

Freeze-dried collagen nanocomposite sponges loaded with nicaraven-incorporated gelatin nanofibers for excisional diabetic wound healing: *In vitro* and *in vivo* studies

Research Article

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Abstract: Diabetic foot ulcers (DFUs) are a frequent and severe complication of diabetes, leading to chronic wounds, infections, and even amputations. The treatment of DFUs poses a significant challenge to healthcare professionals, and therefore new therapeutic approaches are necessary. In the current research, nicaraven was loaded into gelatin nanofibers using the electrospinning method. Then, these fibers were crushed and dispersed in the matrix of a collagen hydrogel to prepare a nanocomposite sponge for diabetic wound healing applications. The produced scaffolds protected skin cells against oxidative stress and increased their migration activity. An *in vivo* study was performed in a rat model of diabetic wound. The study revealed that the sponges loaded with nicaraven accelerated the diabetic wound closure and improved tissue epithelialization and collagen deposition. The enzyme-linked immunosorbent assay showed that the tissue concentration levels of interleukin-6 and tumor necrosis factor alpha cytokines were significantly reduced in diabetic wounds treated with the nicaraven-loaded nanocomposite sponge.

Keywords: Nanocomposite • Diabetic wound • Nicaraven • Drug delivery

1. Introduction

Diabetic foot ulcers (DFUs) are a frequent and severe complication of diabetes, leading to chronic wounds, infections, and even amputations. The treatment of DFUs poses a significant challenge to healthcare professionals, and therefore new therapeutic approaches are necessary [1,2]. Biomaterial-based therapies, such as nanocomposite sponges, have shown promise in promoting wound healing and tissue regeneration. Collagen is a widely used biomaterial in wound healing and tissue engineering applications due to its biodegradability, biocompatibility, and ability to facilitate cell attachment and proliferation [3,4]. However, the mechanical properties of collagen sponges are not strong enough to be used in load-bearing tissues like skin. Consequently, researchers have investigated the incorporation of other

materials, such as gelatin and nanoparticles, to enhance the mechanical properties and therapeutic effectiveness of collagen-based sponges [5,6]. Wang et al. showed that the chitosan-crosslinked collagen sponge loaded with recombinant human acidic fibroblast growth factor (rh-aFGF) accelerated diabetic wound healing by enhancing collagen generation, tumor growth factor beta 1 (TGF- β 1) expression, and dermal cell proliferation [7]. Wu et al. showed that the taurine-loaded collagen sponge (Tau@Col) enhanced wound healing by promoting granulation, collagen deposition, and re-epithelialization. It improved cell proliferation via upregulation of vascular endothelial growth factor (VEGF) and TGF- β and reduced inflammation by stimulating M2 macrophage polarization [8]. Gelatin is a denatured form of collagen that can form nanofibers via electrospinning, providing a high surface area for drug loading and release [9]. Borges-Vilches et al. showed that poly(ϵ -caprolactone)/gelatin electrospun nanofibers loaded

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with *Pinus radiata* bark extracts (PEs) were fabricated for wound healing. PE addition improved the wettability, degradation rate, and biocompatibility, enhancing cell proliferation and inhibiting bacterial activity, promoting complete wound closure within 72 h [10].

Nicaraven is a drug that exhibits anti-inflammatory and antioxidant properties that can be useful for diabetic wound healing [11]. In a study of Suliman Maashi et al., they produced nicaraven-loaded collagen wound dressings via electrospinning and tested for wound healing. Collagen/4% nicaraven and collagen/6% nicaraven dressings significantly enhanced wound closure, epithelium thickness, and collagen deposition, promoting diabetic wound healing [12]. Thus, the combination of freeze-dried collagen nanocomposite sponges with gelatin nanofibers loaded with nicaraven represents a novel strategy for treating DFUs. The nanocomposite sponges can serve as a scaffold for tissue regeneration, while the gelatin nanofibers can act as a drug delivery system for nicaraven. Furthermore, freeze-drying can maintain the function and structure of both materials and enhance the mechanical properties of the resulting sponges.

Previous studies have investigated the use of collagen-based sponges and gelatin nanofibers for tissue regeneration and wound healing [13,14]. However, no prior research has been conducted on the effectiveness of freeze-dried collagen nanocomposite sponges containing nicaraven-loaded gelatin nanofibers in treating excisional diabetic wounds. This study seeks to bridge this gap in the literature by evaluating the efficacy of this novel biomaterial-based therapy in a rat model of DFUs. The findings of this study will offer crucial insights into the development of novel therapeutic approaches for DFUs using biomaterial-based therapies.

2. Materials and methods

2.1 Preparation of nicaraven-loaded nanocomposite hydrogels

To prepare nicaraven-loaded nanocomposite sponges, first, a solution of gelatin and nicaraven was prepared. Gelatin was dissolved in acetic acid at 35 wt% with constant stirring until it completely dissolved. Nicaraven (Sigma Aldrich, USA) was then added to the solution and stirred for 30 min until it was evenly dispersed. The resulting solution was electrospun to form nanofibers using a high voltage power supply and a syringe pump. The electrospinning conditions were optimized to obtain uniform

and continuous nanofibers. The electrospinning parameters used were a voltage of 20 kV, a distance of 15 cm between the needle tip and the collector, and a flow rate of 0.5 mL/h. The gelatin nanofibers loaded with nicaraven were then crushed and dispersed in a solution of collagen. The collagen solution was prepared by dissolving collagen (type 1 from the rat tail) in 0.1 M acetic acid with stirring at 4°C for 24 h. The concentration of collagen in the solution was optimized to obtain a suitable consistency for the sponge. The crushed gelatin nanofibers loaded with nicaraven were added to the collagen solution and mixed thoroughly to obtain a homogeneous mixture. The mixture was then poured into a mold and freeze-dried using a freeze dryer. The freeze-drying process was optimized to obtain a porous and sponge-like structure. The parameters used for the freeze-drying process were a temperature of −20°C for 24 h, followed by a secondary freezing phase at −80°C for 24 h. The resulting sponge was then freeze-dried for 48 h. Nicaraven-loaded and nicaraven-free nanocomposite sponges were named NICGELCOL and GELCOL, respectively.

2.2 MTT assay

To assess the viability of L929 cells cultured on NICGELCOL and GELCOL sponges using the MTT assay, the L929 cells were first grown in culture medium until they reached the desired density. Next, the NICGELCOL and GELCOL sponges were cut into small pieces that would fit into the wells of a 96-well plate. The sponges were then sterilized by soaking them in 70% ethanol for 30 min and washing them three times with sterile phosphate-buffered saline (PBS). The sterilized sponges were then placed in the wells of a 96-well plate. The L929 cells were seeded onto the sponges at a density of 5,000 cells per well, and the plate was incubated at 37°C and 5% CO₂ for 24 h to allow the cells to attach and spread. The cells were cultured for 7 days. On days 2, 3, and 5, the cell viability assay was performed. To prepare the MTT assay solution, MTT powder was dissolved in PBS at a concentration of 0.5 mg/mL. The MTT assay was then performed by adding 100 µL of the MTT solution to each well of the 96-well plate and incubating the plate for 4 h at 37°C and 5% CO₂. After incubation, the MTT solution was removed, and 100 µL of dimethyl sulfoxide was added to each well to dissolve the formazan crystals. The absorbance of each well was then measured at 570 nm using a microplate reader. The viability of L929 cells cultured on NICGELCOL and GELCOL sponges was assessed by comparing the absorbance values of the experimental wells to the absorbance values of control wells containing cells cultured on standard

tissue culture plates. The viability of the cells was considered to be proportional to the absorbance values.

2.3 Swelling assay

In order to evaluate the swelling characteristics of NICGELCOL and GELCOL sponges, the sponges were initially sliced into equally sized pieces. The weight of each sponge piece in the dry state was measured and documented. These sponge pieces were then submerged in distilled water and placed in an incubator set at 37°C for a designated duration of time, with 24 h being a commonly used period. Following the incubation period, the sponge pieces were removed from water, and any extra water on the surface of the sponge was carefully blotted away with filter paper. The wet weight of the swollen sponge pieces was measured and documented. The swelling ratio was assessed by using the following formula: swelling ratio = (wet weight – dry weight)/dry weight, where the swelling ratio shows the weight increase of the sponge after swelling with respect to its initial dry weight. To compare the swelling characteristics of NICGELCOL and GELCOL sponges, the swelling ratio was computed and compared for each type of sponge. The experiment was carried out repeatedly to ensure reliability. In addition to the swelling ratio, the water uptake capacity of the sponges could be determined by dividing the weight of water absorbed by the dry weight of the sponge. This measure provides extra information on the water-retention capability of the sponges. Overall, the swelling properties of NICGELCOL and GELCOL sponges were evaluated successfully using an immersion approach. The swelling ratio and water uptake capacity calculations are essential for determining the performance of sponges in numerous applications, such as drug delivery and tissue engineering.

2.4 MTT assay under oxidative stress

To assess the protective role of the sponges against oxidative stress, NICGELCOL and GELCOL sponges were ground and converted into powder. Then, L929 cells were cultured in 96-well plates to reach 80% confluence. Then, the sponge's powder was added to culture media at 50 mg/mL concentration, and cells were cultured for 24 h. Then, 1% v/v H₂O₂ was added to the culture media, and cells were incubated for 1 h. Finally, the MTT assay was performed using the same method as described above.

2.5 DPPH assay

To evaluate the radical scavenging potential of NICGELCOL and GELCOL sponges using the DPPH assay, the first step involved grinding the sponges into a fine powder and extracting them with an appropriate solvent to obtain an extract for use in the assay. A stock solution of DPPH was then prepared by dissolving it in ethanol at a concentration of 0.1 mM. From the stock solution, a working solution was prepared by diluting the stock solution in ethanol to a concentration of 20 µM. For the assay, various amounts of sponge extract were mixed with the working solution of DPPH, and the mixture was incubated in the dark for 30 min at room temperature. After incubation, the mixture's absorbance was measured at a wavelength of 517 nm using a spectrophotometer. A control sample containing only the working solution of DPPH and the solvent was used as a reference. The radical scavenging potential of the sponge extract was determined by comparing the absorbance of the sample containing the extract to that of the control sample. The percentage inhibition of DPPH radicals by the sponge extract was calculated using the following formula:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100,$$

where *A* control is the absorbance of the control sample and *A* sample is the absorbance of the sample containing the sponge extract.

To ensure the validity of the results, the experiment was repeated multiple times, and the data obtained were analyzed statistically. The radical scavenging potential of NICGELCOL and GELCOL sponges was compared based on the percentage inhibition of DPPH radicals.

2.6 *In vitro* wound closure

To evaluate the effectiveness of NICGELCOL and GELCOL sponges in promoting *in vitro* wound closure, a scratch assay was conducted. Initially, a layer of L929 cells was cultured in a 24-well plate until reaching confluence. Then, a uniform wound was created on the cell layer using a sterile pipette tip (200 µL). The scratched monolayer was treated with either 50 mg/mL NICGELCOL or GELCOL sponges' powder, and the cells were allowed to incubate under standard cell culture conditions for 24 or 48 h. During the incubation period, the cells were observed using a microscope to track the wound closure. The degree of wound closure was calculated by measuring the distance between the edges of the wound at specific

time intervals. The percentage of wound closure was calculated using the formula:

% Wound closure

$$= [(Initial\ scratch\ width - Current\ scratch\ width) / Initial\ scratch\ width] \times 100,$$

where the initial scratch width is the distance between the edges of the wound at time 0, and the current scratch width is the distance between the edges of the wound at a particular time point. To ensure reproducibility, the experiment was conducted multiple times. The wound closure results were compared between the cells treated with NICGELCOL and GELCOL sponges. Statistical analysis was conducted to determine any significant differences between the two treatments.

2.7 Water vapor permeation test

To assess the water vapor permeation of NICGELCOL or GELCOL sponges, they were cut into small pieces. Then, the sponge pieces were placed on top of a glass vial containing 10 mL distilled water and sealed with a thin layer of paraffin to ensure that water vapor only passed through the sponge. After sealing, the vials were placed in a humidity chamber maintained at a specific relative humidity and temperature. The humidity chamber had a constant flow of dry air, which helped to maintain a constant humidity level, and the relative humidity and temperature were recorded at regular intervals. The sponge pieces were allowed to incubate for 24 h. After the incubation period, the sponge pieces were removed from the vials, and the weight loss of the water was recorded. To calculate the water vapor permeation rate of the sponges, the following formula was used:

$$\text{Water vapor permeation rate} = (\text{Water weight loss}) / (\text{Area} \times \text{Time}).$$

Here, the area refers to the surface area of the sponge piece and the time is the duration of incubation. To ensure reproducibility of the results, the experiment was performed multiple times.

2.8 In vivo study

To assess the wound healing potential of NICGELCOL or GELCOL sponges in a rat model of diabetic wound, a surgical procedure was performed. First, streptozotocin was used to induce diabetes in male Wistar rats. Diabetes

was confirmed by measuring the blood glucose levels using a glucometer. Rats with blood glucose levels above 250 mg/dL were included in the study. Next, full-thickness excision wounds were created on the back of the rats using a biopsy punch. The wounds were approximately 1.5 cm in diameter and were made under sterile conditions. The rats were then randomly assigned to three groups: a control group, a group treated with NICGELCOL sponges, and a group treated with GELCOL sponges. The sponges were cut into appropriate sizes and sterilized using UV light. The sponge pieces were then placed on the wounds and secured in place using sterile surgical adhesive tape. The wounds were dressed with sterile gauze and bandages. The rats were housed in individual cages and were allowed to move freely. The wounds were examined daily for signs of infection and inflammation. The dressings were changed every 2 days. The rats were euthanized at predetermined time points, typically at day 7 and day 14 post-wounding. The wound healing progress was assessed by measuring the wound closure rate, which was calculated by measuring the wound area using digital calipers and analyzing the images obtained from the photographs. The percentage of wound closure was calculated using the following formula:

$$\% \text{ Wound closure} = [(Initial\ wound\ area - Current\ wound\ area) / Initial\ wound\ area] \times 100,$$

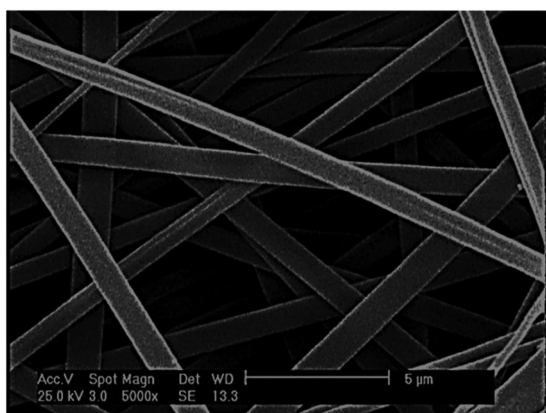
where the initial wound area is the area of the wound at time 0, and the current wound area is the area of the wound at a specific time point.

On day 14, the rats were humanely killed via ketamine overdose, and their wound tissues were harvested for histopathological examination. The tissues were processed and sectioned into 5 µm tissue slides and then stained with hematoxylin and eosin and Masson's trichrome. Finally, the levels of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), were measured in the wound tissue samples using enzyme-linked immunosorbent assay (ELISA) kits.

2.9 Statistical analysis

Data were analyzed using GraphPad Prism software, employing Student's *t*-test for comparisons between groups. For certain datasets, one-way ANOVA was utilized to assess differences among multiple groups. The assumptions for parametric tests were verified, confirming normality of distribution and homogeneity of variance through appropriate tests (e.g.,

Nicaraven-loaded gelatin nanofibers



Nicaraven-free gelatin nanofibers

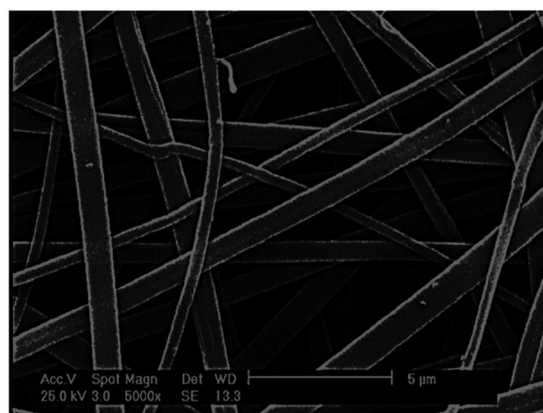


Figure 1. SEM images of nicaraven-loaded and nicaraven-free gelatin nanofibers.

Shapiro–Wilk test for normality and Levene’s test for homogeneity). Each experiment was repeated at least three times to ensure consistency and reliability of the results. The sample sizes for each group were clearly defined, and the power of the tests was calculated to ensure the robustness of the findings. This comprehensive approach to statistical analysis supports the validity of the results obtained.

3. Results and discussion

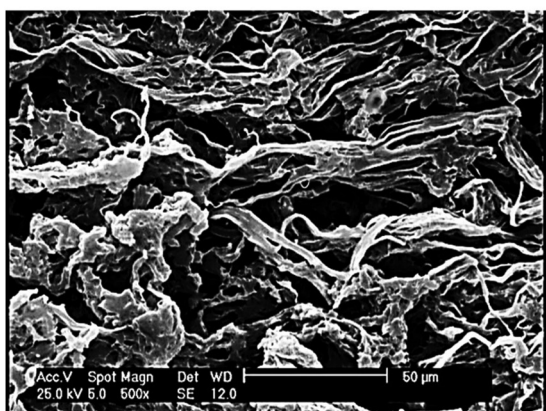
3.1 SEM imaging

This study investigated the characteristics of nicaraven-loaded and nicaraven-free gelatin nanofibers. The results,

presented in Figure 1, indicate that both types of constructs had highly fibrous structures without any beads. The fibers appeared web-like and had smooth surfaces. Furthermore, the average fiber size of nicaraven-loaded and nicaraven-free gelatin nanofibers was measured and found to be around 856.12 ± 168.20 nm and 893.31 ± 244.57 nm, respectively.

The porous structure of wound dressings is a crucial characteristic that can influence their effectiveness in promoting wound healing. In this investigation, it was found that the NICGELCOL and GELCOL sponges had similar pore morphology, indicating that they may have comparable properties in terms of aiding wound healing. The void spaces observed in Figure 2 may have arisen due to the freeze-drying process used to prepare the sponges [15,16].

NICGELCOL



GELCOL

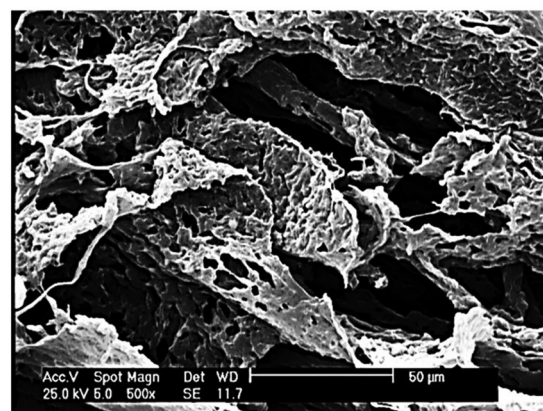


Figure 2. SEM images of NICGELCOL and GELCOL sponges.

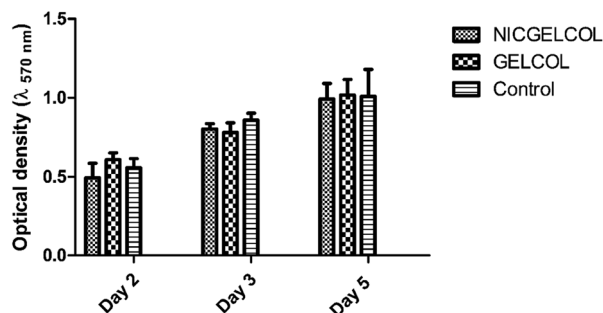


Figure 3. MTT assay with L929 cells cultured on NICGELCOL and GELCOL scaffolds during 5 days of culture on the scaffolds.

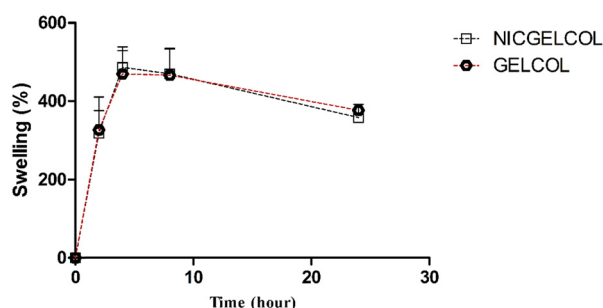


Figure 4. Swelling properties of NICGELCOL and GELCOL sponges incubated in PBS for 24 h.

3.2 MTT assay

MTT assay results (Figure 3) showed that at any of the studied time points, NICGELCOL and GELCOL sponges were not toxic against L929 fibroblasts, as shown by the lack of significant difference between the control and sponge groups, with a p -value of >0.05 .

3.3 Swelling assay

Swelling assay results (Figure 4) showed that NICGELCOL and GELCOL sponges had a similar pattern of swelling while being incubated in PBS. Both sponges underwent a

fast swelling in the initial 4 h period. Then, the percentage of swelling almost stayed steady for the sponges. There was a slight decline observed in the swelling percentage at 24 h.

3.4 MTT assay under oxidative stress

The results of this study, as depicted in Figure 5, demonstrated that NICGELCOL sponges had significantly higher cell viability compared to GELCOL and control groups. The p -value of less than 0.05 indicates that this difference is statistically significant. On the other hand, there is no significant difference between the GELCOL and control groups with a p -value of greater than 0.05.

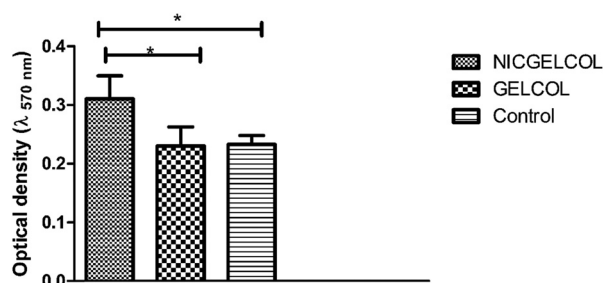


Figure 5. MTT assay with L929 cells cultured with 1% H_2O_2 and NICGELCOL and GELCOL sponges. * shows p -value <0.05 .

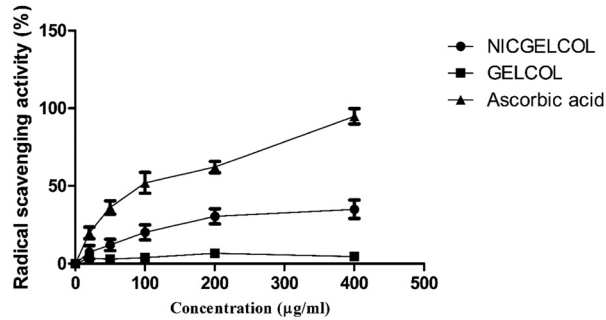


Figure 6. Radical scavenging activity of NICGELCOL and GELCOL sponges compared with ascorbic acid as the control group.

3.5 DPPH assay

DPPH assay results (Figure 6) showed that nicaraven-loaded sponges had significantly higher radical scavenging potential than GELCOL sponges, indicating that nicaraven has imparted antioxidant potential to the sponges.

3.6 *In vitro* wound closure assay

The assay results (Figure 7) showed that at 48 h, the fibroblasts cultured with NICGELCOL sponges had significantly higher percentage of *in vitro* wound closure compared with GELCOL and control groups. At 24 h, statistically no significant difference was found between the groups, with a *p*-value of >0.05 . Therefore, we can assume that nicaraven has increased the wound size reduction.

3.7 Water vapor permeation results

This study showed that NICGELCOL and GELCOL sponges had around 135.16 ± 26.59 mg/h cm² and 139.30 ± 15.28 mg/h cm² of water vapor permeation, respectively.

3.8 *In vivo* study

The *in vivo* study results (Figure 8) showed that on day 14, the NICGELCOL group had a significantly higher rate of wound size reduction than GELCOL and control groups, with a *p*-value of <0.05 . Differences between GELCOL and control groups were not significant, with a *p*-value of >0.05 . The wound closure assay results showed that for the NICGELCOL group, the wound closure was $44.59 \pm 4.99\%$ on day 7, and it improved to $92.74 \pm 3.73\%$ on day 14. In the GELCOL group, wound closure was $43.27 \pm 6.64\%$ on day 7, and it increased to $73.13 \pm 9.08\%$ on day 14. The control group had a wound closure of $40.54 \pm 7.63\%$ on day 7, which improved to $66.20 \pm 6.20\%$ by day 14. Histopathological examinations (Figure 9) showed that the wounds treated with NICGELCOL sponges had the lowest number of pro-inflammatory cells at the wound bed. In addition, the formation of granulation and epithelial tissues in this group was evident. Organized collagen fibers had filled the defect site, and the overall tissue structure was partly restored. On the other hand, wound tissues treated with GELCOL scaffolds had a slightly better healing response than the control group. In both groups, the

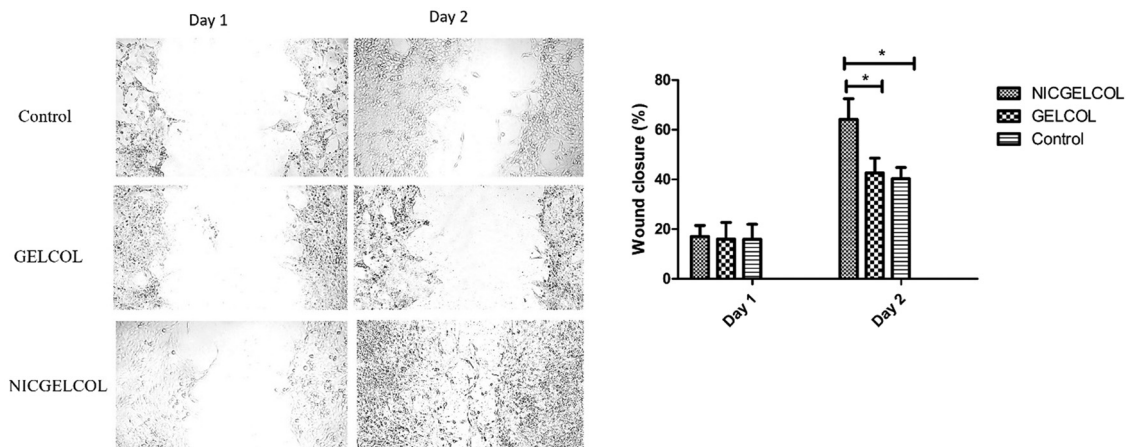


Figure 7. *In vitro* wound closure with L929 cells cultured with NICGELCOL and GELCOL sponges on days 1 and 2. * shows *p*-value <0.05 .

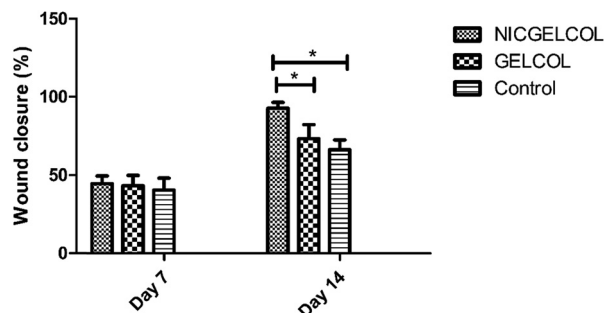


Figure 8. *In vivo* wound closure assay with NICGELCOL and GELCOL sponges in diabetic rats on days 7 and 14 after wounding. * shows p -value <0.05.

polymorphonuclear cells had been infiltrated at the defect site, and the tissue was covered by a crusty scab. Histomorphometric studies showed that the NICGELCOL group had significantly higher percentage of collagen deposition and thickness of epithelium, with a p -value of <0.05 (Figure 9).

ELISA assay results (Figure 10) showed that the concentrations of IL6 and TNF- α cytokines in the wound tissues treated with NICGELCOL sponges were significantly lower than those in the wound tissues in GELCOL and control groups, with a p -value of <0.05. Therefore, we can assume that the presence of nicaraven in the matrix of NICGELCOL sponges reduced the tissues' inflammatory responses.

4. Discussion

Electrospun wound dressings enable controlled drug delivery, promoting healing by mimicking the extracellular matrix and

releasing therapeutic agents. In the current research, we loaded nicaraven into a nanocomposite wound dressing to promote wound healing. Our SEM images showed that the addition of nicaraven did not significantly affect the size of the fibers. A potential reason for the minimal change in fiber size with the addition of nicaraven could be that the molecular structure and weight of nicaraven do not significantly interfere with the electrospinning process of gelatin. Nicaraven may integrate uniformly within the polymer matrix without affecting the viscosity or surface tension of the gelatin solution, which are critical parameters influencing fiber size during electrospinning. Additionally, the chemical interactions between nicaraven and gelatin might not be strong enough to cause noticeable alterations in fiber formation, leading to a similar size distribution in both nicaraven-loaded and nicaraven-free fibers [17,18]. The presence of pores in the sponges can be beneficial in facilitating the exchange of oxygen and nutrients between the wound

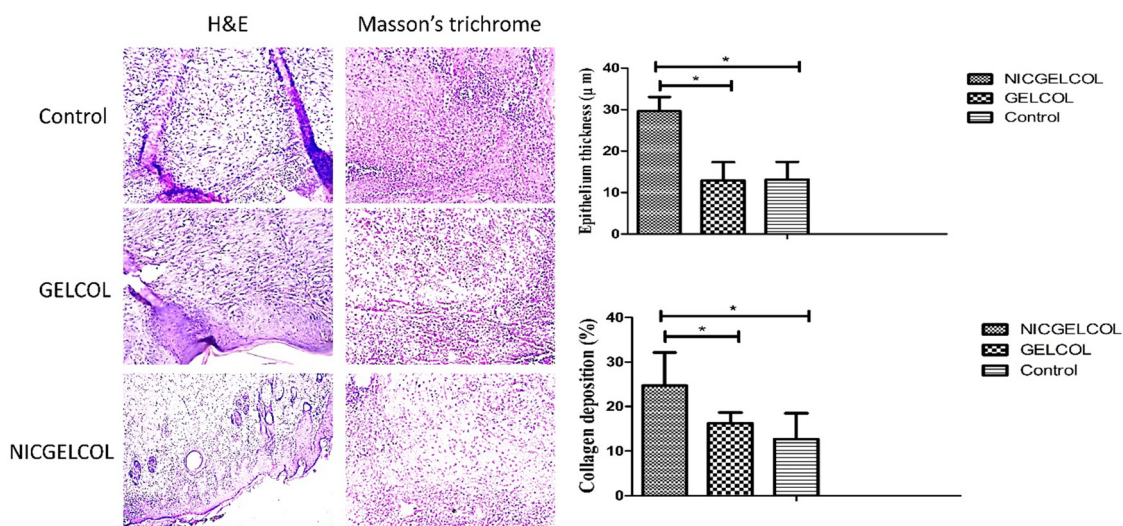


Figure 9. Histopathological studies in diabetic wounds treated with NICGELCOL and GELCOL sponges and histomorphometric analysis. * shows p -value <0.05.

bed and the surrounding environment, which is critical for promoting healing. Moreover, the porous structure of the sponges may enhance their ability to absorb the exudate and encourage the formation of the granulation tissue [19,20].

The superior cell viability observed in NICGELCOL sponges compared to GELCOL and control groups can be attributed to the presence of nicaraven in the sponge structure. Nicaraven is a small molecule drug that has been shown to have antioxidant and anti-inflammatory properties. It could be that nicaraven protected the fibroblasts against oxidative stress, a condition that can damage cell membranes and cause cell death. Suliman Maashi et al. showed that nicaraven was not toxic to L929 fibroblasts and enhanced their migration activity [12]. The high antioxidant capacity of nicaraven can lead to the scavenging of free radicals and prevent their damaging effects on cells. Additionally, nicaraven can reduce the production of reactive oxygen species (ROS), which can contribute to oxidative stress and cellular damage. Therefore, the presence of nicaraven in the NICGELCOL sponges may have helped to protect the cells from oxidative stress, resulting in higher cell viability [11,21]. This theory was validated by the results of DPPH assay. The scavenging activity of the nicaraven-loaded sponges may have effectively prevented oxidative stress and damage caused by free radicals generated in the system. The ability of wound dressings to scavenge free radicals is crucial in promoting efficient wound healing. Free radicals are highly reactive molecules that can cause damage and oxidative stress to cells, which can hinder the wound healing process [22,23]. Xu et al. showed that nicaraven mitigated radiation-induced oxidative stress in the lung tissue by downregulating the NF- κ B and TGF- β /Smad pathways, thereby suppressing the inflammatory response [24]. Nicaraven, a small molecule drug, has been shown to increase the migration activity of fibroblasts. One possible mechanism for this effect is that nicaraven can upregulate the expression of certain growth factors and

cytokines that play a role in cell migration. For example, nicaraven has been shown to increase the expression of VEGF, which is known to stimulate the migration of fibroblasts. Additionally, nicaraven may enhance the production of extracellular matrix components, such as collagen and fibronectin, which are important for cell adhesion and migration [25–27]. The water vapor permeation of wound dressings is an important factor to consider in their effectiveness in managing wounds. The results of this study show that both NICGELCOL and GELCOL sponges have relatively high water vapor permeation rates, with no significant difference between them. This suggests that both sponges have the potential to effectively manage moisture levels in wounds. Effective moisture management is important in wound healing because excessive moisture can lead to maceration, which can delay healing and increase the risk of infection. On the other hand, too little moisture can lead to dryness and hinder the healing process [28,29]. The results of this study suggest that the pore morphology of NICGELCOL and GELCOL sponges may have contributed to their similar water vapor permeation rates [30,31]. The porosity of the sponges likely allows for the exchange of moisture between the wound bed and the surrounding environment, leading to the observed high water vapor permeation rates [32,33]. Our *in vivo* study results showed that incorporation of nicaraven into the matrix of GELCOL sponges augments the sponge's ability to improve diabetic wound healing. Nicaraven has the potential to enhance the healing of diabetic wounds through two key mechanisms. First, it can modulate the inflammatory response, which is crucial for wound healing. In diabetic individuals, inflammation often becomes unbalanced and prolonged, leading to impaired healing [34,35]. Nicaraven, known for its strong antioxidant and anti-inflammatory properties, shows promise in regulating this inflammatory response. Zha et al. showed that nicaraven reduced inflammation in endotoxemia by activating the AMPK/Sirt1 pathway, suppressing

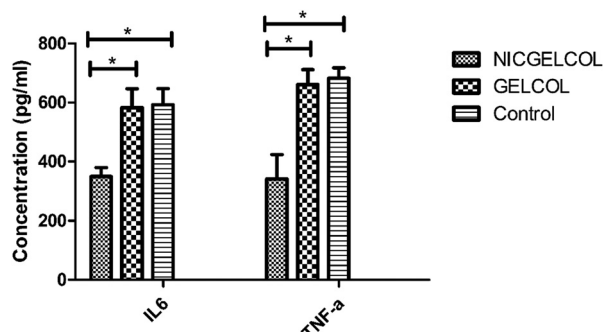


Figure 10. ELISA results demonstrating the tissue concentrations of IL6 and TNF- α in wound tissues treated with NICGELCOL and GELCOL sponges. * shows p -value <0.05.

pro-inflammatory cytokines, and inhibiting NF- κ B activation [25]. By incorporating nicaraven into GELCOL sponges, it can be directly delivered to the wound site, allowing for sustained release and targeted modulation of inflammation. This controlled modulation helps restore the balance between pro-inflammatory and anti-inflammatory processes, thereby promoting more effective wound healing. Second, diabetic wounds are characterized by increased oxidative stress, which further hampers the healing process. Nicaraven possesses potent antioxidant properties that can alleviate oxidative stress in these wounds [36]. Nicaraven, a hydroxyl radical scavenger, demonstrated strong antioxidant capacity by protecting hematopoietic stem/progenitor cells from radiation-induced damage. It significantly reduced DNA oxidation, as indicated by lower urinary levels of 8-oxo-2'-deoxyguanosine, and improved cell survival and colony-forming ability in irradiated mice. Despite no significant change in ROS levels in bone marrow cells, nicaraven lowered the inflammatory cytokines IL-6 and TNF- α , further highlighting its protective effects through both antioxidant and anti-inflammatory mechanisms [27]. By incorporating nicaraven into NICGELCOL sponges, it can be gradually released, providing continuous antioxidant activity at the wound site.

5. Conclusions

This study demonstrates the potential of NICGELCOL sponges in enhancing diabetic wound healing through both *in vitro* and *in vivo* assessments. *In vitro*, NICGELCOL sponges significantly improved fibroblast viability under oxidative stress, exhibited superior radical scavenging activity, and accelerated wound closure after 48 h, highlighting the antioxidant and pro-healing effects of nicaraven. Both NICGELCOL and GELCOL sponges had similar swelling and water vapor permeation rates, indicating effective moisture management. *In vivo*, NICGELCOL sponges significantly enhanced wound closure in diabetic rats by day 14. Specifically, the NICGELCOL group achieved $92.74 \pm 3.73\%$ wound closure by day 14, compared to $73.13 \pm 9.08\%$ in the GELCOL group and $66.20 \pm 6.20\%$ in the control group. Histological analysis revealed greater collagen deposition, thicker epithelium, and reduced inflammation compared to GELCOL and control groups. These findings suggest that nicaraven-loaded sponges offer a promising therapeutic approach for diabetic wound healing by combining antioxidant, anti-inflammatory, and tissue-regenerative properties.

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Author contributions

Huanli Hu was responsible for the conceptualization, methodology development, investigation, formal analysis, and drafting of the original manuscript. Hu also supervised the research and secured funding for the study. Xueyang Zheng contributed to the investigation, data curation, and formal analysis, as well as manuscript review, editing, and visualization. Both authors reviewed and approved the final version of the manuscript.

Conflict of interest statement

Authors state no conflict of interest.

Data availability statement

Data will be made available on request.

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