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REVIEW

# Advancements and Challenges of Nanostructured Lipid Carriers for Wound Healing Applications

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**Abstract:** The current treatments for wound healing still exhibit drawbacks due to limited availability at the action sites, susceptibility to degradation, and immediate drug release, all of which are detrimental in chronic conditions. Nano-modification strategies, offering various advantages that can enhance the physicochemical properties of drugs, have been employed in efforts to maximize the efficacy of wound healing medications. Nowadays, nanostructured lipid carriers (NLCs) provide drug delivery capabilities that can safeguard active compounds from environmental influences and enable controlled release profiles. Consequently, NLCs are considered an alternative therapy to address the challenges encountered in wound treatment. This review delves into the application of NLCs in drug delivery for wound healing, encompassing discussions on their composition, preparation methods, and their impact on treatment effectiveness. The modification of drugs into the NLC model can be facilitated using relatively straightforward technologies such as pressure-based processes, emulsification techniques, solvent utilization methods, or phase inversion. Moreover, NLC production with minimal material compositions can accommodate both single and combination drug delivery. Through in vitro, in vivo, and clinical studies, it has been substantiated that NLCs can enhance the therapeutic potential of various drug types in wound healing treatments. NLCs enhance efficacy by reducing the active substance particle size, increasing solubility and bioavailability, and prolonging drug release, ensuring sustained dosage at the wound site for chronic wounds. In summary, NLCs represent an effective nanocarrier system for optimizing the bioavailability of active pharmacological ingredients in the context of wound healing.

**Keywords:** drug delivery, nanocarrier, nanostructured lipid carriers, wound healing

## Introduction

Wound healing encompasses a myriad of intricate physiological processes aimed at regenerating cells and tissues to replace damaged components.[1] Pharmacological agents play a vital role in supporting and expediting the wound healing process. Among these agents, anti-inflammatories are employed to mitigate complications stemming from an excessive immune response at the wound site.[2] Antibacterial agents are equally indispensable to thwart the growth and infiltration of bacteria that may breach the injured skin's surface.[3,4] Furthermore, various biological preparations, including growth factors, have gained widespread application in accelerating the regeneration of damaged tissue.[5,6] Additionally, the application of wound dressings proves beneficial in shielding the wound from environmental conditions that might impede or exacerbate the healing process.[7]

Nevertheless, the challenge remains to identify optimal drug delivery preparations for expediting wound healing.[8] Currently, formulators encounter an array of issues concerning drug delivery to wounds. These issues encompass

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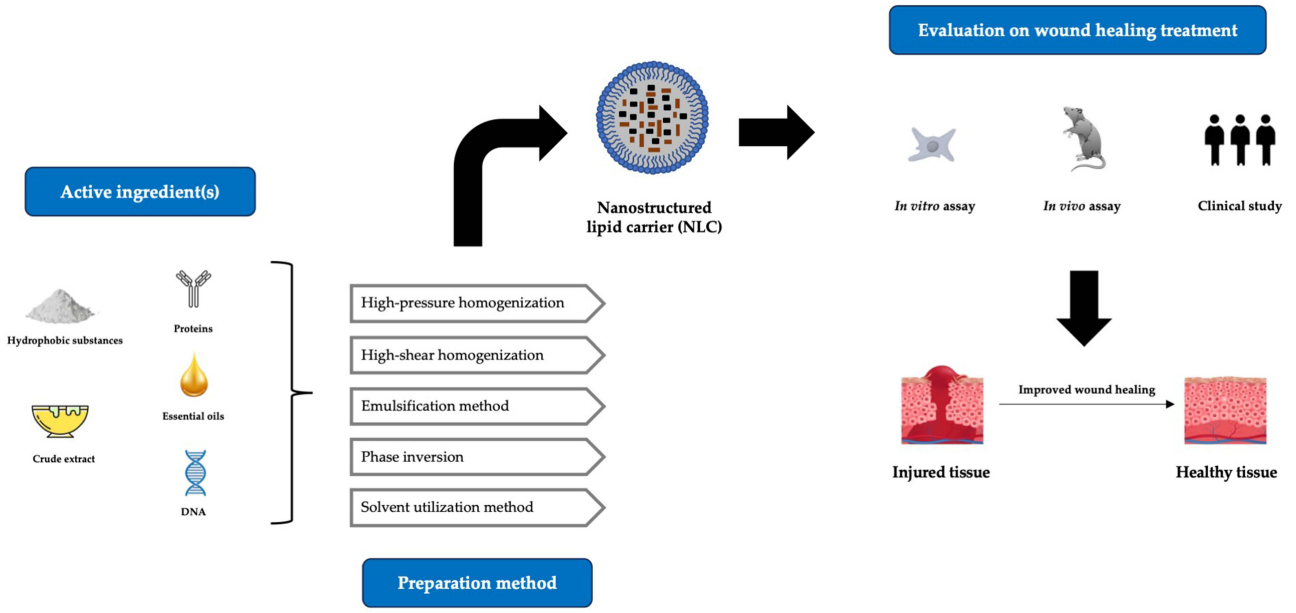
**Graphical Abstract**




mechanical hurdles where maintaining local effects of preparations during patients' daily activities proves arduous.[8] In cases of infected tissues, wounds tend to be chronic, necessitating prolonged therapeutic interventions.[9] Mechanical barriers within the wound necessitate the optimization of an ideal drug delivery system to wound sites, a task complicated by the challenge of maintaining the local effect of therapeutic agents. Additionally, the body's defense system hosts a multitude of protease enzymes, further raising concerns about the effective administration of epidermal growth activator biological agents, typically protein components.[8] Supporting this, Zhang et al's study revealed that topical administration of epidermal growth factor (EGF) failed to significantly reduce the time to improvement in wound conditions compared to controls (6.8 ± 0.12 vs 7.5 ± 0.13 days, respectively).[10] These myriad challenges underscore the importance of developing preparations with extended retention times in the wound area, ensuring long-term release, and providing protection during application at the wound site.

Recent advances in nanotechnology offer a promising avenue for overcoming barriers in drug formulation.[11–15] In the realm of wound healing, nano-delivery systems (nanocarriers) present opportunities to enhance the physicochemical characteristics of preparations.[16–18] Various nanomaterials suitable for drug delivery in wound care encompass inorganic options such as gold and silver nanoparticles, organic choices including polymeric and lipid nanoparticles, as well as nanofibers, hydrogels, nanospheres, and scaffolds.[19–21] Notably, a recent clinical study employing a nano-silver modification technique failed to achieve an optimal timeframe for wound treatment using EGF compared to controls (6.2 ± 0.32 vs 7.5 ± 0.13 days, respectively). Encouragingly, promising results emerged from studies involving wound drug delivery via lipid nanoparticles modified with essential oils. This modification accelerated in vitro wound healing, achieving complete wound closure within 48 hours.[10] Lipid nanoparticles, exemplified by α-Gal liposomes, accelerate wound healing by enhancing macrophage activation and promoting wound closure across various conditions, as evidenced by multiple studies.[22–27] This superiority is attributed to the lipid nanocarriers' composition, consisting of lipids compatible with mucosal and skin components.[28,29]

Lipid nanoparticles are categorized into two types: solid lipid nanoparticles (SLNs), the first generation of lipid nanoparticles, and nanostructured lipid carriers (NLCs), the second generation.[30] The utilization of SLNs has largely been discontinued due to inherent limitations such as a high polymorphic tendency, low stability, and inefficient drug loading capacity.[31,32] In contrast, NLCs, as the second generation, offer numerous advantages.[33] Beyond overcoming the

shortcomings of SLNs, NLCs exhibit controlled release capabilities,[34] are ease of manufacture,[35] and skin hydration properties,[36] thereby enhancing their suitability for wound treatment. Furthermore, NLCs exhibit higher entrapment efficiency compared to SLNs, with NLCs achieving up to 88% drug entrapment, whereas SLNs can only entrap about 80%. Additionally, unlike the burst effect seen with SLNs, the regulated release of NLCs keeps the drug at an efficacious dose, maintaining the therapeutic window and preventing toxic levels.[14] Its advantages are also highly beneficial for wound healing applications, as wounds often require a sustained-release drug mechanism for extended recovery periods.

This review aims to explore the utilization of NLCs as nanocarriers for therapeutic agents in wound therapy. This review was compiled based on a literature search conducted in electronic databases, including PubMed (MEDLINE), Scopus, ScienceDirect, and Google Scholar. The searches utilized the keywords "Nanostructured Lipid Carrier" AND Wound, "Nanostructured Lipid Carrier" AND Ulcer, NLC AND Wound, AND NLC AND Ulcer. Articles published from 2013 to 2023 were considered. The initial section provides an overview of wound pathologies to elucidate the role of therapeutic agents and the significance of NLCs in wound treatment. It subsequently offers a comprehensive examination of NLCs as nanocarriers. Finally, the review summarizes various research findings discussing the application of NLCs in drug delivery for wound treatment, encompassing in vitro, in vivo, and clinical studies. Figure 1 illustrates the strategy employed in structuring this review.

## Wound Pathology

Understanding wound pathology is of paramount importance in the development of drugs and drug delivery strategies for wound treatment. A wound refers to a condition in which the skin becomes damaged or torn due to mechanical stress, violence, trauma, injury, or other factors stemming from physiological processes.[37] Wounds can be categorized based on both the depth

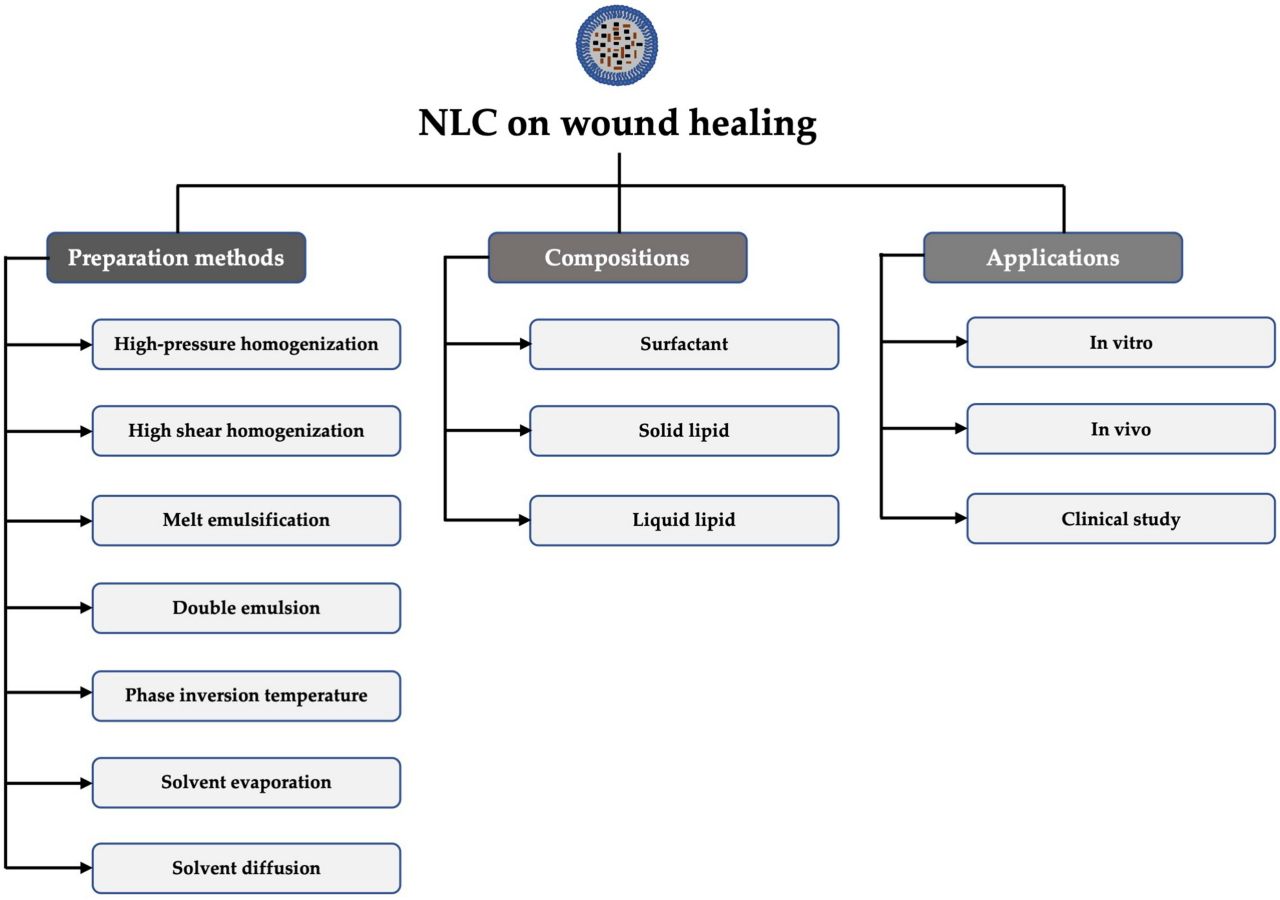



**Figure 1** Reviewing flow chart.

of the affected area and the duration required for the healing process. In terms of depth, wounds fall into three distinct categories,:[38] superficial wounds, where damage is limited to the epidermal layer; partial-thickness wounds, where both the epidermal and deeper dermal layers are affected; and full-thickness wounds, where damage extends to deeper layers, including subcutaneous fat.[39] Furthermore, wounds are classified based on their healing timeframes as acute or chronic wounds.[40] Acute wounds are characterized by a relatively short healing process, while chronic wounds, often associated with pathological conditions such as infection and diabetic ulcers, require a longer healing duration.[38,40,41]

Given the intricacy of wound healing as a physiological process, it comprises four principal stages, as illustrated in Figure 2. These stages encompass the hemostasis phase, inflammation phase, proliferation phase, and remodeling phase.[42,43]

## Haemostasis Phase

This phase initiates immediately following tissue damage, and it holds significant importance in preventing exsanguination by regulating vascularity.[37] Platelets play a pivotal role in stemming exsanguination through the formation of aggregates, a process known as clotting, which effectively obstructs the flow of blood.[44,45] Clot formation involves intricate molecular mechanisms, commencing with the activation of platelet receptors triggered by type I collagen, serving as indicators of the extravascular or extracellular matrix (ECM).[46] Activated platelets subsequently release various substances that collaborate to form a clot. These include adhesive glycoproteins like fibronectin, fibrinogen, fibrin, thrombospondin, and vitronectin, which function akin to glue, binding all components together to form aggregates that impede blood flow.[47] Moreover, growth factors released as a result of platelet activation play crucial roles in various immune recruitment mechanisms.[48,49] These growth factors encompass tumor growth factor-β (TGF-β) and platelet-derived growth factor (PDGF), which participate in recruiting monocytes and neutrophils, as well as tumor growth factor-α (TGF-α), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF), contributing to the activation of endothelial cells, inducing angiogenesis.[49] Additionally, PDGF is known for its role in activating fibroblasts, ultimately initiating collagen and extracellular matrix formation.[50]

Remarkably, platelets also serve to prevent excessive aggregation or clotting,[47] achieved through various molecular mechanisms. This includes the role of prostacyclin, which directly inhibits platelet aggregation, bolstered by antithrombin III's function in inhibiting thrombin activity.[51] Additionally, the complement protein C5a plays a role in curbing excessive aggregation by facilitating the degradation of coagulation factors V and VII.[52]

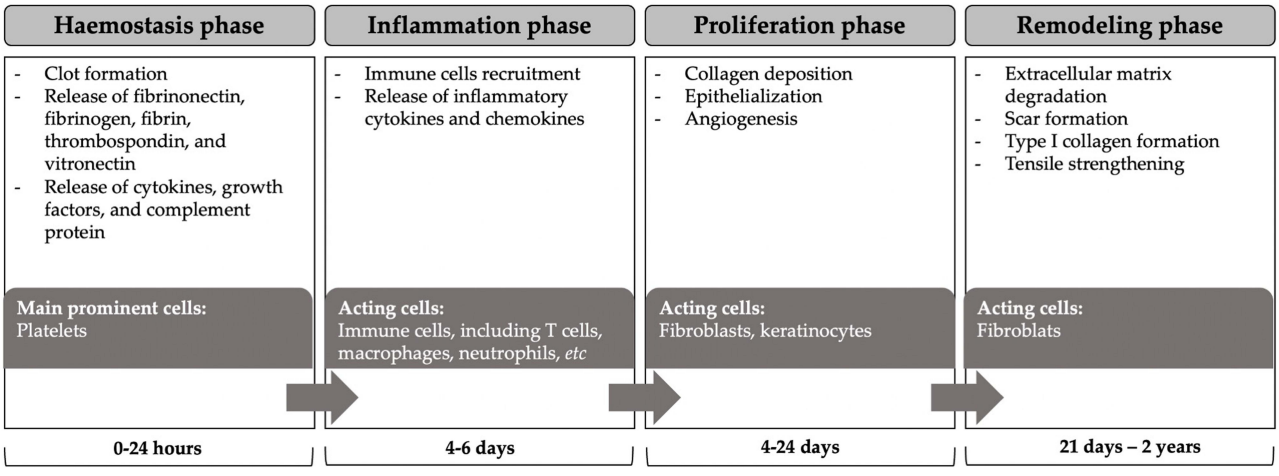



**Figure 2** Phases of wound healing processes.

## Inflammation Phase

The inflammation phase, which is the second phase, typically occurs within a timeframe ranging from 24 hours to 2 weeks following the injury.[37] Innate immunity takes precedence in addressing potential pathogenic agents that may infiltrate exposed skin or mucosal areas due to the wounds.[53] This response is mediated by various immune cells, including macrophages, Langerhans cells, and mast cells.[53] Additionally, T cell activation plays a critical role by releasing pro-inflammatory molecules, contributing to vasodilation, which, in turn, facilitates the migration of neutrophils to the wounded area.[54] Once neutrophils reach the wound site, they embark on eliminating damaged cells and shielding the wound from invading pathogens, achieved through the release of reactive oxygen species (ROS) or phagocytosis.[55]

Furthermore, the role of cytokines and chemokines in adaptive immunity holds significant importance, particularly in cases of chronic infective wounds.[56] Inflammatory cytokines such as IL-1, IL-6, and IL-8 are instrumental in cell signaling processes that facilitate fibroblast infiltration and proliferation, keratinocyte chemotaxis, and collagen synthesis.[57] These processes are vital preparations for the subsequent phases of wound healing. Chemokines, small regulatory proteins, also play a crucial role in recruiting various immune cells.[56] Among the chemokines involved in the inflammatory phase of wound healing are α-chemokines (CXC), β-chemokines (CC), and γ-chemokines (C and CXXXC).[58] Under injury conditions, these chemokines participate in recruiting neutrophils, activated T lymphocytes, eosinophils, basophils, monocytes, and natural killer cells.[58]

## Proliferation Phase

During the proliferation phase, fibroblasts take center stage in the process of wound closure.[37,59] This phase typically commences approximately 12 hours after the initial injury.[37] Migrating fibroblasts transport soluble mediators, forming granulation tissue to replace the fibrin-rich matrix.[59] The migration of these fibroblasts is initiated through interactions with integrin receptors present in the fibrin-rich matrix.[60] The direction of fibroblast movement is contingent upon the composition and concentration of matrix constituents, which include various cytokines, chemokines, and growth factors.[60] Additionally, keratinocytes play a pivotal role in epithelialization.[61,62] They release matrix metalloproteinases (MMPs) to establish a basal membrane that serves as a framework for the extracellular matrix in the formation of new epithelial tissue.[63]

In addition to extracellular matrix production, the proliferation phase also witnesses the formation of new collagen.[37] Primarily, migrating fibroblasts contribute to the production of this new collagen.[59] The collagen generated is utilized by fibroblasts as a component in the formation of granulation tissue, alongside elastin and proteoglycans.[64] The predominant type of collagen synthesized during the proliferation phase is type III collagen.[65] Furthermore, damaged blood vessels undergo repair through angiogenesis during this phase.[66] Vascular endothelial cells are produced by macrophages and various growth factors known as angiogenic factors. This angiogenesis process is specifically regulated by vascular endothelial growth factor (VEGF) as an endothelial cell growth factor and counteracted by endostatin and angiostatin, which function as anti-angiogenic factors.[66]

## Remodeling Phase

The remodelling phase constitutes the final stage of the wound healing process, commencing from the onset of fibrin clot formation and extending until the complete maturation of type I collagen, a process that can span several years.[37] Throughout this phase, fibroblasts continue to play a pivotal role.[67] However, in contrast to their role in the proliferation phase, fibroblasts' involvement in the formation of granulation tissue diminishes during the remodelling phase.[67] Instead, fibroblasts initiate the production and transport of hyaluronan and proteoglycans, thereby replacing the fibrin clot.[68] Additionally, extracellular matrix proteins such as matrix metalloproteinases (MMPs), which also contribute to the proliferation phase, participate in the remodelling process by degrading fibrillar collagen molecules and proteoglycans.[63] The degradation of protein components within the extracellular matrix is mediated by serine proteases, particularly α1-protease inhibitor.[63]

The completion of the remodelling phase serves as an indicator for the type of collagen predominating in the healed tissue. In contrast to the proliferative phase, the predominant collagen type in this phase is typically type I collagen.[69]

Type I collagen undergoes maturation to replace type III collagen, continuing until the ideal composition of healthy tissue is attained. In healthy adult tissues, a typical ratio of type I collagen to type III collagen is 80:10. Notably, type I collagen boasts superior tensile strength compared to type III collagen.[37,70] Consequently, this process reinforces the tender scar until it attains a protective function similar to that of natural skin.[70]

## Nanostructured Lipid Carriers

Subsequent to acquiring an understanding of wound pathology, delving into the nanostructured lipid carriers (NLCs) system becomes imperative for their application in our wound drug candidates. NLCs represent nanocarriers characterized by their formulation as oil-in-water emulsions within the nanoscale range, typically falling within the 10–1000 nm spectrum.[71] NLCs essentially serve as an alternative to solid lipid nanoparticles (SLNs), a concept first introduced by Professor R.H. Müller and Professor M. Gasco in 1990.[30] NLCs offer several notable advantages, including the safeguarding of drugs against unfavorable environmental conditions, facile large-scale synthesis facilitated by the high-pressure homogenization technique, biocompatibility, and biodegradability.[72] Moreover, NLCs stand out due to their ability to encapsulate both solid lipid components and liquid lipid constituents, thereby mitigating the rigidity often associated with nanocarriers, a primary limitation of SLNs.[73] A comprehensive depiction of the general structure of NLCs is provided in Figure 3. Subsequently, a detailed exploration of NLC composition, types, and preparation methods is described below.

### Composition of Nanostructured Lipid Carriers

As previously mentioned, NLCs represent carrier systems comprising both solid and liquid lipids.[73] The distinctions between solid lipids and liquid lipids within NLCs are discernible through their physical properties, melting characteristics, and crystalline attributes. Solid lipids, exemplified by glyceryl behenate, glyceryl palmitostearate, and stearic acid, maintain solidity at both ambient and physiological temperatures, with a melting point typically exceeding 37°C, thus

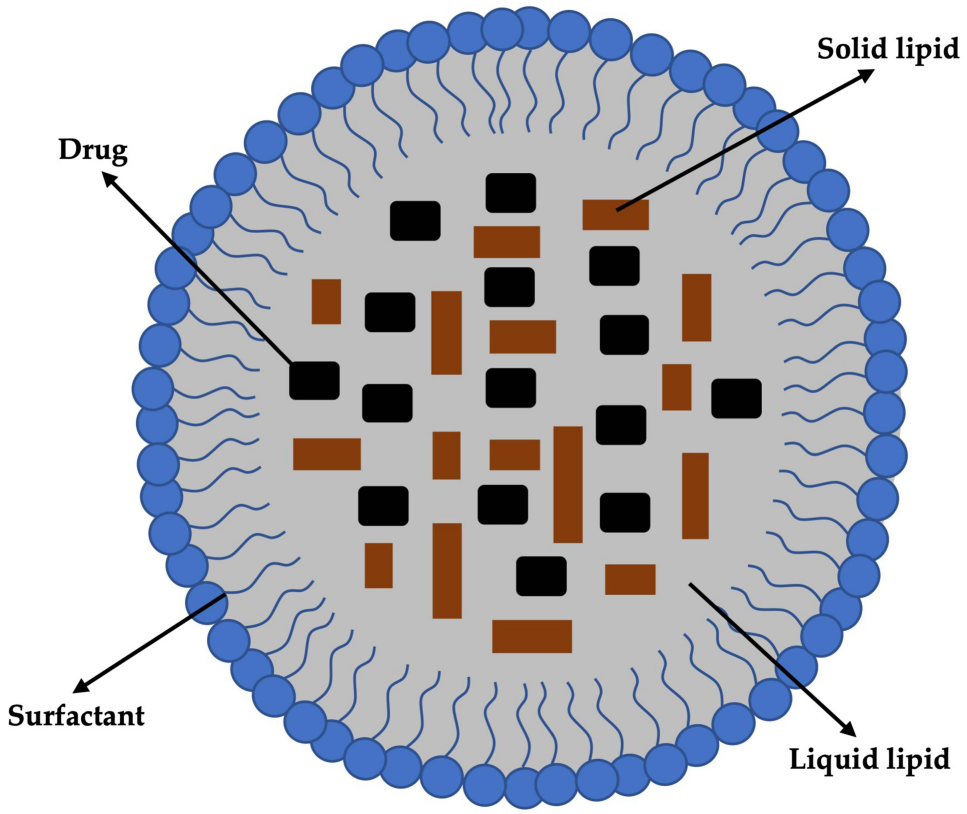



**Figure 3** General structure of nanostructured lipid carrier.

ensuring structural integrity in NLC formulations. In contrast, liquid lipids such as oleic acid, medium-chain triglycerides, and squalene remain fluid at these temperatures, possessing a lower melting point below 37°C, which enhances the lipid matrix's fluidity. Solid lipids exhibit higher crystallinity levels, impacting drug encapsulation efficiency and release kinetics, whereas liquid lipids contribute to a less ordered lipid matrix, promoting enhanced drug loading capacity and modulation of drug release profiles within NLCs.[74,75]

NLCs achieve stability by mitigating the risk of aggregation, a function primarily attributed to surfactants.[76] The drugs are housed within the lipid phase, serving as a reservoir, and are subsequently released as the surfactant layer disintegrates.[77] A compilation of commonly utilized solid lipids, liquid lipids, and surfactants in the development of NLCs for drug delivery in wound healing is presented in Table 1.

## Types of Nanostructured Lipid Carriers

NLCs are categorized into three distinct types: imperfect types, amorphous types, and multiple types. These varying classifications of NLCs exert an influence on the spatial arrangement of the loaded drugs.[79] Each type of NLC possesses a distinctive structure, as depicted in Figure 4.

**Table 1** Common Ingredients in Developing NLC Loaded with Wound Healing's Active Pharmacological Agent

| Component | Name(s) | Ref. |
|---|---|---|
| Solid lipid | Cetyl alcohol | [78] |
| | Cocoa butter | [66] |
| | Glycerol dibehenate (Compritol®) | [67–70] |
| | Glyceryl palmitostearate (Precirol®) | [71–77,79–84] |
| | Hydrogenated coco-glycerides | [85] |
| | Medium-chain triglycerides | [86] |
| | Oleoyl macrogol-6 glycerides and hydrogenated coco-glycerides | [87] |
| | Shea butter | [88] |
| | Soy lecithin | [79,80,89–96] |
| Liquid lipis | Argan oil | [88] |
| | Caprylic / Capric Triglyceride mix | [85] |
| | Coconut oil | [70] |
| | Eicosapentaenoic acid (EPA) or omega 3 | [81] |
| | Glycerol mono-stearate | [86,95,97] |
| | Hydrogenated palm oil, olive oil | [89,91–94] |
| | Isopropyl myristate | [78] |
| | *Lavandula* essential oil | [87] |
| | Olive oil | [66] |
| | Propylene glycol dicaprylate (Miglyol®) | [7,69,72–76,82] |
| | Propylene glycol monocaprylate (Capryol® 90) | [67,68,77,84] |
| | Pumpkin seed oil | [90] |
| | Super refined soybean oil | [96] |

(*Continued*)

**Table 1** (Continued).

| Component | Name(s) | Ref. |
|---|---|---|
| Surfactant | Caprylocaproyl polyoxyl-8 glycerides (Labrasol®) | [67] |
| | d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and polysorbate 80 (Tween® 80) | [90] |
| | Isopropyl myristate | [87] |
| | Lecithin | [66,85] |
| | Oleyl alcohol | [89] |
| | Poloxamer | [68–70,73–77,83,84,86] |
| | Polyethylene glycol 40 stearate (Myrj™) | [96] |
| | Polysorbate 80 (Tween® 80) | [71,72,77,79–84,88,91–95,97] |

## Imperfect Type

The imperfect type, commonly referred to as the imperfect crystal type, is a classification of NLCs characterized by a lower ratio of liquid lipids to solid lipids in its composition.[72,80] Moreover, variations in the composition of the solid lipid constituents within the container have been observed, including differences in the structure of hydrocarbon chains (whether long or branched) and polymorphic forms. These variations result in irregularities in the spatial arrangement, creating more opportunities for drug molecules to occupy the gaps within the lipid matrix.[80] However, this imperfect type has a vulnerability in terms of stability, as the system tends to undergo structural breakdown due to the tendency of solid lipid crystallization.[79]

## Amorphous Type

In the amorphous type, the solid lipid constituents loaded into the system exist in an amorphous state, thus averting the risk of leakage caused by crystallization.[81] Amorphous lipids differ significantly from crystalline lipids as they lack a well-defined, orderly structure. Instead of possessing a regular, repeating molecular arrangement, amorphous lipids

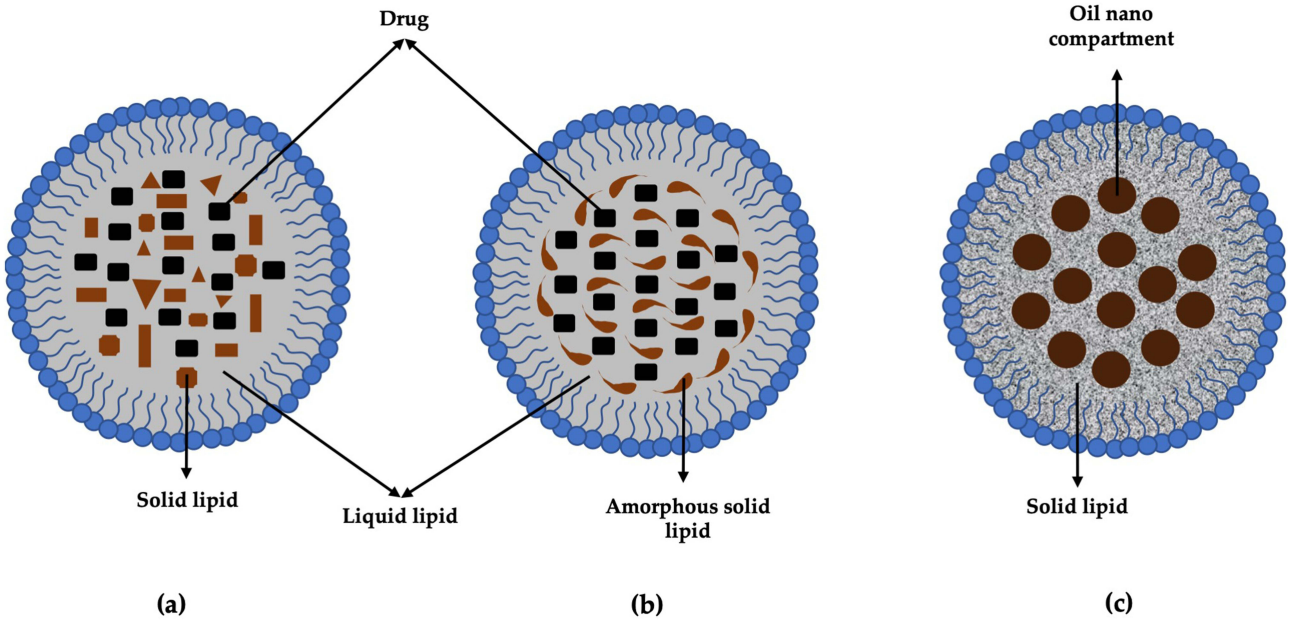



**Figure 4** Types of nanostructured lipid carriers, including (**a**) imperfect type, (**b**) amorphous type, and (**c**) multiple type.

exhibit a random, disordered configuration. Amorphous clusters are predominantly generated through the utilization of the cold homogenization approach, particularly when incorporating highly lipophilic drugs, as opposed to the hot homogenization method.[80] Examples of lipids suitable for creating the amorphous type encompass isopropyl myristate, hydroxy octacosanol hydroxy stearate, dibutyl adipate, and certain triglycerides.[81,82] As this type boasts a more orderly spatial arrangement, the drug loading capacity is generally lower than that of the imperfect type.[81]

### Multiple Type

In this type, liquid oils serve as prominent high-dissolved drug reservoirs, effectively substituting the role of liquid lipids.[76] The utilization of this oil phase has implications for the occurrence of phase separation between solid lipids and oils during the cooling process. Given that drugs are housed within the oil phase, their release proceeds at a slower rate. Consequently, this type is exceptionally well-suited for the administration of controlled-release drugs.[76,79]

## Preparation Method of Nanostructured Lipid Carriers

The preparation methods of NLCs vary and are contingent upon the stability of the active substance and other technical factors. Additional considerations encompass process simplicity, process viability, and the desired particle attributes.[81] NLCs can be prepared using the methods described below:

### High-Pressure Homogenization Method

The high-pressure approach employed in NLCs manufacturing is highly environmentally friendly, particularly as it does not necessitate the use of organic solvents. Furthermore, this method is highly versatile, suitable for both small-scale production and scaling up to higher capacities.[81] This method has been successfully employed to produce NLCs containing the active ingredients thymoquinone, zerumbone, and cinnamon oil. Suitable solid lipids for use in this method include shea butter, while appropriate liquid lipids include argan oil. The process of NLCs production commences with the melting of the lipid component until it reaches a temperature 5–10°C above its melting point.[71] The active substance is prepared as a mixture with the molten lipid. Typically, the hot process is preferred over the cold process. In the hot process, elevated temperatures induce melting, resulting in material dispersion at extremely small particle sizes, often approaching molecular dispersion conditions. In contrast, the cold process involves lowering the temperature to trigger the recrystallization of the mixed ingredients, yielding a stable crystalline state that is still easily broken down into smaller particles. In the next step, the mixture is directly incorporated into the surfactant solution when using the hot temperature method. The surfactant is preheated to the same temperature as in the melting stage.[83] Conversely, in the cold temperature process, the homogeneous melt obtained is first cooled to induce crystallization and form a solid phase. The resultant solid is subsequently pulverized before being encapsulated into a water solution containing surfactants at lower temperatures.[84] Regardless of whether the process involves hot or cold temperatures, the mixture is further homogenized using high pressure through a narrow gap.[81] The flow chart depicting the development of NLCs using high-pressure homogenization in both hot and cold processes is illustrated in Figure 5. Homogenizing the cold-processed mixture generally requires higher pressure and more process cycles. Compared to the cold process, the equipment required for hot-high-pressure homogenization is relatively compact. However, the hot process is not suitable for processing thermolabile active substances.[84]

### High Shear Homogenization Method

In this method, the drug is initially prepared as a mixture within a molten lipid phase. Subsequently, the mixture is homogenized using a high-speed stirrer to create a nano-dispersion.[81] Before pouring, the surfactant solution, which may consist of poloxamers, lecithins, polysorbates, and polyethoxylated monoglycerides, is heated to the melting temperature of the lipid phase. Achieving a homogeneous mixture with smaller particle sizes is contingent upon the gradual integration of the lipid mixture. A higher stirring speed can yield smaller particles. Additionally, this method is frequently combined with ultrasonication techniques, employing probe-type ultrasonication to disintegrate agglomerates or large-sized globules.[85] This method has been effectively utilized to create NLCs with the active compounds Eucalyptus essential oil (EEO) and Rosemary essential oil (REO). In this approach, soya lecithin can serve as both a solid lipid and a liquid lipid.

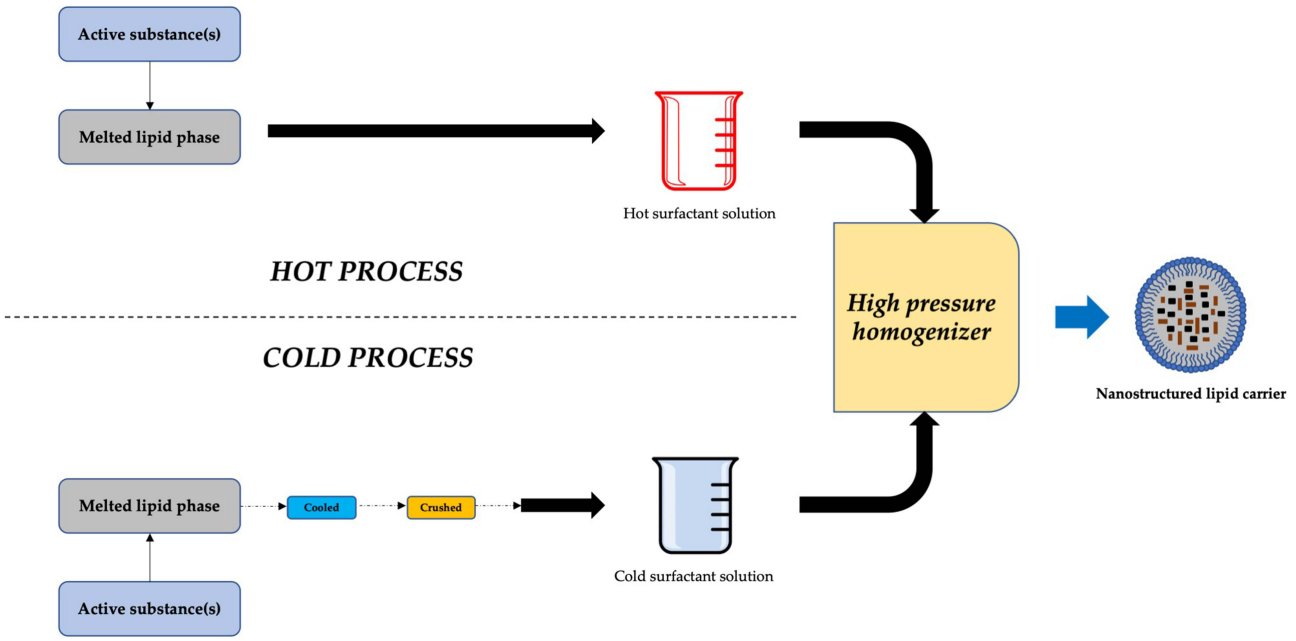



**Figure 5** High pressure homogenization flow chart.

## Melt Emulsification Method

The melt emulsification technique is almost identical to high shear homogenization.[86] The distinction lies in the point at which the nano-dispersion of drugs is formed. This method is suitable for loading peptide-based drugs, such as the LL37 peptide. Initially, the drug is blended with the molten solid and liquid lipids. The mixture of surfactants and co-surfactants, such as lecithin as a surfactant and sodium taurodeoxycholate as a co-surfactant, is then heated to the melting temperature of the lipid phase. These two mixtures are homogenized to produce an oil-in-water (o/w) emulsion phase. Following homogenization, the mixture is promptly immersed in cold water and agitated. Dilution and rapid temperature changes induce emulsions that initially have larger sizes to become smaller particles.[87] This technique is considered a straightforward method for NLC formation. However, it has a drawback in that the particle sizes obtained are often on a micro scale.[88]

## Double Emulsion Technique

This technique is essentially a variation of the melt emulsification method used specifically for incorporating hydrophilic active substances.[30] This method has proven effective in producing NLCs incorporating epidermal growth factor (EGF) and curcumin as active ingredients. Solid lipids such as glyceryl palmitostearate (Precirol®) are suitable for this process, along with liquid lipids like eicosapentaenoic acid (EPA) or omega-3. Initially, the active substances are dissolved in water, which is then dispersed in the melted lipid phase to create a water-in-oil (w/o) emulsion system. Subsequently, this mixture is introduced to a surfactant solution as a stabilizer to produce a nano-water-in-oil-in-water (w/o/w) emulsion phase. The speed of stirring and process temperature are the primary factors influencing the size of the resulting particles.[89]

## Phase Inversion Method

This method can be regarded as a novel approach in the preparation of NLCs. This technique leverages the surfactants' ability to exhibit different hydrophilic-lipophilic balance (HLB) values at varying temperatures.[90] At high temperatures, surfactants will have a relatively low HLB. At a specific temperature threshold, the surfactant undergoes a change in HLB value, which can alter the emulsion phase formed. The formation of the NLCs system with this method begins by mixing the active substances, lipid phase, and surfactant, followed by stirring. The mixture is later subjected to drastic temperature changes for up to three cycles (85°C – 60°C – 85°C – 60°C – 85°C) to produce an oil-in-water (o/w) emulsion phase. Subsequently, the mixture is vigorously introduced to cold water (0°C) to invert the phase into an oil-in-water (o/w) emulsion phase. This technique is highly suitable for thermolabile active substances, especially when

addressing limitations associated with high-temperature organic solvent evaporation.[91] This method has been successfully applied to create NLCs containing the active ingredients ferulic acid (FA) and Lavandula essential oil (LEO). Solid lipids suitable for this method include glyceryl palmitostearate (Precirol®), while appropriate liquid lipids include propylene glycol monocaprylate (Capryol® 90).

## Solvent Evaporation Method

In the solvent evaporation technique, both the active substance and the lipid phase are dissolved in an organic solvent.[92] The organic solvent used must be immiscible with water. The organic phase is subsequently mixed into the surfactant solution to create an oil-in-water (o/w) emulsion system. The resulting emulsion is then heated to facilitate the evaporation of the organic solvent.[93] The remaining lipid phase precipitates into nanoscale particles. This technique is seldom employed due to concerns regarding the potential for organic solvents to leave behind toxic residues.[71] This method has been effectively employed for encapsulating drug such as pioglitazone.

## Solvent Diffusion Method

This technique closely resembles the solvent evaporation method, with the distinction lying in the type of organic solvent and solvent removal approach employed.[84] To clarify, the differing processes involved in developing NLCs using these two methods can be observed in Figure 6. In this method, the organic solvent employed must be water miscible. Initially, both the active substance and the lipid phase are dissolved in the organic solvent. The organic phase is then mixed with a surfactant solution to create an oil-in-water (o/w) emulsion. Subsequently, the organic solvent is removed through dilution in water (at a 1:10 ratio).[71] The organic solvent undergoes diffusion into the water phase, leaving behind nanoprecipitates. Following this, the solid phase can be separated, either through ultrafiltration or lyophilization.[30] This method has been successfully employed to encapsulate drug such as simvastatin.

In NLC research, the incorporation efficiency of active substances (drugs) into the carriers and the handling of non-incorporated substances can vary significantly. Encapsulation efficiency refers to the percentage of drug successfully embedded within the lipid matrix relative to the total amount utilized during formulation, influenced by lipid composition, preparation techniques, and drug-specific properties.

Non-incorporated substances, remaining as free molecules within the suspension post-formulation, can impact the overall stability and therapeutic efficacy of NLCs. Strategies often involve optimizing formulation parameters to enhance drug-lipid

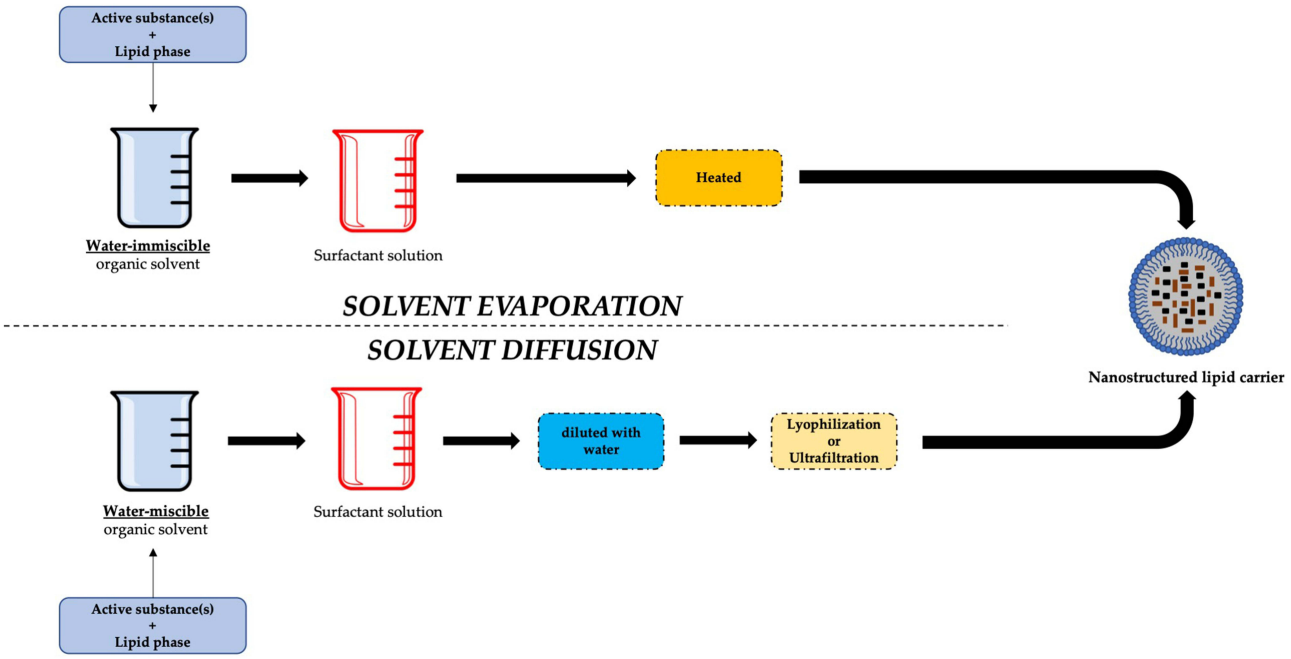



**Figure 6** Solvent evaporation and solvent diffusion method comparation.

interactions and increase encapsulation efficiency. Alternatively, purification methods may be employed to selectively remove non-incorporated substances from the NLC suspension, aiming to concentrate carriers with higher drug content and purity. Approaches to managing non-incorporated substances are tailored to meet specific objectives, such as maximizing drug loading capacity, improving stability profiles, or refining targeted drug delivery strategies within the context of NLC development.

## Application of Nanostructured Lipid Carrier on Wound Healing

Numerous studies have explored the incorporation of active ingredients into NLCs, highlighting the significant role these carriers play in wound drug delivery. The mechanism behind the enhanced wound healing facilitated by NLCs can be attributed to their superior bioadhesive properties. Supporting evidence comes from a study conducted by Saporito et al, which demonstrated that NLCs exhibited the highest level of bioadhesion compared to both control and unloaded drugs. Furthermore, this study revealed that NLCs demonstrated excellent biocompatibility, maintaining fibroblast viability within the range of 90–100%. This can be attributed to the sustained release mechanism of NLCs, ensuring that the drug content does not induce excessive toxicity in fibroblasts.[94] The impact of employing NLCs as nanocarriers in wound treatment has been assessed in vitro, in vivo, and in clinical studies, as summarized in Figure 7. Generally, NLCs have a

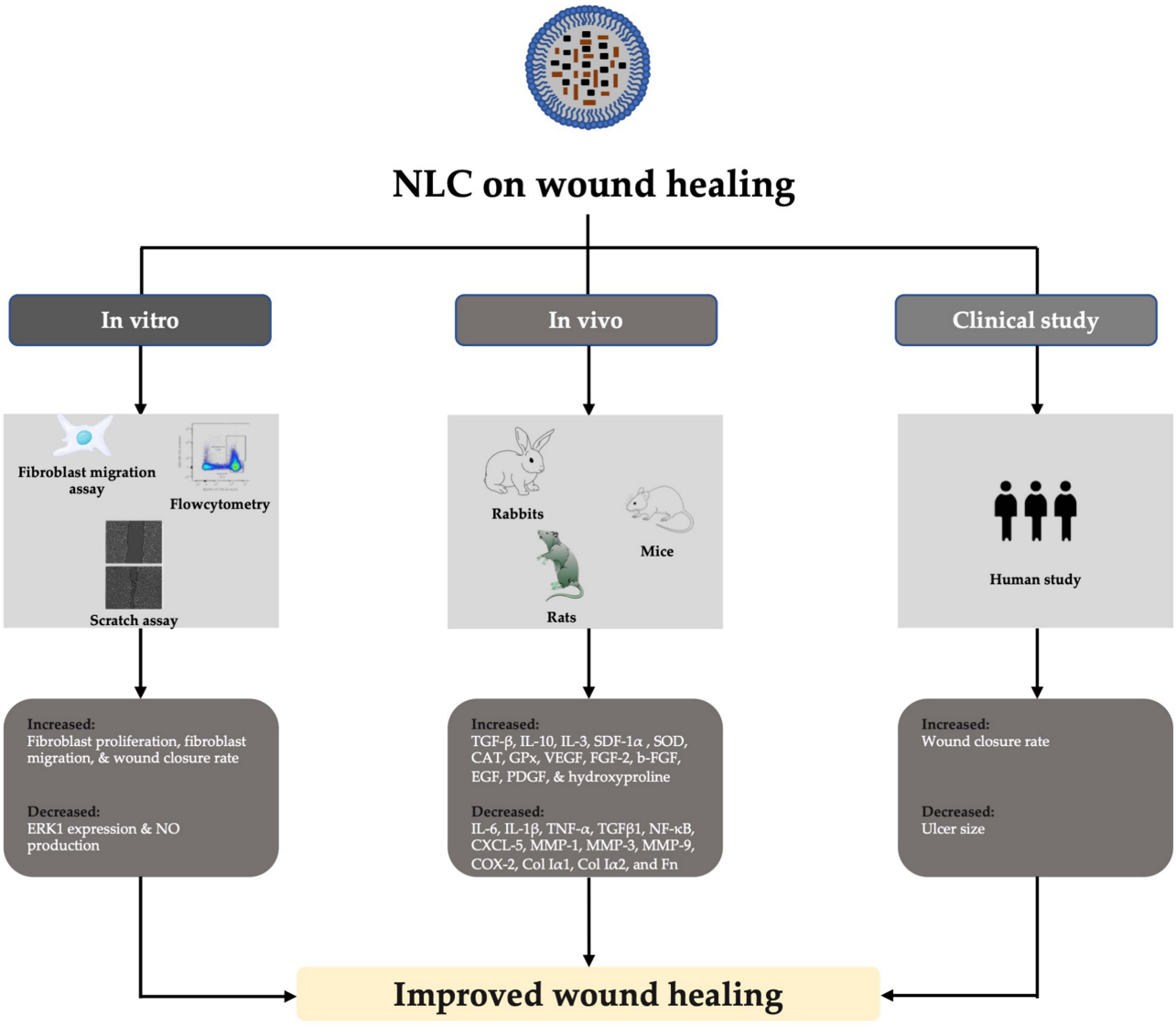



**Figure 7** Benefits of NLC on wound healing based on in vitro, in vivo, and clinical study.

significant influence on enhancing the effectiveness of active pharmacological agents. The application of NLCs for drug delivery in wound healing treatments is summarized in Table 2.

## In vitro Study

In vitro studies have been conducted using various pharmacologically active substances loaded into NLCs. NLCs have proven effective for delivering biologically active agents such as growth factors (eg, epidermal growth factor (EGF) and recombinant human epidermal growth factor (rhEGF)),[95,96] and nucleic acids (small interfering ribonucleic acid (Si-RNA)).[97] The methods employed for constructing biomolecule-loaded NLCs include double emulsion (w/o/w), ultrasonication, and emulsification followed by ultrasonication.[95–97] These methods are particularly suitable for biomolecule agents due to their thermosensitivity, as hot homogenization can degrade bioactive substances.[124] Conversely, thermal techniques are incompatible with the preparation of volatile agents such as essential oil-loaded NLCs, as they can vaporize during the process. An exception is found in the studies by Carbone et al and Costa-Fernandez et al, where a

**Table 2** NLC Development and Application on Delivering Active Agents for Wound Healing

| Experimental Setting | | Active Substance(s) | Method of Preparation | Results | Ref. |
|---|---|---|---|---|---|
| In vitro | Cell migration assay | 20(S)-Protopanaxadiol (PPD) | Emulsion-evaporation-solidification | ↑ HUVEC proliferation, ↑ HUVEC migration, ↑ Wound closure rate | [98] |
| | | Epidermal growth factor (EGF) and Curcumin (CUR) | w/o/w double-emulsion | ↑ Fibroblast proliferation, ↑ Fibroblast migration | [96] |
| | | *Eucalyptus* essential oil (EEO) and *Rosemary* essential oil (REO) | High shear homogenization | ↑ Fibroblast proliferation, ↑ Fibroblast migration | [94] |
| | | Ferulic acid (FA) and *Lavandula* essential oil (LEO) | Phase inversion temperature method | ↑ Fibroblast migration | [99] |
| | | Simvastatin (SV) | Solvent diffusion | ↑ HUVEC proliferation, ↑ HUVEC migration, ↑ Wound confluence rate | [93] |
| | | Tea tree oil (TTO) (antimicrobial agent) and α-tocopherol and quercetin (antioxidants) enriched with sodium alginate | Hot ultrasonication | ↑ Fibroblast migration | [100] |
| | Cellular evaluation of anti-inflammatory effect | Omega-3 fatty acids (OFA) and resveratrol (RES) | Ultrasound technique | ↓ NO production | [101] |
| | Diffusion cell experiment | Recombinant human epidermal growth factor (rhEGF) | Emulsification-ultrasonication | ↑ Availability of drugs until 48 h | [95] |
| | ERK1 expression quantification by flow cytometry method | Small interfering ribonucleic acid (Si-RNA) | Ultrasonication | ↓ ERK1 expression | [97] |
| | Scratch assay | Curcumin (CUR) | Hot emulsification method followed by probe sonication | ↑ Wound closure rate, ↑ Fibroblast migration | [102] |
| | | Melatonin (MEL) | Hot ultrasonication | ↑ Wound closure rate | [103] |
| | | Thymoquinone (TQ) | Hot high-pressure homogenization | ↑ Proliferation and migration of fibroblast, ↓ ROS | [104] |

(*Continued*)

**Table 2** (Continued).

| Experimental Setting | | Active Substance(s) | Method of Preparation | Results | Ref. |
|---|---|---|---|---|---|
| In vivo | Diabetic mice | 20(S)-Protopanaxadiol (PPD) | Emulsion-evaporation-solidification | ↑ Wound closure rate, ↓ TNF-α, ↓ IL-6, ↓ CXCL-5, ↑ VEGF, ↑ Collagen deposition | [98] |
| | | Recombinant human thrombomodulin (rhTM) | Hot homogenization | ↑ Wound closure rate, ↑ Granulation tissue, ↑ Collagen deposition, ↑ Angiogenesis | [105] |
| | Diabetic rats | Epidermal growth factor (EGF) and Curcumin (CUR) | w/o/w double-emulsion | ↑ Wound closure rate, ↑ SOD, ↑ CAT, ↑ GPx | [96] |
| | | Pioglitazone (PIO) | Solvent evaporation technique | ↑ Percentage reduction in wound, ↑ Hydroxyproline, ↓ MMP-9 | [106] |
| | | Zerumbone (ZER) | Hot, high-pressure homogenization | ↑ Wound closure rate, ↑ Hydroxyproline, ↓ IL-1β, ↓ IL-6, ↓ TNF-α, ↑ SOD, ↑ CAT, ↓ LPO | [107] |
| | Rabbit | Propolis extract (PE) | Emulsion-evaporation-solidification | ↑ Wound healing ratio | [108] |
| | Mice | *Caraway* essential oil (CEO) | Hot melt homogenization | ↓ Wound area, ↓ Bacterial colonization, ↓ Edema, ↑ Vascularization, ↑ Fibroblast proliferation, ↑ Granulation tissue, ↑ Collagen deposition, ↓ IL-1β, ↓ TNF-α, ↓ MMP-1, ↓ MMP-3, ↑ TGF-β, ↑ FGF-2 | [109] |
| | | LL37 peptide | Melt emulsification | ↑ Wound closure rate, ↑ Re-epithelialization, ↑ Collagen deposition, ↑ Death bacteria, ↓ TNF-α | [110] |
| | | *Mentha pulegium* essential oil (MEO) | Hot melt homogenization | ↓ Wound area, ↓ Bacterial colonization, ↑ Re-epithelialization, ↑ Collagen production, ↑ vascularization, ↑ Fibroblast infiltration, ↑ Granulation tissue, ↓ Edema, ↓ NF-κB, ↑ IL-10, ↑ TGF-β, ↑ b-FGF | [111] |
| | | Peppermint essential oil (PEO) | Hot melt homogenization | ↑ Wound contraction rate, ↑ fibroblast infiltration, ↑ Re-epithelialization, ↑ Collagen deposition, ↑ Death bacteria, ↑ EGF expression, ↑ FGF-2 expression | [112] |
| | | Rapamycin (RAPA) | Emulsification solvent diffusion and evaporation | RAPA-NLC exhibited promising corneal wound healing effect indicated by no corneal haze or opacity appearance until 21 days compared to healthy subjects<br>↔ Corneal opacity, ↓ TGFβ1, ↓ PDGF, ↓ Col Iα1, ↓ Col Iα2, ↓ Fn, ↓ VEGF | [113] |
| | | *Rosemary* essential oil (REO) | Hot melt homogenization | ↓ Wound size, ↓ Bacterial colonization, ↑ Re-epithelialization, ↑ Collagen production, ↑ vascularization, ↑ Fibroblast infiltration, ↑ IL-3, ↑ IL-10, ↑ VEGF, ↑ SDF-1α | [114] |
| | Rats | Allopurinol (AP) | Emulsion-sonication | ↑ Skin thickness increase | [115] |
| | | Cinnamon oil (CO) | High-pressure homogenization | ↓ Body weight, ↑ Wound contraction area | [116] |
| | | *Eucalyptus* essential oil (EEO) and *Rosemary* essential oil (REO) | High shear homogenization | ↓ Wound area | [94] |
| | | Pumpkin seed oil (PSO) | High-shear homogenization and ultrasonication | ↓ Ulcer index | [117] |
| | | Sesame oil (SO) and miconazole (MZ) | Hot homogenization followed by ultrasonication | ↓ Ulcer index | [118] |

(*Continued*)

**Table 2** (Continued).

| Experimental Setting | | Active Substance(s) | Method of Preparation | Results | Ref. |
|---|---|---|---|---|---|
| | | Simvastatin (SV) | High pressure/ shear homogenization | ↑ Wound closure rate, ↑ Vascularization, ↑ Re-epithelialization | [119] |
| | | Thymoquinone (TQ) | High-pressure homogenization | ↔ Acute hepatotoxic effect, ↑ Pharmacokinetic profile, ↑ Ulcer inhibition | [120] |
| | | Thymoquinone (TQ) and coconut oil (CCO) | Ultrasonication method | ↓ Ulcer index | [121] |
| | | Zerumbone (ZER) | High-pressure homogenization | ↓ Inflammatory cell infiltration and degeneration, ↑ Tissue granulation, ↑ IL-10, ↓ TNF-α, ↓ IL-6, ↓ COX-2 gene expression, ↑ Wound contraction | [122] |
| Clinical study | | Phenytoin (PHT) | Hot homogenization and ultrasonication | ↓ Ulcer size | [123] |
| | | Tretinoin | High-pressure homogenizer | ↓ Acne lesion (↓ black heads, ↓ whiteheads, and ↓ papules) | [78] |

**Notes**: ↑ indicates an increase in the parameter value, ↓ indicates a decrease in the parameter value, ↔ indicates no change in the parameter value.
**Abbreviations**: AP, allopurinol; bFGF, basic fibroblast growth factor; CAT, catalase; CCO, coconut oil; CEO, *Caraway* essential oil; CO, cinnamon oil; Col Iα1, collagen type Iα1; Col Iα2, collagen type Iα2; COX-2, cyclooxygenase 2; CUR, curcumin; CXCL-5, C-X-C motif chemokine ligand 5; DFU, diabetic foot ulcers; ECM, extracellular matrix; EEO; *Eucalyptus* essential oil; EGF, epidermal growth factor; EPA, eicosapentaenoic acid; ERK1, extracellular signal regulated kinase 1; FA, ferulic acid; FGF-2, fibroblast growth factor 2; Fn, fibronectin; GPx, glutathione peroxidase; HLB, hydrophilic-lipophilic balance; HUVEC human umbilical vein endothelial cell; IL-1, interleukin 1; IL-10, interleukin 10; IL-1β, interleukin 1β; IL-3, interleukin 3; IL-6, interleukin 6; IL-8, interleukin 8; LEO, *Lavandula* essential oil; LPO, lipid peroxidation; MEL, melatonin; MEO, *Mentha pulegium* essential oil; MMP-1, matrix metalloproteinase 1; MMP-3, matrix metalloproteinase 3; MMP-9, matrix metalloproteinase 9; MZ, miconazole; NF-κB, nuclear factor κB; NLC, nanostructured lipid carrier; NO, nitric oxide; OFA, omega-3 fatty acids; PDGF, platelet-derived growth factor; PEO, peppermint essential oil; PE, propolis extract; PHT, phenytoin; PIO, pioglitazone; PIT, phase inversion method; PPD, 20(S)-Protopanaxadiol; PSO, pumpkin seed oil; RAPA, rapamycin; REO, *Rosemary* essential oil; RES, resveratrol; rhEGF, recombinant human epidermal growth factor; rhTM, recombinant human thrombomodulin; ROS, reactive oxygen species; SDF-1α, stromal cell-derived factor 1α; Si-RNA, small interfering ribonucleic acid; SLN, solid lipid nanoparticle; SOD, superoxide dismutase; SO, sesame oil; SV, simvastatin; TGF-α, tumour growth factor α; TGF-β, tumour growth factor β; TGF-β1, tumour growth factor β1; TNF-α, tumour necrosis factor α; TPGS, d-α-tocopheryl polyethylene glycol 1000 succinate; TQ, thymoquinone; TTO, tea tree oil; VEGF, vascular endothelial growth factor; ZER, zerumbone.

high-temperature method was employed. For instance, in the manufacturing of tea tree oil-loaded NLCs, tea tree oil was added at 34°C after the melting process at 40°C, preventing the essential oil from evaporating during mixing.[99,100] Similarly, Lavandula essential oil was used in the phase inversion temperature (PIT) method, which involved low energy levels at temperatures of 65–73°C, preventing essential oil degradation during the inversion process.[99] Other agent-loaded NLCs can be developed using high-pressure homogenization, high-shear homogenization, hot emulsification, and solvent diffusion, either followed by or without ultrasonication.[93,94,98,102,104]

Generally, in vitro assays for evaluating the wound-healing effectiveness of NLC preparations have involved cell migration assays, scratch assays, and biomarker expression analyses. The migration cell assay, or scratch assay, assesses wound healing performance based on the ability to induce cell migration on a two-dimensional surface.[125] Some cell lines used in performing these assays include normal human dermal fibroblasts (NHDF), mouse embryonic fibroblasts (NIH-3T3), murine fibroblasts (CCL-3T3), human umbilical vein endothelial cells (HUVEC), and human epidermal keratinocytes (HaCaT).[93,94,96,98,99,104] Effectiveness is measured by the degree of gap closure over a certain time (% wound closure) from the initial scratch area. These assays are performed using fibroblasts, keratinocytes, and endothelial cells. For instance, Tezgel et al evaluated wound healing effectiveness based on its impact on the expression of ERK1 (extracellular signal-regulated kinase 1), a biomarker of the healing process.[97] Inhibition of ERK1 can suppress the inflammatory process, thereby enhancing wound recovery.[126] Gainza et al assessed effectiveness by examining the drug's residence time over approximately 48 hours, given that the preparation is intended as a wound dressing. Residence time was determined through cell diffusion tests using Franz cells, with pharmacological agents evaluated after the diffusion process at specified time points.[95] Additionally, the inhibition of nitric oxide (NO) production, which suppresses inflammatory activity, can serve as a valuable predictor of wound healing efficacy.[127]

Based on in vitro evaluations, NLCs as nanocarriers have demonstrated remarkable efficacy in enhancing wound treatment. Results from cell migration or scratch assays consistently showed that NLCs loaded with various active pharmacological agents exhibited superior wound closure rates compared to control groups and even unloaded active agents or blank NLCs. Notably, Si-RNA-loaded NLCs demonstrated exceptional effectiveness in wound closure, surpassing unloaded Si-RNA and control groups, as evidenced by ERK1 expression levels.[97] NLCs also exhibited prolonged effects due to their controlled release properties, making them suitable for use as wound dressings.[95] Recent studies have shown that NLC systems can effectively encapsulate propolis in combination with α-mangostin, a xanthone isolate compound from mangosteen pericarp.[14,15,128] These findings underscore the NLC system's significant loading capacity for various types of drugs, even though they were not directly confirmed for wound healing treatment. In vitro test results indicated that NLC preparations containing the combination of propolis and α-mangostin produced a significant antioxidant effect compared to unloaded drugs and controls ($p < 0.01$).[14,129,130]

## In vivo Study

Preclinical studies evaluating pharmacologic agent-loaded NLCs for wound treatment were conducted using various animal models, including mice, rats, and rabbits.[108,109,115] These NLCs were applied to both acute and chronic wounds, as indicated by the use of a diabetic animal model. The effectiveness of wound healing was assessed based on parameters such as the percentage of wound closure or reduction in wound area after administering the preparation for a specified period. In cases of ulcer conditions, including diabetic ulcers or gastric ulcers, effectiveness was determined by the preparation's ability to reduce the ulcer index value.[131] The ulcer index is a standardized method for evaluating the presence of lesions, bleeding, and erosions, typically scored on a scale of 0–5 points.[117,118,132] Additionally, the reduction in the ulcer area served as an indicator of ulcer improvement. Preclinical studies also assessed the wound healing effect on animals with corneal injuries.[113] The clarity of the cornea, correlated with lower or absent neovascularization activity, was indicative of the healing ability of the samples in corneal repair.[113] In light of the correlation between wounds and inflammatory conditions, which play a pivotal role in wound healing acceleration, the analysis of inflammatory markers emerged as a valuable indicator of wound healing. The regulation of cytokines and chemokines, in particular, was closely monitored to assess inflammatory activity, which indirectly influences the rate of wound healing.[133] Moreover, antioxidant activity was examined as an indicator of the body's ability to counteract reactive oxygen species (ROS), which can exacerbate wounds and hinder the healing process.[134] Increased expression of growth factors was also a significant focus, as these factors promote the growth and repair of damaged cells and tissues.[49]

Furthermore, in vivo studies demonstrated that the application of NLCs as nanocarriers for wound treatment showed promising potential. Generally, the application of NLCs led to a reduction in inflammatory factors, as indicated by increased levels of TGF-β, IL-10, IL-3, and SDF-1α, along with decreased levels of IL-6, IL-1β, TNF-α, TGFβ1, NF-κB, CXCL-5, MMP-1, MMP-3, MMP-9, COX-2, Col Iα1, Col Iα2, and Fn.[98,106,107,109,111–114,122] Additionally, antioxidant activity increased, as evidenced by elevated levels of SOD, CAT, GPx, and reduced LPO.[96] Furthermore, the growth factors, including VEGF, FGF-2, b-FGF, EGF, and PDGF, were upregulated due to the modulatory role of NLCs.[98,106,107,109,111–114,122] Notably, histopathological examinations of wounds revealed significant repair, as demonstrated by increased levels of hydroxyproline (collagen).[106,122] Furthermore, the delivery of biologic agents such as the LL37 peptide, which has an immunomodulatory effect by inactivating macrophages, resulted in a dose-dependent and superior wound closure rate compared to controls.[135] NLCs were found to be highly compatible with the delivery of various active compounds in the form of essential oils, whether prepared using non-thermal or low thermal energy methods. The nanomodification strategy employed was also effective in optimizing the wound healing ability of anti-inflammatory agents such as thymoquinone and zerumbone,[107,120–122] as well as antibacterial agents like rapamycin.[113] Additionally, NLCs were found to be suitable for enhancing the wound healing properties of extract-based agents like propolis, which contains various antioxidant and anti-inflammatory compounds such as flavonoids and lipid glue-like components.[108] An intriguing discovery was made in a study by Örgul et al, which demonstrated an enhanced wound healing effect of simvastatin, an agent unfamiliar for topical application. NLCs were believed to play a significant role in enhancing the angiogenic properties of simvastatin, resulting in increased vascular endothelial growth factor (VEGF) activity.[119,136]

## Clinical Trial

Clinical studies evaluating the effectiveness of drug delivery using NLCs are still quite limited, with only two study conducted by Samadi et al and Motawea et al utilizing tretinoin (TRE) and phenytoin (PHT), respectively, as the active compound loaded into NLCs.[123] These two studies similarly employed a prospective double-blinded randomized controlled design. Tretinoin, as an anti-acne agent, has been proven to be more effective in acne treatment when modified into an NLC system. The use of tretinoin significantly decreased skin lesions caused by acne vulgaris, including blackheads, whiteheads, and papules. After 8 weeks of treatment, the differences were significant compared to unmodified tretinoin (p value $< 0.001$). Besides its efficacy, tretinoin-loaded NLCs were proven safe in a Phase 1 clinical study, demonstrating that this system is typically safe for short-term application.[78]

In Motawea et al's study, phenytoin, the chosen active compound, was selected due to its established wound healing properties. The mechanism of action of phenytoin in wound treatment is believed to involve nerve restoration,[137] although other potential mechanisms include the stimulation of collagen deposition, enhancement of fibroblast proliferation, glucocorticoid inhibition, and antibacterial action.[123] In this study, the loaded drugs were prepared in a hydrogel formulation (0.5% w/v) and subsequently evaluated in patients with diabetic foot ulcers (DFUs). Following twice-daily application for 8 weeks, patients using PHT-loaded NLCs exhibited the highest percentage of wound healing ($95.82 \pm 2.22$) compared to those using unloaded PHT ($47.10 \pm 4.23$) and blank ($-34.91 \pm 28.33$) formulations ($p < 0.001$).[123] Several factors likely contributed to this advantage, including the small particle size, which enhances solubility and increases surface contact area, as well as the lipid components that facilitate improved drug penetration into the subcutaneous layer of the skin. However, it's important to acknowledge that this study had certain limitations, primarily its relatively small sample size. Therefore, conducting larger-scale clinical studies is highly recommended to further validate the efficacy of NLCs as nanocarriers in wound treatment.[123]

# Challenge and Limitation

The utilization of nanostructured lipid carriers (NLCs) technology for topical wound healing is fraught with various challenges and obstacles. One significant issue is the regulatory framework governing the development of nano-scale pharmaceuticals. Guidelines for testing and quality assurance processes are typically designed for simple, unmodified drugs. The inclusion of modifications within the NLCs system necessitates the standardization of methods, instrumentation, and specifications in the quality assurance process.[138] Furthermore, different formulations and manufacturing methods can introduce variability in the physicochemical properties of the preparations, thereby requiring specific testing methods for each. Additionally, the incorporation of extra materials, processes, and energy to develop preparations within the NLC system inevitably increases production costs, leading to higher product prices. This presents a challenge for developers to carefully balance efficacy and cost, ensuring that the final product remains attractive and affordable to the target users.[139]

The application of NLCs also faces limitations based on the materials used in their formulation. For instance, the use of polysorbate 80 (Tween 80) as a stabilizer is restricted to preparations intended for adult patients, as it poses a risk of skin irritation in pediatric patients.[140] Consequently, formulators must conduct preformulation studies to ensure the compatibility of the materials used in each development. Additionally, given that NLC systems are typically oil-in-water emulsions,[14] it is challenging to formulate drug-loaded NLCs into solid dosage forms. Techniques such as freeze-drying can be employed to obtain powder forms from NLC systems; however, these methods only yield a small amount of powdered product.[141] Therefore, further development of techniques to convert liquid NLCs into solid forms is essential to maximize the potential of NLCs as a drug delivery modification system.

# Future Perspective

NLCs represent highly valuable nanocarrier systems in the realm of drug delivery for wound treatment. The composition of solid lipids and liquid lipids within these systems enables the delivery of hydrophobic compounds and enhances the permeability of the formulation through mucosal and dermal surface membranes.[142] The utility of NLCs extends beyond chemical compounds to encompass the delivery of biologically active substances, such as EGF, rhEGF, rhTM, and siRNA.[95–97,105] Moreover, NLCs for wound treatment can be formulated using various suitable methods, each offering

distinct advantages. Nevertheless, despite the demonstrated effectiveness of NLCs in enhancing the wound healing potential of delivered compounds, there is still a dearth of discussion regarding aspects such as biodistribution, toxicity, and the underlying molecular mechanisms of NLCs systems. Furthermore, clinical studies investigating the application of NLCs in wound treatment remain scarce and typically involve small study populations. Consequently, there is an imperative need for additional clinical studies, especially those involving larger and more diverse populations, to substantiate the efficacy of NLCs as nanocarriers for drug delivery in the context of wound healing.

## Conclusion

Nanostructured lipid carriers (NLCs) present substantial advantages in enhancing the therapeutic efficacy of pharmacologically active substances for wound treatment. Numerous studies have validated the effectiveness of NLCs in delivering hydrophobic chemical compounds, essential oils, crude extracts, and biologically active substances, such as proteins and nucleic acids, for wound healing purposes. NLCs improve the stability, bioavailability, and controlled release of these substances, thereby promoting wound repair and regeneration more effectively. The adaptability of NLC formulations is reflected in the variety of solid and liquid lipids, as well as surfactants used, and the diverse preparation methods available, including high-pressure homogenization, solvent evaporation, and microemulsion techniques. These methods enable the customization of NLCs to address specific therapeutic requirements and optimize the delivery of various pharmacologically active compounds. Since NLCs provide sustained drug release, they are also particularly beneficial for chronic wounds that require long-term drug availability. Nevertheless, further research is necessary to explore the use of NLCs in wound treatment with a broader array of pharmacologically active substances, including newer synthetic and natural agents. Additionally, comprehensive safety evaluations are essential to ensure the secure application of NLCs, especially for chronic wounds, burns, and diabetic ulcers. This involves assessing the long-term effects, potential toxicity, and biocompatibility of NLCs to confirm their safe and effective use in clinical settings.

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## Disclosure

The authors report no conflicts of interest in this work.

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