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REVIEW ARTICLE

LIPID NANOPARTICULATE SYSTEM FOR WOUND HEALING

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1. Introduction

In recent years, the number of people getting affected by chronic diseases such as heart disease or diabetes has increased alarmingly due to unhealthy eating habits and sedentary lifestyle. Among various comorbidities associated with these chronic diseases, a major complication includes delayed wound healing. Currently, with the increased rate of type II diabetes, ageing population, obesity, burns, peripheral vascular disease, and metabolic syndrome; chronic wounds have become a global medical concern. The incredibly complex process of wound healing is dependent on numerous factors working together to restore the normal skin function [(1)]. Dependent on the mechanism involved in wound repair and the time taken for wound healing, wounds can be classified as acute and chronic wounds. Acute wounds heal within a short time interval with minimal or no scarring, while chronic wounds heal at a very slow pace, often taking months to show progress. The chronic wounds are heterogeneous in their etiology and presentation. Many a time the chronic wounds are associated with biofilm formation, high protease activity, hypoxia or ischemia in the tissue, and recurrent injury due to neuropathy. The chronic wounds in obese or diabetic patients often occur as pressure ulcers, foot ulcers, or venous leg ulcers [(2)]. The wound management is a global aspect of medical care that creates an enormous monetary burden due to the rapid increase in the number of wound patients with about 300 million chronic wound and 100 million traumatic wound patients worldwide. The occurrence of chronic wounds has also increased due to the increase in the number of diabetic patients from 108 million in 1980 to 463 million in 2019. About 60% of non-traumatic amputations in United States are performed on diabetic patients. Microvascular angiopathy and neuropathy are the common complications associated with diabetes that contributes to 12-25% lifetime risk in the

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development of diabetic ulcers. The diabetic foot ulcers specifically contribute to 25-50% of total cost in the treatment of diabetes. In the United States alone the financial burden for wound management accounts for over 25 billion dollars every year. Furthermore, about 1,80,000 deaths are caused due to burns each year, the majority of which occur in low- and middle-income countries of South-East Asia and the African region [3].

Currently, an extensive choice of therapy is available by both conventional and modern approaches for wound treatment. Conventional therapy involves debridement and dressing change in proper time intervals. Numerous varieties of dressings are available for wound healing which can be broadly classified into three categories: traditional dressings, biomaterial-based dressings and artificial dressings. A gauze or bandage can be considered as traditional dressing, the allografts, xenografts, and tissue derivatives are biomaterial-based dressings, and film, hydrogel, membrane, scaffolds, spray, hydrocolloids, etc. are artificial dressings. Several materials are of natural sources such as cellulose, collagen, fibrin, chitosan, gelatin, alginate, hyaluronic acid, etc. and used in the preparation of artificial dressings. Several advanced therapeutic approaches include stem cells (mesenchymal stem cells and endothelial progenitor), growth factor therapy, tissue-engineered wound beds (bilayered skin substitutes, fibroblast/keratinocyte seeded scaffolds, decellularized tissue scaffolds). Examples of currently available wound healing products in the market are mentioned in table 1. Hyperbaric oxygen therapy, negative pressure wound therapy, hyperbaric oxygen therapy, electrostimulation, ultrasound noncontact wound healing device, and hydrotherapy are some other type of advanced wound care strategies [4]. The characteristics of ideal wound dressing and its clinical significance to wound healing are described in table 2[5].

Nanotechnology has significantly contributed to this arena by promoting tissue regeneration, controlling the possible infections, preserving skin aesthetics and functions, as well as promoting wound closure. Amongst various types of nanocarriers, lipid nanoparticles are widely investigated by researchers due to their known benefits for topical drug delivery. This review addresses the wound healing application of lipid nanoparticulate system namely nanoemulsion, liposomes, SLNs, and NLCs.

Table 1: Examples of currently available marketed wound healing products:

Type of product	Commercial name	Innovator
Hydrocolloids	Comfeel®	Coloplast
	Aquacel®, duoderm®,	Convatec
	3M™ Tegaderm™ hydrocolloid dressing	3 M Science Applied to Life ™
Films	OPSITE FLEXIGRID, Cutifilm	Smith & Nephew
	Bioclusive	Systagenix
	Polyskin II, Blisterfilm	Covidien
Foams	Lyofoam® Max	Molynlycke
	ALLEVYN	Smith & Nephew
	TIELLE LIQUALOCK ™	3M-KCI
Hydrogels	Regranex gel, Intrasite gel, Flexigel	Smith & Nephew
	Purilon gel	Coloplast
	Granugel	Convatec
	Curasol	Healthpoint
	Aquaflo	Covidien
Alginates	KALTOSTAT®	Convatec
	Algisite M	Smith & Nephew
	Sorbsan	Mylan Bertek
	Seasorb	Coloplast
Tissue-engineered skin substitutes	Apligraf®, Dermagraft®	Organogenesis
	Theraskin®	Misonix®
	Integra Omnigraft ™	Integra Lifesciences
	Alloderm	Biohorizons

Table 2: Characteristics of ideal wound dressings [5]:

Desirable characteristics	Clinical significance to wound healing
Provide/maintain a moist environment	Enhances epidermal migration, favors autolysis by rehydration of desiccated tissues, prevents cell death and desiccation, promotes connective tissue synthesis and angiogenesis
Debridement (wound cleansing)	Supports the accumulation of the enzyme and enhances leucocytes migration into the wound bed. The necrotic tissue and foreign bodies extend the inflammatory phase and also enhances the bacterial growth; therefore, debridement is essential
Gaseous exchange (air and water vapour)	The exudate management gets controlled by water vapour permeability, low tissue oxygen levels stimulates angiogenesis, while raised tissue oxygen stimulates fibroblasts and epithelialization
Absorption (Removal of excess exudate and blood)	The excess exudates in chronic wounds contain tissue degrading enzymes that block cell proliferation and decrease the growth factors. It also breaks down the extracellular matrix (ECM) material and macerates surrounding tissue.
Prevent infection	Infection extends the inflammatory phase, inhibits epidermal migration, delays collagen synthesis, encourages extra tissue damage and also give an unpleasant odour
Low adherence (prevents additional trauma)	It might be difficult to detach adherent dressings and cause pain and additional tissue damage
Provide thermal insulation	Normal tissue temperature enhances blood flow and epidermal migration

2. Pathophysiology of wound healing:

Wounds are majorly classified as acute wounds and chronic wounds. The wound healing is facilitated in a timely manner by the intact biochemical processes. However, the process gets impaired owing to a primary medical disorder such as poor nutrition, diabetes, age, arterial or venous insufficiency that leads to non-healing/chronic wounds. When the wound healing does not occur within 12 weeks after the treatment, then they are usually considered as chronic wounds. The normal pathophysiology of wound healing includes four main stages namely (a) hemostasis, (b) inflammatory phase, (c) proliferation, and (d) remodeling. After the injury, immediately the hemostasis phase gets activated due to the platelets and tissue damage that release several messenger molecules at the site of injury. The clotting factors from the wounded skin activates the extrinsic clotting cascade. Thereafter, the thrombocytes adhere to the surface of sub-endothelium that triggers the cascade of intrinsic clotting factors. The bleeding from the tissue damage site stops due to vasoconstriction and thrombus formation. The signaling molecules like transforming growth factor-β (TGF-β), Platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) gets secreted during this early phase [(6)].

The inflammatory phase begins with vasodilation due to VEGF release, and further results in an influx of various growth factors, enzymes, leucocytes, nutrients and antibodies into the site of injury. The release of cytokines and TGF-β stimulates the infiltration of neutrophils that aids early defense in contrast to microbial invasion. In the inflamed tissue high number of neutrophils remain up to 48 hours. The release of proteinases along with neutrophils provides high phagocytic activity and clears the wound bed from pathogens and debris. The concentration of macrophages reaches to the peak after 48-72 hours post-injury. The release of TGF-β and Epidermal growth factor (EGF) initiates angiogenesis, regulates inflammation process and granulation tissue formation. Three days post-injury, the lymphocytes in wound bed secrete fibroblast growth factor and heparin-binding EGF. They have a key role in ECM formation and collagen remodeling. In non-healing wounds, generally, the inflammatory phase prolongs due to excessive leucocytes infiltration that leads to an elevated concentration of cytokines, proteases, and radicals [(3).]

The proliferation stage begins after 3-5 days post-injury with angiogenesis, granulation tissue formation, deposition of collagen and epithelialization. The neovascularization is initiated in response to hypoxia in the wound bed. During the formation of granulation tissue, a dense complex of leaky capillaries is developed that results in increased volume of wound fluids. During the inflammatory phase, the neutrophils and macrophages release numerous chemokines and cytokines that attract lymphocytes, fibroblasts,

myofibroblasts, endothelial cells, and keratinocytes into the wound microenvironment. Migration of keratinocytes occurs from the wound edge towards the wound bed which aids the restoration of skins barrier function. The fibroblasts secrete metalloproteases (proteolytic enzymes) that digest plasma fibronectin and secretes cellular fibronectin. The fibroblasts proliferation leads to granulation tissue formation that replaces the clot formed initially. Collagen production reaches the maximum after five days of injury that bridges the wound bed edges. The myofibroblasts decrease the volume of tissue needed for healing as well as reduces the scar tissue formation. The last phase of the wound healing is remodeling which is also known as maturation phase. It usually begins after 3 weeks of the initial wound injury and lasts up to 2 years or beyond. Through this phase the gelatinous and soft collagen type-III that was produced during proliferation phase gets substituted by a properly structured collagen type-I. The tissues tensile strength upsurges throughout this phase. It gradually increases by up to 50% within 3 months and extends to about 80% of the original tissue after a lengthy period [7].

The complexity of the wound healing process that includes numerous factors and regulating molecules included in the cascade of wound healing, as described above makes it easier to anticipate that even small aberrations could lead to failure in wound healing. Numerous factors affect wound healing progression such as hypoxia, nutrition, immunosuppression, infection, age, chronic disease, and genetics. The severity and extent of wound infection also affect wound management. Diabetes influences all the four phases of the wound healing process. Long-term hyperglycemia leads to elevation of inflammatory phase. The peripheral neuropathy that leads to lower sensitivity in limbs prevents the detection of ulcers in the early stages. The neuro-immune interactions also get affected which reduces the expression of substances engaged in the inflammatory phase of wound healing. In diabetic patients angiogenesis also gets affected which leads to reduced blood flow and delayed tissue regeneration. The oxygen availability, ATP generation and migration of fibroblasts and keratinocytes to the wound decreases. Thus, hyperbaric therapy improves wound healing. In hyperglycemic conditions, the increased activity of NADPH oxidases causes increased reactive oxygen species (ROS) production. An excess ROS production leads to DNA damage, lipid peroxidation, protein modification, extracellular proteins degradation, and alters the keratinocytes and fibroblasts functioning. The tissue regeneration delays due to cytokine level imbalance i.e. shortage of anti-inflammatory cytokines such as TGF-β and Interleukin (IL-10); and excess of pro-inflammatory molecules such as TNF-α, matrix metalloproteinase-9 (MMP-9), and IL-1β. Also, the generation of advanced glycation end products (AGEs) due to high glucose level leads to

delayed wound healing. A compromised immune system increases the chances of infection and thereby hinders wound recovery. Chronic wounds include diabetic foot ulcers, pressure ulcers, venous ulcers, and arterial ulcers. Burn injury caused by chemicals, heat, electricity or radiation often becomes chronic wounds. it leads to major fluid loss, affects skin integrity and becomes a gateway for bacterial infection [8].

3. Nanotechnology-based wound healing therapy:

Nanotechnology-based therapy has offered an excellent prospect to target the plethora of regulating molecules, cell-type specificity, the complexity of the normal wound healing process and pathophysiology of chronic wounds. Numerous engineered nanotechnologies have demonstrated unique multifunctional properties that have addressed the key problems associated with wound healing mechanisms. Currently, there are two main categories of nanomaterials that can be employed for wound healing therapy, (a) the nanomaterials that provide healing effect owing to the nano-scaled materials feature or, (b) nanomaterials used as a cargo to deliver wound healing agents. Generally, the main categories of nanocarriers include polymeric nanocarriers, carbon-based, metal and metal oxide nanoparticles, and lipid-based nanoparticles [9]. Lipid nanoparticles have gained enormous attention from researchers in the field of topical drug delivery owing to their great functionalities such as proper adhesion to the skin and film formation which provides skin hydration effect and helps to maintain skin integrity. For dermal, cosmetic and pharmaceutical uses, lipid nanocarriers seem to be very promising due to their ease of preparation, biocompatibility, biodegradability, and ability to provide superior drug efficacy as compared to unencapsulated drugs [10]. They have been extensively studied for delivering active moieties, essential oils, growth factors, endogenous molecules, antimicrobial drugs, several herbal extracts, etc. for accelerating wound healing. Generally, lipid nanocarriers are loaded into some dressings such as scaffolds, films, foam, nanofibrous matrix, hydrogels etc. Nanoemulsion, Liposomes, SLNs and NLCs are the main categories of lipid-based nano-systems.

4. Lipid Nanoparticulate Systems:

4.1. Nanoemulsion:

Nanoemulsion is lipid-based thermodynamically stable system composed of oil, water, surfactant, and cosurfactant, having the droplet size in the nanometer range. Over the other unstable dispersions, nanoemulsion represents numerous advantages owing to their nano-sized droplets and thermodynamic stability. Compared to conventional emulsions, nanoemulsion is optically transparent and due to their higher active surface area, they interact in a higher extent with the biological surfaces. This confers high efficiency to deliver active

pharmaceutical ingredients. However, as lipid nanoemulsion comprises of liquid lipids in the core they have limited stability and very fast drug diffusion from the lipid core. For better drug encapsulation in lipid-based nanoemulsion and higher stability, SLNs and NLCs were developed. SLNs and NLCs have solid lipid in the inner core which allows controlled release of drugs as well as increases the long-term stability of the system.

Several essential oils loaded nanoemulsion have been fabricated and evaluated for potential wound healing effects. Alam et al. evaluated the wound healing potential of nanoemulsion comprising clove oil. The optimized nanoemulsion droplet size was 29.10 nm, which was further selected for histopathological examination and collagen determination in rats. As compared to pure clove oil, the nanoemulsion represented significant healing effects in rats. The histopathology results showed no inflammatory cells, suggesting that the nanoemulsion was nontoxic and safe [(11)]. Chitosan-based hydrogels comprising of inclusion compound of thyme oil with cyclodextrin, or thyme oil nanoemulsion, were prepared and characterized by Moradi et al. Freeze-thaw cycling method was employed for the preparation of hydrogels, as this physical method could fabricate hydrogel without using crosslinking agents. The results revealed that higher cell viability was achieved in hydrogels containing thyme oil-nanoemulsion, as compared to cyclodextrin inclusion thyme oil complex containing hydrogels [(12)]. In another study, the effect of lavender essential oil and licorice containing nanoemulsion was evaluated on deep skin wound rat model. Eighty-five rats were divided randomly into five groups; (a) untreated as a negative control, (b) treated with vehicle ointment, (c) treated with licorice extract and lavender essential oil in emulsion form and nanoemulsion form, and (d) treated with phenytoin 1% as the positive control. Thereafter, on 2,7, and 14 days, oxidative stress factors, expression of TGF-β, and collagen type I and type III genes were evaluated. The rats treated with nanoemulsion demonstrated a more significant reduction in wound area compared to other groups. The results of real-time PCR represented that nanoemulsion and phenytoin groups increased the type I and type III collagen, and TGF-β gene expression compared to other groups. The lavender oil and licorice extract containing nanoemulsion cream demonstrated the granular tissue formation and appearance of collagen at a faster rate compared to other groups [(13)]. Morsy et al. developed and evaluated atorvastatin-loaded nanoemulgel, atorvastatin gel, and atorvastatin emulgel for wound healing. The *in vivo* wound healing studies revealed that the highest percentage of wound contraction was represented by atorvastatin-loaded nanoemulgel [(14)]. A novel multifunctional nanofibrous gelatin/cellulose acetate/Zataria multiflora nanoemulsion was formulated by Farahani et al. Nanoemulsion of Zataria multiflora, a natural antibacterial plant was loaded in

the nanofibrous mat. Different weight ratios of gelatin and cellulose acetate were evaluated, in which its lower ratio loaded with zataria multiflora nanoemulsion showed proliferation of L929 fibroblast cells significantly. Also, the rat model experiments represented that the nanoemulsion incorporated nanofibrous dressing depicted accelerated wound healing process compared to other samples [15].

4.2. Liposomes:

Liposomes are the first developed lipid nanocarriers made up of natural or synthetic phospholipids forming the outer lipid bilayer and an aqueous core forming the inner cavity. The structure of liposomes allows encapsulation of hydrophobic drug in the outer lipid bilayer and hydrophilic drug in the inner aqueous cavity. After the approval of first liposomal pharmaceutical formulation (Doxil®) in 1995 for breast cancer, ovarian cancer and Kaposi's sarcoma, till date, there is extensive research and development in the preparation of liposomes for various other diseases. They offer several beneficial properties such as they allow a sustained release, enables the encapsulation of both hydrophilic as well as hydrophobic drug, and their structure mimics the skin's epidermis composition thus they provide higher drug accumulation in the skin. However, some of the limitations also persist such as less encapsulation efficiency, less stability, aggregation on storage, sedimentation, etc. The formulation techniques of liposomes are mentioned in figure 1.

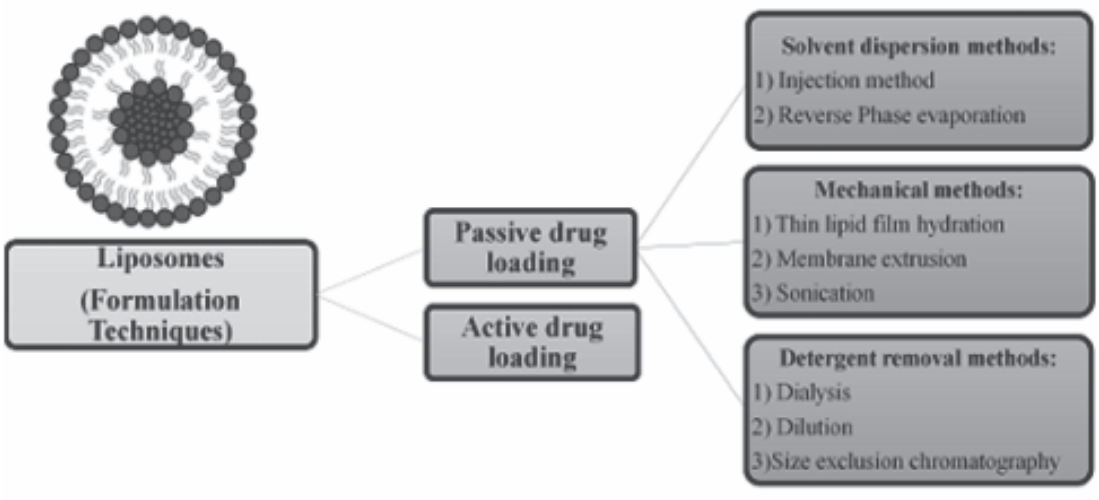

Figure 1 The formulation techniques of liposomes

In 1988, for the first time, Brown and collaborators loaded EGF into liposomes for accelerating tensile strength and promoting wound healing activity. Multilamellar lecithin liposomes were fabricated encapsulating EGF, which was then applied on rats inflicted with 5 cm incisions. The liposomes provided a prolonged EGF exposure which helped to produce about 200% increase in wound tensile strength, increased fibroblast proliferation and collagen formation as compared to EGF solution [16]. In another study, liposomes encapsulating EGF were developed by a film formation method to investigate the effect on second degree burn wounds in rats. Second-degree standard burn wounds induced rats were applied 10 μg/ml EGF containing liposomes, EGF solution, blank liposomes, and silverdine® ointment daily. The observations demonstrated that at the 14th day of therapy, the healing in EGF containing liposomes was fastest. The wound healing efficacy in terms of wound contraction, collagen formation, fibroblast proliferation and epithelial recovery was highest in case of EGF containing liposomes followed by silverdine® ointment and least in case of EGF solution [17]. Another group loaded EGF containing liposomes into chitosan gel and evaluated the effect on second-degree burn wound in rats. Liposomes comprising of 10 μg/ml EGF loaded in 2% chitosan gel, EGF-loaded liposome formulations, and EGF-chitosan gel was smeared on burn wounds on rats and the biopsies were taken thereafter on 3rd, 7th, and 14th day. The immunohistochemical outcomes demonstrated increased cell proliferation in EGF liposomes containing chitosan gel applied group. The epithelialization rate was also observed to be highest in the group applied with EGF liposome comprising chitosan gel, as per the histochemical results. This demonstrated that in order to facilitate the liposomes application, chitosan gel ensured a good delivery option and also provided a good moist healing environment [18]. During wound healing, excessive ECM deposition often results in scar formation that causes skin dysfunctions like hair loss. Basic fibroblast growth factor (bFGF) is useful to promote hair follicle neogenesis and regulate ECM remodeling during wound healing. However, in clinical practice, the repairing quality of bFGF gets hindered due to its inadequate stability in wound fluids and less penetrability in the dense scar formed on the wound. To overcome the stability issue, Xu et al. developed liposome with silk fibroin hydrogel and also added a penetration enhancer (laurocapam) to develop skin-permeable liposomes. The liposomes encapsulation efficiency for bFGF was nearly 90% and it was not affected by laurocapam. The final liposomal formulation represented zeta potential of -2.31 mV and diameter of 103.3 nm. The stability and penetration efficiency of bFGF significantly increased, at the wound zone the hair regrew and the morphology of hair follicle was improved. The skin permeable bFGF encapsulated liposomal formulation with silk fibroin hydrogel core represented to be a potential

option to improve wound healing and promote hair follicle neogenesis by inhibiting the scar formation [19]. Xiang et al. developed and characterized bFGF encapsulated liposomes using a pH gradient method to evaluate its wound healing activities in rats. The bFGF encapsulated liposomes demonstrated fast collagen generation, high dermal cell proliferation, and high TGF-β1 expression [20]. Lu et al. fabricated a dual deformable liposomal ointment comprising of retinoic acid deformable liposomes and EGF cationic deformable liposomes for deep partial-thickness burns treatment. Deformable liposomes show more benefits for topical application due to their high flexibility. They are a new generation of liposomes comprising of phospholipids and an edge activator-like sodium deoxycholate, sodium cholate, and tween-80. The edge activator offers more flexibility and enables the deformable liposomes to move across the stratum corneum and reach to the viable epidermis. The retinoic acid deformable liposomes and EGF cationic deformable liposomes demonstrated higher skin permeability and enhanced the drug accumulation by 2.9 and 18.8 folds, respectively. The combination of two liposomes provided an elevated effect and encouraged cell migration and proliferation. Retinoic acid up-regulated the Heparin-binding epidermal growth factor (HB-EGF) and Epidermal growth factor receptor (EGFR) expression and enhanced the therapeutic efficacy of EGF [21]. A novel formulation was developed by Choi et al., comprising of combinations of growth factors EGF, PDGFA, and insulin-like growth factor-I (IGF-I). The growth factors were genetically modified by conjugating the N-terminal of the growth factors with low-molecular-weight protamine (LMWP) to form LMWP-EGF, LMWP-PDGF and, LMWP-IGF-I. The LMWP fused growth factors were further complexed with hyaluronic acid, and after confirming the synergistic effect of this combination they were loaded into cationic elastic liposomes. The LMWP-fused growth factors loaded cationic liposomes accelerated wound closure rate and decreased the wound size by 65% and 58% on comparison with blank cationic liposomes and growth factors-hyaluronic acid complex, respectively, in a diabetic mouse model [22].

Several phytoconstituents have anti-bacterial, anti-inflammatory, antioxidant, astringent and immunomodulatory activities that promote the wound healing process. Extensive research has been done in the field of developing nanocarriers loaded with phytoconstituents for promoting the wound healing effect and overcome the drawbacks associated with its physicochemical properties, permeability issues, and stability issues. Ternullo et al. developed a liposome in the hydrogel system for delivery of curcumin intended to improve the chronic wound therapy. Curcumin which is a poorly soluble active constituent was incorporated into deformable liposomes which were further loaded into chitosan hydrogel for

proper application on wound site. The effect of liposomal charge on hydrogel properties was determined by incorporating cationic, anionic and neutral deformable liposomes in the chitosan hydrogel. The hydrogel's cohesiveness, hardness, and adhesiveness were affected by the surface charges of the liposomes. The incorporation of cationic deformable liposomes in hydrogel preserved the bio-adhesiveness of the hydrogel to a greater degree compared to anionic and neutral deformable liposomes [23]. Quercetin has a significant wound healing effect due to its antioxidant and anti-inflammatory property. Jangde et al. developed quercetin encapsulated liposomes by thin-film hydration technique and optimized the formulation by employing response surface methodology. The influence of rotary evaporators rotation speed and water baths temperature were studied for preparation of quercetin loaded liposomes. The optimized formulation gave the best results i.e. maximum entrapment efficiency (86.5%) and smallest particle size (146.8) at 75 rpm rotation speed and 46° C temperature. The release profile showed a prolonged release up to 75.09% release during 24 h [24]. Madecassoside is well known for its cell growth-promoting and wound and scar healing effect due to its anti-oxidant, germ-killing, anti-inflammatory, and antiulcer properties. However, the large molecular weight and highly water-soluble property of madecassoside make its permeation through the skin layers very difficult. To surmount this limitation, madecassoside liposomes were formulated by double emulsion technique to increase the permeability and wound healing efficiency. Response surface methodology was implemented to derive the optimum preparation settings of madecassoside liposomes. The skin permeation and wound healing effect of madecassoside liposomes were better than madecassoside film dispersion liposomes. Also, the double emulsion madecassoside liposomes were highly stable with high encapsulation efficiency and smaller particle size and represented the superior performance of burn wound healing effect. The results concluded that the double emulsion method was promising and applicable liposomal preparation method for enhancing the effect of madecassoside [25]. For improving the wound healing effect and surface adhesiveness of madecassoside liposomes, surface modification with poly (ethylene glycol)-poly(□-caprolactone)-poly (ethylene glycol) (PEG-PCL-PEG, PECE) a temperature-responsive and biodegradable copolymer were done. Normal liposomes are less viscous thus they are not able to uphold adequate adhesion on the wound surface. The PECE-modified madecassoside liposomes were able to maintain a hydrogel state up till 43° C temperature which showed better adhesion to the wound site. the modified liposomes also represented superior wound contraction effects compared to unmodified madecassoside liposomes on a rat with second-degree burn [26]. Another research study was done to establish the effect of farnesol encapsulated liposomes

loaded hydroxypropyl methylcellulose (HPMC) gel on third-degree burns induced rat model. The formulation displayed good collagen production and wound healing efficacy in both *in vitro* and *in vivo* studies. After treating the third-degree burns on a rat model with 4 mM liposomes loaded with farnesol and several ratios of 2% HPMC for 7 and 14 days, the histopathological studies were performed. The histopathological results revealed that after treating the third-degree burns on rats with HPMC: farnesol gel with 1:2 and 2:1 ratio, great tissue regenerative effects were observed compared to skin treated with HPMC alone or commercial burn gel. The findings were the same in the *in vivo* quantitative studies such as wound healing scoring, collagen-producing assay, and IL-6 western blot results [27].

Lyophilized liposomal wafers, a novel wound dressing, loaded with gatifloxacin (a fourth-generation fluoroquinolone antibiotic), was designed and evaluated for enhancing wound healing potential. First of all, liposomes were formulated loaded with gatifloxacin, thereafter the liposomes were transformed to gel by using chitosan which was further lyophilized to generate liposomal wafers. The liposomes were formulated by changing the concentration of cholesterol and lipid and the entrapment efficiency, particle size, scanning electron microscopy (SEM), and transmission electron microscopy (TEM), *in vitro* cumulative release was done for its evaluation. The liposomal gel prepared using chitosan was characterized for its clarity, texture, spreadability, viscosity, and *in vitro* drug release. Thereafter, the final lyophilized liposomal wafers were evaluated by SEM, x-ray diffraction spectroscopy (XRD), differential scanning calorimetry (DSC), and drug release studies. The lyophilized liposomal wafers were beneficial as they gave controlled drug release at the wound site because of incorporating liposomes. A moist wound bed was ascertained for faster healing due to swelling of the wafers after absorbing wound exudate. They exhibited good swelling capacity, good strength, hardness and improved adhesion. The wound healing actions were confirmed by histopathological evaluation after conducting *in vivo* experiments in wistar rats. The incorporation of gatifloxacin was beneficial to combat wound infection while the wafers provided better wound healing action because of the sustained release of the drug [28]. Liposomes encapsulated haemoglobin with high O_2 affinity were tested in diabetic dB/dB mice to evaluate its wound healing potential by Fukui et al. Improved tissue perfusion due to significant increase in surface blood flow, suppression in inflammatory cytokines, and accelerated skin wound healing was observed. However, the study concluded that still an optimal O_2 affinity, timing of administration and dose was required to be assessed to determine the optimal application of liposomes encapsulated haemoglobin [29]. Hurler et al. developed mupirocin loaded liposomes and further incorporated them in hydrogel for the treatment of burns. The ability of the

formulation to prevent biofilm formation, its action on mature biofilms and the wound healing potential was determined in *in vivo* mice burn model. The formulation exhibited antimicrobial action against streptococcus aureus biofilms. The *in vivo* studies results depicted that the drug delivery system enhanced the wound healing activity as the healing time was shorter as compared to the marketed product of mupirocin [30]. Bacteriophage therapy to combat drug-resistant microorganisms is a good option for further improving wound healing. However, low phage persistence in situ is the major drawback of bacteriophage therapy. Chhibber et al. developed liposomes entrapped bacteriophage cocktail and evaluated its potential to treat a staphylococcus aureus produced diabetic excision wound infection. MR-5 and MR-10, two potential lytic bacteriophages were characterized alone, in combination (cocktail), or encapsulated in liposomes were evaluated for their efficiency in treating diabetic wound infection. The stability studies (*in vitro*) and the phage titer determination study (*in vivo*) results revealed that the liposomes entrapped with bacteriophage cocktail led to improved persistence of bacteriophages at the site of the wound. The liposomes entrapped bacteriophages represented a 2-log increase in bacteriophage titre at the wound site as compared to free bacteriophage cocktail. This led to an increased rate of infection resolution and enhanced the wound healing rate [31]. Thapa et al. developed collagen mimetic peptide (CMP) -tethered vancomycin liposomes (CMP-vancomycin-liposomes) loaded in fibrin/collagen copolymeric hydrogels (co-gels) for treatment against methicillin-resistant staphylococcus aureus (MSRA) infections in wound infections. Compared to vancomycin loaded co-gels or vancomycin-liposomes loaded co-gels, tethering CMP-vancomycin-liposomes to co-gels represented the sustained release of vancomycin and enhances *in vitro* antibacterial activity in contradiction of MSRA. The *in vivo* results represented that CMP-vancomycin-liposomes retained anti-bacterial effect also after re-inoculating with bacteria, while vancomycin incorporated co-gels and vancomycin-liposomes incorporated co-gels permitted bacterial growth which represents their limited efficacy [32]. Novel unilamellar polyhedral vesicles named santosomes, composed of *Santolina insularis* essential oil and hydrogenated phosphatidylcholine. The essential oil improves the delivery of drug-loaded by two mechanisms: bilayer structure modification and enhances the effect of potent penetration enhancers (mono- and sesquiterpenes), and phospholipids. Phycocyanin a protein found in cyanobacteria having anti-inflammatory, antioxidant, and wound healing activity was encapsulated into santosomes by castangia et al. The santosomes loaded with phycocyanin was more effective compared to free protein to promote internalization into endothelial cells, protecting skin against free radicals, avoiding edema formation, and contributing in the repair of injured skin.

The results of *in vivo* studies performed on mice demonstrated an evident amelioration of skin lesion which confirmed the great wound healing potential of phycocyanin loaded santosomes [33].

4.3. Solid lipid nanoparticles (SLNs) and Nanostructured lipid carriers:

In the beginning of 1990s, SLNs were developed as an alternative to liposomes to overcome several drawbacks of liposomes such as stability issues, low encapsulation efficiency, aggregation on storage, uncontrolled release of drugs, etc. They are a novel pharmaceutical drug delivery system comprising of a solid lipid center which is stabilized by surfactants. They have demonstrated numerous benefits as controlled drug delivery system such as (a) they are biocompatible and biodegradable, (b) they improve the drug targeting and bioavailability, (c) suitable for lipophilic as well as hydrophilic active compounds, (d) due to their size and lipid solubility they promote diffusion through biological barriers, (e) easy scale-up, (f) production process can be free from organic solvents (10). On comparison with liposomes, SLNs present higher flexibility to modulate the drug release and provides better protection to the encapsulated drug. However, the changes in crystal lattice during storage often leads to drug expulsion from SLNs. To overcome this drawback, lipid nanocarriers with amorphous matrix were developed using liquid lipids along with solid lipids which were named as NLCs. The NLCs presents higher drug loading capacity and greater stability compared to SLNs. The Lipids and surfactants widely used in the preparation of SLNs and NLCs are classified in figure 2.

For wound healing, both SLNs and NLCs represent several advantages such as (a) due to their occlusive nature and adhesiveness, they increase the skin hydration effect, (b) they allow controlled release of an encapsulated moiety, (c) the smaller size ensures closer contact with the skin, (d) they enable topical delivery and reduces the systemic exposure. The skin hydration effect is due to two underlying mechanisms suggested by Muller and co-workers: (a) occlusive film formation at stratum corneum that prevents evaporation of water, (b) reinforces the skin lipid natural barrier as nanoparticles adhere to the stratum corneum. From the topical drug delivery perspective, lipid nanoparticles being aqueous dispersions have low viscosity for application. Thus, SLNs and NLCs are further incorporated into some film, hydrogel, scaffold, cream, ointment, etc. or viscosity enhancers are added in the dispersion for making them suitable for topical applicability [34]. The classification of methods used for the preparation of SLNs and NLCs is represented in figure 3.

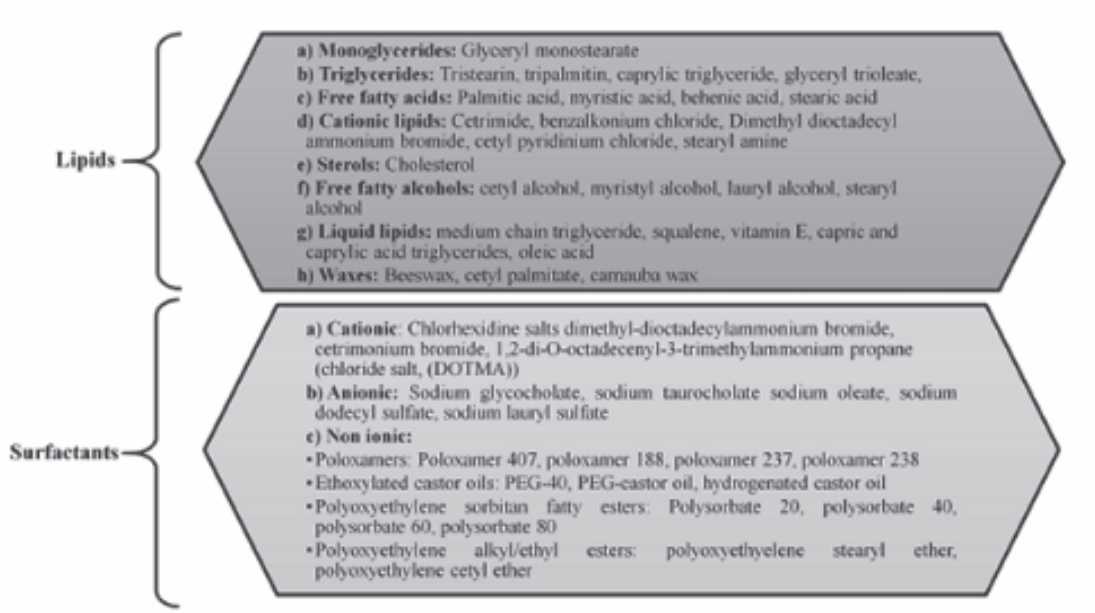

Figure 2: The Lipids and surfactants widely used in the preparation of SLNs and NLCs

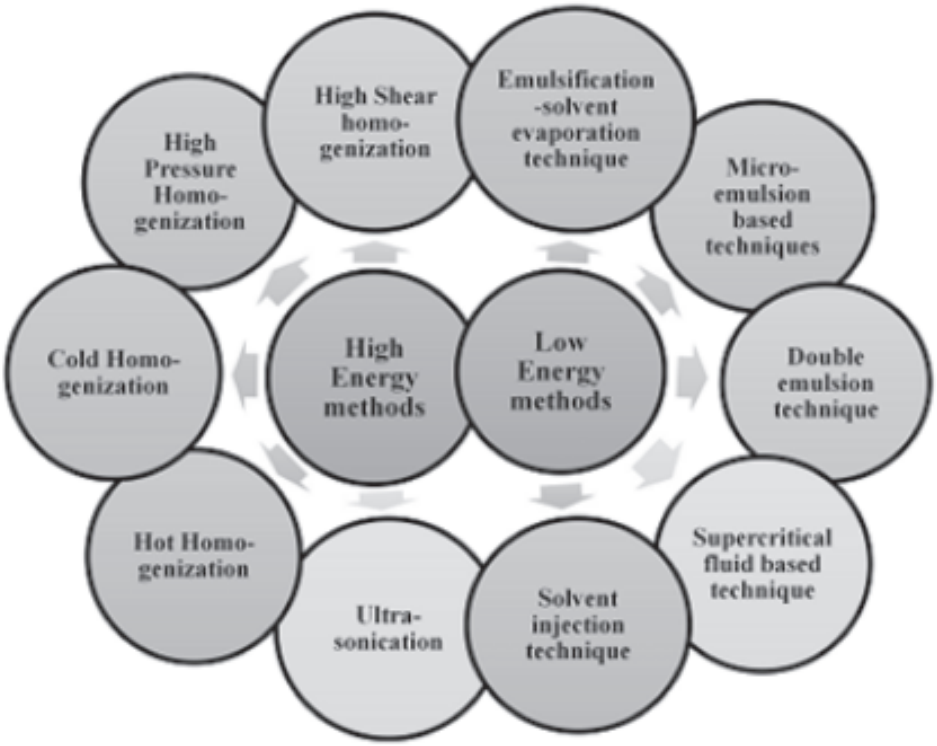

Figure 3: The classification of methods used for the preparation of SLNs and NLCs

Essential oils obtained from several plants have significant antibacterial, anti-inflammatory, and antioxidant properties useful for the cure of nonhealing wounds. *Mentha pulegium* essential oil (MPO), having significant antibacterial and anti-oxidant properties, comprises of piperitone, pulegone, piperitenone, menthone and isomenthone. However, due to the volatile compounds present in essential oils, their use has several limitations like they are not stable and gets easily destroyed by environmental influences. Lipid nanoparticles are reported to protect the essential oils against environmental factors as well as enhance their antimicrobial and wound healing potential. Khezri et al. developed MPO-loaded NLCs and evaluated its efficacy against infected wounds. The effect of MPO-NLCs on wound healing, tissue bacterial count, wound contraction, molecular and histological parameters was assessed at 3, 7 and 14 days after wound creation. MPO-NLCs topical administration shortened the inflammatory phase and enhanced the proliferative phase. The IL-10, b-FGF, TGF-β expression increased and NF-κB decreased after treatment with MPO-NLCs compared to the control group [35]. In another study, Carbone et al. developed NLCs for combined delivery of Lavandula essential oil and ferulic acid, whose potential wound healing action has been reported in several studies. The combined delivery promoted cell migration and represented higher effectiveness in comparison to free drug solution or NLCs without Lavandula essential oil [36]. Chamomile oil has a very significant wound healing action, but its easy degradation and poor tissue permeability limit its topical application. To ameliorate its wound healing potential, it was encapsulated into SLNs by Gad et al. SLNs were formulated by hot homogenization technique using chamomile oil and 20% w/w stearic acid. The in-vivo studies were conducted on 40 rats divided into 5 groups (normal control, wounded rats without treatment, treated with blank SLNs, treated with Camisan® cream, and treated with chamomile SLNs. The results demonstrated that chamomile SLNs treated rats showed improved wound contraction, collagen deposition, TGF-β1 and reduced IL-1β and MMP-9, as compared to other treatment groups [37]. Peppermint essential oil loaded NLCs were fabricated and evaluated for their antibacterial activity and wound healing action by Ghodrati et al. The *in vitro* study on Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa, Listeria monocytogenes, Bacillus anthracis, Staphylococcus epidermidis, and Staphylococcus pneumonia species were tested. The *in vitro* results revealed significant antibacterial activity of peppermint essential oil loaded NLCs. *In vivo* analysis represented increased wound contraction rate, collagen deposition rate and re-epithelialization in peppermint essential oil loaded NLCs group compared to the control group [38]. Another study was done by khezri et al. to evaluate the topical wound healing potential of rosemary

essential oil (REO) into NLCs. The *in vitro* studies represented that REO-NLCs were highly effective against Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, listeria monocytogenes, and pseudomonas aeruginosa. The *in vivo* studies done on mouse induces with full thickness wound on their back which was then treated with REO-NLCs demonstrated reduced bacterial colonization and wound size and increased fibroblast infiltration, vascularization, re-epithelialization, IL-3, IL-10, Stromal cell-derived factor 1 alpha (SDF-1α), VEGF levels [39].

Numerous natural compounds having anti-inflammatory, antibacterial, and wound healing effects have been reported to be loaded in SLNs or NLCs. Zerumbone, a lipophilic compound obtained from Zingiber zerumbet was loaded into NLCs by Albaayit et al. The study was done on excisional wounds in rats, which were treated with zerumbone-loaded NLCs carrier gel (0.5 mg/ml) and silver sulfadiazine 1% w/w (for comparison) for 15 days. Compared to silver sulfadiazine, zerumbone-loaded NLCs carrier gel was more effective for treating the wounds. Increase in IL-10 and decrease in pro-inflammatory tissue necrosis factor-α, IL-6 concentrations, and cyclooxygenase-2 expression was observed after treatment with zerumbone-loaded NLCs [40]. Topical application of opioids for pain reduction in severe chronic wounds was evaluated by Kuchler et al. Morphine-loaded SLNs were developed and evaluated for improvement of wound closure and pain reduction in human-based 3D wound healing model. The human full-thickness skin equivalents were induced by CO_2 laser irradiation. The morphine loaded SLNs showed accelerated wound closure, prolonged release of morphine and enhanced keratinocyte proliferation [41]. A combination approach of an antibiotic with natural wound healing agent was studied by ghaffari et al. in which they developed curcumin-ampicillin SLNs which were further loaded into ointments and gels. Both the gel and ointment and gel represented significant wound healing effect compared to control groups or placebos [42]. Tetrahydrocurcumin (THC), also known as white curcumin, is a stable colourless hydrogenated form of curcumin having superior anti-inflammatory and anti-oxidant properties. THC-SLNs were developed by micro-emulsification technique and further incorporated into the hydrogel. The THC-SLNs gel showed 17 times higher skin permeation compared to free THC gel. The formulation was non-irritating, showed desired occlusivity and stability. The pharmacodynamic evaluation done in excision wound mice model depicted enhanced anti-inflammatory effect by THC-SLNs gel which were confirmed by histopathological and biochemical studies [43]. In recent years, artificial scaffolds performing as skin substitutes have been fabricated for improving wound healing. Collagen is an excellent biomaterial which can be employed to fabricate scaffolds as it has an abundant quantity of bodily proteins, low immunogenicity and high stability. Collagen is known to promote

cell adhesion, migration and differentiation. Curcumin, when loaded as such into collagen hydrogels, tends to form large aggregates which clog the matrix pores of the hydrogel. A strategy to load curcumin-lipid nanoparticles into collagen scaffolds was developed. The homogeneous distribution of curcumin-lipid nanoparticles into the collagen scaffolds was observed. 100% release from lipid nanocarriers was obtained after 25 days when hydrogels were soaked into the saline buffer [44]. The effect of astragaloside IV- loaded SLN enriched hydrogel on the anti-scar formation and wound healing was evaluated by Chen et al. The astragaloside IV-loaded SLNs were prepared by solvent evaporation method and characterized. Furthermore, the optimized SLNs were loaded into carbomer hydrogel to produce astragaloside IV-SLNs enriched gel. The astragaloside IV-SLNs enriched gel enhanced the proliferation and migration of keratinocytes and also increased the uptake of the drug on fibroblasts *in vitro* by caveolae endocytosis pathway. The *in vivo* study represented that the wound closure rate, angiogenesis and collagen formation increased strengthening the wound healing and inhibiting the scar formation after treatment with astragaloside IV-SLNs enriched gel [45].

Melatonin, secreted by the pineal gland is a well-recognized free radical scavenger, anti-inflammatory agent, and stimulator of antioxidant enzymes. Due to the intracellular chelating activity, its potential to fight against bacterial, viral and parasitic infections have been reported. It also promotes cell migration and proliferation during the wound healing process. Romic et al. developed NLCs loaded chitosan microspheres as an innovative dry powder-based wound dressing, in which melatonin was added in both NLCs as well as chitosan matrix. Hot homogenization technique was employed to formulate NLCs, while the microspheres were prepared by spray-drying technique. The dry powder system swelled after coming in contact with simulated wound fluid and turned into the hydrogel. The formulation represented significant antimicrobial activity against S. aureus MRSA strain and staphylococcus aureus [46]. Nucleic acid delivery is an appealing approach for regulating and controlling the production of proteins during wound healing. Downregulation of a protein named fidgetin-like protein 2, improves the wound healing process. The function of the protein is to regulate microtubule assembly which reduced the migration of cells. siRNA knocks down this protein and improved the rate of cell movement, which in turn improves the wound healing. However, siRNA as a therapeutic agent needs an efficient carrier to reach the intracellular site of action. It is unstable in serum and also sensitive to nuclease degradation. The high negative charge, stiffness, and high molecular weight of siRNA impede cellular internalization. Tezgel et al. developed collagen scaffolds loaded with either naked siRNA, siRNA/NLCs complexes forming cationic NLCs including either a DOTAP (1,2-

dioleoyl-3-trimethylammonium-propan) and DOPE (1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine) shell (cNLC) or chitosan coating (CS-NLC). Collagen scaffolds encumbered with cNLC represented the most prolonged release of siRNA and downregulation of ERK-1 protein *in vitro* [47]. Nowadays, numerous studies are done on the exogenous application of growth factors such as EGF, PDGF, FGF, or TGF for wound healing. However, they are having very low stability which necessitates the development of ideal nanocarriers that helps to keep them stable and provide a localized and sustained release. Gainza et al. developed recombinant human epidermal growth factor (rhEGF) loaded lipid nanoparticles (SLNs and NLCs) and evaluated the *in vitro* and *in vivo* effectiveness for wound healing in healing-impaired db/db mice. The encapsulation efficiency of rhEGF-NLCs was higher than rhEGF-SLNs, and the gamma sterilization method was found to be suitable for final sterilization as there was no loss of activity after sterilization. The proliferation assays demonstrated that nanoformulations had higher bioactivity than that of free rhEGF [48]. Another approach was also done by gainza et al. where the lipid nanoparticles encapsulating rhEGF were further loaded into either semi-solid hydrogels or fibrin-based scaffolds [49].

5. Future perspective:

The scientific community is focusing on improving the current therapeutic approaches by developing novel release systems intended to allow a controlled release, avoid recurrent dressing changes, promote cell proliferation, provide ideal wound healing conditions, etc. Lipid nanoparticles are widely investigated due to their non-toxic nature, high penetration in the wound area, ability to provide a controlled release, protect the drug from enzymatic degradation, etc. Although numerous studies are performed to develop lipid nanocarriers based dressings for wound healing which demonstrates encouraging results, it is still necessary to carry out in-depth studies for foreseeing the consequences like their accumulation, elimination and toxicology. A lot of information supported by in vitro and *in vivo* studies is available for use of nanocarriers in wound healing, however, it is still necessary for them to reach clinical application and fulfil the task of improving the life quality of chronic wound patients. There is a great demand for advanced animal models and designs of clinical trials to provide strong evidence and help in regulatory approval of these nanotechnology-based drug delivery approaches. Due to the variable wound causes and types and the complexity of the wound healing process, the studies done on patients by nano-systems are still very limited. Establishment of a single therapy for all the patients becomes difficult due to the variability between patients and comorbidities associated with the wound. Thus, more efforts are required to make these systems available for human use and thereby overcome the current drawbacks associated with chronic wound healing.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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List of abbreviation:

SLNs: Solid lipid nanoparticles

NLCs: Nanostructured lipid carriers

TGF-β: Transforming growth factor-β

PDGF: Platelet-derived growth factor

VEGF: Vascular endothelial growth factor

ROS: Reactive oxygen species

IL: Interleukin

MMP-9: Matrix metalloproteinase-9

AGEs: Advanced glycation end products

EGF: Epidermal growth factor

ECM: Extracellular matrix

bFGF: Basic fibroblast growth factor

HB-EGF: Heparin-binding epidermal growth factor

EGFR: Epidermal growth factor receptor

IGF-I: Insulin-like growth factor-I

PECE: poly (ethylene glycol)-poly(□-caprolactone)-poly (ethylene glycol)

HPMC: Hydroxypropyl methylcellulose

SEM: Scanning electron microscopy

TEM: Transmission electron microscopy

XRD: x-ray diffraction spectroscopy

DSC: Differential scanning calorimetry

CMP: Collagen mimetic peptide

MSRA: Methicillin-resistant staphylococcus aureus

MPO: Mentha pulegium essential oil

REO: Rosemary essential oil

SDF-1α: Stromal cell-derived factor 1 alpha

THC: Tetrahydrocurcumin

cNLC: DOTAP (1,2-dioleoyl-3-trimethylammonium-propan) and DOPE (1,2-Dioleoyl-sn- glycero-3-phosphoethanolamine) shell

CS-NLC: Chitosan coating

rhEGF: Recombinant human epidermal growth factor