Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Review
Volume 13, Issue 5, 2023, 426
https://doi.org/10.33263/BRIAC135.426

Applications of Nanotechnology-Based Drug Delivery System for Delivering Natural Products into Acute and Chronic Wounds: A Review

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Scopus Author ID 25959283200
Received: 26.07.2022; Accepted: 20.09.2022; Published: 15.12.2022

Abstract: Improper treatment of wounds and certain pathophysiological conditions may lead to the onset of chronic injuries. This condition leads to the need for an effective approach to wound treatment. Natural products are a potential alternative for wound treatment. Natural products can help wound healing with their anti-inflammatory, antioxidant, and antimicrobial activities. The issue of natural products, especially high hydrophobicity, poor stability, and the possibility of irritation, can be overcome by formulating them into a nanotechnology-based drug delivery system to increase their efficacy. In addition, nanotechnology-based drug delivery systems can provide ideal dressing characteristics for wound healing, including providing occlusive properties to wounds, improving the stability of natural products, enabling the controlled release of natural products, and encouraging better interaction between natural products and biological targets. Therefore, this study aims to determine the healing effects of natural products once they are included in nanotechnology-based drug delivery systems. Through studies conducted, it is known that nanotechnology-based drug delivery systems can improve the healing effect of wounds with natural products. The combination of the two can be used as an alternative solution to wound healing problems, especially chronic wounds.

Keywords: wound healing; natural product; drug delivery system; nanotechnology.

1. Introduction

Damage to a person's skin will lead to loss of skin function in maintaining the physicochemical homeostasis and body temperature, storing essential body nutrients, defense against microbes, and responding to trauma or injury [1,2]. Wounds can be divided into two categories, namely acute and chronic wounds. Acute wounds often occur due to external injuries such as exposure to sharp objects, scorching temperatures, electric shocks, or corrosive chemical compounds that can heal through an orderly process in a relatively short time. On the other hand, if wound healing fails to occur regularly and takes a relatively long time (up to months or even years), the wound can be classified as chronic [3,4].

Chronic wounds are most commonly associated with pathophysiological disorders that ultimately lead to local tissue damage and the formation of skin ulcers, such as diabetic foot ulcers. Persistent and increased inflammatory cell activity is considered a critical factor in the pathogenesis of chronic wounds. Chronic wounds may reduce a patient's quality of life due to

the pain of an unhealed wound and the amount of money required for its treatment [5]. In addition, the incompatibility of wound treatment can inhibit the healing process, leading to infection and chronic wounds. Wound treatment usually involves preventing infection because the skin is damaged as a body protector. However, topical antimicrobials such as silver particles, classified as heavy metals, have serious side effects and are less effective in wound healing [6]. This condition leads to the need for a more effective approach to wound treatment.

Natural products, especially those derived from plants, are up-and-coming as an alternative to wound care because they have a variety of beneficial biological activities, are relatively safer, easier to obtain, and have competitive prices [7]. However, using natural products in wound healing applications is less effective because most of them have high hydrophobicity, low solubility in water, extensive metabolism, limited penetration to the targets, poor stability, and irritatable, thus limiting their usage [8]. Natural product formulations can be developed to address these issues.

Nanotherapeutic-based approaches can address existing natural product problems and can provide ideal wound dressing characteristics [9,10]. For example, with their submicron size, nanotechnology-based drug delivery systems can provide ideal wound dressing characteristics for delivering natural products. In addition, they can give occlusive properties, improve product stability, enable the controlled release of active substances, and encourage better interaction with biological targets [4].

This study discusses the pathophysiology of wounds, the causes of chronic wounds, the role of natural products in accelerating wound healing, and the usage of nanotechnology-based drug delivery systems for improving the healing effects of natural products. In addition, this study aims to determine the healing properties of natural products before and after they are included in nanotechnology-based drug delivery systems.

2. Acute and Chronic Wound Healing

Maintaining body homeostasis is very important for an organism to survive. Therefore, when damaged, the skin has an effective repair mechanism to restore its normal anatomical structure and function [11]. The acute wound healing process is generally divided into four phases, including hemostasis, inflammation, proliferation, and remodeling phase, that occur overlappingly and involve complex interactions of various components [12,13].

2.1. Hemostasis phase.

Hemostasis is the initial stage that initiates the wound-healing process. At this stage, vasoconstriction, platelet aggregation, and blood clotting occur immediately after the body detects injury, involving a series of coagulation events (fibrin formation) initiated by platelets to stop the bleeding. Platelets become the first component to enter the site of injury. They will be activated by thrombin to release growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), endothelial growth factor (EGF), and fibroblast growth factor (FGF), which will drive the inflammatory processes [5]. These growth factors act as chemotactic molecules to attract white blood cells, macrophages, endothelial, and fibroblast cells [10].

2.2. Inflammatory phase.

The tissue then enters the inflammatory phase to prevent microbial infection at the injury site by forming immune protection [10]. This phase occurs immediately after the hemostasis phase and is characterized by the transmission of inflammatory agents and the migration of cells from the blood vessels to the injury site. Mastocyte cells release inflammatory mediators such as prostaglandins, histamine, and leukotrienes that stimulate angiogenesis and the permeability of blood vessels so that cells and molecules present in the bloodstream can enter the injury site. White blood cells, consisting of neutrophils, monocytes, and lymphocytes, will then occupy the injury site. Neutrophils will fight microbial infections; stimulate angiogenesis by removing TGF-β, vascular endothelial growth factor (VEGF), and FGF; and produce tumor necrosis factor-alpha (TNF-α) that will destroy damaged tissue and facilitate the proliferation of fibroblasts that deposit collagen for tissue granulation [5]. Monocytes will differentiate into mature macrophages that can phagocytize bacterial fragments, contain several growth factors and pro-inflammatory cytokines, and activate endothelial, fibroblast, and keratinocyte cells [10].

2.3. Proliferation phase.

The proliferation phase involves different activities, including angiogenesis by endothelial cells, the formation of granulated tissue by fibroblast cells, and re-epithelialization by keratinocyte cells [14]. Fibroblasts proliferate and produce large amounts of extracellular matrices, such as type III collagen, to form granulated tissue that replaces the damaged tissue [15]. The formation of a new extracellular matrix will replace the matrix formed during hemostasis through degradation by the matrix metalloproteinase (MMP) [16]. Fibroblasts also differentiate into myofibroblast phenotypes during the tissue granulation process with the addition of cytoskeleton alpha-smooth muscle actin (α-SMA), which plays a vital role in wound closure. Tissue re-epithelialization occurs when the wound is covered by new epidermal tissue through a keratinocyte migration, proliferation, and differentiation that provides a superficial covering and protects the tissue underneath from subsequent injury [5].

2.4. Remodelling phase.

The final phase of the wound healing process is the remodeling phase. When the wound has been closed, the granulated tissue with an irregular extracellular matrix arrangement will be actively repaired. Fibroblasts will be reduced through apoptosis mechanisms [17]. Type III collagen will be replaced by a more organized type I collagen mediated by PDGF and TGF-β [5]. The result of wound healing is scarring (fibrosis). Scar tissue has a different texture and biomechanical and functional properties that are reduced compared to normal tissue [18].

2.5. Chronic wound healing.

Wounds that refuse to heal regularly and take a relatively long time to heal are classified into chronic wounds. Chronic wound formation is associated with four factors: local tissue hypoxia, bacterial colonization, recurrent ischemia conditions, and altered cellular and systemic pressure responses [9]. Chronic wounding often occurs in patients suffering from pathophysiological disorders, such as diabetic patients [19]. The state of diabetes affects the entire healing phase of the acute wound, and the causes are multifactorial [9,20]. In diabetic conditions, where the glucose level in the blood fluctuates, there will be abnormalities in

endothelial cell function and smooth muscles due to a decrease in the endothelial vasodilators, causing arterial constriction that leads to peripheral artery disease.

Furthermore, atherosclerosis, thickening of blood capillaries, and hardening of the artery walls will cause a blockade of the major arteries, leading to ischemia [5]. Hyperglycemia can also increase oxidative stress on nerve cells, causing neuropathy and affecting sensory, motor, and autonomic nerves [21]. Peripheral artery disease, ischemia, and neuropathy can cause local hypoxia, increasing the inflammatory response due to increased oxygen radicals [16]. Wounds that a diabetic patient suffers tend to maintain the chronic inflammatory phase without progressing towards the proliferation and remodeling phase [22], thus set-backing the whole process.

3. Wound Treatment by Natural Products

In recent years, there has been a significant increase in the use of natural products that have the potential to cure various types of skin problems, one of which is wound healing. Natural products provide a higher level of safety in chronic wound healing compared to chemical drugs at a more competitive price [23,24].

Acute wounds are able to heal with or without the treatment of the wound. Natural products can help the acute wound to heal with their activity as an antimicrobial that can prevent infection because it is known that infected wounds will be more difficult to heal. The antioxidant activity of natural products can also help reduce tissue damage caused by oxygen radicals. Furthermore, tissue regeneration activity from natural products can accelerate the migration and proliferation of cells that play a role in the wound-healing process [5].

Chronic wounds undergo a prolonged inflammatory phase and do not lead to recovery. In the inflammatory phase, biologically active mediators will attract white blood cells such as neutrophils, leukocytes, and monocytes to the injury site and then attack microorganisms and foreign materials through phagocytosis mechanisms that lead to the production of reactive oxygen species (ROS). ROS, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals, and other reactive oxygen derivatives, is deadly and causes widespread damage to proteins, DNA, and lipids, thus affecting normal cellular function. Because high amounts of ROS can damage cells by oxidizing lipids and proteins, their levels are tightly controlled by the presence of ROS-breaking enzymes and small-molecule antioxidants [5]. A wide variety of anti-inflammatories and antioxidants can control inflammation and free radicals during chronic wound healing, one of which is anti-inflammatory and antioxidant from natural ingredients [8,25]. Chronic wound healing is often a disorder with delayed healing time, generally associated with microbial infections. Contamination by pathogens in wounds can evolve into bacterial colonization, leading to localized and even systemic infections, sepsis, and even multiorgan dysfunction. The presence of biofilms leads to prolonged inflammation due to the stimulation of cytokines and free radicals. To promote healing, effective treatment is necessary to deliver antimicrobial drugs to infected wounds [5,26]. The use of natural products such as plant-derived essential oils is considered safer than synthetic products such as silver particles, which is a heavy metal often used as an antimicrobial [27,28]. Anti-inflammation, antioxidant, and antimicrobial activities of natural products become important in chronic wound healing [29,30].

4. Nanotechnology-based Drug Delivery System in Wound Healing

The nanotherapeutic approach is very promising as a solution for the wound healing process because it can achieve ideal wound dressing characteristics. In addition, a delivery system with a larger surface area to volume ratio allows the encapsulation of more active compounds to accelerate cell regeneration. In general, nanotechnology-based wound healing methods provide advantages such as facilitating local delivery of drugs, providing occlusive effects, cell specificity, and controlled release of drugs over a period that can increase the accumulation of active substances and accelerate wound healing [5,31]. Each particular form of the delivery system also owns other advantages. Thus, wound healing treatment involving a nanotherapeutic approach to the delivery of active compounds opens up opportunities to overcome the complexity of chronic wound healing [19,32]. This study discusses the nanotechnology-based drug delivery systems, focusing on liposomes, lipid nanoparticles, nanofibers, polymeric nanoparticles, nanohydrogels, and nanoemulsions loaded with natural products.

Nanomaterials can encourage and initiate the healing of dermal and epidermal wounds based on biological processes involving different stages and cell types [33]. Nanotechnology-based drug delivery systems possess a good potential to improve the pharmacological effects of natural products and drug molecules [23,34]. Furthermore, nanoparticle-sized drug delivery systems can be incorporated into nanofibers, hydrogels, foams, films, and other bases as nanocomposite systems, creating a new concept in wound dressing that can encourage wound healing. Such dressing can increase the surface porosity-to-volume ratio. Its structure can simulate endogenous extracellular matrix structure, allowing attachment and spread of fibroblast and keratinocyte cells, thereby facilitating collagen synthesis and wound epithelialization [5]. The types and advantages of nanotechnology-based drug delivery systems that can be used as a carrier of natural products for improving the wound healing process are shown in Figure 1.

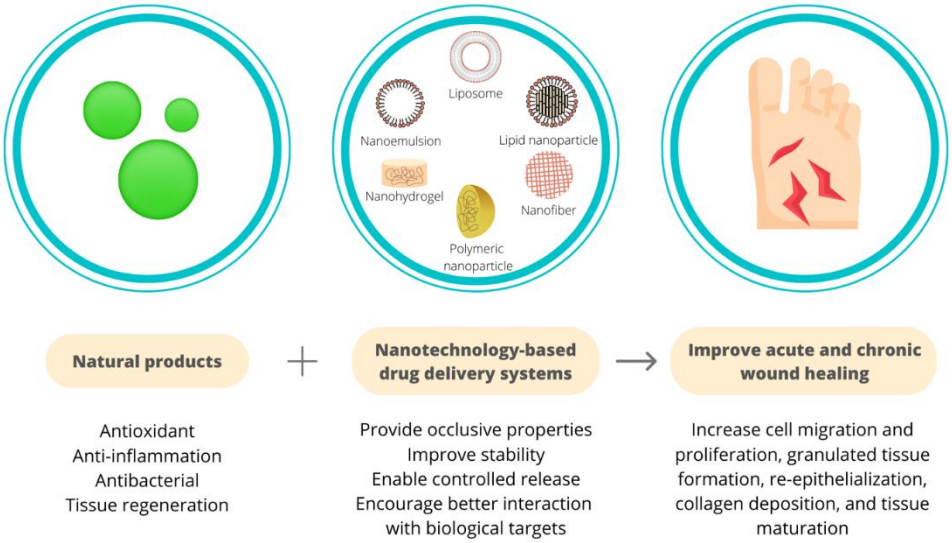

Figure 1. The types and advantages of nanotechnology-based drug delivery systems for delivering natural products to the wound site.

5. Liposome Containing Natural Products

Liposomes are spherical vesicles formed through surfactant hydration, such as phospholipids, mixed with water under low shear force [35]. Liposomes are used as a drug

delivery system for wound healing because they have many advantages, such as good biocompatibility, low toxicity, increased permeability, and the ability to deliver hydrophilic and hydrophobic drugs [36]. Liposomes can protect encapsulated drugs and provide a sustained release of the drug. In addition, liposomes effectively close the wound and create a moist environment on the wound's surface after application [4]. The wound-healing effects of some natural products before and after being included in liposomes with various test models are summarized in Table 1.

Table 1. Wound healing effects of natural products before and after encapsulation with liposomes.

Natural products	Test model	Results on control	Healing effects of natural products		Ref.
			Before encapsulation	After encapsulation	
Curcumin	Inflammation and ulceration of the mice skin by TPA induction	Showed an area of lesion that increased over time	Reduced skin damage compared to controls ($p>0.05$); however, the skin appeared dry and crusted	Prevented and reduced skin damage completely compared to controls ($p<0,05$)	[37]
Madecassoside	The burned wound on Sprague Dawley's rats	Showed wound healing but was not completely covered on day 12	Showed improvements in wound healing compared to controls ($p>0.05$), but remained not fully covered on day 12	Showed significant improvements in wound healing compared to controls ($p<0.001$) and madecassoside solution ($p<0.01$), and the wound was completely covered on day 12	[38]
Aloe vera leaf gel extract	Proliferation in fibroblast and keratinocyte cell cultures	Showed cell proliferation and type I collagen synthesis	Showed an increase in cell proliferation of up to 60% ($p<0.05$) and type I collagen synthesis by 4% ($p>0.05$) compared to controls	Showed an increase in cell proliferation by 101% ($p<0.05$) and type I collagen synthesis by 23% ($p<0.05$) compared to controls	[39]
Epigallo-catechin gallate	The burned wound on mice and infected with MRSA	Showed a specific CFU value in the organ evaluated and 100% mice population died after 10 days	Showed a decrease in CFU values in the organs evaluated compared to controls ($p>0.05$) and an increase in survival by 40%	Showed a significant decrease in CFU values in the organs evaluated compared to controls ($p<0.001$) and an increase in survival by up to 100%	[40]

CFU = colony-forming unit; MRSA = methicillin-resistant *Staphylococcus aureus*; TPA = 12-O-tetradecanoylphorbol 13-acetate

The results showed that natural products could provide a better wound healing effect than control. Furthermore, when these natural products were included in liposomes, there was a significant increase in the wound-healing effect compared to the controls. Manca *et al.* (2015) explained that the increased healing effect of curcumin wounds after being included in hyalurosomes (liposomes added with hyaluronic sodium in its formulation) could be attributed to the synergistic effect of all three components of the formulation (curcumin, phosphatidylcholine, and hyaluronic sodium) that allow the vesicles to survive in the application site, thus facilitating the penetration of curcumin into tissues and cells. In addition, the drug delivery ability of phospholipid vesicles that provide a continuous release and increase drug deposition in the affected tissues may support the therapeutic activity of hyaluronic sodium and curcumin [37]. Similar observations were also found in the study by Li *et al.* (2016), where liposomes can improve the delivery of madecassoside in wounds due to the fluidity of their membranes and can deposit madecassoside in wound tissue, thus accelerating the healing process [38]. Takahashi *et al.* (2009) research suggests liposomes may improve aloe vera leaf gel extract's ability to encourage fibroblast and keratinocyte cell proliferation and type I collagen synthesis. Liposome particle size plays an important role in the speed of

absorption into the cell, especially liposomes measuring about 200 nm in size. This can encourage membrane fusion with the target cells and efficiently deliver the drug into the cells [39]. Liposomes may also increase the antimicrobial activity of natural products in infected wounds, as shown in the Gharib *et al.* (2013) study, especially positively charged liposomes. It may be attributed to the electrostatic interactions between the outer membrane of bacteria and cationic liposomes that can increase the entry mechanism of the natural products into the bacterial cells [40].

6. Lipid Nanoparticles Containing Natural Products

Lipid nanoparticles are represented as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). SLNs are made from one type or a mixture of solid lipids. The advantages that SLNs have include: (1) enabling controlled drug release and drug targeting; (2) avoiding the use of organic solvents; (3) being able to encapsulate drugs that are lipophilic or hydrophilic; (4) allowing large-scale production and sterilization; (5) safe and non-toxic; and (6) improving the stability of the drug [35]. NLCs are the latest generation of lipid nanoparticles in which liquid lipids are added to reduce the crystal structure. NLCs exhibit some advantages that SLNs do not possess, and these advantages can minimize or prevent several problems related to SLNs, including: (1) the load capacity for some drugs that are too low; (2) the release of the drug from the encapsulation system during storage; and (3) high water content at SLNs dispersion [41,42].

Lipid nanoparticles are widely considered a suitable delivery system for improving wound healing because they have occlusive properties that can improve skin hydration and increase drug penetration [43]. Lipid nanoparticles are an interesting strategy for treating chronic wounds because they can reactivate the wound-healing process [44]. The wound-healing effects of several natural products before and after being included within lipid nanoparticles with various test models are summarized in Table 2.

Table 2. Wound healing effects of natural products before and after encapsulation with lipid nanoparticles.

Natural products	Test model	Results on control	Healing effects of natural products		Ref.
			Before encapsulation	After encapsulation	
Tetrahydro-curcumin	Excision wound on mice	Showed the delayed healing process of the wound	Showed a decrease in wound size compared to controls	Showed a significant decrease in wound size compared to controls	[45]
Thymol	Acute inflammation of the mice's skin by induction of croton oil	Showed inhibition of inflammation almost 100% (positive control of betamethasone)	Showed inhibition of inflammation by ± 30% compared to controls ($p<0.001$)	Showed inhibition of inflammation by ± 60% compared to controls ($p<0.01$)	[46]
Calendula extract	Scratch/migration test on conjunctiva epithelial cells	Showed scratch closure after 48 hours	Increased scratch closure by 20% compared to controls ($p<0.05$) after 48 hours	Increased scratch closure by up to 56% compared to controls ($p<0.05$) and only extracts ($p<0.01$) after 48 hours	[47]
Ferulic acid	Scratch/migration test on fibroblast cells	The scratch was closed after 48 hours, but a residual scar was still observed	The scratch was not closed after 48 hours and showed worse results compared to the controls	The scratch was closed after 48 hours, and no residual scar was observed	[48]
Rosemary oil	Excision wound infected with *P. aeruginosa* and *S. aureus* on mice	The wound was not completely covered until day 12	The wound closed completely compared to the controls ($p<0.05$) on day 12	The wound closed perfectly on day 12 but did not differ significantly from rosemary oil, not in formulation ($p>0.05$)	[49]

Natural products	Test model	Results on control	Healing effects of natural products		Ref.
			Before encapsulation	After encapsulation	
Astragalosid e IV	Excision wound in Sprague Dawley rats	The wound almost completely closed on day 12	The wound almost completely closed on day 12 but did not differ significantly from the controls ($p>0.05$)	The wound almost completely closed on day 12 and differed significantly from the controls ($p<0.05$)	[50]
Thymoquin one	Scratch/migration test on fibroblast cells 3T3 (normal) and 3T3-L1 (diabetes)	Showed scratch closure	Showed better migration in 3T3 cells compared to controls ($p<0.001$) but did not differ significantly from NLCs formulation ($p>0.05$)	Showed better migration in 3T3-L1 cells compared to controls ($p<0.001$) and free thymoquinone ($p<0.001$) after 48 hours	[51]
Protopanax-adiol	Excision wound in genetically diabetic mice with hyperglycemia	The cumulative wound contraction rate was 71.67 ± 12.4%	The cumulative wound contraction rate was 86.52 ± 9.92% but did not differ significantly from the controls ($p>0.05$)	The cumulative wound contraction rate was 94.72 ± 6.48% and significantly higher than the controls ($p<0.01$) and protopanax-adiol solution ($p<0.01$)	[52]
Curcumin (+EGF)	Excision wound in streptozotocin-induced diabetic Sprague Dawley rats	Showed a wound closure of 10.39 ± 6.91% on day 3	Showed a wound closure of 29.00 ± 16.76% on day 3 and significantly higher than controls ($p<0.05$)	Showed a wound closure of 42.11 ± 10.83% on day 3, significantly higher than controls ($p<0.05$) and free active substances ($p<0.05$)	[53]

In general, the encapsulation of natural products into lipid nanoparticles can increase their wound-healing activity. Kakkar *et al.* (2018) showed that when tetrahydrocurcumin was encapsulated into SLNs, its wound closure activity increased compared to before encapsulation and control. The increase in the activity of tetrahydrocurcumin is related to the size of the submicron particles that can increase the penetration into the cell [45]. Pivetta *et al.* (2018) also showed that when thymol was encapsulated into NLCs, its anti-inflammatory activity increased compared to before encapsulation and control. It can be caused by the advantages that NLCs have as an active carrier of substances, namely the ability to increase drug penetration into the skin and encapsulation that can reduce drug toxicity. In addition, calendula oil, as a liquid lipid component in NLCs, which also has anti-inflammatory activity, can provide a synergistic effect in wound healing along with thymol [46]. The effect of calendula extract on wound healing has been proven by Arana *et al.* (2015) [47]. The ability of lipid nanoparticles to lower the toxicity of natural products was observed in research conducted by Carbone *et al.* (2020). Free ferulic acid at the same test concentrations as the NLCs formulation cannot induce wound closure in the *in vitro* test but exerts a worse effect than the untreated cells. These results can be attributed to NLCs' ability to control the release of ferulic acid. The release of ferulic acid in the form of a solution will be massive and may interfere with cell metabolism [48]. However, in experiments conducted by Khezri *et al.* (2019), it was observed that there was no significant difference between the activity of rosemary oil before and after encapsulation in NLCs. Only a slight increase in activity was observed in the NLCs encapsulated oil group. This discovery demonstrates the importance of observing drug release behavior from lipid nanoparticles, which will also affect the healing activity of wounds that have been given dosage formulations to aid the healing process [49].

In a research done by Chen *et al.* (2013), SLNs containing astragaloside IV were examined for wound healing activity in the rat excision wound model. As a result, astragaloside IV included within SLNs significantly contributed to wound healing more than the astragaloside IV solution group, controls, and carriers. Compared to the solution form, SLNs are more beneficial in wound healing because it provides a continuous release of the drug, thus extending the duration of the drug contact in the skin, providing good compatibility, and

moisturizing effect [50]. A few years later, Luo *et al.* (2016) conducted research using the compound astragaloside IV to accelerate the healing of diabetic wounds in streptozotocin-induced mice. The results showed that topical application of astragaloside IV contributed to the closure of the diabetic wound by reducing the wound area and showing faster re-epithelization. In addition, collagen accumulation was also accelerated, affecting the extracellular matrix synthesis. Interestingly, a noticeable increase in the infiltration of inflammatory cells, especially alternatively activated macrophages to M2 phenotypes (anti-inflammatories), occurred in mice wounds given with astragaloside IV. This discovery was proposed as an accelerated mechanism for healing diabetic wounds given by astragaloside IV [54]. Both findings suggest that astragaloside IV, including lipid nanoparticles, may show good activity in curing the diabetic wound, but its efficacy should still be tested in further research.

Lipid nanoparticles, especially NLCs, are an efficient and non-toxic drug delivery system for various active compounds. It has been reported that NLCs protect the drug from oxidation or hydrolysis while providing a controlled release. In particular, NLCs are suitable for topical administration of wounds, especially chronic wounds, because they can provide appropriate concentrations of the drug on the damaged skin. Research comparing the efficacy of thymoquinone contained in NLCs and free thymoquinone *in vitro* on the 3T3 and 3T3-L1 fibroblast cells was conducted by Alexander *et al.* (2019). The result showed that the formulation of thymoquinone NLCs could increase both cells' migration and proliferation better than free thymoquinone, which depends on a specific dose level and time. Thymoquinone NLCs work better on 3T3-L1 cells than free thymoquinone, and vice versa. Although there is no explicit explanation, it can be estimated that the encapsulated form of thymoquinone in NLCs contributes to the differential effect caused, especially in reducing the toxicity and number of active substances on target that can improve healing effects. The tween 80 in the NLCs formulations is also considered an advantage for improving thymoquinone's performance [51]. Sun *et al.* (2020) created an NLC system containing protopanaxadiol to improve diabetic ulcer healing. The formulation of protopanaxadiol NLCs can encourage regular healing of diabetic wounds, as shown by the higher rate of wound contraction than the treated groups of protopanaxadiol solution and control, can suppress inflammation, encourage angiogenesis, and regulate collagen deposition. It is mainly due to the controlled release of the NLCs matrix. Initially, NLCs provide the release of active substances in large quantities to provide enough concentration for the active substance to act on the wound site and then observe the continuous release of active substances to maintain the effective concentration of active substances at the wound site [52]. Lee *et al.* (2020) created NLCs containing curcumin and EGF to treat diabetic wounds. EGF is a key regulator capable of stimulating the migration and proliferation of fibroblast and keratinocyte cells and collagen deposition. Curcumin was added to the formulation to obtain its antioxidant effect. Both provide synergistic effects for treating diabetic wounds. As a result, NLCs containing curcumin and EGF can accelerate wound healing in the early healing process. However, over time the activity of the NLCs formulation did not differ significantly from the activity of EGF and curcumin that were not encapsulated. This increase in activity can be due to: (1) encapsulation processes increasing the stability of EGF by protecting it from proteases and oxidative stress; (2) controlled release of EGF and curcumin provides a continuous concentration of the drug; and (3) NLCs formulation showed higher levels of antioxidants from the non-encapsulated or controlled group. Meanwhile, the similar healing effect of encapsulated and unencapsulated active substances as the healing process went on is hypothesized due to the imperfect release of the active substance from the

NLC system, rendering a higher concentration of the active substance on groups given free-form unencapsulated active substances [53].

7. Nanofiber Containing Natural Products

The nanofiber-based wound dressing has garnered much attention because it can match the skin's characteristics, deliver nutrients, exchange gases, absorb exudate, have a porous structure, and have a high surface area. Replicating the skin characteristics can encourage the migration and proliferation of fibroblast and keratinocyte cells and increase collagen synthesis [55]. Although there are several techniques for constructing nanofibers, electrospinning is currently the only technique that allows the manufacture of continuous fibers with diameters ranging from several micrometers to several nanometers that are architecturally similar to the structure of the extracellular matrix [56–58].

Nanofibers have a structure resembling the natural fiber tissue of the extracellular matrix that provides favorable conditions for cell attachment and enhances cell interaction with drugs [4]. The nanofiber's high surface area allows efficient fluid absorption and sends agents that can promote wound healing, such as natural products. Small pores and a highly effective surface area make nanofibers useful for improving the hemostasis process. The high porosity of the nanofiber surface allows gas exchange across the wound and prevents the wound from drying. The fine fibers in the nanofiber enable it to adapt better to the wound's contours than other wound dressings [10]. Nanofibers containing natural products are more widely researched for their effects on chronic wound healing, especially diabetic wounds, than other nanotechnology-based drug delivery systems. After being included with nanofibers, the healing effects of wounds from some natural products are summarized in Table 3.

Table 3. Wound healing effects of natural products after loading within nanofibers.

Natural products	Test model	Results on control	Healing effects of natural products included nanofibers	Ref.
Bixin	Excision wound in streptozotocin-induced diabetic mice	Showed a wound closure of ± 70% on day 7	Showed a wound closure of ± 80% on day 7 and differed significantly from the controls ($p<0.001$)	[59]
Curcumin	Excision wound in streptozotocin-induced diabetic Sprague Dawley rats	The wound area was only reduced by 20.96 ± 1.35% on day 15	Improved wound healing significantly compared to controls ($p<0.05$) and completely closed wounds on day 15	[60]
Sesamol	Excision wound in streptozotocin-induced diabetic mice	Showed a wound closure of ± 65% on day 9	Showed a wound closure of ± 95% on day 9 and differed significantly from controls ($p<0.05$)	[56]
Malva sylvestris extract	Excision wound in streptozotocin-induced diabetic Wistar rats	Showed a wound closure of 32.1 ± 0.2% on day 14	Showed a wound closure of 95.11 ± 0.2% on day 14 and differed significantly from the controls ($p<0.05$)	[55]
Astragalus polysaccharides	Excision wound in streptozotocin-induced diabetic Sprague Dawley rats	Showed an average wound healing of 46.6 ± 5.4 days	Showed faster-wound healing compared to controls ($p<0.05$) and an average healing time of 17.1 ± 3.4 days	[57]
Asiatic acid	Excision wound in streptozotocin-induced diabetic mice	Showed a wound closure of 50.25% on day 14	Showed a wound closure of 97.37% on day 14 and differed significantly compared to controls ($p<0.001$)	[61]
β-glucan	Excision wound in genetically diabetic mice	Showed a wound closure of ± 50% on day 24	Showed significant improvement in wound closure ($p<0.05$) and wound closure of ± 95% on day 24	[62]

Natural products	Test model	Results on control	Healing effects of natural products included nanofibers	Ref.
Berberine	Excision wound in streptozotocin-induced diabetic Wistar rats	Showed minimum re-epithelization, collagen synthesis, and angiogenesis after 16 days	Showed the highest re-epithelization, collagen synthesis, and angiogenesis compared to controls and empty nanofibers ($p<0.05$) after 16 days	[63]
Cod liver oil	Excision wound in streptozotocin-induced diabetic rats	Showed a healing percentage of < 50% on day 14	Showed a healing percentage of 94.5% on day 14 and differed significantly compared to controls ($p<0.05$)	[64]

Nanofibers containing natural products positively affect the wound healing process, as shown in the experiments above, exhibiting faster wound closure rates compared to the control groups. The positive effect of wound healing comes from the advantages provided by nanofiber structures supported by natural products with their activities [65]. The advantages that can be observed in the nanofiber research on wound healing carried out are: (1) helping the hemostasis phase of wound healing; (2) absorbing the exudate of the wound; (3) maintaining the humidity of the wound environment; (4) providing controlled release of the drug; and (5) avoiding the formation of wound marks/scar [55,59].

8. Polymeric Nanoparticles Containing Natural Products

Biopolymers such as starch, cellulose, silk fibers, collagen, gelatin, albumin, and chitosan-based nanomaterials provide synthetic nanoparticles with good biocompatibility, biodegradability, and low toxicity [66].

Table 4. Wound healing effects of natural products before and after encapsulation with polymeric nanoparticles.

Natural products	Test model	Results on control	Healing effects of natural products		Ref.
			Before encapsulation	After encapsulation	
Epigallo-catechin gallate and ascorbic acid	Excision wound in streptozotocin-induced diabetic mice	The wound closure rate was ± 83% on day 10	The wound closure rate was ± 88% on day 10, and significantly higher than the controls ($p<0.05$)	The wound closure rate was ± 94% on day 10, and significantly higher than the controls ($p<0.001$) and the solution of the active substance ($p<0.001$)	[66]
Ferulic acid	Excision wound in streptozotocin-induced diabetic Wistar rats	The wound closure rate was ± 29% on day 14	The wound closure rate was ± 60% on day 14	The wound closure rate was ± 79% on day 14, and significantly higher than the controls ($p<0.001$)	[67]
Berberine	Excision wound in streptozotocin-induced diabetic Wistar rats	The wound closure rate was ± 50% on day 14	The wound closure rate was ± 64% on day 14, and significantly higher than the controls ($p<0.05$)	The wound closure rate was ± 98% on day 14, and significantly higher than the controls ($p<0.05$) and the solution of the active substance ($p<0.05$)	[68]
Curcumin	Excision Wound in streptozotocin-induced diabetic Sprague Dawley rats	The wound closure rate was 70% on day 7 and 84.2% on day 14	The wound closure rate was 79.7% on day 7 and 92.4% on day 14, but did not differ significantly from controls ($p>0.05$)	The wound closure rate was 90.6% on day 7 and 98.3% on day 14, and differed significantly from controls ($p<0.05$)	[69]

When included within nanoparticles, natural products can be protected from endogenous enzymes or wound environmental states that can degrade natural products and provide a controlled release of substances to reduce the frequency of administration and increase patient acceptance [4]. The wound healing effects of some natural products before and

after being included with polymeric nanoparticles with various test models are summarized in Table 4.

Several studies have been conducted on polymeric nanoparticles containing natural products to study their effects on wound healing. Sun *et al.* (2020) studied gelatin and chitosan nanoparticles containing epigallocatechin gallate and ascorbic acid for their effects on diabetic wound healing by using their potent anti-inflammatory and antioxidant activity. Epigallocatechin gallate has eight phenolic hydroxyl groups that cause them to be unstable and easily oxidized. Therefore, several efforts to increase their stability are being made, one of which is nanoparticle formulations. As a result, on the final day of observation (day 10), diabetic wounds treated with nanoparticle formulations were able to be cured by way of the increased new blood vessel, thick granulation tissue, less inflammatory cell infiltration, intact and thick regenerated epithelial cells, and greater amount of collagen deposition than the control group and the ones treated with only epigallocatechin gallate [66]. Research conducted by Lin *et al.* (2016) also showed similar results where epigallocatechin gallate nanoparticles were able to accelerate the healing process in acute wounds through inflammatory prevention and facilitating epithelialization and tissue regeneration [70]. Bairagi *et al.* (2018) produced PLGA nanoparticles containing ferulic acid to study their effects on diabetic wound healing. As a result, PLGA nanoparticles containing ferulic acid, when administered orally (in dispersion form) and topically (on a hydrogel basis) in wounded diabetic rats, showed faster epithelialization so that the wound was able to close on day 14, faster compared to the diabetic wound control group [67]. Panda *et al.* (2021) also produced chitosan and lecithin nanoparticles containing berberine to study their effects on diabetic wound healing. As a result, chitosan and lecithin nanoparticles containing berberine, when administered topically in wounded diabetic rats, showed a faster wound closure on day 14 compared to the free berberine and control group. Polymeric nanoparticles can give a sustained release that prolongs the healing effect of berberine. In addition, the combination of berberine and chitosan provides synergetic effects to reduce inflammation, promote blood vessel and fibroblast proliferation, and promote collagen deposition [68].

In chronic wounds such as diabetes-related wounds, activated macrophages secrete more pro-inflammatory cytokines, causing excessive inflammation. It keeps the wound in the inflammatory phase, which inhibits healing. Curcumin has major activity as an anti-inflammatory that can drive the healing process from the inflammatory phase to the proliferation and remodeling phase. Therefore, curcumin is widely researched in wound healing. Li *et al.* (2019) produced chitosan nanoparticles containing curcumin to stop inflammation and promote diabetic wound healing. The results showed that chitosan nanoparticles containing curcumin significantly inhibited macrophage-induced inflammatory responses by lowering the number of pro-inflammatory cytokines (TNF-a and IL-6) as shown in the *in vitro* test. In addition, nanoparticles containing curcumin also promote angiogenesis by increasing cell migration and the formation of blood vessels in endothelial cells with high glucose levels, as shown in the *in vitro* test. Injecting curcumin-loaded nanoparticles around diabetic wounds in mice showed fewer inflammatory cell infiltration at the injury site, and more new blood vessels were observed in the early healing period. Collagen formation and fibroblast distribution also increased significantly. It suggests that curcumin nanoparticles promote the healing of wounds in diabetes. Curcumin intracellular absorption test in macrophage cells showed that the cellular fluorescence of nanoparticles containing curcumin was significantly higher than the cellular fluorescence of free curcumin. This discovery

suggests that nanoparticles accelerate the internalization of curcumin in macrophages compared to free curcumin. Free curcumin relies on passive diffusion to internalize into the cell. At the same time, the nanoparticle surface has a positive charge that the cell surface can absorb to improve curcumin's capture rate and efficacy [69].

9. Nanohydrogel Containing Natural Products

A hydrogel is a three-dimensional series of water-soluble polymer that binds to each other and expands in an aqueous environment. The main obstacle in the usage of hydrogels is the rapid elucidation of drugs from the expanding hydrogel matrix. This obstacle can be overcome using nanohydrogels, and hydrogel particles with nanoscale dimensions. Nanohydrogels have characteristics shared with hydrogels and nanosystems [71].

The porous three-dimensional structure of the nanohydrogels allows them to absorb fluids, preventing wounds from becoming dehydrated and creating a moist environment that is beneficial for wound healing. Nanohydrogels also has a smooth texture, so they are comfortable treating wounds. Furthermore, nanohydrogels can encapsulate various drugs with good biocompatibility and efficacy properties. This advantageous nano hydrogel biocompatibility is attributed to its high fluid retention capacity, physicochemical properties, composition, and mechanical similarity with the body's extracellular membranes [71]. The wound-healing effects of several natural products before and after being included in nanohydrogels with various test models are summarized in Table 5.

Table 5. Wound healing effects of natural products before and after encapsulation with nanohydrogels.

Natural products	Test model	Results on control	Healing effects of natural products		Ref.
			Before encapsulation	After encapsulation	
Baicalin	TPA-induced mice skin inflammation	The skin appeared very damaged with the loss of the epidermis	Wound formation was partially reduced and indicated the presence of inhibition of inflammatory markers	Wound formation was greatly prevented and showed a significant increase in inhibition of inflammatory markers compared to controls ($p<0.05$) and baicalin suspension ($p<0.05$)	[72]
Aloe vera leaf gel extract	Wound excision in rats	Showed wound healing, but did not cure until days 14 and 21; wound healed completely on day 28	Showed improved wound healing compared to controls on days 3 and 7 but was insignificant ($p>0.05$), and the wound healed completely on day 14	Showed significant improvements in wound healing ($p<0.002$) on days 3 and 7 compared to controls and completely healed on day 14	[73]
Berberine	Excision wound in streptozotocin-induced diabetic Sprague Dawley rats	The wound was not closed until day 13	Wounds were not closed until day 13, but wound healing improved significantly compared to controls ($p<0.05$)	Wounds closed completely on day 13, and wound healing improved significantly compared to controls ($p<0.05$) and berberine suspension ($p<0.05$)	[74]

Gellan-cholesterol nanohydrogels containing baicalin were studied for their effect on TPA-induced mice skin inflammation by Manconi *et al.* (2018). The nanohydrogels produced exhibited good viscosity characteristics, improved skin retention, and expected biocompatibility. When applied to inflamed skin, nanohydrogels containing baicalin showed

the optimal performance to completely repair the skin and inhibit specific inflammatory markers (edema, myeloperoxidase, and TNF-α) compared to controls and baicalin only in the *in vivo* experiments. Nanohydrogels have been shown to enhance the healing and inhibitory effects of inflammatory markers by baicalin, which can occur due to the effects of nanohydrogels in hydrating the skin and providing more accumulation of baicalin in the deeper layers of the skin, where baicalin can work against oxidative stress, regulate inflammatory processes, and improve the skin repair process [72]. Ashouri *et al.* (2019) produced a chitosan nanohydrogel containing aloe vera leaf gel extract to speed up wound healing. In general, it was observed that the effect of aloe vera leaf gel extract on the skin regeneration process was anti-inflammatory. However, combining chitosan nanohydrogels with aloe vera leaf gel extract can modulate the response of M1 pro-inflammatory macrophages and M2 anti-inflammatory macrophages, which leads to improved wound healing. The M1 decreased after three days, and the M2 increased after 14 days. The balance between M1 and M2 plays an important role in the healing and remodeling process of the affected tissue. The synergistic effect between chitosan and aloe vera leaf gel extract can cause this beneficial effect. Each of these components can provide a wound-repair effect. However, their combination in nanohydrogel preparations enhances each of its healing effects through macrophage modulation that can increase re-epithelization, formation of new blood vessels, granulation tissue, fibroblast proliferation, and extracellular matrix production [73].

Nanohydrogel is considered very potent in curing chronic wounds, such as diabetic wounds, with various complications. The research was conducted by Zhang *et al.* (2020) to study the healing activity of nanohydrogels containing berberine in diabetic rats. As a result, nanohydrogels containing berberine were able to cure diabetic wounds only after 13 days. In contrast, the inferior healing effect was observed in the group that was given free-form berberine as well as the untreated test group. This good healing effect of diabetic wounds is hypothesized to stem from the increased berberine solubility, more uniform distribution, moisturizing effects exerted by nanohydrogels, and extended berberine discharge from the nanohydrogel matrix at the wound site [74].

10. Nanoemulsion Containing Natural Products

An emulsion is a system consisting of two unmixed phases and at least three components, namely the water phase, oil phase, and surfactant [75]. A nanoemulsion is an emulsion with a very small droplet size with an average nanoscale diameter. Droplets in nanoemulsion are stabilized by a layer consisting of a mixture of surfactants and cosurfactants [35]. Nanoemulsions can protect drugs from hydrolysis and oxidation, improve the efficacy of drugs, which leads to a decrease in the required dose, minimize side effects, and increase the solubility and bioavailability of the drug [24,27]. In addition, nanoemulsion characteristics are biocompatible and biodegradable, have good physicochemical properties, and have relatively low production costs, so they are suitable to be used as an alternative to wound healing [33,76].

Several studies have been conducted on nanoemulsions containing natural products and their effects on wound healing. Ahmad *et al.* (2019) produced eucalyptus nanoemulsions that contain curcumin for wound and inflammatory treatment. As a result, eucalyptus nanoemulsions containing curcumin showed a significant increase in wound healing and anti-inflammatory activity compared to controls ($p<0.01$) and curcumin suspension in topical applications with rat models. This increase in curcumin efficacy can be attributed to the increased penetration of curcumin into the cell when encapsulated with nanoemulsions. In

addition, the observed healing effect of wounds on eucalyptus can provide a synergistic combination with curcumin in nanoemulsion formulations [34]. The efficacy of eucalyptus-containing nanoemulsions was confirmed in another study by Sugumar *et al.* (2014) that showed higher antimicrobial activity and wound contraction rates than controls and treatment groups using neomycin antibiotics in mouse models. Nanoemulsions also showed no symptoms of irritation or cause inflammation, so it is safe and non-toxic [27]. Previously, Thomas *et al.* (2017) also produced nanoemulsions containing curcumin, and the results showed faster wound healing than controls in rat models [77]. Better efficacy of β-caryophyllene after being included in nanoemulsions was also observed in the Parisotto-Peterle *et al.* (2020) study using the excision model in rats. The group treated with nanoemulsions containing β-caryophyllene showed wound contractions of 48.82% on day 4, while the group treated with only β-caryophyllene showed 29.17%. Wounds treated with only β-caryophyllene showed the onset of skin irritation with erythema around the wound. However, no more skin irritation was observed when included in the nanoemulsion system. It suggests that nanoemulsions may reduce the side effects of using natural products on wounds [78].

11. Conclusions

The results showed that nanotechnology-based drug delivery systems containing natural products could improve and accelerate wound healing by increasing cell migration and proliferation, granulation tissue formation, re-epithelialization, collagen deposition, and tissue maturation. The improvement in the wound healing process is attributed to the antioxidants, anti-inflammatories, antimicrobials, and tissue regeneration characteristics of natural products, which were enhanced in efficacy by nanotechnology-based drug delivery systems. In addition, nanotechnology-based delivery systems can provide ideal dressing characteristics for wound healing, including providing occlusive properties, improving the stability of natural products, enabling the controlled release of active substances, and encouraging better interaction with biological targets. The information in this literature study may provide insights for further research on natural products and nanotechnology product-based preparations to cure chronic wounds.

Funding

The authors would like to thank the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology, the Republic of Indonesia, for funding research through the Master's Thesis Research grant scheme year 2022 (Contract number: 083/E5/PG.02.00.PT/2022).

Acknowledgments

The authors would like to thank the School of Pharmacy from Institut Teknologi Bandung for facilitating this collaborative work.

Conflicts of Interest

The authors declare no conflict of interest.

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