loss modulus (LM).

stability and gel-like characteristics of the samples under dynamic conditions.[26] Formulations such as SM7, SM9, and SM10 exhibited higher absolute G' values indicating a denser crosslinked structure and stronger elastic behavior. These hydrogels showed minimal frequency dependence, suggesting mechanical robustness and integrity even under varying shear conditions. Among all formulations SM8 demonstrated a particularly balanced viscoelastic profile, with moderate but stable G' and G'' values across the frequency range. Unlike the overly rigid SM10 or the more fluid-like LM8, SM8 maintained sufficient mechanical strength to ensure structural integrity, while preserving enough flexibility to conform to the irregular geometry of wound sites. This balance between elasticity and viscosity is a key requirement for wound dressing materials, as it ensures both resilience to external stress and patient comfort. Furthermore, SM8 exhibited relatively low frequency dependence in its moduli, indicating stable mechanical performance under dynamic physiological conditions such as skin movement.

3.2. Chemical and thermal characterization

3.2.1. ATR-FTIR spectral analysis of hydrogel. Fourier transform infrared (FTIR) spectroscopy was employed to investigate the chemical composition and possible interactions between the components of the hydrogel formulations. The first set of spectra included those for the individual constituents of betaine, aloe vera, and gelatin along with those of two hydrogel formulations, S1 and S2 (Fig. 5A). The characteristic broad absorption band observed at 3290 cm^{-1} in all spectra corresponds to the O–H stretching vibrations, confirming the presence of hydroxyl groups, likely from aloe vera and gelatin. The bands at 2938 cm^{-1} and 2870 cm^{-1} are attributed to aliphatic C–H stretching, indicating the presence of –CH_2 groups. A prominent absorption peak around 1634 cm^{-1} was observed in gelatin and hydrogel formulations, corresponding to the C═O stretching vibration (amide I), which is indicative of a protein backbone structure. This peak showed a slight shift in the hydrogel spectra, suggesting possible hydrogen bonding or electrostatic interactions between gelatin and other components. The bands appearing at 1414 cm^{-1} and 1330 cm^{-1} in the spectra of betaine and the hydrogel formulations correspond to C–N stretching and N–O stretching vibrations, respectively. These peaks indicate the retention of zwitterionic functionality and support the formation of amide linkages, likely through interaction between betaine and gelatin. The presence of a peak at 1031 cm^{-1} in all

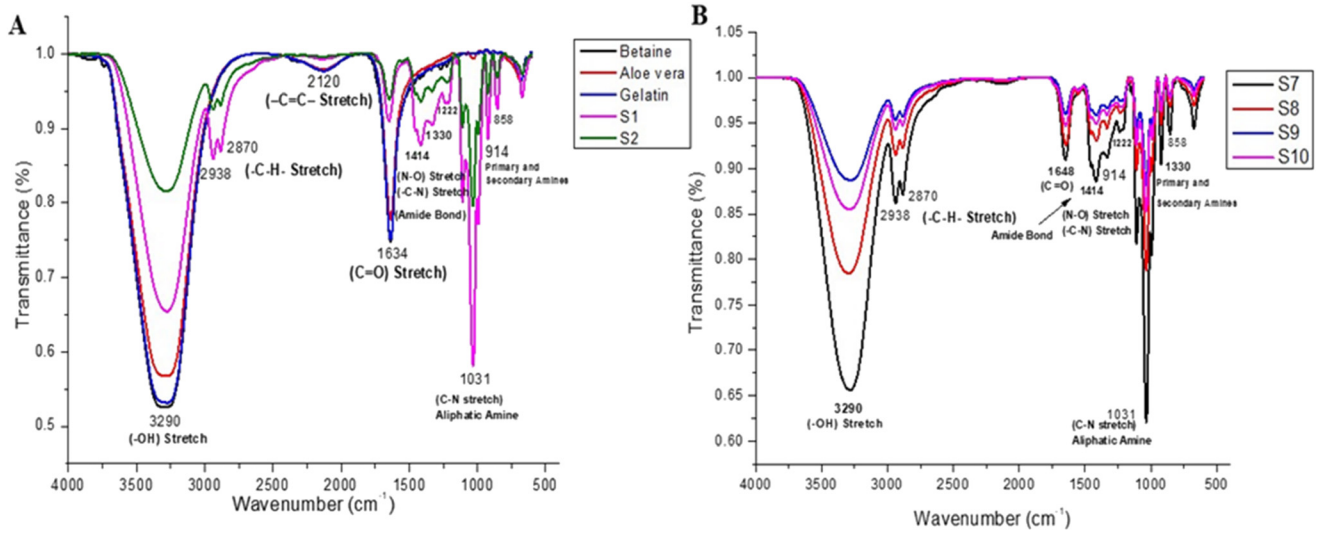

Fig. 5 FTIR spectra of hydrogel samples. Characteristic peaks indicate hydroxyl, amide, carbonyl, and amine groups, confirming the presence of functional groups and successful crosslinking in the hydrogel matrix.

samples corresponds to the aliphatic amine (C–N stretch) while bands near 914 cm^{-1} and 858 cm^{-1} are associated with primary and secondary amines confirming the structural preservation of functional groups in the hydrogel network. Collectively the FTIR data for S1 and S2 suggest successful incorporation of all components, with the observed peak shifts and intensities supporting interaction between the functional moieties of betaine, aloe vera, and gelatin. In the second set of spectra (Fig. 5B), hydrogel formulations S7 to S10 were analyzed to assess any differences arising from compositional or concentration variations. These samples also showed the characteristic broad bands of O–H stretching at 3290 cm^{-1} and C–H stretching bands at 2938 cm^{-1} and 2870 cm^{-1}. Interestingly, a shift in the carbonyl stretching frequency was observed from 1634 cm^{-1} in the earlier formulations to 1648 cm^{-1} in S7–S10. This shift suggests stronger amide bond formation, likely due to enhanced crosslinking or more efficient interaction among components in these formulations. The consistent presence of bands at 1414 cm^{-1}, 1222 cm^{-1} (C–N and N–O stretching), and 1031 cm^{-1} (aliphatic amine) across all spectra indicates that the chemical integrity of the hydrogel network was maintained.

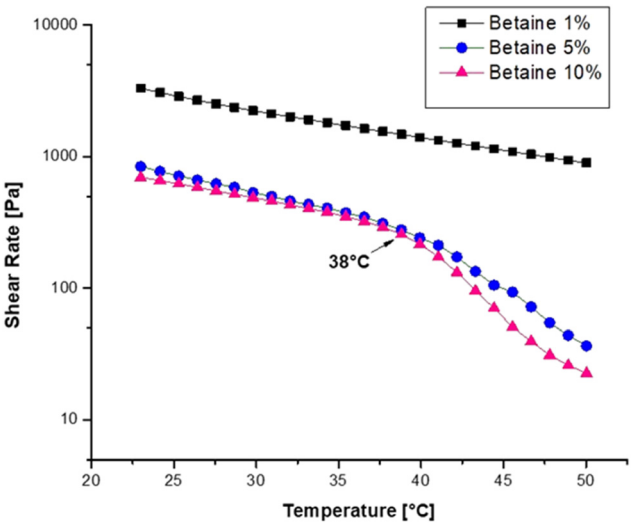

Fig. 6 Temperature ramp study. Temperature-dependent shear rate profiles of betaine solutions at different concentrations (1%, 5%, and 10%). The plots exhibit marked decreases in shear rate beyond 38 °C, particularly for the solution of higher concentration, indicating temperature-sensitive rheological behavior of the betaine formulations.

3.2.2. Temperature ramp analysis of the hydrogel gel–sol transition. A temperature ramp test was conducted to assess the thermal response and viscoelastic stability of hydrogel formulations containing varying concentrations of betaine (1%, 5%, and 10%). The evolution of shear rate as a function of increasing temperature (25–50 °C) is presented in Fig. 6. This test simulates physiological and pathological temperature conditions to evaluate the suitability of the hydrogels for biomedical applications, particularly in wound healing. At baseline (25 °C) all formulations exhibited relatively stable shear rates indicative of a well-formed hydrogel matrix. As the temperature increased, a gradual decrease in shear rate was observed for all concentrations suggesting a progressive weakening of the gel network due to thermal agitation. A notable inflection point was identified at approximately 38 °C beyond which all samples exhibited a sharp decrease in shear rate. This transition reflects the onset of gel melting or gel–sol disintegration particularly evident in the 5% and 10% betaine formulations. Such behavior is characteristic of thermo-responsive hydrogels undergoing a phase transition near physiological temperature. Among the tested samples the 1% betaine hydrogel exhibited the most thermally stable behavior maintaining a relatively consistent shear rate across the temperature

range. In contrast the 5% and 10% formulations demonstrated shear thinning behavior especially beyond 38 °C. This phenomenon is attributed to the disruption of hydrogen bonding and electrostatic interactions within the hydrogel matrix resulting in decreased viscosity and enhanced molecular mobility under thermal stress. The observed shear thinning behavior at elevated temperatures is advantageous for biomedical applications as it implies spreadability and conformability upon application to body surfaces. In particular the 5% betaine formulation represents a balanced profile, with sufficient structural integrity at ambient temperature and responsiveness near physiological temperature suggesting optimal behavior for on-skin application and *in situ* adaptability.

4. Physicochemical characterization

4.1. Zeta potential

The zeta potential analysis revealed notable differences in surface charge among the hydrogel formulations. The AG (aloe vera–gelatin) hydrogel exhibited a negative zeta potential of −9.374 mV, indicating the presence of anionic groups, likely derived from aloe vera's polysaccharides and organic acids. In contrast, the BG (betaine–gelatin) hydrogel showed a positive zeta potential of +14.02 mV, while the BAG (betaine–aloe vera–gelatin) hydrogel exhibited the highest positive value of +17.42 mV. The incorporation of betaine, a zwitterionic molecule, shifted the surface charge toward positive values, suggesting electrostatic interactions with the gelatin matrix and enhanced stability of the hydrogel network. The significantly higher zeta potential of the BAG hydrogel indicates improved colloidal stability, which is essential for maintaining formulation homogeneity and preventing aggregation. Furthermore, the positively charged BAG hydrogel may facilitate better interaction with negatively charged biological membranes, enhancing cellular adhesion and promoting more effective wound healing outcomes. These results support the role of betaine in modulating the physicochemical characteristics of the hydrogel system and enhancing its therapeutic potential.

4.2. Particle size analysis using dynamic light scattering

Dynamic light scattering (DLS) was conducted to monitor the temporal evolution of particle size in hydrogel formulations betaine–gelatin (BG), aloe vera–gelatin (AG), and the composite formulation containing betaine, aloe vera, and gelatin (BAG) across various time points (0 to 36 hours). The results depicted in Fig. S1 reveal significant changes in the particle size profile indicating dynamic molecular interactions and network stabilization processes occurring within the hydrogel matrices. At time zero, the BAG formulation exhibited the largest initial particle size followed by AG and BG. This larger size in BAG is likely due to the initial presence of transient aggregates or incomplete interaction within its multicomponent system. Over time, all formulations exhibited a reduction in particle size suggestive of internal molecular reorganization and enhanced dispersion. The BAG formulation despite its initially higher particle size demonstrated a consistent and progressive reduction reaching ~300 nm by 24 hours and maintaining a relatively stable size thereafter. This trend indicates the establishment of a thermodynamically stable colloidal system. The transient peak observed at 6 hours may reflect intermediate rearrangement states prior to achieving a more compact and uniform hydrogel network. In contrast, the AG formulation exhibited the fastest initial drop in particle size, reaching its lowest point at 12 hours. The minor fluctuations observed between 24 and 36 hours suggest slight instability or ongoing hydration effects. BG showed a relatively slower but steady decline in particle size stabilizing at ~240 nm by 24 hours indicative of gradual self-assembly into a consistent structure. Among the three hydrogels, the BAG formulation stands out due to its ability to undergo self-organization into a stable nanoscale dispersion over time. The plateauing of particle size after 24 hours suggests the formation of a robust and stable hydrogel network likely attributed to synergistic interactions between betaine and aloe vera within the gelatin matrix. These interactions may promote uniform crosslinking and enhanced structural integrity making the BAG formulation particularly suitable for applications requiring prolonged stability and consistent nanoscale behavior, such as in wound healing and controlled drug delivery systems. The hydrophilic composition of the hydrogel, primarily due to gelatin and aloe vera, also suggests favourable swelling and moisture retention behavior, which are essential for maintaining a moist wound environment and supporting effective healing.

5. *In vivo* study

All treatment groups exhibited significantly enhanced wound closure rates compared to the control. Four animals from each group were included in the study. The BAG hydrogel group exhibited the most rapid and consistent wound healing progression throughout the experimental period. On day 1, the BAG group achieved approximately 30% wound closure compared to 18% in the AG group, 15% in the BG group, and only 10% in the control. This early onset of wound healing in the BAG group suggests superior anti-inflammatory and tissue-protective activity likely attributable to the synergistic effects of the BAG hydrogel components. By day 11 the BAG group reached over 80% ($P < 0.001$) closure, significantly surpassing that of the AG and BG groups, both of which achieved around 60–65% while the control group lagged behind at 40%. By the end of the study on day 21, the BAG-treated wounds achieved nearly complete closure (98%) ($P < 0.001$) while the BG and AG groups demonstrated 95% and 94% closure, respectively (Fig. 7B) ($P < 0.01$). The control group exhibited the slowest healing, with only 70% closure, indicating suboptimal epithelialization and tissue remodelling. All groups started with an initial wound area of approximately 70 mm^2. A marked decrease in wound area was observed in the BAG group as

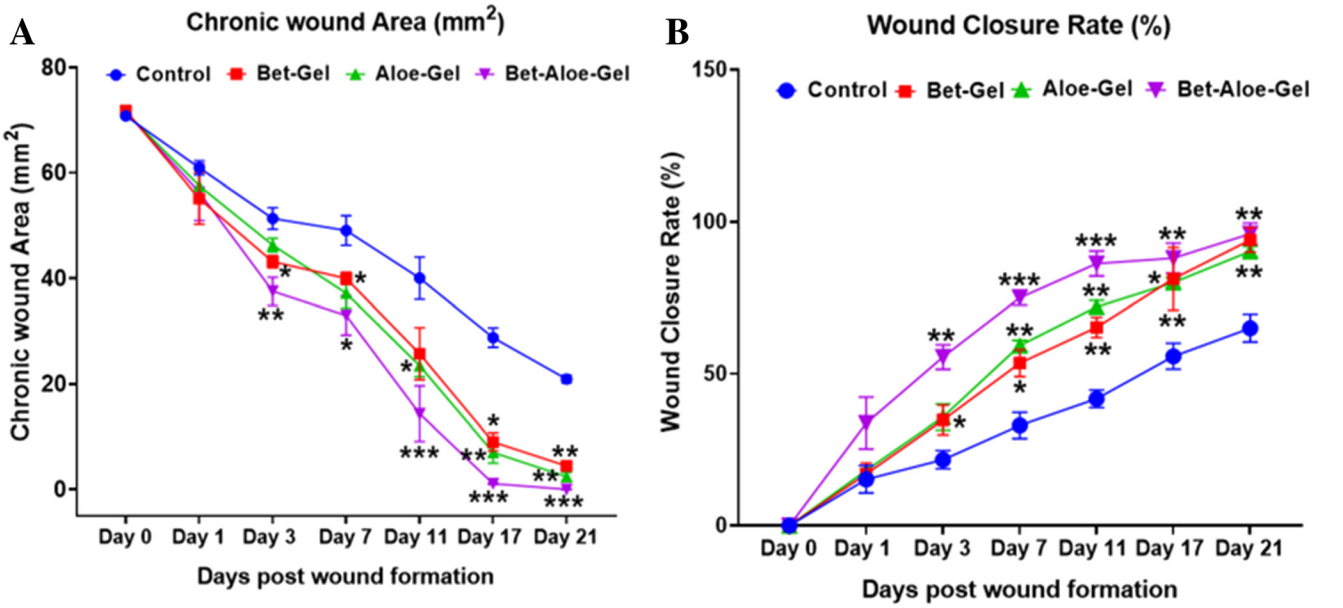

Fig. 7 Measurement of wound closure rate and chronic wound area post-wound formation. (A) Chronic wound area (mm^2) and (B) quantitative analysis of wound closure rate (%) measured over 21 days post-burn injury in different treatment groups: Control, Bet–Gel (betaine–gelatin), Aloe–Gel (aloe vera–gelatin), and Bet–Aloe–Gel (betaine–aloe vera–gelatin). Data are presented as mean ± SEM (n = 4). Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparison test. $^{**}p < 0.01$ and $^{***}p < 0.001$ indicate significance *versus* the control group. The BAG group showed significantly enhanced wound closure and reduced chronic wound area compared to other groups, indicating superior wound healing efficacy.

early as day 3, where the wound size dropped to 38 mm^2, compared to 45 mm^2 in AG and BG and 55 mm^2 in the control group (Fig. 7A). By Day 11 the BAG group showed an accelerated reduction to 20 mm^2 ($P < 0.001$), while the AG and BG groups remained at around 28–30 mm^2, and the control group around 42 mm^2. By day 21 the wound area in the BAG group had almost completely resolved (2 mm^2) ($P < 0.001$), indicating robust tissue regeneration, while residual wound areas remained in the BG (5 mm^2), AG (4 mm^2), and control (20 mm^2) groups ($P < 0.01$). These data collectively confirm that the BAG hydrogel not only accelerated wound closure but also significantly reduced chronic wound burden, suggesting enhanced granulation tissue formation and re-epithelialization. The progression of macroscopic wound healing over a period of 21 days in a burn wound model under four different treatment conditions involving the hydrogel formulations, namely Burn Wound Control, Burn Wound + BG (betaine–gelatin), Burn Wound + AG (aloe vera–gelatin), and Burn Wound + BAG (betaine–aloe vera–gelatin) is shown in (Fig. 8). Photographs were taken on days 1, 3, 7, 11, 17, and 21 to monitor wound closure and tissue regeneration. In the control group, burn wounds exhibited persistent inflammation, scabbing, and incomplete epithelialization even by day 21, with noticeable scar formation and delayed wound contraction. In contrast, wounds treated with the BG and AG hydrogels showed moderately improved healing patterns, with reduced eschar formation and partial re-epithelialization over time. However, complete wound closure was not consistently observed by day 21, and residual wound areas remained visible. The BAG hydrogel-treated group demonstrated a significantly enhanced healing trajectory. As early as day 7, the wounds showed reduced necrotic tissue and a clean wound bed. By day 11, signs of granulation tissue and contraction were prominent. Complete wound closure with minimal scarring and restored skin architecture was observed by day 21, indicating superior therapeutic efficacy. The enhanced healing in the BAG group can be attributed to the synergistic effects of betaine's anti-inflammatory and antioxidant properties, aloe vera's regenerative and anti-microbial activity, and the biocompatible scaffold function of gelatin.

5.1. Histopathological evaluation of burn wound tissue

Histological analysis of skin tissues collected on day 7 and day 21 post-burn injury was performed to assess the wound healing efficacy of different treatments. Haematoxylin and eosin (H&E) staining revealed distinct differences in the regenerative response among the treatment groups (Fig. 9). On day 7, the burn wound control group showed disrupted epidermal architecture, inflammatory cell infiltration, and incomplete re-epithelialization. In contrast, the BG- and AG-treated groups exhibited early signs of re-epithelialization and reduced inflammation. The BAG group demonstrated enhanced tissue regeneration with initial epithelial bridging and early collagen deposition, indicating accelerated wound healing. By day 21, the BAG-treated group showed almost complete re-epithelialization, well-organized collagen fibers, and restored dermal integrity. The BG and AG groups also showed improved tissue architecture compared to the control but were less organized

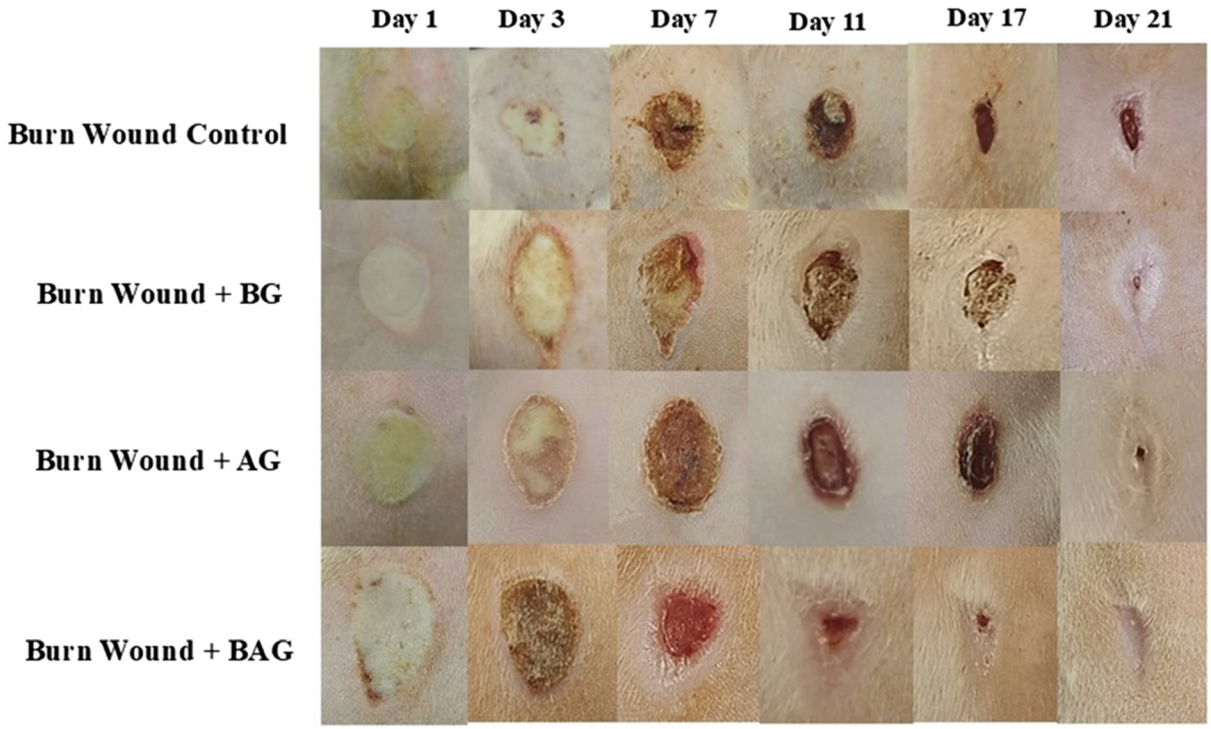

Fig. 8 Macroscopic evaluation of burn wound healing. Representative macroscopic images of burn wound healing in rats treated with different hydrogel formulations over a 21 day period. The groups include Burn Wound Control, Burn Wound + BG (betaine–gelatin), Burn Wound + AG (aloe vera–gelatin), and Burn Wound + BAG (betaine–aloe vera–gelatin). Photographs were taken on days 1, 3, 7, 11, 17, and 21 post-wound to assess the progression of wound closure.

than BAG. The control group retained signs of inflammation and incomplete epidermal regeneration. To evaluate the extent of epidermal regeneration during wound healing, histomorphometric analysis of total epidermal thickness was performed on tissue sections obtained on day 7 and day 21 post-treatment (Fig. 10).

On day 7, minimal epidermal thickness was observed in the control group indicating delayed epithelialization. In contrast, the treated groups (BG, AG, and BAG) demonstrated markedly enhanced epidermal proliferation, with the BAG formulation showing the greatest increase, followed by AG and BG. This early enhancement of epidermal growth in the BAG group ($P < 0.001$) suggests a synergistic effect and promoting accelerated re-epithelialization and cellular migration. By day 21 further improvement in epidermal thickness was evident across all groups. The BAG formulation exhibited the highest thickness (~310 μm) significantly exceeding that of AG, BG, and control. These results underscore the superior regenerative potential of the BAG hydrogel, which likely facilitates enhanced keratinocyte proliferation and differentiation critical for effective barrier restoration and wound closure. The pronounced epidermal thickening observed in the BAG group may be attributed to the bioactive contributions of its constituents. Betaine, which is known for its osmoprotective and anti-inflammatory properties, may contribute to cellular hydration and stress resilience, while aloe vera enhances fibroblast activity and collagen deposition, and gelatin provides a biocompatible scaffold supporting cellular adhesion and tissue remodelling.

6. Discussion

The present study offers a comprehensive evaluation of a novel betaine–aloe vera–gelatin (BAG) hydrogel developed for enhanced burn wound healing. The success of this hydrogel system can be attributed to the synergistic integration of three naturally derived bioactive components each contributing unique and complementary therapeutic effects. Betaine, a zwitterionic osmolyte, plays a crucial role in maintaining cellular homeostasis under stress-like conditions such as inflammation and oxidative damage, both of which are hallmarks of burn injuries.[27] Its anti-inflammatory activity is thought to arise from the suppression of pro-inflammatory cytokines and stabilization of cell membranes, which collectively contribute to the attenuation of the acute inflammatory response and prevention of further tissue damage. Aloe vera, long recognized for its wound healing properties, contributes through multiple mechanisms including the stimulation of fibroblast proliferation, enhancement of angiogenesis, promotion of extracellular matrix (ECM) production and inhibition of microbial colonization.[28] The gelatin provides a structurally supportive scaffold that mimics the native ECM facilitating cellular

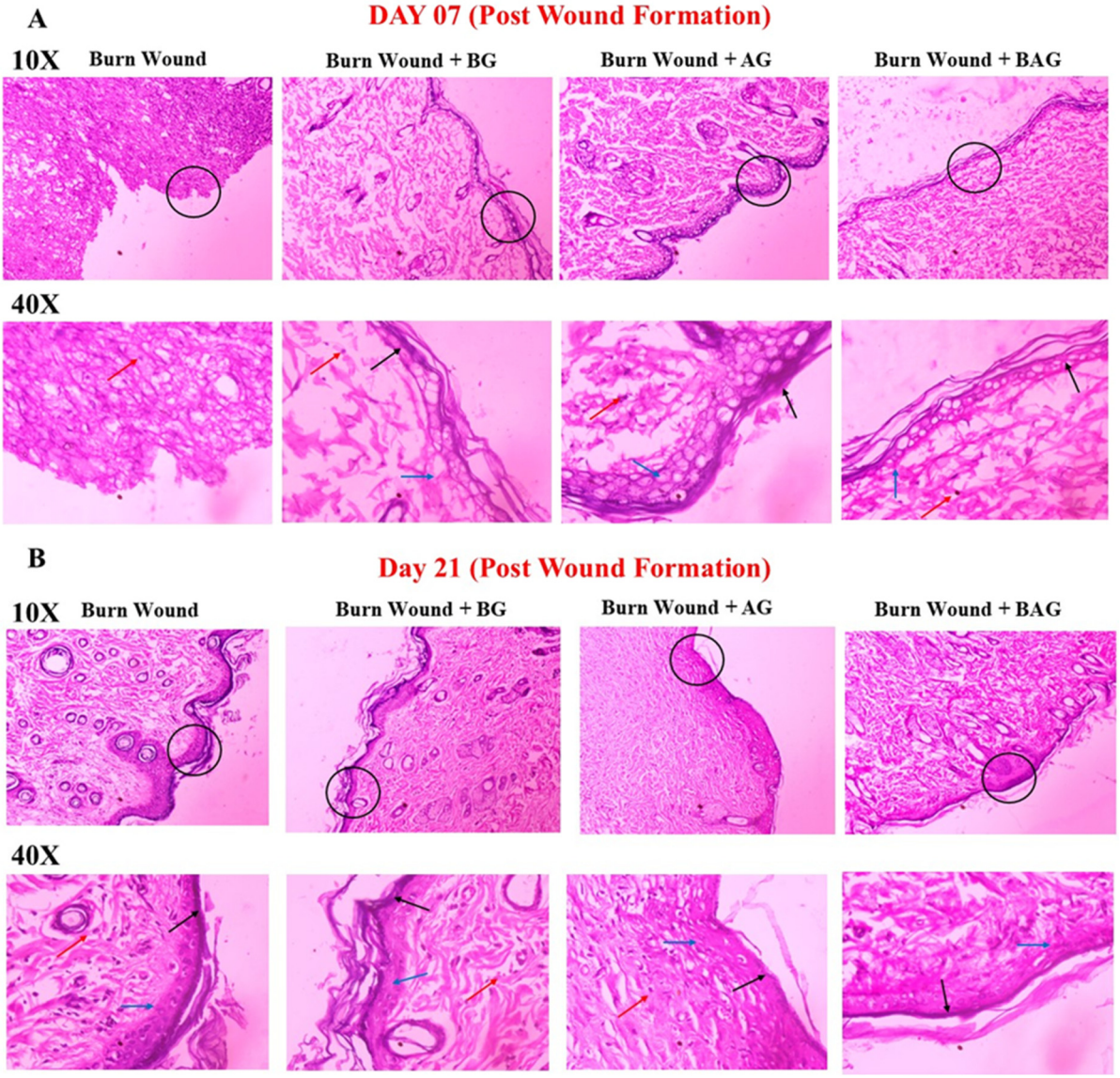

Fig. 9 Histopathological evaluation of burn wound tissue. Images captured at 10× and 40× magnifications show the progression of tissue regeneration in untreated burn wound (Control) and treatment groups: BG (betaine–gelatin), AG (aloe vera–gelatin), and BAG (betaine–aloe vera–gelatin). On day 7 (A) the BAG-treated group displayed early signs of re-epithelialization (black arrows), moderate collagen deposition (blue arrows), and fibrosis (red arrows) compared to other groups. On day 21 (B), the BAG group showed prominent and continuous re-epithelialization, dense collagen matrix, and minimal fibrosis, indicating advanced healing.

adhesion, proliferation, and directed migration necessary for tissue regeneration and repair.[29] The hydrogel prepared using the solvent-casting method exhibited uniform morphology and consistent physicochemical characteristics thereby ensuring reproducibility and potential scalability, two essential prerequisites for clinical translation. ATR-FTIR analysis revealed distinct absorption peaks corresponding to hydroxyl, amide, and carbonyl groups, which confirmed the presence of inter- and intramolecular hydrogen bonding and chemical interactions among the three components. These molecular interactions are indicative of crosslinking within the hydrogel matrix, which contributes to its structural stability, integrity, and sustained performance upon application to the wound surface. *In vivo* assessment using a rat model of second-degree thermal burn injuries further validated the superior wound healing potential of the BAG hydrogel. The use of low glutaraldehyde concentration (0.06–0.1% v/v) with extended curing minimizes residual free aldehyde groups, thereby reducing potential cytotoxicity.[30] In addition to the observed physicochemical properties, the hydrophilic composition of the BAG hydrogel suggests favorable swelling and moisture retention, which are essential for maintaining a moist wound environment and supporting tissue regeneration. Gelatin-based networks are also known to undergo gradual biodegradation under physiological conditions, aiding tissue remodeling. Although not quantitatively evaluated in this study, such behavior is consist-

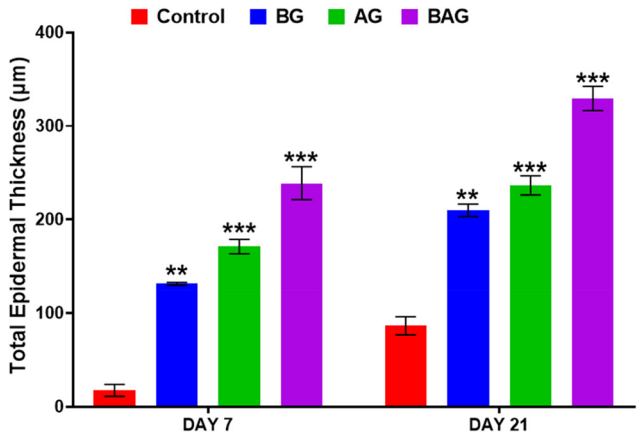

Fig. 10 Histomorphometric analysis of epidermal thickness on days 7 and 21. The histogram represents the mean total epidermal thickness (µm) measured in different treatment groups: Control, BG (betaine–gelatin), AG (aloe vera–gelatin), and BAG (betaine–aloe vera–Gelatin). A significant increase in epidermal thickness was observed in all treatment groups compared to the control at both time points. The BAG-treated group exhibited the highest epidermal thickness, indicating enhanced re-epithelialization and skin regeneration. Data are presented as mean ± SEM (n = 4). Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparison test. **p < 0.01 and ***p < 0.001 indicate significance *versus* the control group.

ent with similar hydrogel systems.[31,32] Over a 21-day treatment period the BAG-treated group showed significantly accelerated wound contraction and re-epithelialization compared to the control and other formulation groups of AG and BG. The enhanced wound closure observed in the BAG group can be attributed to the combined effects of betaine-like osmoprotective and anti-inflammatory actions, aloe vera's regenerative and antimicrobial functions, and the ECM-mimicking support of gelatin for cell anchorage and migration. The BAG hydrogel-treated wounds demonstrated minimal eschar formation and reduced wound exudate suggesting an effective modulation of the wound environment and inflammatory response. Histological analyses further reinforced these macroscopic findings. H&E staining revealed that the BAG hydrogel facilitated early granulation tissue formation, increased neovascularization, and higher fibroblast density. These features are critical for delivering oxygen and nutrients to the regenerating tissue thereby supporting cellular metabolism and matrix synthesis. The dermis exhibited organized collagen bundles with minimal gaps indicating effective tissue remodelling, while the epidermis displayed a well-formed basal layer suggesting complete epithelial restoration. The reduced presence of inflammatory cells in the BAG-treated tissues suggests an accelerated transition from the inflammatory to the proliferative phase, a key determinant of faster and scar-free wound healing. Comparatively, the AG and BG groups also showed healing but to a lesser extent. The observed healing response also aligns with the known biocompatibility of the constituent materials. Gelatin supports cell adhesion, aloe vera enhances fibroblast activity, and betaine exhibits cytoprotective effects, as reported in previous studies.[12,33,34] Consistently, no signs of irritation or adverse tissue response were observed *in vivo*, further indicating good biocompatibility of the hydrogel. The AG group demonstrated moderate re-epithelialization consistent with aloe vera's healing potential, although its relatively lower mechanical stability and hydration retention likely limited its long-term efficacy. The BG hydrogel, while structurally supportive, lacked the strong regenerative signals provided by aloe vera and it therefore exhibited a slower healing profile than the BAG formulation. The enhanced performance of the BAG hydrogel thus underscores the importance of multi-component, synergistically formulated systems for comprehensive wound care. These findings suggest that the BAG hydrogel not only accelerates the closure of burn wounds but also improves the quality of regenerated tissue, addressing both functional recovery and aesthetic outcomes. The observed biological effects of the hydrogel are a result of its multi-targeted therapeutic action encompassing inflammation resolution, promotion of angiogenesis, support for fibroblast function, collagen remodelling, and epithelial barrier restoration. Such a holistic approach is especially valuable in managing complex wounds like burns that require coordination of multiple biological processes for effective recovery. From a translational perspective, the ease of preparation, cost-effectiveness, and natural origin of the hydrogel components make this formulation particularly attractive for large-scale manufacturing and clinical application especially in resource-constrained settings. While the present study demonstrates promising therapeutic efficacy and favorable physicochemical characteristics of the BAG hydrogel, further investigations are warranted to deepen understanding of its functional performance and clinical applicability. In particular, detailed evaluation of swelling behavior, degradation kinetics, and moisture retention would provide additional insight into its behavior under physiological conditions. Moreover, further studies exploring the molecular mechanisms underlying the observed therapeutic effects, along with long-term safety, biocompatibility, and validation in larger animal models, will be essential to fully establish its potential for clinical wound care applications.

7. Conclusion

The formulated betaine–aloe vera–gelatin hydrogel demonstrated significant potential as an effective wound healing agent for burn injuries. The synergistic combination of natural bioactive components resulted in enhanced wound contraction, improved tissue regeneration, and accelerated healing as confirmed by both macroscopic and histological evaluations. ATR-FTIR analysis validated the successful integration and crosslinking of components within the hydrogel matrix. Overall, the favourable physicochemical, rheological, and stable nanoscale properties establish this biocompatible and multifunctional hydrogel as a promising candidate for advanced burn wound management, meriting further clinical investigation.

Conflicts of interest

The authors declare no competing interests.

Data availability

Data from this study are available upon reasonable request to the corresponding author.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d6pm00049e.

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