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Bacterial nanocellulose/honey composite impact on excisional wound healing in mice

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Abstract

The rising demand for natural wound dressings has spotlighted bacterial nanocellulose (BNC), produced by *Komagataeibacter xylinus*, for its biocompatibility, biodegradability, and high crystallinity. Combined with honey, renowned for antimicrobial, anti-inflammatory, and regenerative properties, this composite offers enhanced therapeutic potential. This study evaluated the efficacy of BNC/honey composites in healing excisional wounds in mice. Twenty-four mice were divided into four groups, including untreated control, honey-only, BNC-only, and BNC/honey composite. An 8.00-mm full-thickness wound was created dorsally, and healing was monitored over 14 days. Planimetry showed accelerated wound contraction in the composite group. Histopathology (days seven and 14) revealed superior tissue regeneration, increased angiogenesis, and fibroblast proliferation. Biochemical assays demonstrated significantly elevated total anti-oxidant capacity and glutathione peroxidase activity, alongside reduced oxidative stress markers (total oxidant status and malondialdehyde) in the composite group. Hydroxy-proline levels, indicating collagen synthesis, were also the highest in this group. Collectively, the BNC/honey composite markedly enhanced wound healing by promoting collagen deposition, reducing inflammation, and boosting anti-oxidant defense. These findings position the BNC/honey composite as a highly promising, natural biomaterial for advanced wound care with strong translational potential in clinical settings.

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Introduction

Bacterial cellulose, or nanocellulose (BNC), is a nanostructured polysaccharide produced through fermentation by Gram-negative bacteria, such as *Komagataeibacter xylinus* and *Gluconacetobacter hansenii*, along with other genera, like *Aerobacter* and *Pseudomonas*.[1] Unlike plant cellulose, its fibrous, nanoporous structure imparts superior properties, including high crystallinity, thermal and mechanical stability, large specific surface area, optical transparency, and high liquid absorption capacity.[2] This lightweight, flexible, and moldable biomaterial is renewable, non-toxic, biodegradable, and biocompatible, showing no allergenic activity.[3,4] Its high porosity and ability to maintain moisture make it ideal for wound healing, as it mimics the extracellular matrix, promoting cellular infiltration, proliferation, and tissue regeneration.[5,6] Bacterial cellulose dressings outperform conventional treatments in healing burns, chronic wounds, and surgical wounds in animal models. While BNC itself does not possess intrinsic anti-microbial activity, its nanoporous structure and high liquid absorption capacity allow efficient loading and sustained release of antimicrobial agents, making it an effective delivery vehicle for bio-active compounds, such as honey, silver nanoparticles, and antibiotics. This property has been exploited to address antibiotic-resistant wound infections.[7,8] Its tunable mechanical properties also support applications in drug delivery, tissue engineering for bone and cartilage, and veterinary uses, like fracture repair.[9] Honey complements bacterial cellulose with its broad-spectrum antimicrobial, anti-inflammatory, and

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tissue-regenerative properties.[10] Its antibacterial effects, attributed to the low pH, osmotic activity, and hydrogen peroxide production, are particularly pronounced in Manuka honey, inhibiting pathogens, like methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*.[11-13]

Honey's anti-bacterial effects are partly attributed to the sustained production of hydrogen peroxide at low, non-cytotoxic concentrations, minimizing damage to host tissues while retaining antimicrobial activity.[14,15] By stimulating macrophages and up-regulating cytokines (tumor necrosis factor alpha, interleukin-1β, and interleukin -6), honey enhances granulation tissue formation and epithelialization.[16,17] However, clinical trials suggest inconsistent outcomes, with weak study designs limiting confidence in honey efficacy for superficial wounds and burns.[10,18] Honey is most effective during the inflammatory and early repair phases of wound healing.[17]

While previous studies mentioned above have explored BNC-honey composites primarily for their physicochemical and anti-bacterial properties, the present study will provide novel insights into the *in vivo* wound healing mechanisms through comprehensive assessment of oxidative stress biomarkers, collagen synthesis, and temporally resolved histopathological changes, establishing a foundation for clinical translation.

This study evaluated BNC/honey composites for excisional wound healing in mice, leveraging their combined properties to enhance therapeutic outcomes.

Materials and Methods

Chemicals. All consumables and reagents used in this study were purchased from Sigma-Aldrich (St. Louis, USA) and were of research-grade purity unless otherwise stated. Any materials not supplied by Sigma-Aldrich are explicitly indicated in the text, along with their respective suppliers and grades.

Animals. Twenty-four mice, weighing approximately 25.00 ± 5.00 g and 6 - 8 weeks of age, were obtained from the Animal House of Urmia University, Urmia, Iran. The animals were housed under standard conditions (natural light/dark cycle, 25.00 ± 2.00 ˚C, and 50.00 ± 10.00% humidity) with free access to the food and water. All experimental procedures were approved by the Veterinary Ethics Committee of Urmia University, Urmia, Iran (IR-UU-AEC-3/60), and conducted in strict accordance with the 3R principles (replacement, reduction, and refinement). Intraperitoneal injections of ketamine (100 mg kg^{-1}; Alfasan, Woerden, The Netherlands) and xylazine (10.00 mg kg^{-1}; Alfasan) were used to anesthetize the animals. Animals were also euthanized by intraperitoneal injection of ketamine (100 mg kg^{-1}) and xylazine (10.00 mg kg^{-1}), in accordance with standard veterinary protocols for rodent euthanasia.

Preparation of bacterial nanocellulose (BNC)-honey composite. Bacterial nanocellulose was produced using *K. xylinus* ATCC 700178 from the microbial collection at the Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. The bacterium was cultured in a static medium, containing 1.91% gluten, 5.38% molasses, 1.38% ethanol, 0.63% ammonium sulfate, 0.12% citric acid, and 0.27% Na_2HPO_4 at 30.00 ˚C for 3 days. Following production, the BNC was rigorously purified to remove bacterial cells, culture medium residues, and, critically, to reduce endotoxin levels for biomedical safety. The synthesized BNC was first transferred to distilled water to remove loosely bound medium residues. It was then immersed in a 1.00% (w/v) NaOH solution at 80.00 ˚C for 1 hr. This alkaline treatment was repeated with fresh NaOH solution to ensure the effective lysis and removal of bacterial cells, and to neutralize acidic by-products. The BNC was subsequently washed repeatedly with large volumes of sterile distilled water until the pH of the washings was neutral. This extensive washing step is crucial for eliminating the solubilized bacterial debris and NaOH. The synthesized BNC was transferred to distilled water to remove culture medium residues and then, immersed in 0.10 M NaOH to eliminate bacteria and adjust pH. It was washed with distilled water until neutral pH and stored at 4.00 ˚C until use.[19] Honey was added to BNC at an optimized concentration *via* the *ex-situ* method, and the composites were refrigerated for subsequent use.

Optimization of honey concentration. Honey powder (Mirnajmi, Urmia, Iran) was selected over natural liquid honey to ensure batch-to-batch consistency, precise concentration standardization, and uniform incorporation into the BNC matrix. This approach minimized variability in bio-active compound content (*e.g.*, hydrogen peroxide, phenolic compounds, and flavonoids), typically occurring with natural honey due to the floral source and geographical variations, thereby enabling reproducible experimental conditions. One kilogram of powdered honey was obtained from Mirnajmi Honey Factory. Solutions of 10.00, 20.00, 30.00, 40.00, 50.00, 60.00, and 70.00% honey in distilled water were prepared in separate Falcon tubes. Tubes were vortexed for 2 min and sonicated using a Sonica 2200MH S3 (Soltec, Milano, Italy) at 60.00 ˚C for 15 min to ensure dissolution. Absorbance was measured at 400 nm using a T80+ spectrophotometer (PG Instruments, Leicestershire, UK) at time zero to generate a standard curve. The BNC sheets (8.00-mm diameter) were dehydrated and immersed in 10.00 mL of each honey solution. Tubes were shaken at 110 rpm for 24 hr using a TM 52 E shaker (Fan Azma Gostar, Tehran, Iran). Absorbance was measured at 400 nm using a T80+ spectrophotometer (PG Instruments) at time zero to generate a standard curve. The 400 nm wavelength was selected as a validated proxy for total soluble components and phenolic content in honey solutions, providing a

simple and reproducible measure of honey concentration in solution. The 50.00% concentration showed maximum loading, indicating optimal interaction with BNC (Fig. 1).

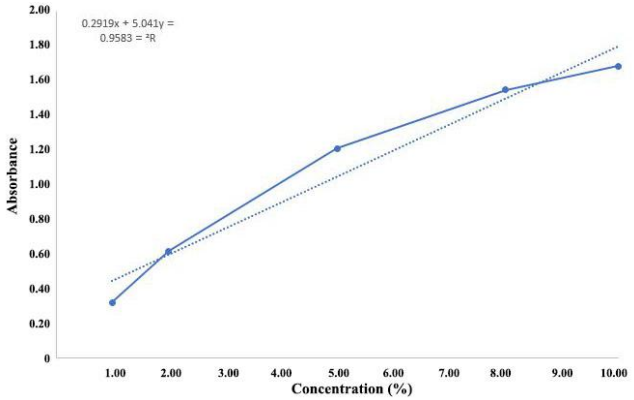


Fig. 1. Standard curve for selecting optimal honey concentration.

Full-thickness excisional wound induction. After anesthesia induction, mice were positioned dorsally on a surgical table. The dorsal thoracic region was prepared and scrubbed. Four circular wounds (8.00-mm diameter) were created using an 8.00 mm skin punch, removing the epidermis, dermis, and hypodermis completely, following the excision wound model.[20]

Grouping. The mice were randomly divided into four groups (n = 6) post-wounding. Four wounds were created on each mouse. In each mouse, one wound was designated for planimetric evaluation, one wound for histopathological assessment on day seven, one wound for histopathological examination on day 14, and one wound for biochemical assays on day seven. The wounds that were kept until day 14 for planimetric evaluation were subsequently used, after imaging, for histopathological examinations on day 14 and for determining hydroxyl-proline levels. The groups were as follows: Group 1 (sham) with open wounds and no intervention, group 2 with wounds treated with honey, group 3 with wounds treated with BNC, and group 4 with wounds treated with BNC-honey composite. The BNC/honey composite was applied as a pre-formed biofilm sheet (8.00 mm in diameter) placed directly onto the excisional wound. Due to its inherent adhesive and moisture-retentive properties, the biofilm adhered to the wound bed without the need for an additional secondary dressing. The BNC-only and honey-only groups received similar topical applications, while the sham group received no treatment. The BNC sheets were standardized to match the wound size (8.00 mm), and the honey loading was optimized at 50.00% concentration *via* an *ex-situ* immersion method.

Planimetry. Wound contraction was assessed in six mice *per* subgroup using digital photography on days 0, 3, 6, 9, 12, and 14. Wound areas were calculated *via* planimetry, using Adobe Acrobat Professional (version 2024; Adobe Inc., San Jose, USA).

Histopathology. Samples from six mice *per* subgroup were collected on days seven and 14, including wound margins plus 2.00 mm of healthy skin. Samples were immediately fixed in 10.00% buffered formalin, processed, embedded in paraffin, sectioned at 5.00 µm thickness, and stained with Hematoxylin and Eosin. Histopathological evaluation assessed distribution of inflammatory cells, fibroblasts, and angiogenesis (number of newly formed blood vessels *per* mm^2 in the wound area). All histopathological evaluations were performed by two independent pathologists blinded to the treatment groups. For each sample, five random microscopic fields were examined at 400× magnification. The following parameters were assessed using a semi-quantitative scoring system: Angiogenesis (number of new blood vessels *per* high-power field): Score 0: 0 - 5 vessels, score 1: 6 - 10 vessels, score 2: 11 - 15 vessels, and score 3: > 15 vessels, fibroblast activity (cellular density in granulation tissue): Score 0: Absent or minimal, score 1: Mild (scattered fibroblasts), score 2: Moderate (moderate fibroblast proliferation), and score 3: Marked (dense fibroblast infiltration), and neutrophil infiltration (*per* high-power field): Score 0: 0 - 5 neutrophils, score 1: 6 - 20 neutrophils, score 2: 21 - 50 neutrophils, and score 3: > 50 neutrophils. Inter-observer agreement was assessed using Cohen's kappa coefficient ($\kappa > 0.80$). The mean scores from both pathologists were used for statistical analysis.

Biochemical analysis. Approximately 1.00 g of regenerating tissue was collected on days seven and 14. Samples were homogenized (1.00 g in 4.50 mL buffer; 15 min on ice), filtered, and centrifuged at 4.00 ˚C, and the supernatant was used for assays at room temperature. Total anti-oxidant capacity (TAC), total oxidant status, malondialdehyde, and glutathione peroxidase (GPx) were measured using commercially available kits, according to the manufacturers' protocols. Total anti-oxidant capacity was measured using 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) decolorization method. Briefly, 10.00 µL of sample supernatant was mixed with 1.00 mL of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical solution and incubated for 6 min at room temperature, and absorbance was measured at 660 nm. Results were expressed as nmol Trolox equivalents *per* liter (Rel Assay Diagnostics, Gaziantep, Türkiye). Total oxidant status was determined by the colorimetric method, using ferrous ion oxidation with xylenol orange. Sample (25.00 µL) was mixed with 225 µL of reagent and incubated for 30 min at 37.00 ˚C, and absorbance was measured at 560 nm. Results were expressed as µmol H_2O_2 equivalents *per* liter (Rel Assay Diagnostics). Malondialdehyde was assessed using the thiobarbituric acid reactive substances method. Sample (100 µL) was mixed with 200 µL of thiobarbituric acid reagent (0.38% thiobarbituric acid, 15.00% trichloroacetic acid, and 0.25 N HCl), heated at 95.00 ˚C for 30 min, cooled, and extracted

with butanol. Absorbance was measured at 532 nm (Rel Assay Diagnostics). Glutathione peroxidase was measured using the coupled enzymatic method with glutathione reductase. Decrease in nicotinamide adenine dinucleotide phosphate absorbance at 340 nm was monitored for 5 min at 37.00 ˚C. One unit of GPx activity was defined as 1.00 µmol nicotinamide adenine dinucleotide phosphate oxidized *per* min (Rel Assay Diagnostics). All samples were analyzed in duplicate, and the intra-assay coefficient of variation was less than 5.00%.

Hydroxyproline determination. Hydroxyproline, a collagen marker, was quantified from day-14 samples of equal weights. Tissues were dried at 60.00 ˚C, hydrolyzed in 6 N HCl at 130 ˚ C in sealed tubes, neutralized to pH: 7.00, oxidized with Chloramine T for 20 min, terminated with 4.00 M perchloric acid, and color developed with Ehrlich reagent at 60.00 ˚ C. Absorbance at 557 nm was measured *via* the ultraviolet-visible spectrophotometer. Day-21 right-side wounds were used for histopathology-linked assessment.

Statistical analysis. All statistical analyses were performed using SPSS Software (version 11.5; SPSS Inc., Chicago, USA). Data are presented as mean ± standard deviation unless otherwise specified. The normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Since the data did not meet the assumptions of parametric testing, non-parametric methods were employed throughout. For inter-group comparisons across all four treatment groups (sham, honey-only, BNC-only, and BNC/honey composite), the Kruskal–Wallis H test was applied. When a statistically significant difference was detected ($p < 0.05$), *post-hoc* pairwise comparisons were performed using Mann–Whitney U test with Bonferroni correction to adjust for multiple comparisons. The adjusted significance level was set at $p < 0.05$ for pairwise comparisons to control the family-wise error rate. To comparie between two time points (day seven *versus* day 14) within the same treatment group, the Mann–Whitney U test was used, as the samples were independent (different animals were euthanized at each time point).

Results

Wound contraction. Statistical analyses revealed significant differences ($p < 0.05$) between the BNC/honey composite and other treatments (sham, honey alone, and BNC alone), underscoring the synergistic effect of this composite in accelerating wound closure (Fig. 2).

Histopathological evaluation. The BNC/honey composite group exhibited the most effective therapeutic dynamics, characterized by early resolution of inflammation, robust angiogenesis, and high fibroblast activity, by day 14. The BNC alone group also demonstrated notable therapeutic benefits, though slightly less pronounced than the combined treatment. In contrast, the sham (open wound and no intervention) and honey-only groups displayed lower efficacy in promoting healing, with prolonged inflammatory responses, delayed angiogenesis, and reduced fibroblast activity. The BNC/ honey composite group exhibited the highest levels of angiogenesis on day seven, indicating its strong capacity to promote early blood vessel formation, being critical for initial wound healing. By day 14, a reduction in angiogenesis in this group may reflect enhanced healing, where vascular formation stabilizes as tissue repair progresses (Fig. 3A). The sham group showed a delayed but sustained angiogenic response, potentially indicative of slower progression through the healing stages. The honey-only group appeared relatively effective in promoting angiogenesis, while the BNC-alone group demonstrated slightly better results than honey, particularly on day seven. The BNC/honey composite group exhibited the highest fibroblast activity on day seven, indicating enhanced wound repair during the early stages. A significant reduction in fibroblast activity by day 14 aligns with tissue maturation, reflecting efficient healing (Fig. 3B). The BNC alone group also demonstrated robust fibroblast activity, though slightly less than the combined treatment, highlighting its effectiveness in promoting cellular repair. The honey-only group showed moderate fibroblast activity, whereas the sham (open wound and no intervention) group lagged in fibroblast levels on day seven, indicating a slower onset of tissue repair. An increase in fibroblast levels by day 14 in the sham group suggests prolonged or slower healing dynamics.

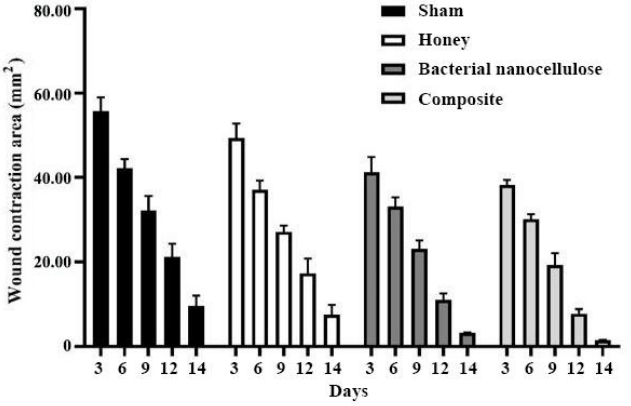


Fig. 2. The graph illustrates the wound contraction area (mm^2) measured on different days, highlighting the progression of wound closure across the experimental groups.

The sham and honey-only treatments exhibited higher levels of neutrophil infiltration, indicative of a sustained inflammatory response or less effective resolution of inflammation during the healing process. The BNC/honey composite treatment demonstrated the most significant reduction in neutrophil infiltration by day 14, suggesting

enhanced anti-inflammatory effects or improved resolution of inflammation compared to the other treatments (Fig. 3C). The BNC alone group showed moderate effects, indicating it is beneficial but less effective than the combined treatment (Figs. 4 and 5).

Biochemical assays. The results demonstrate that the BNC/honey composite significantly increases TAC and GPx levels, while reducing total oxidant status and malondialdehyde levels. These findings indicate a positive effect of the composite in mitigating oxidative damage and enhancing anti-oxidant activity. The data highlight the substantial potential of this composite in reducing oxidative stress and promoting tissue repair processes under injury conditions (Table 1).

Hydroxyproline levels determination. The hydroxyproline levels in the sham, honey, BNC, and BNC/honey groups were recorded as 52.55 ± 3.89, 67.34 ± 8.23, 83.58 ± 4.61, and 94.21 ± 4.19 mg g^{-1}, respectively. Concentrations in the BNC/honey group were significantly higher than other groups ($p < 0.05$).

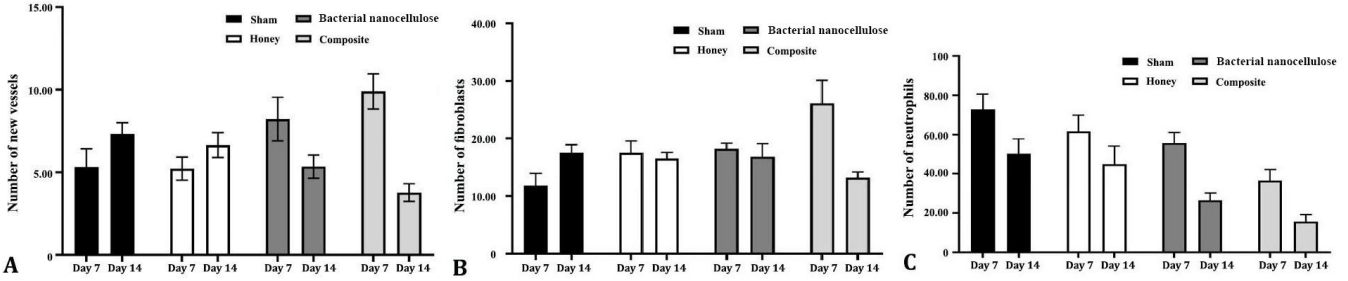


Fig. 3. Graphs showing; **A)** Changes in angiogenesis levels (number of new vessels); **B)** Comparative assessment of fibroblast activity levels (number of fibroblasts); **C)** Neutrophil infiltration levels (number of neutrophils) on days seven and 14 across different groups.

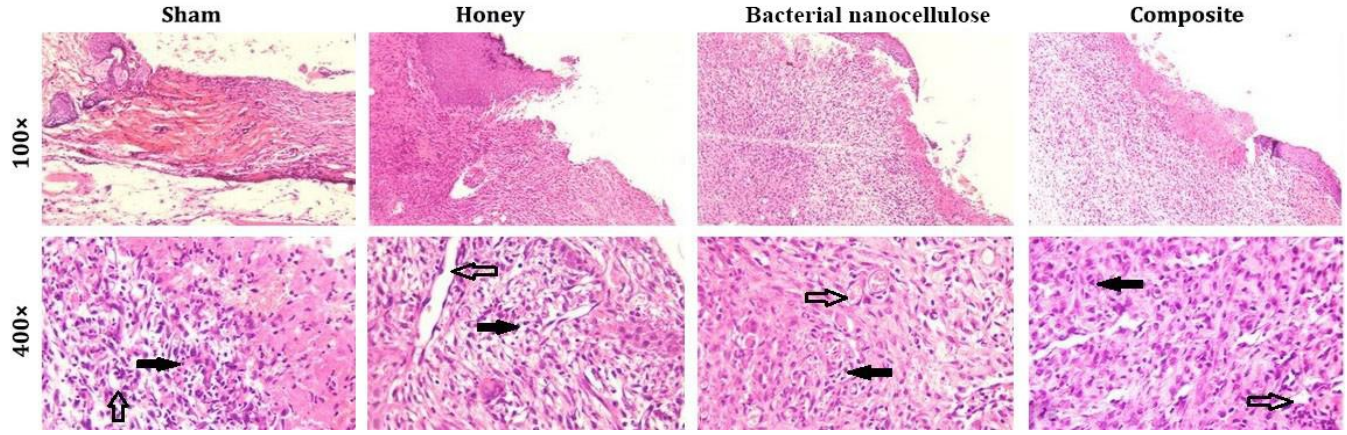


Fig. 4. Histopathological section images of the effects of different treatments on the wound healing process on day seven. The sham group, which received no therapeutic intervention, shows granulation tissue at the wound site, accompanied by a significant amount of inflammatory cells infiltration (thick arrow) and new blood vessels (blank arrow); Images related to the honey group indicate, as visible in the images that the epidermis at the wound site has lost its continuity, with granulation tissue accompanied by inflammatory cells infiltration (thick arrow) and angiogenesis (blank arrow) being observed in the cross-section; Images related to the nanocellulose group on day seven show granulation tissue filled with inflammatory cells (thick arrow) and new blood vessels (blank arrow) occupying the wound site; Images related to the combined nanocellulose and honey group demonstrate that on day seven, the wound site contains granulation tissue filled with blood vessels (blank arrow) and infiltrating cells (thick arrow), but the level of neutrophil infiltration in this group is reduced compared to the other groups (Hematoxylin and Eosin staining).

Table 1. Comparison of total anti-oxidant capacity (TAC), total oxidant status (TOS), malondialdehyde (MDA), and glutathione peroxidase (GPx) activities in tissue samples collected from experimental groups on days seven and 14.

Parameters	Days	Sham	Honey	Bacterial nanocellulose	Composite
TAC (nmol L^{-1})	7	0.56 ± 0.04	0.85 ± 0.05	1.30 ± 0.05**	1.82 ± 0.02*
	14	0.64 ± 0.09	0.91 ± 0.07	1.65 ± 0.07**	1.89 ± 0.07*
TOS (nmol L^{-1})	7	106.35 ± 14.85	55.48 ± 6.49	49.04 ± 0.03**	31.63 ± 0.66*
	14	115.27 ± 15.17	69.03 ± 7.63	55.81 ± 0.39**	35.58 ± 0.39*
MDA (nm *per* mg protein)	7	0.97 ± 0.05	0.53 ± 0.43	0.43 ± 0.05**	0.33 ± 0.05*
	14	0. 87 ± 0.08	0.49 ± 0.83	0.41 ± 0.04**	0.29 ± 0.04*
GPx (IU L^{-1})	7	0.11 ± 0.05	0.23 ± 0.05	0.43 ± 0.21**	0.50 ± 0.03*
	14	0.16 ± 0.05	0.27 ± 0.07	0.45 ± 0.03**	0.67 ± 0.09*

* and **: Differences were significant in each row compared to the other groups at the $p < 0.05$ level

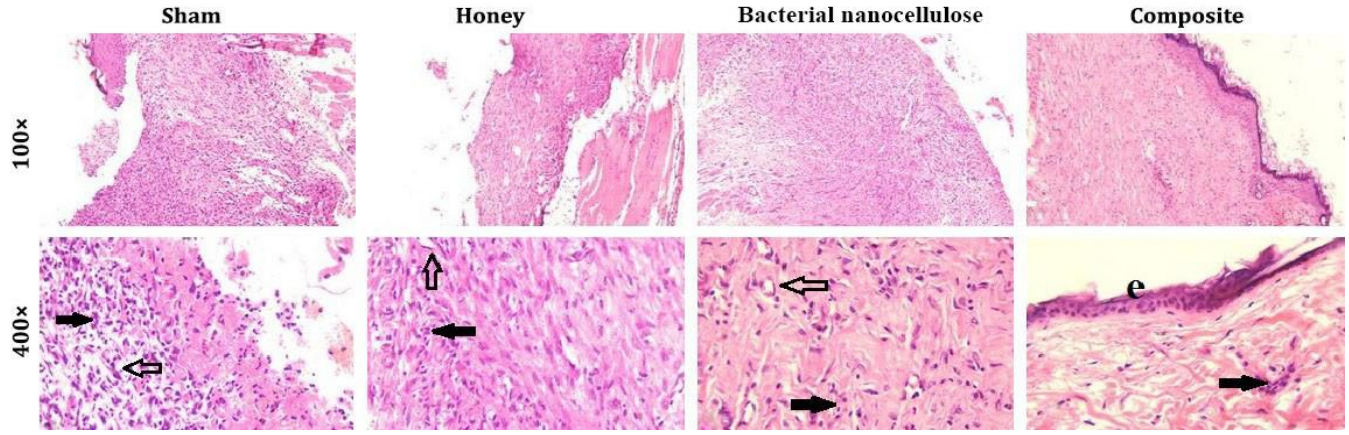


Fig. 5. Histopathological section images of the effects of different treatments on the wound healing process on day 14. Images related to the sham group, which received no pharmacological intervention, show that, on day 14, the epidermis at the wound site remains disrupted, and the granulation tissue is filled with inflammatory cells (thick arrow), particularly neutrophils, with the healing process progressing slowly in this group and new blood vessels (blank arrow); The honey-treated group indicates that honey alone performed better than the sham group in reducing neutrophil infiltration; however, the wound site still contains granulation tissue with inflammatory cells (thick arrow), new blood vessels (blank arrow)and a new epidermis has not formed; The nanocellulose-treated group performed better than the sham group, but the healing process remains slow, with granulation tissue observed at the wound site, though the number of inflammatory cells (thick arrow) and new blood vessels (blank arrow) have decreased; The combined nanocellulose and honey treatment group achieved the best results in the wound healing process. The images clearly demonstrate that the combined group exhibited a high capacity to reduce inflammatory cells infiltration (thick arrow) and the number of blood vessels, with the granulation tissue fully matured and a new epidermis (e) formed at the wound site (Hematoxylin and Eosin staining).

Discussion

The present study demonstrates the appropriate efficacy and synergistic effect of combining BNC with honey in promoting the healing of full-thickness excisional wounds in mice. This aligns closely with findings from Meftahi *et al.*, who explored BNC-honey composites and reported notable anti-bacterial activity alongside enhanced wound healing in medical applications, mirroring the current study demonstration of reduced inflammation and faster closure, though their work emphasized physicochemical properties rather than detailed *in vivo* biochemical assays, like hydroxyproline measurement.[21] Similarly, Abu *et al.* investigated BNC as a delivery system for honey in anti-bacterial dressings, finding enhanced therapeutic effects that reduced infections and accelerated healing, paralleling the significant decreases in neutrophil infiltration and oxidative stress observed in our study, particularly the composite superiority over honey alone in promoting early angiogenesis and fibroblast proliferation.[22] In comparison, Nezhad-Mokhtari e*t al.*,[23] developed a BNC-gelatin-guar gum-honey film that exhibited enhanced mechanical strength, biocompatibility, and anti-bacterial effects against Gram-positive and Gram-negative bacteria, with moisture-retention properties accelerating wound healing; while their focus was more on *in vitro* biocompatibility and cell growth support without cytotoxicity, the current study *in vivo* results extend this by quantifying synergistic improvements in tissue maturation and collagen synthesis, suggesting the simpler BNC-honey formulation achieves comparable or even superior anti-inflammatory outcomes in a mouse model.[23] Oprică *et al.*,[24] examined plant-derived nanocellulose with anti-bacterial agents, confirming strong anti-bacterial activity, reduced infections, and accelerated healing with decreased inflammation; although their material differs (plant *versus* bacterial source), the inflammation reduction aligns with this study histopathological data, showing lower neutrophil levels in the BNC/honey group by day 14, highlighting nanocellulose broad potential but with honey adding anti-oxidant benefits evident in the elevated TAC and GPx levels here.[24] Jančič *et al.*,[25] studied BNC loaded with bromelain and nisin for wound debridement, demonstrating effective bacterial inhibition and facilitated healing; this study use of honey instead of enzymes yields similar infection control inferred from reduced oxidative damage, but excels in promoting robust early fibroblast activity and collagen deposition, positioning BNC-honey as a more reparative option for non-debrided excision wounds.[25] Prasathkumar and Sadhasivam,[26] combined chitosan, hyaluronic acid, alginate, and honey, showing significant anti-bacterial activity and improved repair; the polymeric synergies there echo the enhanced wound contraction and anti-inflammatory effects in the current BNC-honey composite; though, this study isolates BNC role more clearly, with statistical significance over controls in all metrics, emphasizing honey value in natural dressings without additional polymers.[26] Nezhad-Mokhtari *et al.*,[23] reviewed honey-based hydrogels, highlighting anti-

bacterial, anti-inflammatory, and reparative properties with high moisture absorption; this resonates with the current findings of reduced malondialdehyde and total oxidant status levels, indicating mitigated oxidative damage, and supports the composite efficacy in moisture-dependent healing, as seen in the rapid transition to matured granulation tissue by day 14.[27] Aderibigbe,[28] explored nanocellulose in wound dressings, noting biocompatibility, moisture absorption, and infection control; the BNC-alone group in this study showed moderate benefits, but the honey addition amplified these, leading to higher hydroxyproline and faster closure, affirming nanocellulose value while demonstrating honey role in boosting anti-oxidant and anti-inflammatory capabilities.[28] Yang *et al.*,[29] developed a BNC-methylglyoxal nanocomposite with broad-spectrum anti-bacterial activity and enhanced healing for infected wounds; similar to the anti-bacterial inference from lowered inflammation here, but our study natural honey integration provides additional reparative edges, such as peak angiogenesis on day seven, offering a comparable natural solution without synthetic additives.[29] Finally, Shaheen *et al.* produced silver nanoparticles with BNC, showing strong anti-oxidant properties, low cytotoxicity, and accelerated healing; the anti-oxidant alignment is evident in this study (GPx and TAC increases), but the BNC-honey composite achieves this without nanoparticles, potentially reducing cytotoxicity risks while maintaining superior collagen outcomes in the *in vivo* model.[30]

The BNC/honey composite exhibits remarkable therapeutic benefits for wound healing. By promoting collagen synthesis and increasing hydroxyproline levels, this composite supports tissue repair and regeneration. It also fosters an optimal healing environment by enhancing anti-oxidant defenses and reducing oxidative stress. At the cellular level, it speeds up wound closure, mitigates inflammation, and promotes angiogenesis to ensure proper tissue nourishment and oxygenation. Furthermore, it boosts fibroblast activity and enhances extracellular matrix production, facilitating faster healing and efficient progression to tissue regeneration. The synergy between BNC structural properties and honey anti-inflammatory qualities positions this composite as a highly promising solution for clinical wound management. These findings strongly endorse its ability to accelerate healing, reduce inflammation, and optimize tissue repair.

While our histopathological assessment provided valuable semi-quantitative data on angiogenesis and collagen deposition, immunohistochemical analysis using specific markers, such as CD31 for endothelial cells, α-SMA for vascular smooth muscle, and collagen types I and III would have provided more definitive evidence of vascularization and extracellular matrix remodeling. The absence of immunohistochemical data is acknowledged as a limitation that should be addressed in future investigations. Another significant limitation of this study was the absence of physicochemical characterization of the BNC/honey composite, including scanning electron microscopy for morphological analysis, Fourier-transform infrared spectroscopy for chemical interaction assessment, X-ray diffraction for crystallinity evaluation, and porosity measurements. Such characterization would provide critical insights into the structural basis of the composite superior wound healing properties. Future studies should incorporate these analytical techniques to establish structure-activity relationships.

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Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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