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ORIGINAL RESEARCH

Propolis-Based Nanostructured Lipid Carrier of α-Mangostin for Promoting Diabetic Wound Healing in Alloxan-Induced Mice

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Introduction: Diabetic wounds present a significant challenge due to delayed healing and susceptibility to infection. Conventional therapies often fall short of achieving complete and timely wound repair. This study investigates the potential of α-mangostin (αM) and its propolis-based nanostructured lipid carrier (NLC-P-αM) formulation as novel therapeutic agents for diabetic wound healing.

Purpose: To evaluate the release profile, safety, and efficacy of NLC-P-αM in promoting wound repair in an in vitro and in vivo diabetic wound model.

Methods: The NLC-P-αM formulation was prepared using a melt-emulsification technique with ultrasonication. In vitro release studies were conducted using a dialysis bag method and analyzed using kinetic models. Cytotoxicity was assessed using the WST-8 assay on NIH-3T3 fibroblast cells. In vivo diabetic wound healing was evaluated using alloxan-induced diabetic Swiss Webster mice. The treatments were applied topically for 14 days, and wound closure was monitored quantitatively. Histological analysis was performed to assess the inflammatory cell infiltration, epidermal thickness, and tissue regeneration.

Results: NLC-P-αM demonstrated a significantly enhanced release profile, with 85.55 ± 4.25% of αM released at 360 min compared to 19.82 ± 6.78% for free αM, following a non-Fickian diffusion mechanism. Both formulations exhibited excellent safety, with cell viabilities of 94.76 ± 4.95% for NLC-P-αM and 102.16 ± 7.98% for αM in NIH-3T3 cells. In vivo, NLC-P-αM achieved the highest wound closure rate (85.83 ± 3.33%) by day 14, outperforming αM and the controls. Histological analysis confirmed reduced inflammation, a thinner epidermis, and advanced tissue regeneration in the NLC-P-αM group, highlighting its superior therapeutic efficacy.

Conclusion: NLC-P-αM demonstrated enhanced release, excellent safety, and superior efficacy in promoting diabetic wound healing compared to free αM and other controls. This nanoformulation offers a promising therapeutic strategy for accelerating wound repair in diabetic patients.

Keywords: α-Mangostin, propolis, nanostructured lipid carrier, (NLC), diabetic wound, wound healing

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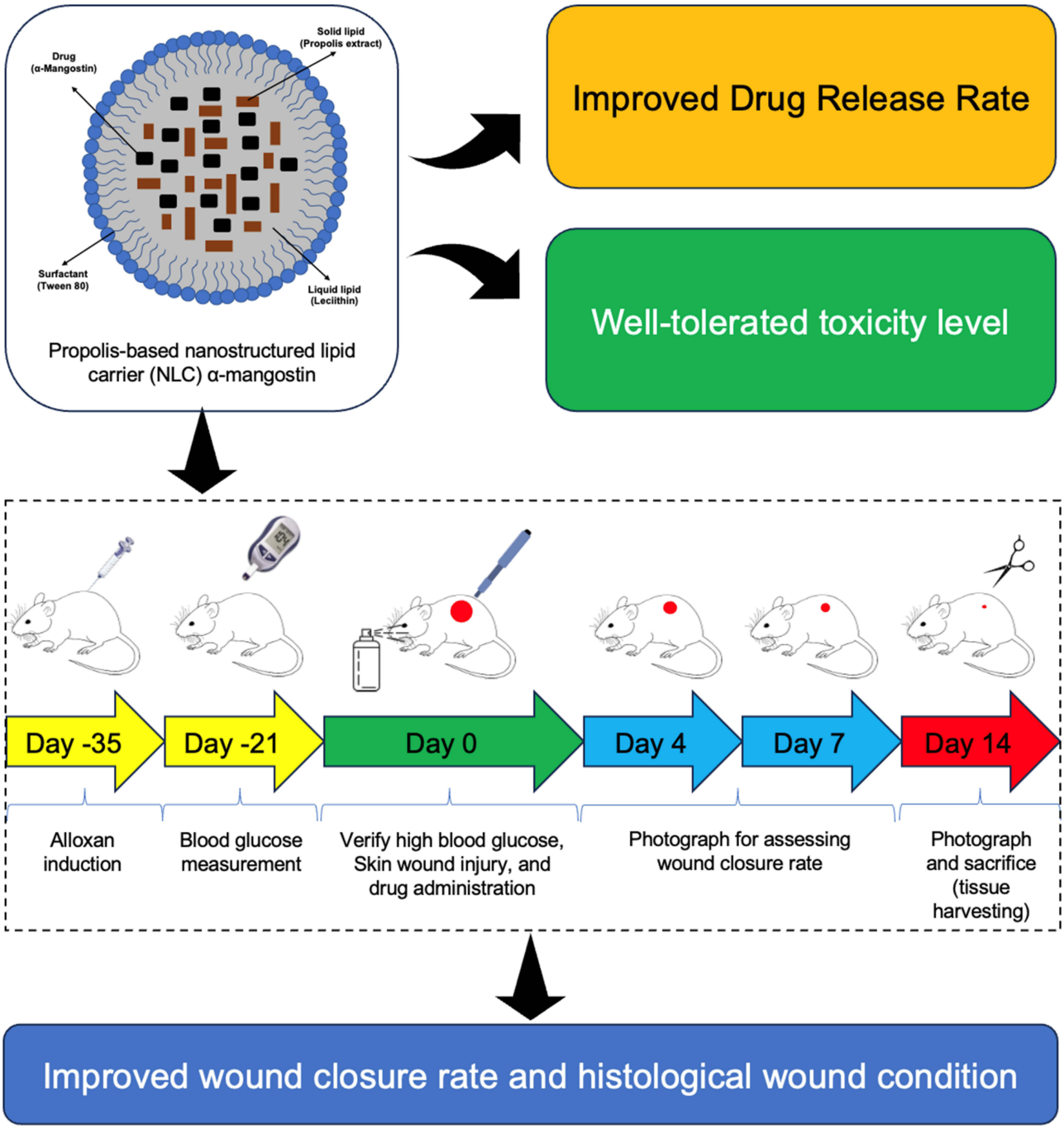
Graphical Abstract



Introduction

Diabetic wounds, a common complication of diabetes mellitus, are marked by delayed healing, persistent inflammation, and a high risk of infection.[1,2] Affecting approximately 11.6% of individuals with diabetes annually, these wounds can lead to infection in up to 60% of cases, with 20% progressing to amputation.[3,4] The five-year mortality rate among affected patients reaches 30%, underscoring the critical nature of this condition.[5] Pathophysiological mechanisms such as

impaired angiogenesis, heightened oxidative stress, and abnormal inflammatory responses contribute to prolonged wound healing and secondary complications.[6–9]

Due to their susceptibility to infection, diabetic wounds have traditionally been treated with topical antibiotics, especially silver-based formulations.[10] However, clinical evidence has not demonstrated superior healing outcomes with such treatments.[11,12] On the contrary, current guidelines increasingly advise against the use of topical antibiotics, citing limited therapeutic benefit alongside potential drawbacks, including adverse reactions and the emergence of antibiotic resistance.[13] The inability of conventional treatments to adequately address the complex biology of diabetic wound healing highlights the urgent need for innovative therapies.

α-Mangostin (αM), a xanthone compound derived from the pericarp of *Garcinia mangostana* (mangosteen), is a plant-based secondary metabolite with promising pharmacological activity. It possesses strong anti-inflammatory, antioxidant, and antimicrobial properties, which make it a potential candidate for managing oxidative stress, inflammation, and infection in diabetic wounds.[14–18] Notably, αM modulates key inflammatory pathways, including nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK), thereby reducing levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β).[19–21] Additionally, it suppresses cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), resulting in decreased production of prostaglandin E2 (PGE2) and nitric oxide (NO), both of which are central to the inflammatory response.[20,22] By regulating these pathways, αM promotes tissue regeneration and accelerates wound closure. However, its clinical use is limited by poor water solubility and low bioavailability, necessitating the development of an effective delivery system.[23,24] One potential solution is its incorporation into nanostructured lipid carriers (NLCs).

Previous topical formulations that combined αM with other antioxidants, such as resveratrol, enhanced antioxidant efficacy but were constrained by formulation challenges.[25] Although resveratrol contributes therapeutic benefits, it suffers from poor chemical stability and is prone to photodegradation, limiting its usefulness in topical applications.[26,27] Moreover, it lacks the lipid components needed to form a stable NLC matrix.[25] In contrast, propolis—a resinous substance rich in flavonoids and phenolic compounds—provides both therapeutic benefits and a waxy composition suitable for use as a natural solid lipid.[28–30] This dual function makes it ideal for constructing NLCs capable of both delivering and stabilizing active compounds.

By integrating αM into a propolis-based nanostructured lipid carrier (NLC-P-αM), our formulation addresses solubility and stability limitations while potentially enhancing therapeutic effects through synergistic interactions. Recent studies confirm the advantages of NLC systems in wound therapy, especially when incorporating bioactives like propolis, which contributes antimicrobial, anti-inflammatory, and antioxidant activities.[28–31] Additionally, its wax content enables propolis to act as a lipid matrix, supporting its role in carrier formation.[32–34] The combined properties of αM and propolis, delivered via NLCs, present a compelling strategy to address the complex pathophysiology of diabetic wounds.

In our previous work, we developed and characterized NLC-P-αM (Figure 1) to improve the limitations of unformulated αM.[35] The system showed significantly enhanced antioxidant activity, attributed to improved solubility, stability, and controlled release. NLCs facilitate higher bioavailability of encapsulated agents and enable targeted delivery into wound tissue, supporting their therapeutic potential.

This study builds upon our previous work by evaluating the in vivo efficacy of NLC-P-αM in promoting diabetic wound healing in alloxan-induced mice model. By leveraging the synergistic effects of αM and propolis within an advanced delivery system, this study aims to provide a novel therapeutic strategy that addresses the limitations of conventional treatments and offers a targeted, biocompatible solution for enhancing diabetic wound repair.

Materials and Methods

Materials

The materials used in this study included distilled water, α-mangostin (Chengdu Biopurify Phytochemicals Ltd., Chengdu, China), propolis (CV Efi Maju Sejahtera, Jakarta, Indonesia), ethanol 95% (Sigma-Aldrich Co., St. Louis, MO, USA), lecithin (Sigma-Aldrich Co., St. Louis, MO, USA), potassium dihydrogen phosphate (KH_2PO_4) (Sigma-

Figure 1 Physical appearance of NLC-P and NLC-P-αM preparation.

Aldrich Co., St. Louis, MO, USA), polysorbate 80 (Tween 80®) (Sigma-Aldrich Co., St. Louis, MO, USA), sodium hydroxide (NaOH) (Sigma-Aldrich Co., St. Louis, MO, USA), alloxan monohydrate (Sigma-Aldrich Co., St. Louis, MO, USA), NIH-3T3 fibroblast cells (ATCC, Manassas, VA, USA), Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich Co., St. Louis, MO, USA), fetal bovine serum (FBS) (Sigma-Aldrich Co., St. Louis, MO, USA), penicillin-streptomycin (Sigma-Aldrich Co., St. Louis, MO, USA), WST-8 reagent (Sigma-Aldrich Co., St. Louis, MO, USA), and cellulose acetate dialysis tubing (MWCO 12,000–14,000 Da) (Sigma-Aldrich Co., St. Louis, MO, USA). The anesthetic agent's ketamine and xylazine were sourced locally. All chemicals and reagents were of analytical grade and used without further purification.

Preparation of NLC-P-αM

Propolis extract was obtained by maceration using 95% ethanol as a solvent at a 1:10 (w/v) ratio of raw propolis to solvent. The process was conducted at room temperature (25 °C) for 48 hours with intermittent shaking, followed by filtration and solvent evaporation to yield a concentrated extract. NLC-P-αM were prepared via melt-emulsification and ultrasonication. αM and propolis extract were incorporated into the lipid phase, which was emulsified with an aqueous phase containing polysorbate 80 and phosphate buffer (pH 7.4). The resulting pre-emulsion was ultrasonicated to obtain nanosized particles. Detailed methods for formulation and characterization were reported in our previous study.[35]

In vitro Release Study

The release of αM from NLC-P-αM was assessed using a dialysis bag method.[36,37] The dialysis tubing (MWCO 12,000–14,000 Da) was pre-soaked in phosphate buffer (pH 7.4) for 24 h prior to use. An aliquot of NLC-P-αM, equivalent to 1.25 mg of αM, was placed into the dialysis bag, which was securely tied at both ends. The bag was then immersed in 40 mL of phosphate buffer (pH 7.4) in a beaker, maintained at 37 ± 0.5 °C with continuous stirring at 50 rpm. An aliquot of NLC-P-αM, equivalent to 1.25 mg αM, was added to the donor compartment. At predetermined intervals (15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, and 360 min), 1 mL of the receptor medium was withdrawn and replaced with an equal volume of fresh phosphate buffer to maintain sink conditions. The concentration of αM in the collected samples was determined using UV-Vis spectrophotometry at its maximum absorption wavelength (λ_{max} = 281 nm). The cumulative percentage of drug release was calculated and plotted as a function of time.

To evaluate the release mechanism, the data were analyzed using R software by fitting the release data to four commonly used kinetic models: zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. Zero-order release and first-order release models were tested to determine whether the drug release followed a constant rate or exponential decay, respectively.[38] The Higuchi model was applied to assess diffusion-controlled release, while the Korsmeyer-Peppas

model was used to determine the release exponent (n) and classify the release mechanism.[39,40] A nonlinear regression analysis was performed using the "nls" function in R, with the cumulative release data modeled against time for each of the four models.[41] The release mechanism was further categorized based on the value of n from the Korsmeyer-Peppas model: $n \leq 0.5$ indicates Fickian diffusion-controlled release, $0.5 < n < 1$ suggests anomalous (non-Fickian) diffusion, and $n = 1$ corresponds to zero-order release.[42]

In vitro Safety Assay

The safety of NLC-P-αM was assessed using NIH-3T3 fibroblast cells via the WST-8 assay.[43,44] NIH-3T3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to adhere overnight. The cells were treated with varying concentrations of NLC-P-αM and unformulated αM (25, 50, 75, 100, and 125 µg/mL) for 24 hours. After incubation, 10 µL of WST-8 reagent was added to each well, and the plates were incubated for an additional 4 h. The absorbance was measured using a microplate reader at dual wavelengths of 450 and 620 nm. Cell viability was calculated as a percentage relative to that of the untreated control group.

In vivo Diabetic Wound Healing Assay

Animal Model

Adult male Swiss Webster mice (25–30 g) were procured and maintained under standard laboratory conditions (12-hour light/dark cycle, temperature 22 ± 2 °C, relative humidity 40–60%, and free access to food and water). Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline. After 7 days, fasting blood glucose (FBS) levels were measured using a glucometer (YASEE® Blood Glucose Meter, model GLM-76). Mice with FBS levels ≥200 mg/dL were categorized as diabetic and included in the study.[45]

Wound Induction and Treatment Protocol

The study protocol was approved by the Universitas Padjadjaran Research Ethics Committee (Approval Code: 757/UN6.KEP/EC/2024) and was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (8th Edition, National Research Council, 2011). A full-thickness excisional wound was created on the dorsal area of each Swiss Webster mouse using a 6 mm biopsy punch under anesthesia (ketamine 100 mg/kg and xylazine 10 mg/kg, intraperitoneally).[46] Mice were divided into four groups (n = 5 per group):

- Group I: Diabetic control (treated with phosphate buffer saline (PBS))
- Group II: Diabetic wound treated with αM
- Group III: Diabetic wound treated with blank propolis-based NLC (NLC-P) as (vehicle control)
- Group IV: Diabetic wound treated with NLC-P-αM

Treatments were applied topically, with one puff per day every morning for 14 days. Wound closure was assessed on days 0, 4, 7, and 14 by photographing each wound. The area of the wound was measured quantitatively using ImageJ software, and the percentage of wound closure was calculated using the following formula:

$$\text{Wound Closure } (\%) = \left(\frac{\text{Initial Wound Area} - \text{Remaining Wound Area}}{\text{Initial Wound Area}} \right) \times 100$$

Histological Analysis

On day 14, the wound tissues were harvested and fixed in 10% formalin for histological analysis. Tissue sections were stained with hematoxylin and eosin (H&E) to qualitatively evaluate inflammatory cell infiltration, hair follicle growth, and sebaceous gland formation.[47] The analysis was conducted using a light microscope with a magnification of 100x to observe these parameters. Further analysis was performed using ImageJ software to measure the thickness of the epidermal layer.[48]

Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

In vitro Release Study

The in vitro release profiles of both αM and the NLC-P-αM formulation exhibited distinct patterns over time (Figure 2). The αM group showed a gradual increase in cumulative release, starting at 5.12 ± 1.48% at 15 minutes and reaching 19.82 ± 6.78% by 360 minutes. In contrast, the NLC-P-αM formulation demonstrated significantly enhanced release, with 11.15 ± 1.09% at 15 minutes and 85.55 ± 4.25% at 360 minutes, indicating the NLC system's capacity to facilitate αM release more effectively.

Fitting models were applied to describe the release kinetics of the studied compounds (Table 1). For αM, the zero-order model provided the best fit with an R^2 value of 0.930, indicating a relatively constant release rate. The Korsmeyer–Peppas model also showed a good fit ($R^2 = 0.871$), with a release exponent (n) of 0.566 ± 0.068, suggesting an anomalous (non-Fickian) diffusion mechanism. The Higuchi model exhibited a moderate fit for αM with an R^2 value of 0.849, indicating diffusion-controlled release. For NLC-P-αM, the release profile was best described by the Korsmeyer–Peppas model with an R^2 value of 0.997 and an n value of 0.643 ± 0.046, further supporting the involvement of non-Fickian diffusion mechanisms. The zero-order and Higuchi models also demonstrated excellent fits ($R^2 = 0.985$ and 0.990, respectively), suggesting that the release was governed by both diffusion through the lipid matrix and gradual erosion of the carrier system.

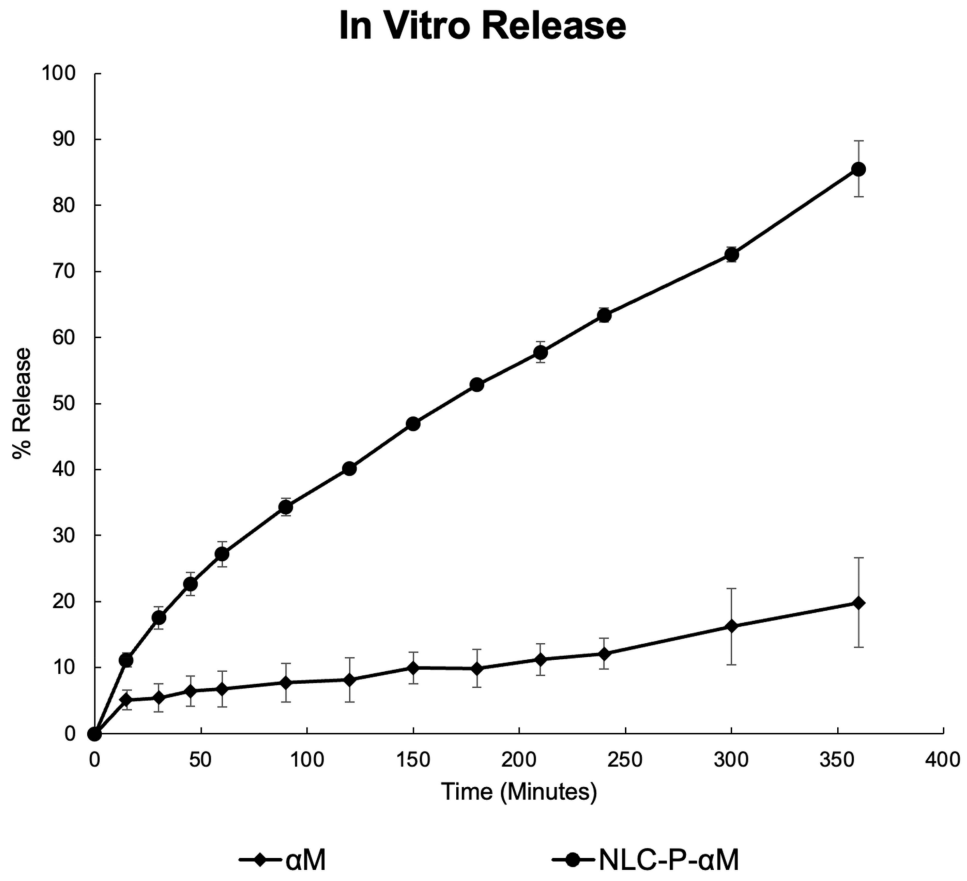


Figure 2 In vitro release profile of NLC-P-αM compared to unloaded α-mangostin. Data are presented as mean ± standard deviation (n=3).

Table 1 Curve Fitting Results for Drug Release Mechanisms

Sample	Zero Order		First Order		Higuchi		Korsmeyer-Peppas		
	K_0	R^2	K_1	R^2	K_H	R^2	K_P	n	R^2
αM	0.039 ± 0.011	0.930	NA	0.00	0.868 ± 0.236	0.849	0.657 ± 0.307	0.566 ± 0.068	0.871
NLC-P-αM	0.206 ± 0.011	0.985	NA	0.00	4.764 ± 0.233	0.990	1.919 ± 0.459	0.643 ± 0.046	0.997

Abbreviation: NA; not applicable.

In vitro Safety Assay

Cytotoxicity evaluation on NIH-3T3 fibroblasts (Figure 3) revealed high cell viability for both αM and NLC-P-αM. Cells treated with αM exhibited 102.16 ± 7.98% viability, while those treated with NLC-P-αM showed 94.76 ± 4.95%. The difference was not statistically significant (P = 0.244), indicating that both formulations are biocompatible and safe at the tested concentrations.

In vivo Diabetic Wound Healing Assay

Wound Closure Rate

The wound closure rate was evaluated for different treatments, including PBS, αM, NLC-P, and NLC-P-αM (Figures 4 and 5). After 14 days, the PBS-treated group exhibited a wound closure of 73.39 ± 2.05%, serving as a control. The αM treatment showed a slightly higher wound closure rate of 75.76 ± 4.70%, indicating a moderate effect on wound healing. Similarly, the NLC-P group resulted in wound closure of 74.69 ± 7.36%, demonstrating an effect comparable to that of αM. However, the NLC-P-αM formulation exhibited the highest wound closure rate of 85.83 ± 3.33%, suggesting that the nanoformulation of αM significantly enhanced the wound healing process compared to both free αM and NLC-P treatments.

Figure 5 highlights the progression of wound closure across different time points, with a particular focus on the differences between the NLC-P-αM and the other treatment groups. On day 4, the NLC-P-αM group exhibited a significantly higher wound closure rate than the PBS group (P < 0.01), but no significant differences were observed when compared to the αM and NLC-P groups (P > 0.05). By day 7, the NLC-P-αM group maintained a significantly

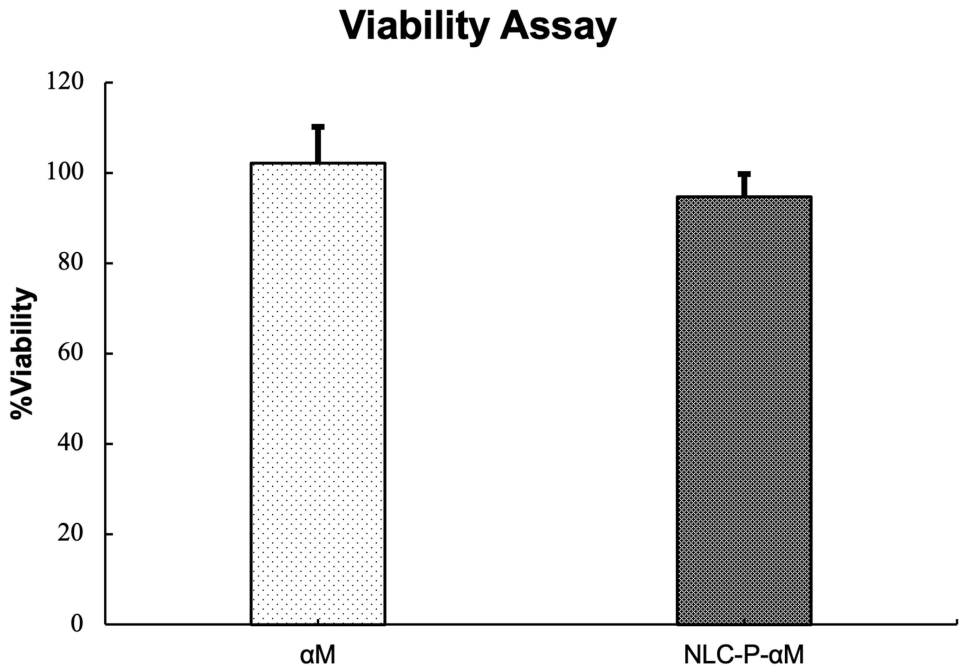


Figure 3 Viability of NIH-3T3 fibroblast cells after 24-hour exposure to NLC-P-αM and unloaded α-mangostin. Data are presented as mean ± standard deviation (n=3).

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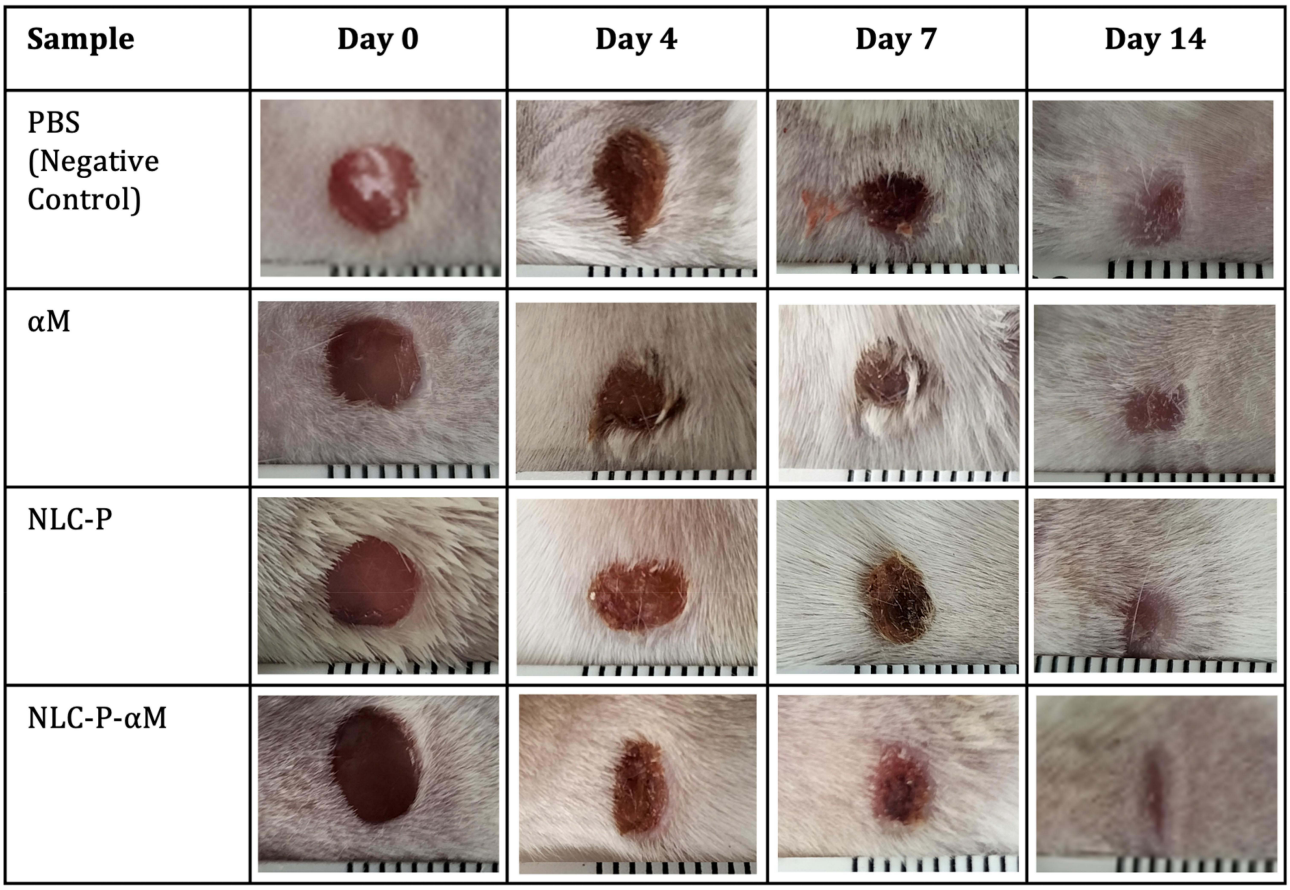

Figure 4 Representative images of wound closure at days 0, 4, 7, and 14 post-treatment, showing the progression of healing over time.

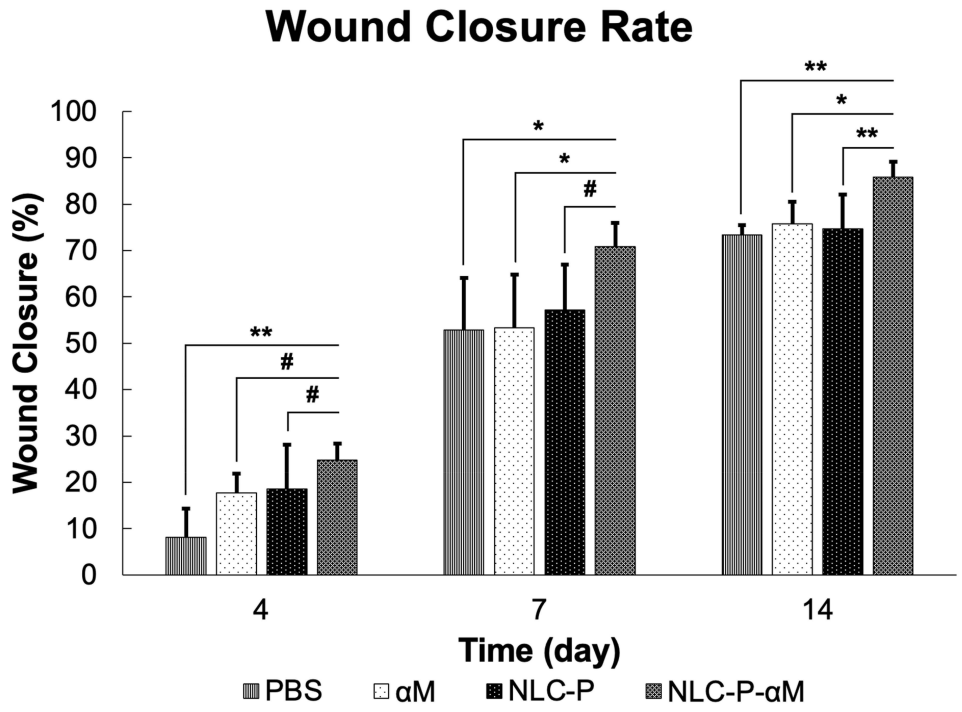

Figure 5 Wound closure rate (%) over days 0, 4, 7, and 14 post-treatment, showing the healing progression in different treatment groups. Data are presented as mean ± standard deviation (n=5); * significance level of $P < 0.05$; ** significance level of $P < 0.01$; and # insignificance level of $P > 0.05$.

higher wound closure rate than the PBS and αM groups ($P < 0.05$), while the difference with the NLC-P group remained insignificant ($P > 0.05$). On day 14, the NLC-P-αM group showed superior wound closure compared to the PBS ($P < 0.01$) and NLC-P ($P < 0.01$) groups, as well as a significantly higher rate than the αM-treated group ($P < 0.05$). These findings emphasize that while NLC-P-αM consistently outperformed PBS, its advantages over free αM and NLC-P became more pronounced over time, demonstrating its potential as an effective therapeutic agent for wound healing in diabetic rats. These findings indicate that NLC-P-αM may offer improved therapeutic potential for wound healing.

Histological Analysis

Histological analysis of skin samples revealed significant differences between the treatment groups. Both the PBS and NLC-P groups exhibited noticeable inflammatory cell infiltration, suggesting ongoing inflammation at the wound site (Figure 6A and C). In contrast, the αM and NLC-P-αM groups showed no signs of inflammatory cell infiltration, indicating reduced inflammation (Figure 6B and D). Furthermore, the αM and NLC-P-αM groups displayed hair follicles and sebaceous glands, indicative of more advanced stages of wound healing and tissue regeneration. Regarding epidermal thickness (Figure 7), the PBS group showed an epidermal thickness of 20.94 ± 0.66 μm, while the αM and NLC-P-αM groups had significantly thinner epidermis, with values of 4.65 ± 1.08 μm and 6.31 ± 1.34 μm, respectively. The NLC-P group showed intermediate epidermal thickness of 15.01 ± 2.52 μm.

Discussion

This study presents compelling evidence for the efficacy of the NLC-P-αM formulation in enhancing diabetic wound healing. Across multiple assays, the nanoformulation consistently outperformed both the free compound and other controls, fulfilling essential criteria for an effective wound healing agent. The in vitro release data demonstrated a sustained and controlled release of αM from the NLC-P-αM system. Unlike our previous study,[35] where

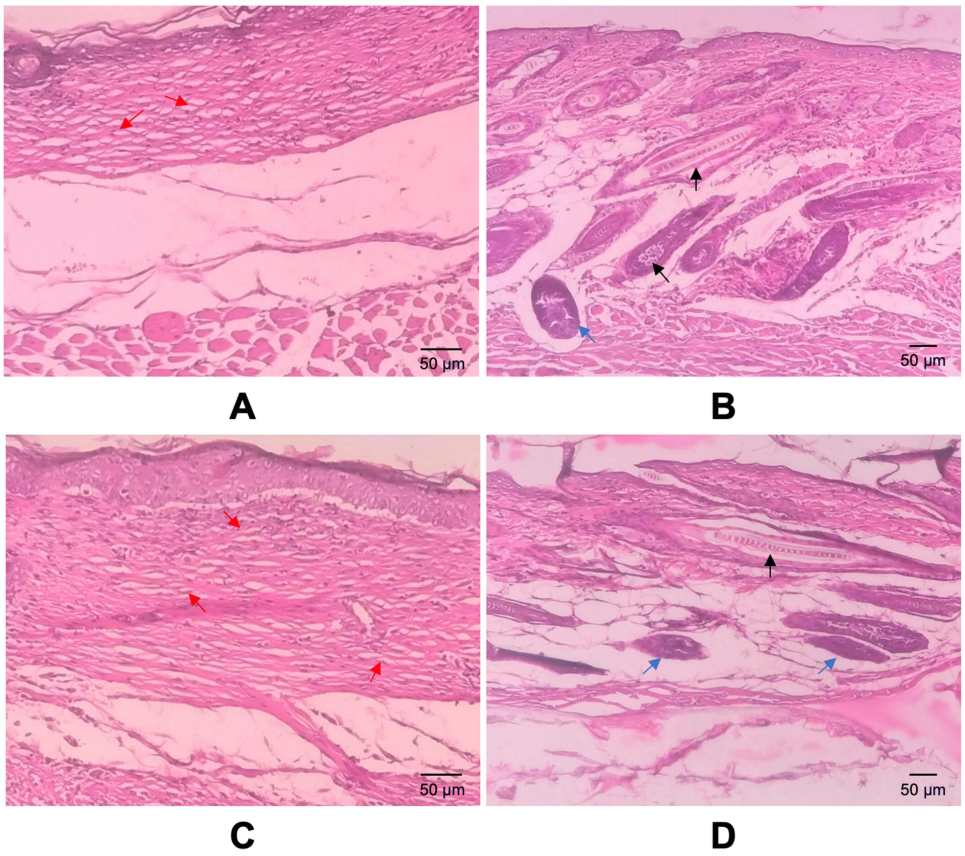

Figure 6 Histological evaluation of skin tissue on day 14 post-treatment with PBS (**A**), αM (**B**), NLC-P (**C**), and NLC-P-αM (**D**), stained with H&E. The images highlight sebaceous gland formation (black arrow), hair follicle growth (blue arrow), and inflammatory cell infiltration (red arrow).

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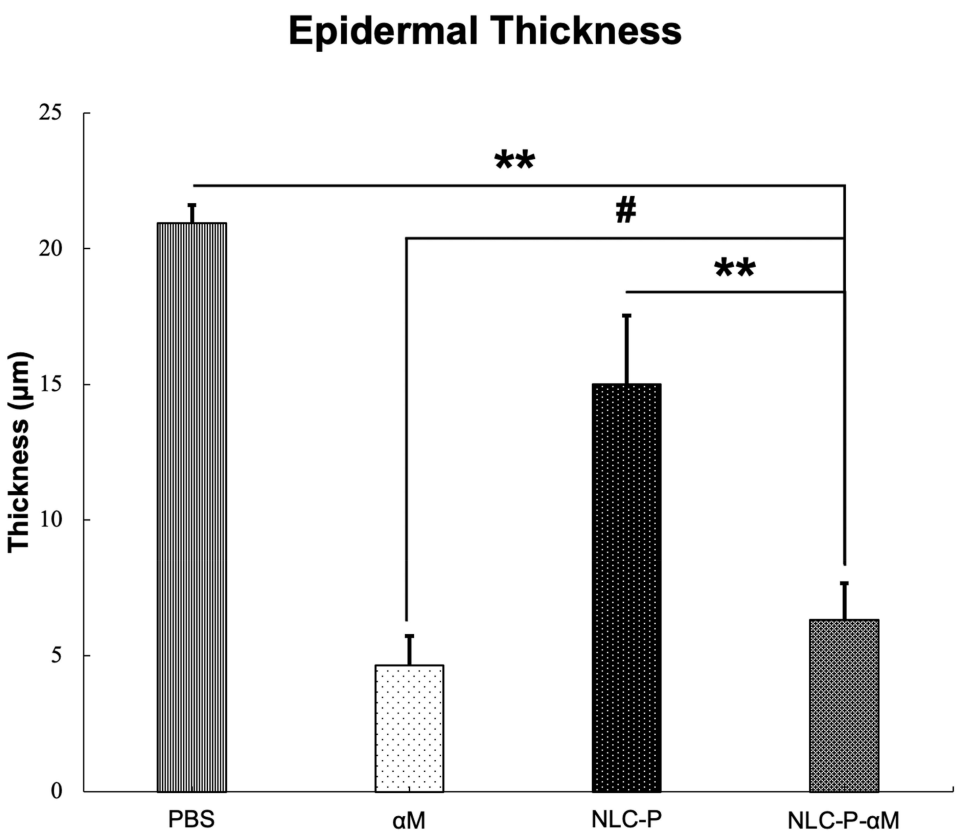

Figure 7 Epidermal thickness (μm) of skin tissue on day 14 post-treatment, measured from H&E-stained sections. Data are presented as mean ± standard deviation (n=5); ** significance level of $P < 0.01$; and # insignificance level of $P > 0.05$.

a dissolution method was used primarily to assess solubility enhancement of αM, the current work employed a membrane-based in vitro release test (IVRT). This method was chosen to better simulate drug release behavior under biorelevant conditions for topical wound application.

Furthermore, while 32 °C is often used to reflect the temperature of healthy skin, we conducted the IVRT at 37 °C to more accurately mimic the diabetic wound microenvironment, which typically exhibits elevated temperatures due to inflammation, increased perfusion, and infection.[49,50] This approach aligns with practices in other wound-healing studies that simulate pathological conditions.[51,52] At this temperature, the NLC-P-αM system achieved a significantly higher cumulative release of αM (85.55% at 360 min) compared to free αM. This aligns with the principle that an ideal delivery system should provide controlled and sustained release, avoiding rapid depletion of the drug while maintaining therapeutic levels over time.[53–55] The observed non-Fickian (anomalous) diffusion mechanism (n = 0.643) suggests a complex release process driven by both diffusion and matrix erosion, enabling prolonged and targeted delivery at the wound site.[56,57] This improved release profile effectively addresses αM's inherent limitations, such as poor solubility and bioavailability, thereby enhancing its therapeutic potential in diabetic wound healing.

Studies have shown that incorporating αM into lipid-based nanoparticles is highly promising for enhancing its therapeutic efficacy. For instance, Bonafè et al demonstrated the potential of αM in a solid lipid nanoparticle (SLN) system for improving wound healing outcomes.[58,59] However, SLN systems have become less favorable due to several limitations, including a high tendency for crystallization, which may lead to system instability and potential breakdown during storage.[60,61] As a next-generation lipid-based nanocarrier, NLC offer a superior alternative by incorporating liquid lipids into the matrix.[62] This innovation reduces crystallization tendencies, enhances drug loading capacity, and improves long-term stability, making NLC systems particularly suitable for applications requiring sustained and efficient drug delivery, as demonstrated in the current study.[63,64]

In addition to its well-documented anti-inflammatory, antioxidant, and antibacterial properties, αM is also recognized for its cytotoxic effects, which have been extensively utilized in cancer treatment, particularly for breast cancer.[65] Previous studies have shown that oral administration of αM falls under toxicity class 5, with an LD_{50} of 2000–5000 mg/kgBW, indicating relatively low systemic toxicity.[66] However, despite the lack of established studies on αM's cytotoxic effects on skin cells or in topical applications, these effects should not be overlooked. This is particularly important when using nano-delivery systems that can potentiate the overall effects of αM, even at very low doses, as employed in the present study. For example, a study that incorporated αM into chitosan-based nanoparticles demonstrated enhanced cytotoxicity against breast cancer cell lines (MCF-7), highlighting the potential of nanoformulation to amplify αM's biological activity.[67]

Therefore, an effective drug delivery system must also demonstrate biocompatibility to ensure safety in cellular environments. Both αM and NLC-P-αM exhibited high cell viability (>90%) in NIH-3T3 fibroblasts, satisfying this fundamental criterion. While αM showed slightly higher viability (102.16%) than NLC-P-αM (94.76%), the small reduction observed with NLC-P-αM can be attributed to the gradual and sustained release of αM, which may result in prolonged cellular exposure. Importantly, the absence of cytotoxicity supports the potential for safe application of the formulation in vivo. An ideal wound healing agent should minimize toxicity while maximizing efficacy, which was observed for both tested systems.[68,69]

The wound closure rate is a direct measure of therapeutic efficacy, with faster and more complete closure reflecting improved healing outcomes.[70] The NLC-P-αM formulation consistently outperformed the other groups, demonstrating superior wound closure on day 14 (85.83 ± 3.33%) compared to PBS (73.39 ± 2.05%), free αM (75.76 ± 4.70%), and NLC-P (74.69 ± 7.36%). This result meets the criteria for an advanced wound healing agent, which should enhance repair by accelerating the closure of chronic wounds, reducing inflammation, and promoting tissue regeneration.[71,72] The enhanced therapeutic efficacy of NLC-P-αM is attributed to both pharmacological and pharmaceutical mechanisms.

Pharmacologically, αM plays a major role as an antioxidant and anti-inflammatory agent, mitigating oxidative stress and suppressing excessive inflammatory responses at the wound site. Additionally, the phenolic and flavonoid compounds in propolis synergistically contribute to these effects by further reducing inflammation and enhancing tissue regeneration. Pharmaceutically, nanosized lipid carrier systems improve the physicochemical properties and bioavailability of αM by increasing the surface area, enhancing water solubility, and promoting a controlled release profile, ensuring sustained therapeutic action. Notably, the gradual but significant improvement in NLC-P-αM over time, particularly compared to free αM and NLC-P, suggests that the formulation effectively leverages the bioactive properties of both αM and propolis while optimizing drug delivery through NLC, resulting in superior wound healing outcomes.

Furthermore, statistical analysis provides crucial insights into the progression of healing. On day 4, NLC-P-αM showed a significant advantage over PBS ($P < 0.01$); however, it did not show a significant difference compared to αM or NLC-P, indicating early compatibility with free αM. However, by day 7, it exhibited superior efficacy compared to PBS and αM ($P < 0.05$), emphasizing its sustained activity. By day 14, NLC-P-αM significantly outperformed PBS ($P < 0.01$), αM ($P < 0.05$), and NLC-P ($P < 0.01$), fulfilling the key criterion for long-term effectiveness.

The wound closure rate observed for NLC-P-αM was comparable to that observed in similar studies utilizing other advanced delivery systems. For example, one study used αM complexed with hydroxypropyl-β-cyclodextrin (HP-β-CD) and formulated into a hydrogel preparation.[21] Similar to the results presented here, that study reported an enhanced wound closure rate compared to unmodified αM, likely attributed to an identical release mechanism, namely Fickian diffusion. In this mechanism, drug release is concentration-independent and primarily driven by the system's ability to enhance bioavailability.[38] Despite significant improvements in wound closure rates after 14 days, complete wound closure was not achieved with NLC-P-αM treatment alone. This may be attributed to the limited retention of αM at the wound site, despite enhanced bioavailability, leading to insufficient persistence of the released αM to sustain its therapeutic effects. However, it is important to note that complete wound closure may take longer than 14 days, particularly under diabetic conditions. Future studies with extended observation periods could provide further insights into the long-term efficacy of NLC-P-αM in promoting complete wound healing.

Histological findings further validated the therapeutic benefits of NLC-P-αM, meeting the criteria for reduced inflammation, effective tissue regeneration, and restored skin integrity.[73] Inflammatory cell infiltration, a hallmark of

delayed healing, was significantly reduced in the NLC-P-αM group, aligning with its anti-inflammatory properties. In contrast, the PBS and NLC-P groups exhibited persistent inflammation, indicating incomplete resolution of the wound healing process.

The presence of hair follicles and sebaceous glands in the NLC-P-αM-treated wounds indicates advanced tissue remodeling, a critical criterion for complete wound healing.[74] These findings suggest that NLC-P-αM not only accelerates closure but also promotes dermal regeneration, restoring the functional and structural integrity of the skin.

Epidermal thickness is an additional marker of healing quality. The significantly thinner epidermis observed in the αM (4.65 ± 1.08 μm) and NLC-P-αM (6.31 ± 1.34 μm) groups compared to the PBS group (20.94 ± 0.66 μm) reflects effective re-epithelialization, reducing hyperproliferation commonly associated with impaired healing.[75,76] The intermediate thickness in the NLC-P group (15.01 ± 2.52 μm) highlights the partial benefits of the lipid carrier, although αM's presence is essential for optimal outcomes.

The modification of αM into NLC-P-αM addresses multiple challenges in diabetic wound management and meets the key criteria for safety, efficacy, and mechanistic advancement. The enhanced release profile overcomes the limitations of αM in terms of solubility and bioavailability, while the in vivo results emphasize its dual role in reducing inflammation and promoting regeneration. Compared to free αM or NLC-P, NLC-P-αM demonstrated clear advantages in achieving consistent and advanced wound healing, making it a promising candidate for clinical translation. Future research should aim to elucidate the molecular mechanisms driving these benefits, such as the modulation of oxidative stress markers, inflammatory cytokines, and growth factors involved in tissue repair processes. Additionally, formulating NLC-P-αM into hydrogel-based preparations would be highly beneficial for enhancing retention time on wound surfaces and improving efficacy. This approach may provide superior local retention, sustained release, and enhanced therapeutic outcomes for chronic wound management.

Conclusion

This study demonstrates the superior potential of a propolis-based nanostructured lipid carrier containing α-mangostin (NLC-P-αM) in enhancing drug release, safety, and therapeutic efficacy compared to free α-mangostin (αM). The in vitro release study revealed a significantly improved release profile for NLC-P-αM, with sustained and higher cumulative release than αM, attributed to a super case-II transport mechanism. Both formulations exhibited minimal cytotoxicity, confirming their biocompatibility. Furthermore, the in vivo diabetic wound healing assay showed that NLC-P-αM significantly accelerated wound closure and promoted advanced tissue regeneration, as evidenced by histological findings, including reduced inflammation and restored normal skin architecture. These results suggest that NLC-P-αM is a promising therapeutic strategy for wound healing, particularly in diabetic conditions, due to its enhanced release kinetics, biocompatibility, and regenerative capabilities.

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Disclosure

The authors report no conflicts of interest in this work.

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