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Review Article

Vesicular Systems Used for Wound Healing

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ABSTRACT

When the skin is injured through physical, chemical, mechanical, and/or thermal damage, a spontaneous series of events start to happen, often called the "cascade of healing," to restore the injured tissues, replace the damaged structures and prevent the invasion of pathogens into the damaged tissues. Many traditional products are available for wound healing such as gels, creams, ointments, dressings, and solutions, which depend mainly on moisture intake to help tissue repair, yet they do not provide optimal conditions to permit recovery of the wounds. Nanocarriers play a significant role in wound healing since they are reported to improve drug delivery into the skin through the alternation of pharmacokinetics and biodistribution of drugs, hence they increase the bioavailability of drugs. Vesicular systems such as liposomes, niosomes, transfersomes, penetration enhancer containing vesicles (PEVs), and ethosomes are among the carriers proven to enhance the therapeutic action of drugs applied for wound healing. In this review, we summarize and discuss different vesicular systems used for wound healing, their composition, their advantages and disadvantages, their methods of preparation, and their mechanisms of skin penetration.

Keywords: *Skin; skin structure; wound healing; wound treatment; vesicular systems.*

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1. INTRODUCTION

Topical preparations are formulations that are applied directly to an external body surface by spreading **[1]**, rubbing, spraying, or installation such as creams, ointments, lotions, and gels **[2]**. The topical route has acquired much attention for many years, owing to its impressive advantages compared to other routes of drug delivery. Topical formulations have high patient compliance as they are non-invasive, can be self-administered **[1]**, and offer the flexibility to terminate the drug administration through the removal of formulations from the skin**.** The topical route has advantages in treating various skin infections like fungal infections **[3]** as it allows for deposition of antifungal drugs on the site of infection, enhancing their therapeutic efficacy and decreasing their undesirable systemic side effects. The most important problem encountered with topical drug delivery is the low penetration of most drugs through the skin, owing to the barrier function of the skin **[4-7]**. Conventional formulations for topical drug delivery have weak percutaneous permeability and poor deposition in the skin. In search of improved topical products, scientists either design new vehicles or explore novel

nanocarriers to ensure adequate penetration, and more importantly, localization of the drug within the deeper layers of the skin **[8-10].**

2. The skin: structure and barrier properties

The skin is the largest part of our body with a surface area of about 1.7 m^2. The skin acts as an effective obstacle **[11]** against the loss of water and electrolytes. Additionally, the skin is impermeable to most substances as it prevents the entry of harmful substances present in the environment and the locally applied drugs.

2.1. Skin structure

Human skin is composed of four main regions **[11]** as shown in **Fig. 1**, stratum corneum, epidermis, dermis, and subcutaneous fat. Many appendages are present in the skin such as hair follicles and sweat glands.

2.1.1. The stratum corneum (SC)

It is the uppermost constituent of the skin as shown in **Fig. 1**. It is responsible for the barrier function of the skin against permeation (over 80% of the skin resistance) despite being very thin. It is composed of the corneocytes, joined together with desmosomes **[7]**, and present in a lipid matrix composed of free fatty acids, cholesterol, cholesteryl esters, and ceramides **[12]**.

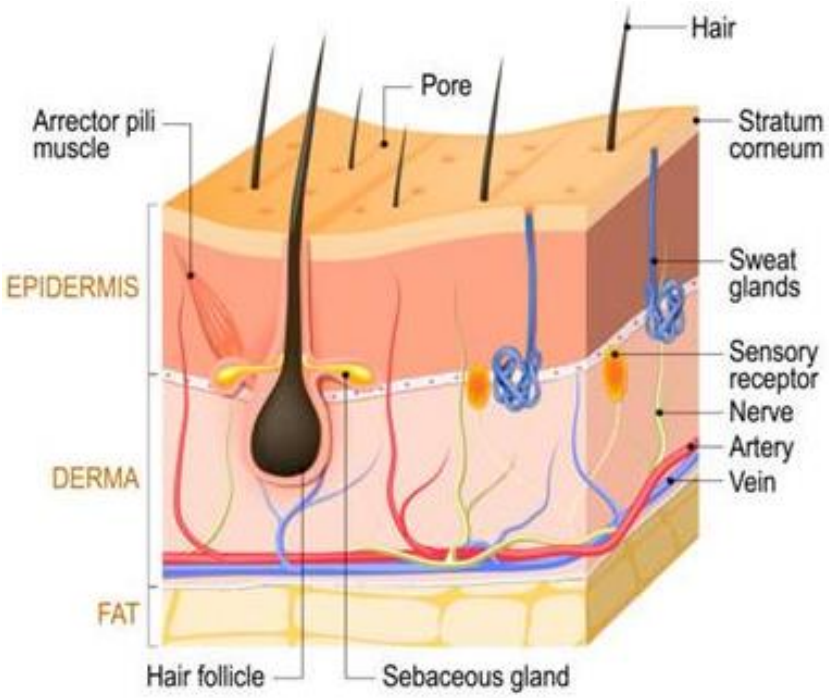

Fig. 1. represents skin structure

(https://freerangestock.com) accessed on 8 April 2021

2.1.2. Epidermis

It is the thin, relatively dry, tough outer barrier against the penetration of foreign substances from the outside or the wastage of water and electrolytes from the body.

2.1.3. Dermis

The dermis is the skin's supporting tissue. Its specialized cells (fibroblasts) produce collagen and elastin fibers. The dermis is a large region of connective tissue that contains blood vessels **[7]**, hair follicles, nerve endings, smooth muscles,

sweat, and oil glands **[11]**. The dermis acts as the body's primary temperature regulating mechanism and controls blood pressure. The dermis is also rich in nerve endings, which are sensitive to touch; pain, and temperature, making the human skin a sensorial organ **[13]**.

2.1.4. Subcutaneous fat

It is the innermost layer of the skin as shown in **Fig. 1**. The fat stored in this layer acts as an energy source for the body and also plays an important role in insulating the body against the changes in the outside temperature by monitoring heat gain and loss. This layer has no role in the barrier function of the skin because the drug is taken up by the capillaries before it reaches this layer **[14]**.

The skin acts as an effective barrier for drug passage. The passage of drugs across the skin can occur only *via* the following pathways **[15]**:

(1) The appendageal route; the permeation of drugs in this route occurs *via* the hair follicles and the sweat glands. It is a favorable route for highly hydrophilic molecules and electrolytes, as well as large molecules with low diffusion coefficients.

(2) The transcellular route; the permeation of drugs in this route occurs *via* the corneocytes and the surrounding lipid matrix. This route is favorable for hydrophilic drugs ($\log k < 1$).

(3) The intercellular route; the permeation of drugs in this route occurs between the corneocytes. This route is very important for highly lipophilic drugs ($\log k > 3$).

3. Wound healing

When the skin is injured through physical, chemical, mechanical, and/or thermal damage **[8]**, a spontaneous series of events begin to happen, often called the "cascade of healing," to restore the injured tissues **[16]**, replace the damaged structures and prevent the invasion of microorganisms into the damaged tissues. The healing process is composed of four overlapping phases: Hemostasis, Inflammatory, Proliferative, and Maturation phases **[17-19]**. The healing process is remarkable and complex **[16]**, and it is also hindered by local factors including moisture and infection as well as systemic factors including age and nutritional status. When the right healing environment is established, the body starts to heal and restore the devitalized tissues **[20]**.

3.1. Phase 1: Hemostasis Phase

Hemostasis; the first phase of healing, starts immediately after injury to stop blood loss and to limit the invasion of microorganisms **[16]**. This phase is characterized by vascular constriction, platelet aggregation, and formation of fibrin clot through activation of enzyme thrombin which supports platelet clumps into the stable clot to stop bleeding. In this stage, platelets come into contact with collagen, resulting in activation and aggregation of platelets **[10]**. In addition, they release several growth factors **(Table 1)** which are responsible for inflammation at the site of injury including the transforming growth factor-β (TGF-β), epidermal growth factor (EGF), insulin-like growth factor-1, and platelet-derived growth factor (PDGF) **[10, 16]**, **[18, 20]**.

3.2. Phase 2: Defensive/Inflammatory Phase

The defensive/Inflammatory phase happens at the same time as homeostasis **[16]**, and it aims to eradicate microorganisms, clear debris, and essentially prepare the wound bed for the growth of new tissues. In this phase, white blood cells mainly neutrophils, macrophages, and lymphocytes release several proinflammatory cytokines, cationic peptides, proteases, reactive oxygen species, and growth factors allowing for wound decontamination. Growth factors like TGF-β, recombinant human Granulocyte macrophage-colony Stimulating Factor **(**GM-

CSF), PDGF, fibroblast growth factor, basic fibroblast growth factor (BFGF), and EGF **[10, 16]** have a critical role in the communication between cells, stimulation of keratinocytes, fibroblast, and angiogenesis, as well as attraction of immune cells into the wound to destroy bacteria, clean debris and repair damaged tissues **(Table 1)**. When these cells end their role, unneeded cells undergo apoptosis, leading to a reduction in their number. At that time, specialized cells (macrophages) **[17]** continue clearing debris. Moreover, macrophages secrete growth factors and proteins that entice immune cells to the wound to restore the devitalized tissues and efferocytosis. Efferocytosis is the elimination of apoptotic neutrophils before they undergo secondary necrosis by macrophages to limit tissue damage and assist its recovery but a prolonged inflammatory phase may lead to cell destruction and changing composition of the extracellular matrix that delay epithelization **[17, 18, 20]**. This phase is often characterized by edema, erythema, heat, and pain.

Table 1. Different types of growth factors and their roles in wound healing [21]

Growth factors	Role in wound healing
EGF	They accelerate epidermal regeneration through activation of keratinocytes and fibroblasts propagation, discrimination, and transmigration.
PDGF	They increase the structural integrity of vessels, extracellular matrix deposition, and re-epithelialization through promoting neutrophils, macrophages, and fibroblast proliferation.
BFGF	They promote collagenase production, extracellular matrix deposition by promoting keratinocytes and fibroblasts proliferation and re-epithelialization.
TGF-β	They promote the formation of granulation tissue; re-epithelialization; matrix formation through activation of keratinocytes, macrophages, lymphocytes, and fibroblasts proliferation and remodeling.
GM-CSF	They help wound contraction and promote local recruitment of inflammatory cells through the stimulation of keratinocytes, endothelial cells, macrophages, and eosinophils proliferation and differentiation.

3.3. Phase 3: Proliferative Phase

This phase consists of granulation, contraction, and re-epithelialization **[22]**. Once the wound is washed out, the wound enters the proliferative phase **[16]** which aims to fill the wound with new tissues. This phase is characterized by three different steps: 1) filling the wound with granulation tissues; 2) shrinkage of the wound edges; and 3) re-epithelialization to cover the wound **[16]**. During the first stage (filling the wound), epithelial cells begin to replace dead cells and fibroblasts produce collagen, hyaluronan, fibronectin,

glycosaminoglycan, and proteoglycan. During this phase, shiny, deep red granulation tissue fills the wound bed with connective tissue and new blood vessels are formed (angiogenesis). Then, the wound margins shrink towards the center of the wound and so decrease in size by the movement of epidermal and dermal cells. In the third stage, epithelial cells begin to proliferate and replace dead cells to cover the wound area with epithelium (epithelization) **[18, 20]**.

3.4. Phase 4: Maturation stage (remodeling phase)

In this stage, the newly formed tissue begins to gain strength and flexibility until it becomes the initial healthy tissue. Here, collagen fibers reorganize, the tissue remodels and matures and there is an overall increase in tensile strength (though maximum strength is limited to 80% of the pre-injured strength). The maturation phase differs greatly from a wound to wound **[18, 20]**.

Wound treatment aims to keep the wound away from bacteria to prevent infection **[10]**, reduce pain, and at the same time induce fast healing to achieve optimum healing and quality of wound closure **[23]**. The slow healing process converts acute wounds that require 7 to 10 days for healing into chronic wounds which require a long period of treatment and hospitalization **[24]**. Some commercial products are available such as gels, creams, ointments, dressings, and solutions, which are mainly based on the principle of moisture intake that is intended to support tissue repair, yet they are not anti-infective and sometimes even cannot be used in the presence of a potential infection **[25]**. Additionally, healing of wounds with conventional treatments often requires a longer duration and thus does not provide optimal conditions to permit recovery of the wounds, and dressings usually cause injuries upon removal. Moreover, they require frequent application and wound coverage to ensure sterility of wounds, the therapeutic action of drugs, and to avoid wound drying **[9]**. Therefore, they decrease patient compliance and acceptance **[10]**. Nanocarriers play an important role in wound healing since they are reported to improve therapeutic action more than drugs themselves **[8, 9]** as they act as new entities that differ from free drugs **[26]**. They improve drug delivery into the skin through enhancement of the pharmacokinetics and biodistribution of drugs, hence they increase the bioavailability of drugs **[10]**. Moreover, they promote wound closure, reduce wound pain, enhance tissue regeneration and control wound infection and inflammation. Vesicular nanocarriers are the most commonly reported systems for wound healing, and hence they will be the focus of this review.

4. Vesicular delivery systems for wound healing

Vesicular delivery systems such as liposomes and niosomes have been intensively studied as carrier systems for topical delivery of drugs and cosmetic agents. They can enhance the penetration of hydrophilic and hydrophobic drugs into the skin, increase their shelf life, decrease the serious side effects like skin irritation, and act as a depot for controlled drug release **[27, 28]**. Vesicular systems can be classified into two categories: rigid vesicles such as liposomes and niosomes, and flexible or ultra-flexible vesicles such as transfersomes. Rigid vesicles were reported to be inefficient for transdermal drug delivery because they remain on the upper layer of the stratum corneum and do not deeply penetrate the skin **[29]**.

4.1. Liposomes

They are self-assembled bilayers made of phospholipids, which enclose a distinct aqueous space **[30, 31]**. The main components of these vesicles are phospholipids such as lecithin, phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA),

and phosphatidylinositol (PI) with or without cholesterol which used to impart rigidity for the lipid bilayer.

Liposomes are widely used as drug delivery carriers owing to their biodegradability, non-toxicity, and biocompatibility **[30]**. Additionally, they can encapsulate both hydrophilic and hydrophobic constituents **[31]** as hydrophilic drugs get entrapped in their aqueous core while hydrophobic compounds get entrapped in the bilayers. Moreover, liposomes can stabilize therapeutic compounds **[30]** and overcome barriers to cellular and tissue uptake. Also, surface modification of liposomes by binding to site-specific ligands (active targeting) allows them to improve the delivery of drugs to sites of diseases and consequently reduce non-specific toxicity **[32]**. Also, liposomes increase skin penetration of hydrophilic drugs because of their lipidic composition that mimics the composition of percutaneous tissue **[31]**.

Liposomes can also be categorized according to their lamellarity into uni-, oligo-, and multi-lamellar vesicles **[33]**. Unilamellar liposomes have one lipid bilayer and generally are 50–250 nm in size, whereas multilamellar liposomes have several concentric lipid bilayers and have diameters of 1–5 μm. Also, they are categorized according to their size into small, intermediate, or large vesicles and based on the method of preparation into reverse-phase evaporation vesicles, thin-film hydration vesicles, and solvent dispersion vesicles **[33]**.

Also, liposomes are categorized according to their surface characteristics **[32]** into four main types: classical liposomes, stealth liposomes, ligand-targeted liposomes, and theranostic liposomes. PEGylated liposomes which also called stealth liposomes **[33]** create steric hindrance to augment the circulation half-life of the delivery system following its systemic administration **[32]** through decreasing mononuclear phagocytic system (MPS) uptake **[32, [33].** Additionally, stealth liposomes decrease the non-specific drug toxicity through increasing passive targeting **[33]** whereas, theranostic liposomes are used for cancer imaging.

Among the drawbacks of liposomes is that they are liable to rancidity upon exposure to metals, light, and high temperatures because of their phospholipid constituents **[34]**. Therefore, they have a limited shelf life **[35]**. They are also thermodynamically unstable carriers as they are susceptible to aggregation upon storage **[36]** and they are liable to leakage of encapsulated drug molecules after preparation **[37]**.

Moreover, classical liposomes have been rarely used as carriers for percutaneous drug delivery, as they do not penetrate deeply into skin layers, but rather exist on the upper layers of the stratum corneum **[38]**. Also, their cost of preparation is high due to the high cost of organic solvents used for the dissolution of phospholipids **[34, 35]**.

4.1.1. Mechanisms for liposomes' skin penetration

Regarding their mechanism of skin penetration, liposomes were suggested to deliver drugs into the skin through different mechanisms; the first mechanism is that liposomes act only as local drug carriers, hence they cannot pass deeply into the skin layers, but rather exist on the upper layers of the stratum corneum **[38, 39]**. They were also suggested to enhance percutaneous drug penetration by acting as penetration enhancers through modifying the lipid bilayers of the SC **[36]**. Another proposed mechanism is that liposomes exhibit permeation enhancer effect through their direct fusion and adsorption on the stratum corneum **[40]**. Some liposomes were also suggested to make use of the transfollicular route for drug delivery where the whole vesicles enter

the hair follicles then penetrate the dermis **[40]**.

Liposomes have been extensively used as drug bearers for wound healing. Vogt et al. formulated Polyvinyl pyrrolidone-iodine (PVP-I) liposome hydrogel using Polyacrylic acid as a hydrogel base **[23]**. PVP-I has a broad-spectrum antibacterial activity which made it useful for treating infected wounds. PVP-I liposome hydrogel was found to have less tissue toxicity compared to the conventional PVP-I ointment. Also, the clinical study showed that PVP-I liposome hydrogel was more efficacious for both wound healing and prevention of infection in comparison to 10% PVP-I ointment, since it exhibited a synergistic effect of the moist wound treatment of the hydrogel and antisepsis of PVP – I for achieving better wound healing.

In addition, epidermal growth factor (EGF) liposome formulation was used for treating burn wounds in rats **[30]**. EGF is a polypeptide of 53 amino acids that is present in a variety of tissues and body fluids. EGF has been beneficial for wound healing through promoting keratinocyte division and epidermal tissue regeneration. The results obtained from the *in vivo* study indicated that collagen synthesis and complete wound contraction were observed in the EGF-containing liposome formulation treated group compared to the EGF solution treated group, the liposomal preparation devoid of EGF treated group and the commercial silver sulfadiazine cream treated group.

In addition, Nada et al. prepared ricinoleic acid liposomal chitosan hydrogel for the treatment of wounds **[9]**. Liposomes were prepared by the thin-film hydration method and then incorporated into a crosslinked chitosan solution which acted as a hydrogel base. The *in vivo* study showed that chitosan hydrogel formulation infused with ricinoleic acid empty liposomes or in liposomal chitosan hydrogel achieved faster contraction rates of the wound area compared to Vaseline gauze fabric and Garamycin commercial cream. The histopathological analysis proved that the skin tissues were histologically intact and showed normal healing with the ricinoleic acid liposomal chitosan hydrogel.

Furthermore, madecassoside loaded liposomes were prepared by double emulsion technique and applied on burn wounds in rats **[36]**. Madecassoside promotes wound healing by promoting cell growth and owing to its antioxidant, antiulcer, sedative, neuroprotective activities as well as its antibacterial and anti-inflammatory properties. The results obtained from the *in vivo* study showed that madecassoside loaded liposomal formulation exhibited greater wound closure after surface coating with polyethylene glycol (PEG) compared with conventional liposomes, normal saline control group, and the drug solution.

In addition, a clinical assessment of wound healing and chondrolysis following the administration of a liposomal preparation of bupivacaine was conducted **[41]**. Bupivacaine is a local anesthetic used for post-surgical injury. The clinical study showed no chondrolysis following the application of liposomal bupivacaine. The efficacy of bupivacaine-loaded liposomes was found to be similar to that of bupivacaine HCl in the wound healing process.

A formulation of chitosan-coated liposomes loaded with the neuropeptide substance P (SP) was also attempted **[42]**. SP contains 11 amino acids and has a wound healing activity owing to its vasodilator effect, promoting angiogenesis and enhancing the release of nitric oxide (NO). However, it has a short half-life that limits its application. Hence, chitosan was used for coating liposomes to increase the stability of SP-loaded liposomes. The *in vitro* study on HaCaT cells showed that SP-encapsulated chitosan-coated liposomes offered greater wound closure

compared to SP-loaded liposomes and free SP solution due to the controlled release of SP.

Moreover, Dawoud et al. formulated insulin-loaded liposomes using chitosan as a hydrogel base **[43]**. Insulin is a peptide hormone with an excellent wound-healing property and can restore the integrity of the broken skin. Chitosan was used in this study to form a final hydrogel base and to provide bio adhesion, stop bleeding and enhance wound healing. The clinical study revealed that the insulin-loaded liposomal chitosan hydrogel achieved a significant reduction in the erythema on the seventh day, which may be due to the anti-inflammatory effect of the insulin and due to its vasodilatory effect. Additionally, an improvement in the wound healing rate in the test group that received insulin-loaded liposomal chitosan hydrogel was about 16 times more than the control group that received liposomal chitosan gel without insulin and there were no signs of hypoglycemia.

Additionally, quercetin and curcumin were incorporated in liposomes **[26]**. Quercetin and curcumin are polyphenols and they have antioxidant and anti-inflammatory activities that are beneficial for wound healing. The vesicles inhibited myeloperoxidase accumulation and leukocyte infiltration in damaged tissues with greater suppression of edema.

Shailesh and Kulkarni formulated a mupirocin liposomal hydrogel that was used as a diabetic wound dressing using sodium alginate and gelatin as a crosslinked gel **[44]**. Mupirocin is an antibiotic that is used to treat wound pathogens while hydrogel provides moist wound treatment. The *in vitro* drug release study revealed that formulations containing higher cholesterol amounts achieved a controlled and slow-release profile due to the rigidity of these vesicles. The *in vitro* stability study was evaluated after storage for 6 months at room temperature (20 °C) or 4 °C. The results obtained after every 1 month of storage showed that the mupirocin liposomal hydrogel was stable in terms of entrapment efficiency and drug release.

Moreover, preparation of fibroblast growth factor (bFGF)-loaded liposome (LIP) with silk fibroin (SF) as a hydrogel core was developed **[19]**. Fibroblast growth factor (bFGF) promotes fibroblast proliferation and angiogenesis, so it enhances the wound healing process. However, it has a short half-life of about 1.5 min, lower stabilization; as it loses its activity through enzymatic degradation of proteases in wound exudates and undergoes rapid clearance by wound exudates before reaching the wound area. Hence, silk fibroin hydrogel was used to coat bFGF to increase its stability, sustain its release and further enhance its wound healing activity. The *in vivo* study on deep, second-degree burn revealed that bFGF-SF-LIP showed accelerated wound closure compared to the control group that received normal saline, the group that received bFGF alone, the group that received bFGF-LIP, and the group that received SF-LIP. Additionally, bFGF-SF-LIP showed better angiogenesis, collagen deposition, and re-epithelization than the aforementioned control groups.

Additionally, Gauthier *et al*. prepared liposomes loaded with biologically inactive dexamethasone-phosphate (pro-drug) and surface-modified with either (PEG) or phosphatidylserine (PS) **[45]**. Dexamethasone has a powerful anti-inflammatory activity through several mechanisms. This study utilized dexamethasone phosphate (DexP) to check its uptake by macrophages where it is activated into dexamethasone (Dex). Also, PS liposomes were developed to mimic PS-bearing apoptotic cells which are substrates for efferocytosis; an essential pro-resolution function. The *in vitro* cell culture showed that both DexP liposomes with surface PEG and DexP liposomes with surface PS were uptaken by macrophages; resulting in

the decrease of both interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFa) release and the increase of thrombospondin 1 efferocytosis activity. However, DexP liposomes with surface PS showed a faster uptake than DexP liposomes with surface PEG.

In addition, quercetin-loaded liposomes were formulated then subsequently optimized using a 3-level factorial-response surface methodology (3LF-RSM) **[46]**. Quercetin was used in this study owing to its potential anti-inflammatory and antioxidant properties. Quercetin showed a biphasic release behavior, with a burst release at the initial stage followed by sustained release. The quercetin-loaded liposomes showed acceptable stability under the storage conditions tested (at 4°C and 25°C) for a total period of 3 months in terms of the particle size, zeta potential, and encapsulation efficiency of the liposomes.

Also, a gelatin-based membrane containing usnic acid liposomes (UAL) was formulated **[47]**. Usnic acid is formed due to the secondary metabolism of algae and fungi and has an antibiotic, antibacterial, and wound healing activity. A gelatin-based membrane containing usnic acid/liposome showed a biphasic release pattern that started with initial burst release followed by controlled release, providing a chance to decrease the number of administrations. The results obtained from *in vivo* study on the healing of burns revealed that UAL treated group showed higher collagen deposition compared to the duoDerme® (GDU) treated group and silver sulfadiazine (SDZ) treated group. Additionally, UAL treated group displayed formation of granulation tissue and repair of the scar that was comparable to that produced by the GDU treated group but higher than SDZ treated group.

4.2. Niosomes

Niosomes are also called (non–ionic surfactant-based vesicles) **[34]** as they are vesicular structures composed of bilayers made of a nonionic surfactant such as alkyl or dialkyl polyglycerol ether, alkyl esters as sorbitan esters or span, and Alkyl Amides as Galactosides and glucosides with an aqueous core.

Being one of the vesicular systems, niosomes have the same advantages of liposomes as biodegradability, biocompatibility, stability, and nontoxicity **[34]** due to the presence of nonionic surfactants. However, they have more penetrating power than conventional liposomes owing to the nonionic surfactant that acts as a penetration enhancer. Despite overcoming the instability of phospholipids' oxidation, niosomes still suffer from vesicles' aggregation upon storage and low drug encapsulation **[37]**.

Regarding their mechanism of percutaneous skin penetration, niosomes increase skin hydration and modify the characteristics of the SC by reducing transepidermal water loss (TEWL). Additionally, the nonionic surfactant acts as a penetration enhancer that allows the penetration of niosomes **[48]**. Also, Cationic niosomes enhance topical drug delivery through electrostatic interaction with negatively charged skin surface to increase drug permeation rates **[49]**.

Niosomes have been used as carriers for drugs for enhancing wound healing owing to the aforementioned advantages. Dharashivkar et al. formulated silver sulfadiazine (SSD) liposomal gel using carbopol 934 as a gelling agent by the thin film hydration method **[50]**. The *in vivo* study on burn wounds showed that the (0.5% w/w) SSD liposomal gel was more advantageous than the marketed product (1% w/w) SSD cream, as the former required only 16 days for reduction of burn area while the latter required 19 days.

Additionally, (0.5% w/w) SSD liposomal gel required 31 days for complete cure of burn wound whereas (1% w/w) SSD cream required 37 days. The SSD liposomal gel showed superior antibacterial activity against *S. aureus* than SSD cream.

Also, a preparation of pentoxifylline (PTX) liposomal cream and its subsequent application on full-thickness incised wounds of the BALB/c mice was conducted **[51]**. Pentoxifylline (PTX) facilitates tissue repair owing to its hemorheological and immunomodulation properties. Results obtained from the *in vivo* study showed that the PTX liposomal cream accelerated epithelization and collagen synthesis higher than PTX-conventional cream, with faster wound closure.

Additionally, Priprem et al. prepared mucoadhesive polymeric gels encapsulating anthocyanin-niosomes to promote oral wound healing using sodium polyacrylate and carbomer 934P as gelling agents **[52]**. Anthocyanin (AC) has an anti-inflammatory activity which makes it beneficial for wound healing. An *in vivo* study was done on cut wounds in the buccal cavities of Wistar rats. The results obtained from the *in vivo* study showed that both AC gel and AC liposomal gel reduced wound sizes after 3 days. However, AC liposomal gel (10%) showed greater wound contraction compared to fluocinolone acetonide gel due to the enhanced mucosal permeability and controlled release of AC from the liposomal gel.

Also, El-Ridy *et al*. formulated metformin HCl liposomal gel using Span 60, Span 40, and Tween 80 as nonionic surfactants **[53]**. Owing to the anti-hyperglycemic effect of metformin – HCl, it was supposed to enhance wound healing in diabetic mice. The results obtained from the *in vivo* study showed that metformin liposomal gel formulation demonstrated moderate acceleration of the wound healing process**.**

Moreover, a formulation of methylene blue liposomal gel applied on full-thickness surgical excisions of male Wistar rats was performed **[54]**. Methylene blue was hypothesized to enhance wound healing due to its ability to enhance cell proliferation and fibroblast immortality, in addition to its antioxidant activity. The results obtained from the *in vivo* study revealed that the methylene blue-loaded liposomal gel-treated group showed the highest collagen deposition, granulation tissue formation, and epithelization compared to other groups that treated either with a gel containing free methylene blue or gel without methylene blue.

Also, Un *et al*. studied the wound healing activity of Marigold-loaded niosomes by an *in vitro* scratch assay **[55]**. Marigold has powerful antioxidant, antifungal, antibacterial and anti-inflammatory activities. The results obtained from the *in vitro* scratch assay indicated that marigold-loaded niosomes containing Tween 60 showed greater wound healing (higher ability to close the created gap) compared to marigold extract, plain niosomes, and control.

Ali *et al*. formulated a phenytoin sodium liposomal gel using sodium alginate as a gelling agent **[56]**. The topical wound healing activity of this liposomal gel was evaluated using guinea pigs with induced wounds. The results obtained from the *in vivo* study revealed that by the ninth day, the niosomes-treated lesions were completely healed, in contrast to the vehicle-treated control group.

Additionally, a bioadhesive system of moxifloxacin (MFX) loaded niosomes in chitosan gel was formulated **[57]**. Chitosan was used to achieve the antibacterial effect and to localize drug release while moxifloxacin has a broad-spectrum antibacterial activity against wound pathogens. The antimicrobial test against *pseudomonas aeruginosa* showed that MFX loaded niosomes had a higher antibacterial effect

than free (MFX), plain niosomes, and a mixture of free MFX and plain niosomes. The incorporation of MFX niosomes into chitosan gel exhibited a higher antibacterial effect against *Staph. aureus* than gel containing free MFX.

Also, the wound healing activity of *Hypericum perforatum*-loaded niosomes was evaluated on adult mongrel dogs **[58]**. *Hypericum perforatum* has wound healing and anti-inflammatory activities by enhancing the migration of fibroblasts and collagen deposition. The results obtained from the *in vivo* study showed that by the seventh day, the liposomal gel (1.5%) treated group showed remarked angiogenesis and fibroblastic proliferation while the control group and panthenol treated group showed marked edema, hemorrhage, and necrosis.

A formulation of *Narcissus tazetta* extract loaded niosomes was tested by an *in vitro* wound healing evaluation using scratch assay on human dermal fibroblasts (HDFs) **[48]**. Narcissus extract promotes wound healing owing to its antibacterial, anti-inflammatory, antiviral, and antioxidant activities. A formulation containing span 60: tween 60 in a ratio of 25:25, 5% Narcissus tazetta extract, and 50 mg/ml of cholesterol caused a significant reduction in gap width compared to *Narcissus tazetta* extract.

4.3. Transfersomes

Transfersomes are also called deformable liposomes, which were first introduced by Cevc and Blume **[59]**. Transfersomes like liposomes in their vesicular structure but with more tailored membrane characteristics. They are phospholipid bilayers with an edge activator which is usually a single chain surfactant with a high radius of curvature, destabilizing the lipid bilayer of the vesicle and making it ultra-flexible, self-adaptable, and much more elastic than classic liposomes **[60, 61]**. Sodium cholate, sodium deoxycholate, Spans, and Tweens are very commonly used for this purpose **[62]**. Similar to liposomes, transfersomes are chemically unstable because they are liable to oxidative degradation **[37]**.

Transfersomes can overcome the skin barrier *via* opening intercellular pathways, then they undergo deformation to fit themselves through these openings **[60]**. They have the capability of squeezing themselves through skin pores 5-10 times smaller than their diameters owing to their low pore penetration resistance **[63]**. In addition to the mechanisms of penetration enhancement proposed for liposomes, transfersomes can pass to deeper skin layers intact, owing to their deformability, driven by their ability to avoid dry surroundings. Hence, in addition to their topical delivery potential, transfersomes are usually used for transdermal drug delivery owing to their capability to penetrate deeply through skin layers reaching systemic circulation without the risk of vesicle rupture **[63]**. Transfersomes are among elastic vesicles that can penetrate the skin deeply due to the presence of edge activators. Hence, they are tailored carriers for better wound healing.

For example, self-assembled transfersomes loaded with baicalin in a gallon –cholesterol nano hydrogel was prepared **[64]**. Baicalin (7-glucuronic acid 5, 6-dihydroxyflavone) is one of the main active constituents of the dried roots of *Scutellaria baicalensis Georgi*, which has an anti-inflammatory effect. The *In vivo* anti-inflammatory study on inflamed skin showed that baicalin transfersomes exerted higher wound contraction than that provided by baicalin in phosphate buffer saline. Additionally, all baicalin-loaded transfersomes showed greater suppression of the inflammatory markers (such as myeloperoxidase (MPO), edema, TNFα, and Il-1β) and better wound healing than that obtained with betamethasone cream.

Also, Avadhani *et al*. co-encapsulated EGCG (Epigallocatechin-3-gallate) and hyaluronic acid (HA) in a single nano-transpersonal drug carrier system for obtaining synergistic antioxidant and anti-aging benefits of both compounds **[62]**. EGCG acts as an effective antioxidant, anti-inflammatory, antiviral and antibacterial agent; which is available in green tea. Hyaluronic acid is present in connective tissues and is the main component of the extracellular matrix. It is a biocompatible polymer that has been widely used as an effective anti-aging agent because of its properties like wound healing, skin repair, skin hydration, and protection against skin wrinkling. Transfersomes were formulated first by the thin film hydration method than by the high-pressure homogenization technique. The *in vitro* permeation study showed that the co-encapsulation of HA in the formulation increased both skin permeation and deposition of EGCG. Moreover, EGCG-HA-TF transfersomes were able to suppress malondialdehyde (MDA) levels and reactive oxygen species (ROS) levels to a significant extent in human keratinocytes.

Additionally, formulations of tocopherol-loaded transfersomes using (tween 20, 40, 60, and 80) surfactants as edge activators were assessed for their efficacy in skin regeneration **[63]**. Alpha-tocopherol is the most abundant form of vitamin E which has a strong antioxidant activity and can accelerate wound repair by promoting cell polarization and migration in human keratinocytes via phosphatidylinositol kinase/protein kinase C signaling cascade. The *in vitro* study showed that tocopherol-loaded transfersomes containing tween 80 were able to suppress the tissue damage by capturing free radicals generated during injury, due to the antioxidant activity of tocopherol. Moreover, these transfersomes stimulated the proliferation and migration of skin cells and accelerated the wound healing process.

Furthermore, Chen *et al*. prepared papain elastic liposomes (PEL) using tween-80 as an edge activator by reverse-phase evaporation method **[65]**. Papain; a cysteine protease isolated from the latex of Carica papaya fruit was used in this study due to its anti-inflammatory, anti-bacterial, and antioxidant activity. *The in vitro* skin deposition study revealed that elastic liposomes bearing papain showed greater skin deposition compared to papain solution. The *in vivo* study showed that PEL decreased the hypertrophic scar by inhibiting angiogenesis in rabbit ears, so PEL could be an efficient cure for topical scars.

A fabrication of mangiferin-loaded transfersomes and glycotransfersomes that were further modified with mucin as an innovative strategy for wound healing was attempted **[66]**. Mangiferin is a natural antioxidant isolated from mango leaves. Glycotransfersomes were prepared by the addition of both glycerols as a skin-hydrating agent and propylene glycol which increases skin penetration of transfersomes. Whereas, mucin (MUC) which is a large glycoprotein, was added to increase the vesicle adhesion to the skin. Glycotransfersomes and muc-glycotransfersomes were able to stimulate fibroblasts' proliferation and migration. Also, they were able to decrease inflammation and promoted skin regeneration.

Also, El-Gizawy prepared deferoxamine (DFO) loaded transpersonal gel for evaluating its efficacy in treating diabetic non-healing ulcers **[67]**. DFO is an effective Hypoxia-inducible factor-1 alpha (HIF-1α) stabilizer by inhibiting Prolyl Hydroxylase Domain (PHD) enzyme through the prevention of iron release in diabetic tissue. The prepared vesicles were able to increase the rate of neovascularization and collagen deposition thanks to their ability to sustain the release of deferoxamine and to increase its skin penetration. The *in vivo* study

proved that the DFO-loaded transpersonal gel was promising as a potential therapy for the treatment of human diabetic ulcers.

4.4. Penetration enhancer containing vesicles (PEVs)

Penetration enhancers containing vesicles contain one or more penetration enhancers (PE) in their structure. PEs differing in their chemical structure and properties can be utilized, such as oleic acid, translator, and labrasol **[18, 68]**.

Regarding their topical mechanism of action, PEVs were reported to penetrate as a whole down to the epidermis, and then keep penetrating to the deeper layers owing to the bilayers' fluidity enhancement caused by the penetration enhancer. In addition, the free penetration enhancer exerts a synergistic effect through interaction with the skin lipids, which leads to the perturbation of the intercellular skin lipid pathway, hence improving the accumulation of drugs in the deeper skin layers **[69]**.

Penetration enhancer containing vesicles (PEVs) of oryzanol utilizing two penetration enhancers (Transcutol and Labrasol), with bisabolol as a co-penetration enhancer were prepared using the thin film evaporation method **[18]**. Oryzanol is the main active constituent of rice bran oil that was used in this study owing to its antioxidant and anti-inflammatory activity. The group treated with PEVs formulation containing both oryzanol and bisabolol showed collagen deposition by day 14 compared to 21 days with other groups treated with PEVs containing oryzanol only or Healosol® spray for wound healing. Moreover, the former group showed faster angiogenesis by day 5 than the group treated with PEVs containing oryzanol only due to the presence of bisabolol that augmented the wound healing activity of oryzanol.

Moreover, baicalin-loaded sorbitol-PEVs showed a proliferative effect which was significant even at the lower concentrations tested **[6]** and could be successfully used for protecting the skin from UV radiation, and stimulating cell proliferation.

Also, curcumin-loaded propylene glycol (Cur-PGL) PEVs were prepared and investigated for skin burn **[70]**. The biocompatibility assessment study showed that Cur-PGL nanovesicles were less toxic than free curcumin due to the controlled release of curcumin from the vesicles. Additionally, 0.3% Cur-PGL vesicles were able to inhibit the bacterial growth as the silver sulfadiazine (SSD) cream 1%. After application of Cur-PGL for 18 days on rats with induced burns, complete wound closure was occurred compared to other groups that received free curcumin (0.3%), SSD cream, or liposomes alone.

4.5. Ethosomes

Ethosomes are ethanolic liposomes **[71]**. They are vesicular systems that consist of a relatively large amount of alcohol (20-50%) such as ethanol and isopropyl alcohol, phospholipids, and water. They can be prepared using the hot method, cold method, active loading, and passive loading **[37].** The most important drawback encountered with the use of ethosomes is skin irritation due to the presence of ethanol in high amounts **[49]**.

Ethosomes can penetrate deeply into the skin layers due to the presence of ethanol that is responsible for interaction between skin lipids and ethosomes, whereby the intercalation of the ethanol into intercellular lipids increases skin fluidity and decreases the density of lipid layers. Moreover, the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids improves inter-lipid penetration and permeation thus resulting in drug release into deep layers of the skin **[49]**.

Razavi *et al*. relied on ethosomal penetrating power and formulated (SSD) nanoethosomal gel using carbopol 974 as gelling agent **[72]**. SSD nanoethosomal gel was tested on rats with a second degree of burn. Results revealed that topical application of SSD nanoethosomal gel was able to accelerate wound healing compared to SSD solution, SSD free nanoethosomal gel, and commercial SSD cream (1%). Also, the antibacterial activity of SSD nanoethosomal gel was found to be higher than commercial SSD cream.

Also, Partoazar *et al*. tested the wound healing activity and antibacterial potential of curcumin-loaded ethosomes on second-degree burn wounds in rats. The results obtained from the in vivo study showed that the curcumin-loaded ethosomes exhibited faster collagen deposition, angiogenesis, and granulation tissue formation than the free curcumin. The encapsulation of curcumin in nano-range allowed for better penetration of curcumin through the skin and sustained its release. Also, the results showed that the antibacterial activity of curcumin-loaded ethosomes was higher than the free curcumin but similar to SSD cream 1% **[73]**.

Somwanshi *et al*. evaluated the wound healing activity of the Sesamum Indicum L. Seed Extract loaded ethosomal gel using carbopol 934 as a gel base **[74]**. The results obtained from the *in vivo* studies revealed that the Sesamum loaded ethosomal gel received group showed better re-epithelization and re-vascularization compared to the betadine ointment received group and control group. Also, the Sesamum-loaded ethosomal gel received group showed faster wound contraction compared to the aforementioned groups.

4.6. Hyaluosomes

Hyaluosomes, also called (gel core-filled liposomes), are liposomes like vesicles entrapping hydrogel polymer inside their core **[75]**. Therefore, they combine the advantages of the high penetration of liposomes and the viscosity of hydrogels. In addition, the polymer incorporated inside the core provides stability for the encapsulated drugs against decomposition in the body and early drug release. **[76]**.

Curcumin-loaded gel-core hyalurosomes (Curc–GC-HS) were prepared by the thin-film hydration method using hyaluronic acid as a gel core **[76]**. The burn-wound healing study showed that Curcumin-loaded gel-core hyalurosomes exhibited marked improvement on the seventh day of treatment. On the 11^{th} day, Circ–GC-HS treated wounds showed almost normal skin with no scar formation, confirmed by the histological analysis. Curc–GC-HS showed five-folds higher skin deposition compared to the conventional curcumin-loaded transpersonal pluronic gel (Curc–T-Pl gel).

An *in vitro*/*in vivo* study of liposomes and hyalurosomes loaded with liquorice extract and glycyrrhizin against oxidative stress damage in wounds was performed **[77]**. Glycyrrhizin is the main active constituent of liquorice roots and it has glucocorticoid-like actions like anti-inflammatory, anti-viral, anti-tumor, and hepatoprotective activities. The *in vitro* scratch assay revealed that liquorice extract-loaded vesicles (especially hyalurosomes) showed greater wound closure compared to the liquorice extract and glycyrrhizin solution due to the synergism of the anti-inflammatory effect of glycyrrhizin and the moist treatment of hyaluronate. The *in vivo* assessment of edema showed that both liquorice extract formulations and glycyrrhizin formulations significantly reduced edema compared to the liquorice extract and glycyrrhizin solution.

Conclusion

This review sheds the light on the nanocarriers used for achieving better wound

healing, with special emphasis on vesicular systems, being promising biocompatible systems for topical delivery of both hydrophilic and lipophilic drugs. It can be concluded the state of the art is in continuous improvement, and we expect that they may eventually lead to the replacement of conventional wound healing modalities.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

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5. REFERENCES

1. Singla V, Saini S, Joshi B, Rana A. Emulgel: A new platform for topical drug delivery. International Journal of Pharma and Bio Sciences 2012; 3(1): 485-98.
2. Buhse L, Kolinski R, Westenberger B, Wokovich A, Spencer J, Chen CW, Turujman S, Gautam-Basak M, Kang GJ, Kibbe A. Topical drug classification. International journal of pharmaceutics 2005; 295(1-2): 101-12.
3. Fasolo D, Pippi B, Meirelles G, Zorzi G, Fuentefria AM, von Poser G, Teixeira HF. Topical delivery of antifungal Brazilian red propolis benzophenones-rich extract using cationic lipid nanoemulsions optimized using Box-Behnken Design. Journal of Drug Delivery Science and Technology 2020; 56: 101573.
4. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. Advanced Drug Delivery Reviews2002; 54: S77-S98.
5. Herkenne C, Alberti I, Naik A, Kalia YN, Mathy F-X, Préat V, Guy RH. In vivo methods for the assessment of topical drug bioavailability. Pharmaceutical research 2008; 25(1): 87.
6. Manca ML, Mir-Palomo S, Caddeo C, Nacher A, Díez-Sales O, Peris JE, Pedraz JL, Fadda AM, Manconi M. Sorbitol-penetration enhancer containing vesicles loaded with baicalin for the protection and regeneration of skin injured by oxidative stress and UV radiation. International journal of pharmaceutics 2019; 555: 175-83.
7. Nagula RL, Wairkar S. Recent advances in topical delivery of flavonoids: A review. Journal of Controlled Release 2019; 296: 190-201.
8. Pachuau L. Recent developments in novel drug delivery systems for wound healing. Expert opinion on drug delivery 2015; 12(12): 1895-909.
9. Nada AA, Arul MR, Ramos DM, Kroneková Z, Mosnáček J, Rudraiah S, Kumbar SG. Bioactive polymeric formulations for wound healing. Polymers for Advanced Technologies 2018; 29(6): 1815-25.
10. Bernal-Chávez S, Nava-Arzaluz M, Quiroz-Segoviano R, Ganem-Rondero A. Nanocarrier-based systems for wound healing. Drug development and industrial pharmacy 2019; 45(9): 1389-402.
11. Bouwstra J, Honeywell-Nguyen P. Skin structure and mode of action of vesicles.

Advanced Drug Delivery Reviews 2002; 54: S41-S55.

12. Elias PM. Structure and function of the stratum corneum extracellular matrix. Journal of investigative dermatology 2012; 132(9): 2131-3.
13. Okano J, Kojima H, Katagi M, Nakae Y, Terashima T, Nakagawa T, Kurakane T, Okamoto N, Morohashi K, Maegawa H. Epidermis–dermis junction as a novel location for bone marrow-derived cells to reside in response to ionizing radiation. Biochemical and Biophysical Research Communications 2015; 461(4): 695-701.
14. Römgens AM, Bader DL, Bouwstra JA, Baaijens FP, Oomens CW. Diffusion profile of macromolecules within and between human skin layers for (trans) dermal drug delivery. Journal of the mechanical behavior of biomedical materials 2015; 50: 215-22.
15. Bolzinger M-A, Briançon S, Pelletier J, Chevalier Y. Penetration of drugs through skin, a complex rate-controlling membrane. Current opinion in colloid & interface science 2012; 17(3): 156-65.
16. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. Journal of International Medical Research 2009; 37(5): 1528-42.
17. Mohanty C, Sahoo SK. Curcumin and its topical formulations for wound healing applications. Drug discovery today 2017; 22(10): 1582-92.
18. Aldalaen S, Nasr M, El-Gogary RI. Angiogenesis and collagen promoting nutraceutical-loaded nanovesicles for wound healing. Journal of Drug Delivery Science and Technology 2020; 56: 101548.
19. Xu HL, Chen PP, Zhuge DL, Zhu QY, Jin BH, Shen BX, Xiao J, Zhao YZ. Liposomes with Silk Fibroin Hydrogel Core to Stabilize bFGF and Promote the Wound Healing of Mice with Deep Second-Degree Scald. Advanced healthcare materials 2017; 6(19): 1700344.
20. Guo Sa, DiPietro LA. Factors affecting wound healing. Journal of dental research2010; 89(3): 219-29.
21. Wang W, Lu K-j, Yu C-h, Huang Q-l, Du Y-Z. Nano-drug delivery systems in wound treatment and skin regeneration. Journal of nanobiotechnology 2019; 17(1): 1-15.
22. Chereddy KK, Coco R, Memvanga PB, Ucakar B, des Rieux A, Vandermeulen G, Préat V. Combined effect of PLGA and curcumin on wound healing activity. Journal of Controlled Release 2013; 171(2): 208-15.
23. Vogt PM, Hauser J, Roßbach O, Bosse B, Fleischer W, Steinau HU, Reimer K. Polyvinyl pyrrolidone iodine liposome hydrogel improves epithelialization by combining moisture and antiseptic. A new concept in wound therapy. Wound Repair and Regeneration2001; 9(2): 116-22.
24. Ibrahim NI, Wong SK, Mohamed IN, Mohamed N, Chin K-Y, Ima-Nirwana S, Shuid AN. Wound healing properties of selected natural products. International journal of environmental research and public health 2018; 15(11): 2360.
25. Reimer K, Vogt P, Broegmann B, Hauser J, Rossbach O, Kramer A, Rudolph P, Bosse B, Schreier H, Fleischer W. An innovative topical drug formulation for wound healing and infection treatment: in vitro and in vivo investigations of a povidone-iodine liposome hydrogel. Dermatology 2000; 201(3): 235-41.
26. Castangia I, Nácher A, Caddeo C, Valenti D, Fadda AM, Díez-Sales O, Ruiz-Saurí A,

Manconi M. Fabrication of quercetin and curcumin nanovesicles for the prevention and rapid regeneration of full-thickness skin defects on mice. Acta biomaterials 2014; 10(3): 1292-300.

27. Nasr M, Mansour S, Mortada ND, Elshamy A. Vesicular aceclofenac systems: a comparative study between liposomes and niosomes. Journal of microencapsulation 2008; 25(7): 499-512.

28. Nasr M, Taha I, Hathout RM. Suitability of liposomal carriers for systemic delivery of risedronate using the pulmonary route. Drug delivery 2013; 20(8): 311-8.

29. Manosroi A, Jantrawut P, Manosroi J. Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with diclofenac diethyl ammonium. International journal of pharmaceutics 2008; 360(1): 156-63.

30. Alemdaroğlu C, Denim Z, Celebi N, Şengezer M, Alömeroglu M, Nacar A. Investigation of epidermal growth factor containing liposome formulation effects on burn wound healing. Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials 2008; 85(1): 271-83.

31. Medina-Cruz D, Saleh B, Vernet-Crua A, Ajo A, Roy AK, Webster TJ. Drug-delivery nanocarriers for skin wound-healing applications. Wound Healing, Tissue Repair, and Regeneration in Diabetes: Elsevier; 2020. p. 439-88.

32. Robson A-L, Dastoor PC, Flynn J, Palmer W, Martin A, Smith DW, Woldu A, Hua S. Advantages and limitations of current imaging techniques for characterizing liposome morphology. Frontiers in pharmacology2018; 9: 80.

33. Immordino ML, Dosio F, Cattel L. Stealth liposomes: a review of the basic science, rationale, and clinical applications, existing and potential. International journal of nanomedicine 2006; 1(3): 297.

34. Sharma V, Anandhakumar S, Sasidharan M. Self-degrading niosomes for encapsulation of hydrophilic and hydrophobic drugs: an efficient carrier for cancer multi-drug delivery. Materials Science and Engineering: C 2015; 56: 393-400.

35. Kumar GP, Rajeshwarrao P. Nonionic surfactant vesicular systems for effective drug delivery—an overview. Acta pharmaceutica sinica B 2011; 1(4): 208-19.

36. Li Z, Liu M, Wang H, Du S. Increased cutaneous wound healing effect of biodegradable liposomes containing madecassoside: preparation optimization, in vitro dermal permeation, and in vivo evaluation. International journal of nanomedicine 2016; 11: 2995.

37. Singh D, Pradhan M, Nag M, Singh MR. Vesicular system: Versatile carrier for transdermal delivery of bioactive. Artificial cells, nanomedicine, and biotechnology 2015; 43(4): 282-90.

38. Gillet A, Lecomte F, Hubert P, Ducat E, Evrard B, Piel G. Skin penetration behavior of liposomes as a function of their composition. European Journal of Pharmaceutics and Biopharmaceutics 2011; 79(1): 43-53.

39. Bragagni M, Mennini N, Maestrelli F, Cirri M, Mura P. Comparative study of liposomes, transfersomes, and ethosomes as carriers for improving topical delivery of celecoxib. Drug delivery 2012; 19(7): 354-61.

40. Sinku S, Sharma GK, Kushwaha RS, Chandrul KK. Review on Assessment of

Permeability of Enhancement Property of Hyaluronic Acid as Compare to Peg in Core Gel Liposome. 2019.

41. Baxter R, Bramlett K, Onel E, Daniels S. Impact of local administration of liposome bupivacaine for postsurgical analgesia on wound healing: a review of data from ten prospective, controlled clinical studies. Clinical therapeutics 2013; 35(3): 312-20. e5.

42. Mengoni T, Adrian M, Pereira S, Santos-Carballal B, Kaiser M, Goycoolea FM. A Chitosan—Based Liposome Formulation Enhances the In Vitro Wound Healing Efficacy of Substance P Neuropeptide. Pharmaceutics 2017; 9(4): 56.

43. Dawoud MH, Yassin GE, Ghorab DM, Morsi NM. Insulin mucoadhesive liposomal gel for wound healing: a formulation with sustained release and extended stability using quality by design approach. Maps Pharmscitech 2019; 20(4): 158.

44. Shailesh T, Kulkarni P. Development and evaluation of mupirocin-loaded liposomal hydrogels for diabetic wound healing properties. Indian Journal of Advances in Chemical Science 2014; 2: 42-5.

45. Gauthier A, Fisch A, Seuwen K, Baumgarten B, Ruffner H, Aebi A, Rausch M, Kiessling F, Bartneck M, Weiskirchen R. Glucocorticoid-loaded liposomes induce a pro-resolution phenotype in human primary macrophages to support chronic wound healing. Biomaterials 2018; 178: 481-95.

46. Jangde R, Singh D. Preparation and optimization of quercetin-loaded liposomes for wound healing, using response surface methodology. Artificial cells, nanomedicine, and biotechnology 2016; 44(2): 635-41.

47. Nunes PS, Rabelo AS, de Souza JCC, Santana BV, da Silva TMM, Serafini MR, dos Passos Menezes P, dos Santos Lima B, Cardoso JC, Alves JCS. The gelatin-based membrane containing usnic acid-loaded liposome improves dermal burn healing in a porcine model. International journal of pharmaceutics 2016; 513(1-2): 473-82.

48. Rameshk M, Sharififar F, Mehrabani M, Pardakhty A, Farsinejad A, Mehrabani M. Proliferation and in vitro wound healing effects of the Microniosomes containing Narcissus tazetta L. bulb extract on primary human fibroblasts (HDFs). DARU Journal of Pharmaceutical Sciences 2018; 26(1): 31-42.

49. Muzzalupo R, Pérez L, Pinazo A, Tavano L. Pharmaceutical versatility of cationic niosomes derived from amino acid-based surfactants: Skin penetration behavior and controlled drug release. International journal of pharmaceutics 2017; 529(1-2): 245-52.

50. Dharashivkar SS, Sahasrabuddhe SH, Saoji AN. Niosomally encapsulated silver sulfadiazine gel for burn treatment. Journal of microencapsulation 2015; 32(2): 137-42.

51. Aghajani A, Kazemi T, Enayatifard R, Amiri FT, Narenji M. Investigating the skin penetration and wound healing properties of niosomal pentoxifylline cream. European Journal of Pharmaceutical Sciences 2020; 151: 105434.

52. Priprem A, Damrongrungruang T, Limsitthichaikoon S, Khampaenjiraroch B, Nukulkit C, Thapphasaraphong S, Limphirat W. Topical niosome gel containing an anthocyanin complex: a potential oral wound healing in rats. Aaps Pharmscitech 2018; 19(4): 1681-92.

53. El-Ridy MS, Yehia SA, Elsayed I, Younis MM, Abdel-Rahman RF, El-Gamil MA. Metformin hydrochloride and wound healing: from nanoformulation to pharmacological

evaluation. Journal of liposome research 2019; 29(4): 343-56.

54. Farmoudeh A, Akbari J, Saeedi M, Ghasemi M, Asemi N, Nokhodchi A. Methylene blue-loaded niosome: preparation, physicochemical characterization, and in vivo wound healing assessment. Drug delivery and translational research 2020: 1-14.

55. Un RN, Barlas FB, Yavuz M, Ag Seleci D, Seleci M, Gumus ZP, Guler E, Demir B, Can M, Coskunol H. Phyto-niosomes: in vitro assessment of the novel nanovesicles containing marigold extract. International Journal of Polymeric Materials and Polymeric Biomaterials 2015; 64(17): 927-37.

56. Ali A, Sarhan HA, Magdy T. Preparation and characterization of phenytoin sodium niosomes for enhanced closure of skin injuries. Int J Pharm Pharm Sci 2014; 6: 542-6.

57. Sohrabi S, Haeri A, Mahboubi A, Mortazavi A, Dadashzadeh S. Chitosan gel-embedded moxifloxacin niosomes: an efficient antimicrobial hybrid system for burn infection. International journal of biological macromolecules 2016; 85: 625-33.

58. Ali M, Abdel Motaal A, Ahmed MA, Alsayari A, El-Gazayerly ON. An in vivo study of Hypericum perforatum in a niosomal topical drug delivery system. Drug delivery 2018; 25(1): 417-25.

59. Cevc G, Blume G. Lipid vesicles penetrate intact skin owing to the transdermal osmotic gradients and hydration force. Biochimica et Biophysica Acta-Biomembranes 1992; 1104(1): 226-32.

60. Omar MM, Hasan OA, El Sisi AM. Preparation and optimization of lidocaine transpersonal gel containing permeation enhancers: a promising approach for enhancement of skin permeation. International journal of nanomedicine 2019; 14:1551.

61. Fernández-García R, Lalatsa A, Statts L, Bolás-Fernández F, Ballesteros MP, Serrano DR. Transferosomes as nanocarriers for drugs across the skin: Quality by design from lab to industrial scale. International journal of pharmaceutics 2020; 573: 118817.

62. Avadhani KS, Manikkath J, Tiwari M, Chandrasekhar M, Godavarthi A, Vidya SM, Hariharapura RC, Kalthur G, Udupa N, Mutalik S. Skin delivery of epigallocatechin-3-gallate (EGCG) and hyaluronic acid loaded nano-transfersomes for antioxidant and anti-aging effects in UV radiation-induced skin damage. Drug delivery 2017; 24(1): 61-74.

63. Caddeo C, Manca ML, Peris JE, Usach I, Diez-Sales O, Matos M, Fernàndez-Busquets X, Fadda AM, Manconi M. Tocopherol-loaded transfersomes: in vitro antioxidant activity and efficacy in skin regeneration. International journal of pharmaceutics 2018; 551(1-2): 34-41.

64. Manconi M, Manca ML, Caddeo C, Valenti D, Cencetti C, Diez-Sales O, Nacher A, Mir-Palomo S, Terencio MC, Demurtas D. Nano design of new self-assembling core-shell gellan-transfersomes loading baicalin and in vivo evaluation of repair response in the skin. Nanomedicine: Nanotechnology, Biology and Medicine 2018; 14(2): 569-79.

65. Chen Y-Y, Lu Y-H, Ma C-H, Tao W-W, Zhu J-J, Zhang X. A novel elastic liposome for skin delivery of papain and its application on the hypertrophic scar. Biomedicine & Pharmacotherapy 2017; 87: 82-91.

66. Allaw M, Pleguezuelos-Villa M, Manca ML, Caddeo C, Aroffu M, Nacher A, Diez-Sales O, Saurí AR, Ferrer EE, Fadda AM. Innovative strategies to treat skin wounds with mangiferin: fabrication of transfersomes

modified with glycols and mucin. Nanomedicine 2020; 15(17): 1671-85.

67. El-Gizawy SA, Nouh A, Saber S, Kira AY. Deferoxamine-loaded transfersomes accelerate healing of pressure ulcers in streptozotocin-induced diabetic rats. Journal of Drug Delivery Science and Technology 2020; 58: 101732.

68. Manconi M, Caddeo C, Sinico C, Valenti D, Mostallino MC, Lampis S, Monduzzi M, Fadda AM. Penetration enhancer-containing vesicles: composition dependence of structural features and skin penetration ability. European Journal of Pharmaceutics and Biopharmaceutics 2012; 82(2): 352-9.

69. Manconi M, Sinico C, Caddeo C, Vila AO, Valenti D, Fadda AM. Penetration enhancer containing vesicles as carriers for dermal delivery of tretinoin. International journal of pharmaceutics 2011; 412(1-2): 37-46.

70. Kianvash N, Bahador A, Pourhajibagher M, Ghafari H, Nikoui V, Rezayat SM, Dehpour AR, Partoazar A. Evaluation of propylene glycol nanoliposomes containing curcumin on burn wound model in rat: biocompatibility, wound healing, and anti-bacterial effects. Drug delivery and translational research 2017; 7(5): 654-63.

71. Aggarwal D, Nautiyal U. Ethosomes: A review. Int J Pharm Med Res 2016; 4(4): 354-63.

72. Razavi S, Partoazar A, Takzaree N, Fasihi-Ramandi M, Bahador A, Darvishi MH. Silver sulfadiazine nanoethogel for burn healing: characterization and investigation of its in vivo effects. Nanomedicine 2018; 13(11): 1319-31.

73. Partoazar A, Kianvash N, Darvishi M, Nasoohi S, Rezayat S, Bahador A. Ethosomal curcumin promoted wound healing and reduced bacterial flora in second-degree burn in the rat. Drug research 2016; 66(12): 660-5.

74. Somwanshi SB, Hiremath SN. In-vivo Evaluation of the Wound Healing Activity of the Sesamum Indicum L. Seed Extract in Novel Ethosomal Vesicular System. Journal of Drug Delivery and Therapeutics 2018; 8(5): 411-20.

75. Kawar D, Abdelkader H. Hyaluronic acid gel-core liposomes (hyaluosomes) enhance skin permeation of ketoprofen. Pharmaceutical development and technology 2019; 24(8): 947-53.

76. El-Refaie WM, Elnaggar YS, El-Massik MA, Abdallah OY. Novel curcumin-loaded gel-core hyaluosomes with promising burn-wound healing potential: development, in-vitro appraisal, and in-vivo studies. International journal of pharmaceutics 2015; 486(1-2): 88-98.

77. Castangia I, Caddeo C, Manca ML, Casu L, Latorre AC, Díez-Sales O, Ruiz-Saurí A, Bacchetta G, Fadda AM, Manconi M. Delivery of liquorice extract by liposomes and hyalurosomes to protect the skin against oxidative stress injuries. Carbohydrate Polymers 2015; 134: 657-63.