(Phospho)lipid-based Nanosystems for Skin Administration

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Running title: ***Nanosystems and skin***

Keywords: skin, drug delivery nanosystems, nanopharmaceuticals, nanotechnology, skin therapy

Formatted: Norwegian (Bokmål)

Abstract

Nanotechnology and nanomedicine provide a platform for advanced therapeutic strategies for dermal and transdermal drug delivery. The focus of this review is on the current state-of-art in lipid-based nanotechnology and nanomedicine for (trans)dermal drug therapy. Drug delivery nanosystems based on the (phospho)lipid constituents are characterized and compared, with the emphasis on their ability to assure the controlled drug release to the skin and skin appendages, drug targeting and safety. Different types of liposomes, biphasic vesicles, particulate lipid-based nanosystems and micro- and nano-emulsions are discussed in more details. Extensive research in preclinical studies has shown that numerous parameters including the composition, size, surface properties and their combinations affect the deposition and/or penetration of carrier-associated drug into/through the skin, and consequently determine the therapeutic effect. The superiority of the most promising nanopharmaceuticals has been confirmed in clinical studies. We have selected several common skin disorders and provided overview over promises of nanodermatology in antimicrobial skin therapy, anti-acne treatment, skin oncology, gene delivery and vaccines. We addressed the potential toxicity and irritation issues and provided an overview of registered lipid-based products

Key words: skin, drug delivery nanosystems, nanopharmaceuticals, nanotechnology, skin therapy

(Graphical abstract)

INTRODUCTION

The skin conditions were reported to be the fourth leading cause of nonfatal disease burden at the global level. The skin disease prevention and treatment are to be included in the global health strategies of the future [1]. In parallel, recent trends in the pharmaceutical development of topical drug products are moving from a search for a new chemical entity towards development of new drug products based on the already approved drugs [2]. Nanotechnology, one of the fastest progressing fields in various research arenas, can enable development of superior drug products and is often seen as an intelligent design to treat complex diseases based on the inbuilt ability to perform temporal and spatial site-specific delivery [3-5].

Nanodermatology, nanotechnology applied to dermatology, represents one of the most advanced fields both from the scientific as well as economic point of view. Nanodermatology offers novel directions in the medical diagnosis, monitoring and treatment of skin diseases [6]. These emerging drug delivery technologies that often involve self-assembled phospholipid-based vesicular carriers (liposomes, ethosomes), particulate carriers (lipid nanoparticles), emulsion-based nanosystems (micro- and nano-emulsions) and similar and are in the focus of this review. We attempted to provide an unbiased overview of these nanosystems and comment on their advantages and limitations. Due to a limited space, we could not cite all of the reported findings and tried to cite the representative articles reporting the original delivery systems, those including mechanistic studies and *in vivo* data. Similarly, we could not include the nanocosmeceuticals destined for skin administration. This review encompasses all the lipid-based nanosystems (vesicular, particular, emulsion-based) intended for either topical skin delivery (dermal) or systemic (transdermal) delivery of drugs and provides the state-of-the art in the field.

BARRIERS FOR SKIN DELIVERY

Numerous reviews have characterized the skin anatomy and physiology and the readers are referred to for example Bouwstra and Ponec [7], Prow and colleagues [8] and Banarjee [9].

The barrier property of the skin is the synergy between the positive cooperation and interactions between *stratum corneum* macro and micro-structure, bi- and three-dimensional supramolecular organization of the lipid matrix and the whole composition of *stratum corneum* [10]. The macrostructure represents corneocytes cross-sectional organization often simplified as a "brick and mortar" model [11]. The microstructure refers to supramolecular organization of the intercorneocyte lipids [7]. Non-uniform cellular packing of *stratum corneum* representing the permeability barrier of intact skin was confirmed by Schätzlein and Cevc [12]. The surface of the healthy *stratum corneum* is of slightly acidic pH. pH gradient increases up to the central layers of the *stratum corneum* where the pH reaches the values identical to that in viable epidermis [11]. It is expected that lipid-based nanopharmaceuticals may, to a different extent, interact with the skin lipids, presumably through the fusion and mixing [13]. Their composition and physicochemical properties may enhance or limit the ingress and diffusion of drug into/through the skin [10]. The sweat glands and pilosebaceous units open on the skin surface representing the potential penetration pathway for nanosystems. However, the low density of those appendages and intrinsic epithelization should be taken into the consideration [11].

The hydration and/or occlusion of *stratum corneum* will reduce the barrier properties and assist in the penetration. When water saturates the skin its permeability significantly increases. Hydration may result from water diffusing from underlying epidermal layers or from perspiration that accumulates after the application of an occlusive vehicle or dressing [14]. These effects can be

achieved by the proper choice of nanosystem as well as a vehicle (base) often applied to improve the viscosity and achieve applications properties of liquid nanosystems [15-17]The interactions between the vehicle (nanosystem and/or base), skin and drug affect the release of the drug, its penetration through the *stratum corneum*, permeation through the skin layers leading to drug deposition (dermal delivery) or absorption into the blood (transdermal delivery). Release of the drug from the vehicle and uptake into the *stratum corneum* is dependent on the relative solubility in each skin layer and hence, the *stratum corneum*-vehicle partition coefficient. The diffusion coefficient (speed at which the drug moves within each skin layer) is dependent on the drug properties including the molecular weight, solubility, melting point, ionization and potential for binding within the environment (epidermal layers). General rule is that drugs less than 500 Da, with low melting point and those having log P of 2-3 can permeate via both the lipid and polar microenvironments within the intercellular route and are good candidate for (trans)dermal delivery [14].

The effects of the nanosystem and base on the skin penetration of the drug are described in more details in the very recent review by our group [18].

NANODERMATOLOGY

. In respect to the skin and wound therapy, the potential of nanopharmaceuticals in treating local skin and systemic diseases has yet to be fully realized, however the extensive research efforts are expected to result in improved therapy outcome [19,20]. Nanosized delivery systems offer an opportunity for extensive innovation in nanomedicine, making them an attractive target in drug product development. For more details on the skin properties relevant for the interaction between nanoparticles and skin the readers are referred to the extensive review published by Prow and

colleagues [8]. Nanosized drug delivery systems designed for improved skin therapy are expected to exhibit all or at least some of the desired features, namely to be able to protect drug from degradation as well as improve penetration of drug into/through the skin [3]. The limitations in the analytical tools and instrument sensitivity to detect nanoparticles, together with the variety of applied *ex vivo* and *in vivo* models, accompanied by the physiological factors (variations in epidermal thickness and hair follicle density among species and anatomical site) limit broader consensus in the field [21].

Although ~~nanotechnology~~ nanosystems may provide means to modulate the packing and phases of the lipid component of the *stratum corneum* by fluidization, the potential toxicity issues should not be neglected particularly for non-lipid based nanodermatologicals [9].

SKIN PENETRATION OF NANOSYSTEMS

In spite of the fact that the size limit for nanosystem able to penetrate *stratum corneum* remains to be debatable, it is accepted by the Scientific Committee on Consumer Products that only very small particles, size range below 10 nm, are detectable in *stratum spinosum* in the epidermal layer [8]. DeLousie [21] proposed that the skin is a formable barrier to particle penetration; that the hair follicles serve as the collection sites for topically applied nanosystems and that their surface charge plays an important role in their penetration pattern, as the differences in the penetration have been observed for neutral and negatively charged particles. It seems that the nanocarriers penetrate preferably into the hair follicle canals rather than through *stratum corneum*. If this is the case, the skin penetration of nanocarrier-associated drugs will be the result of the carriers' accumulation within the hair infundibulum, the release of the drug within the hair follicle canal

and finally diffusion of the substance. Nanosystems are expected to increase both the penetration depth and permeation rate of the substance [22]. Unsaturated fatty acids and phospholipids containing higher portion of unsaturated fatty acids (e.g. soy lecithin) play a role in the fluidization of the *stratum corneum* [9].

Several parameters including lamellarity, lipid composition, surface charge of the nanosystem, presence of the edge activators and/or penetration enhancers and total lipid concentration determine drug deposition into the deeper skin layers [18, 23,24].

The required size of nanosystems for successful trans(dermal) drug delivery remains to be extensively discussed [10]. For example, deformable liposomes with an average size of 120 nm, have been shown to enhance the penetration of hydrophilic fluorescent compound for more than 5-fold into the deeper skin layers, as compared with larger vesicles (>190 nm) of the same lipid composition [25]. Decreasing the particle size of solid lipid nanoparticles has been confirmed to increase the occlusive effect on the skin consequently affecting skin hydration and drug permeability [26]. Besides the size effect, the effect of the nanosystem's rigidity/elasticity is important, as this feature will influence interaction of nanosystem with the *stratum corneum* and skin in general. Considering the (trans)dermal drug delivery. The distinction between particles which are soft and rigid in their nature is the feature that will affect nanosystems interaction with the *stratum corneum* and skin in general [22]. Typical examples of soft nanoparticles are elastic liposomes, whereas solid lipid nanoparticles (SLNs) represent rigid nanoparticles [26].]. It has been shown that the type and concentration of edge activator had great effect on the drug penetration via deformable liposomes through decreasing the particle size and increasing bilayer elasticity[24,27]. Furthermore, the surface charge of nanosystem has been also demonstrated to play a role in skin drug delivery [28].

NANOSYSTEMS (NANOPHARMACEUTICALS)

Nanosystems, often referred to as nanoparticles, nanovesicles, or nanopharmaceuticals, are in general expected to increase the bioavailabilty, biocompatibility and safety profiles of associated drug molecules, serving as carrier systems with specific properties related to their nanosize. Their ability to prolong and, at least to certain extent, control the release of associated drugs would potentially decrease the doses and dosing frequency while assuring the desired therapeutic effect [29].

Among different drug delivery systems investigated for improving (trans)dermal therapy, lipid-based nanosystems are of particular interest. They are commonly composed of physiologically acceptable lipids, usually non-toxic and degraded to non-toxic residues [10]. A unique advantage of lipid nanocarriers lies in the compatibility of their ingredients with the physiologically occurring compounds, i.e. *stratum corneum* constituents. The overview of the nanosized lipid-based systems classified as vesicular, particular or emulsion-based nanosystems is provided in the following chapters. In the second part on the review, the selected skin diseases and the tested nanopharmaceuticals are discussed in more details.

PHOSPHOLIPID-BASED VESICULAR DRUG DELIVERY NANOSYSTEMS

LIPOSOMES

General considerations

Liposomes are fully physiologically acceptable nanovesicles consisting of one (unilamellar liposomes), several (oligolamellar liposomes) or many (multilamellar liposomes) concentrically arranged lipid bilayers surrounding inner aqueous compartment(s). In addition, they may have a multicompartmental structure (multivesicular liposomes) [30]. The compatibility between their constituents (phospholipids, cholesterol and water) and skin constituents makes them superior skin drug delivery nanosystems. They have been investigated for the skin drug delivery for over 30 years [31]. Due to the specific structural properties liposomes are able to encapsulate/incorporate drugs of different sizes and lipophilicity. Hydrophilic drugs will be encapsulated into the aqueous compartment(s), lipophilic inside the bilayer, while amphiphilic will partition themselves between these two regions. Liposomal characteristics are determined by their lipid composition, membrane rigidity/elasticity, particle size, surface charge, number of lamellae and inner/outer aqueous phases [32]. Phospholipids were proposed to act as a penetration enhancers enabling alteration of the intercellular lipid matrix within the skin; therefore encapsulation of hydrophilic drugs in liposomes can increase their penetration into/through the skin [33]. Furthermore, liposomes may provide targeted delivery to skin appendages and assure localized depot of the lipophilic drug in the skin [33]. In addition, liposomes are able to incorporate poorly soluble drugs and alter their pharmacokinetics and skin bioavailability. Even empty liposomes have been shown to increase the skin hydration level which is of high importance in the treatment of *xerosis cutis* and atopic dermatitis [34]. Regarding the membrane properties, i.e. presence of surfactant or co-solvent in the bilayers, liposomes are categorized as conventional liposomes (with more rigid bilayers) and elastic liposomes (with pronounced bilayers elasticity), later including i) deformable liposomes, ii) ethosomes and iii) permeation enhancer containing vesicles, i.e. propylene glycol liposomes and invasomes (Figure 1). Considering the liquid nature of liposomal suspension and need for their

retention at the skin as administration site, liposomes are usually incorporated in the suitable bases such as gels, creams.

Conventional liposomes

Conventional liposomes are the first generation of liposomes comprising of the neutral phospholipids (phosphatidylcholine), or the combination of neutral and charged phospholipids, originating either from natural or synthetic sources with or without addition of cholesterol (Figure 1A). Mezei and Gulasekharam [35] were the first to propose liposomes for skin application. They have claimed that triamcinolone acetonide incorporated in liposomes composed of dipalmitoylphoshatidylcholine and cholesterol increased drug deposition in rabbit's epidermis and dermis, while concentrations of the drug in thalamic region (potential place for side-effects) and urinary excretions were significantly reduced in comparison to control formulation. Similar fate of liposomally encapsulated drug has been shown for hydrocortisone [36,37] and local anesthetics tetracaine [38] and lidocaine [39]. Liposomal delivery increases the efficacy of treatments even at the significantly lower concentrations of active substances as compared to the conventional formulations. For example, replacing the moisturizing cream with liposomes reduced the therapeutic concentration of 5-aminolevulinic acid in photodynamic therapy by a factor of 40 while maintaining the same effect [40]. Additional advantage of using liposomes in dermatological treatments is the reduced skin irritation caused by substances like retinoids [41]. Conventional liposomes increased the skin delivery of vitamin D3 and dithranol in the treatment of psoriasis [42]. In the case of the plaque type psoriasis, the entrapment of dithranol in liposomes has promoted its epidermal bioavailability and enabled the dose lowering, resulting in

the reduction of consequent dose-dependent side-effects [43]. Conventional liposomes have also been examined for the targeting to the skin appendages [44,45], for improved treatment of hair follicle disorders such as alopecia. The liposomes comprising of dimyristoyl phosphatidylcholine, cholesterol and dicetylphosphate (8:2:1, mole ratio) significantly increased the deposition of finasteride in the hair follicles compared to hydroalcoholic drug solution [46]. Similar findings have been reported for liposomal minoxidil [47].

However, most of the studies confirmed that the conventional liposomes have negligible or no penetration potential across the *stratum corneum* and can therefore only slightly improve the delivery of drug to the deeper layers of skin and transdermally [32,33,48,49].

Deformable (elastic) liposomes

In the early 90th of the last century Cevc and Blume [23] introduced a new type of liposomes with increased bilayer elasticity, namely deformable liposomes (highly fluid vesicles), which become known as elastic, flexible, ultradeformable liposomes or under the trade name Transferosomes® [50,51]. Deformable liposomes are commonly composed of lipids and an edge activator that destabilizes the liposome bilayers thus increasing the membrane deformability/elasticity (Figure 1B). As a consequence, the vesicles should be able to squeeze through the pores in the *stratum corneum* smaller than 1/10 of the vesicle diameter and transport entrapped drug deeper into the skin when applied under the non-occlusive conditions. In such a setting the osmotic gradient operating from the dry skin surface (15% water) towards wet viable tissues (75% water) drives vesicles through *stratum corneum* [52]. Honeywell-Nguyen and colleagues [53] confirmed that the intact elastic vesicles penetrate into the *stratum corneum* under the non-occlusive dressing; however, only few intact vesicles were found in the deeper horny

layers. Cevc and Blume [54] confirmed the penetration of diclofenac through the skin using Transferosomes® as a carrier system; the therapeutically relevant concentrations of the drug were obtained in the target tissue. When hydrocortisone, dexamethasone or triamcinolone-acetonide in the deformable liposomes have been administered epicutaneously the drug levels were found to be comparable to those obtained by a subcutaneous injection of the same drugs. Interestingly, Transfersome®-based corticosteroids were biologically active at doses several times lower than those currently used in the dermatologic formulations for the treatment of skin diseases [55]. Deformable liposomes have also been investigated as carriers for large molecular weight drugs, such as insulin. Applied under the non-occlusive dressing, Transferosomes® have been shown to deliver insulin through the non-compromised skin barrier with reproducible pharmacokinetics and the pharmacodynamic effects comparable to the subcutaneous injection [56]. The efficacy of deformable vesicles in skin delivery of drugs has also been evaluated by other research groups. Jain and colleagues [57] tested deformable liposomes for transdermal delivery of levonorgestrel. The peak plasma concentration of the drug was achieved after 4 hours and was maintained for 48 hours after a single topical application of deformable liposomes. Using deformable liposomes, the same group has also reported on the increased transdermal flux of poorly soluble zidovudine. The AUC (24 h) for elastic liposomes was found to be almost 12-fold higher than the control formulation; higher accumulation of antiviral drug has been found in target organs of reticuloendothelial system [58].

Although the main target of deformable liposomes has been the increased transdermal delivery of numerous drugs [59], they have been also studied as means for improved dermal therapy, such as the treatment of deep dermal infections. The elastic liposomes containing neomycin sulphate assured the enhanced skin deposition while histological studies showed complete eradication of

Staphylococcal infections within 7 days [60]. Pandit and colleagues [61] demonstrated better penetration of miconazole nitrate across the skin and better antifungal activity *in vivo* in comparison to the conventional liposomes.

Cadena and co-workers [62] proposed deformable liposomes with flavonoides for the weight loss treatment. Quercitin and resveratrol were encapsulated into phosphatidylcholine liposomes containing sodium deoxycholate as edge activator. This innovative phospholipid nanosystem was suggested as a novel approach for dissolving the subcutaneous fat when applied as a subcutaneous injection. However, no *in vivo* data on the efficacy of the system are available up to now.

Trotta and co-workers [63] investigated topical administration of methotrexate, potent antipsoriatic drug known to cause numerous side-effects and hepatotoxicity when applied orally. Moreover, the drug is hydrophilic and mostly in a dissociated form, therefore its topical application is limited. The encapsulation of methotrexate in deformable liposomes resulted in the enhanced penetration of drug into the skin. In another study, methotrexate was entrapped in deformable vesicles containing oleic acid as an edge activator. Enhanced skin penetration of the drug through the *stratum corneum* with increased accumulation in the epidermis and dermis layers has been ascribed to the elasticity of vesicles and the penetration enhancing effect of oleic acid [64].

Despite the generally accepted mechanism of intact vesicle penetration, there is still substantial discussion whether deformable liposomes penetrate intact through the skin or act as the penetration enhancer. El Maghraby and co-workers [65] compared the deformable and conventional liposomes as the carriers for skin delivery of 5-fluorouracil. Better *in vi*tro skin delivery of the drug was obtained via deformable vesicles as compared to the conventional liposomes. However, due to limited drug partitioning inside the skin the authors suggested that

deformable liposomes are not penetrating intact into the skin rather then that the vesicle components act as the penetration enhancers promoting the skin deposition of the drug; contrary to claims proposed originally by Cevc and collaborators [23,52]. The similar speculations regarding the mechanism involved in the improved skin delivery of drug by elastic vesicles, i.e. penetration enhancing effect, has been suggested by Gillet's group. Bethametasone entrapped in the aqueous compartment of the vesicles via cyclodextrin complexation was released and diffused as free molecules through the *stratum corneum* thus partitioning itself into the viable skin tissue [24]. These results are in accordance with those reported by Bahia and co-workers [66]. Using calcein as hydrohophilic marker entrapped in the deformable liposomes they proved the penetration-enhancing effects of sodium cholate and ethanol. *Ex vivo* skin permeation and *in vivo* transdermal studies on hairless mice showed the reduced transdermal flux of calcein in comparison to the solution forms, suggesting that the transdermal absorption of calcein from the deformable vesicles is controlled by the release of the drug from the formulation deposited onto the skin surface. Moreover, fluorescence measurements of the receptor fluid after the addition of Co^{2+} quencher have revealed that permeated calcein existed essentially in the free form, thus contradicting the proposed penetration of intact vesicles [66].

Ethosomes

Ethosomes are soft phospholipid vesicles originally developed by Touitou and her group [67] as a novel skin delivery system. They are composed of phospholipids and water as the conventional liposomes, but in addition include high ethanol content (20-45%, v/v) (Figure 1). Due to a well-known skin permeation enhancing effect of ethanol, ethosomes are also categorized as the skin permeation-enhancing vesicles. The presence of ethanol enables the entrapment/incorporation of

drugs with limited water solubility with rather high efficiency. Compared to the conventional liposomes of the same phospholipid composition, ethosomes are of significantly smaller size that can be attributed to high ethanol content. Ethanol may affect dissociation degree of a partially charged molecule at bilayer surface that can subsequently reflect to increased negative net surface charge of vesicles affecting decreased particle size of ethosomes [68]. The encapsulation efficiency of lipophilic drugs in ethosomes is higher in comparison to the conventional and especially the deformable liposomes. This is a consequence of the solubilizing effect of ethanol and the multilamellar morphology of ethosomes confirmed by Touitou and co-workers [69]. Moreover, ethosomes exhibited improved intracellular delivery into fibroblasts [70]. A proposed mechanism of improved skin drug delivery by ethosomes involves the dual fluidizing effects of ethanol on both the ethosomal lipid bilayers and the intercellular lipid matrix of *stratum corneum*. Compared to the deformable liposomes, which are able to increase the skin delivery only when applied under the non-occlusive dressing [51], ethosomes are efficient both under the non-occlusive [71,72] and occlusive conditions [71,73]. *In vitro,* animal and clinical studies have reported superiority of ethosomes in the skin delivery of minoxidil [67], trihexyfenidil HCl [71], cannabinoids [74], bacitracin [75], erithromycin [76,77] and testosterone [73].

Ainbinder and Touitou (73) compared the efficiency of ethosomes in transdermal delivery of testosterone across the rat skin *in vivo* to a registered gel formulation of the drug. The AUC of the drug from ethosomal formulation was found to be 64% greater than with the gel formulation. However, their study lacks reliable evidence supporting the claim of carrier mediated, rather than facilitated transport. In a recent study with surfactant-modified testosterone propionate ethosomes, the higher transdermal flux and lower lag time were obtained in comparison to the conventional liposomes and ethanolic solution of the drug [78]. *Ex vivo* studies on dermatomed

human cadaver skins revealed the enhanced transdermal permeation of the ethosomal melatonin as compared to the conventional liposomes and alcoholic solution of the drug [79].

Potential of ethosomes in the transdermal delivery of anti-inflammatory drugs (or isolated plant medicals) has been reported by several research groups. The assessment of transdermal delivery of diclofenac sodium from different phospholipid vesicles demonstrated similar transdermal flux of both ethosomes and deformable liposomes in comparison to the conventional liposomes [80]. Using the isolated human epidermis, Chourasia and co-workers [81] investigated potentials of ethosomes for transdermal delivery of ketoprofen. Paolino and colleagues [82] studied the penetration of ethosomal ammonium glycyrrhizinate through the isolated *stratum corneum* and viable epidermis as well as anti-inflammatory effect on human volunteers. Ethosomes have also been proposed for transdermal delivery of phytochemicals, such as capsaicin [83].

Ethosomes have been able to increase the effectiveness of topical anesthesia. Transdermal flux of lidocaine ethosomes was significantly greater than those obtained with the conventional liposomes and ethanolic solution of the drug [84].

They were also studied for improved skin therapy in dermatology providing increased drug solubility and enhanced penetration through the *stratum corneum*. Clinical investigation of ethosomal acyclovir in the treatment of recurrent *herpes labialis* demonstrated advantages of using ethosomes over the commercial product; time necessary for crusting of the lesions and loss of crusts were significantly reduced by applying ethosomal formulation [85].

Verma and Fahr [86] reported on potential of phospholipid vesicles embodying 10-20% (v/v) of ethanol in delivering the skin-impermeable drugs such as cyclosporine A for the treatment of skin inflammatory diseases, i.e. psoriasis and atopic dermatitis. Dubey and colleagues [87] tested ethosomes with methotrexate for the topical treatment of psoriasis. *Ex vivo* skin permeation

studies on dermatomed human cadaver skin showed better flux of the drug and skin deposition by ethosomes than with other vesicles. However, studies based only on *in vitro* and *ex vivo* penetration assessments do not always correlate to *in vivo* conditions as reported by Cevc and colleagues [88].

For the treatment of alopecia, Meidan and Touitou [89] studied ethosomes with minoxidil *in vitro*. The quantity of the drug accumulated into the skin of nude mice after the application of ethosomes was 2.0, 7.0 and 5.0 fold higher as compared to the ethanolic phospholipid dispersion, hydroethanolic solution and ethanolic solution of the drug.

Ethosomes have also been suggested for topical therapy of different skin allergies including urticaria, pollinosis and atopic dermatitis. *Ex vivo* skin permeation studies in the mice model showed high penetration potential of cetirizine compared to the conventional liposomes, while *in vivo* pharmacodynamic study proved the reduction in starching and erythema scores, skin hyperplasia and dermal eosinophil count [90]. The application of ethosomal tacrolimus in the treatment of atopic dermatitis enabled greater *ex vivo* penetration of the drug than with conventional liposomes in the mice skin model. Furthermore, pharmacodynamic study displayed the lowest ear swelling compared to the conventional liposomes and commercial ointment, and effectively impeded accumulation of mast cells in the ear of the mice, suggesting efficient suppression for the allergic reactions [91].

Encapsulation of psoralen into ethosomes resulted in the increased penetration through the *stratum corneum* and skin deposition. *In vivo* skin microdialysis study showed that the peak concentration and area under the curve of psoralen from ethosomes were approximately 3 and 2 times higher than those of psoralen from the tincture formulation indicating potential of ethosomal psoralen for improved treatment of vitiligo [92].

Penetration enhancer-embodying liposomes (PEVs)

This term refers to elastic liposomes composed of the phospholipids and penetration enhancer as bilayer building compounds (Figure 1D). Propylene glycol or diethylene glycol monoethyl ether (Transcutol®) have been commonly used as the penetration enhancers [93,94]; however, some authors used the cineole and capryl-caproyl macrogol 8-glyceride (Labrasol®) [95]. The elastic vesicles containing terpens as the penetration enhancer also known as *invasomes* [96] are presented separately at the end of this chapter.

Propylene glycol-containing liposomes (PG liposomes) have been proposed by Elsayed and colleagues [93] as a new type of phospholipid vesicles for improved skin drug delivery; however it is worth noting that these vesicles were investigated earlier for topical drug delivery via mucosal route under different name, i.e. the polyol dilution liposomes [16,97,98,]. Those liposomes were composed of phospholipids, propylene glycol (PG) and water and characterized by the increased entrapment efficiency for poorly soluble drugs due to the solubilizing effect of PG [17,99]. PG liposomes can be prepared using PG as a solvent for the phospholipids and lipid drug [93,97-99-87] or as a part of the aqueous phase of formulation [94,100]. The presence of PG or other penetration enhances in the phospholipid bilayer significantly increases the elasticity of vesicles [100,101]. Preliminary *in vivo* skin deposition study using an animal model has shown superiority of PG liposomes over the conventional, deformable liposomes and ethosomes in the skin delivery of local anesthetic cinchocaine [93]. Moreover, the PG liposomes were superior to deformable liposomes in delivery of diclofenac sodium in the permeation studies performed on the artificial *stratum corneum*-mimicking membranes [100].

Manconi et al. [101] evaluated diclofenac (both as acid and salt form) loaded liposomes containing Transcutol®. Increasing the concentration of penetration enhancer resulted in the

better drug encapsulation, while rheological experiments revealed that Transcutol® was able to improve the bilayer fluidity. Compared to commercial gel, Transcutol®-containing vesicles enabled enhanced penetration of the both drug forms into and through the skin. In another study PG liposomes were investigated for transdermal delivery of curcumin as anti-inflammatory agent. Comparison pf PG liposomes with other elastic vesicles, i.e. ethosomes and deformable liposomes revealed the highest entrapment of curcumin in the PG liposomes (> 90%). The same formulation also exhibited the highest transdermal flux across the rat skin. The superiority of PG liposomes was confirmed in *in vivo* anti-inflammatory study measuring the inhibition of the paw edema [102]. Evaluation of different penetration enhancers used for the preparation of PEVs with quercitin confirmed that PG and polyethylene glycol 400 enable similarly high drug accumulation into and through the skin [103]. The same group also reported on the potentials of PEVs for cutaneous delivery of minoxidil. Among different penetration enhancers examined, Labrasol®- and cineole- containing PEVs were found to be able to deliver a higher amount of the drug than controls [104].

The recent study by Wang and colleagues [105] reports on the use of novel penetration modifiers embodied into the bilayers of phosphatidylcholine liposomes, namely 1,2-hexanediol and 1,4-cyclohexanediol, able to enhance the targeted delivery of ketoconazole into the skin.

Invasomes

Invasomes are a novel type of elastic phospholipid vesicles composed of phosphatidylcholine, ethanol and a mixture of terpenes as penetration enhancers. Invasomes containing 3.3% ethanol and 1% of the terpene mixture (cineole:citral:d-limonene=45:45:10) have shown to significantly

enhance the skin penetration and deposition of the highly hydrophobic photosensitizer temoporfin (mTHPC) in comparison to the vesicles without terpenes and conventional liposomes [96]. Invasomes could provide the efficient delivery of mTHPC in photodynamic therapy [106]. The precise mechanism of the penetration enhancing ability of invasomes is debatable and should be further investigated. It is hypothesized that synergistic effect of phospholipids, terpens, ethanol might play a role in increased delivery of active substances into the skin [106].

Penetration enhancer-embodying deformable liposomes

Hiruta and colleagues [107] formulated bleomycin-loaded ultra-deformable liposomes composed of egg phosphatidylcholine and sodium cholate and additionally containing beta-sitosterol 3-beta-D-glucoside (Sit-G) as a penetration enhancer. The presence of Sit-G increased drug entrapment, *in vitro* stability, and significantly increased the distribution of bleomycin in the epidermis and dermis as compared to the ultra-deformable liposomes without Sit-G.

The overview of main characteristics, advantages and limitations of described systems in provided in Table 1.

OTHER PHOSPHOLIPID VESICLES FOR IMPROVED SKIN THERAPY

pH-sensitive liposomes

pH-sensitive liposomes become destabilized in acidic pH such as in endosome inside the cells, enabling the release of the entrapped material into the cytoplasm. Although mostly studies to deliver the genetic material via parenteral route, two decades ago Yarosh and co-workers applied these type pf liposomes in dermatology. Yarosh and co-workers designed and evaluated pH-sensitive T4N5 liposomes containing a DNA repairing enzyme (T4 endonuclease 5), specific for solar UV-induced skin DNA damage [108-109-100]. T4N5 liposomes applied after the UV exposure, penetrated human skin and delivered DNA repairing enzyme into keratinocytes and epidermal Langerhans cells in 15 volunteers with preceding skin cancers [111]. Moreover, daily applications of T4N5 liposome-based lotion for a period of one year to the 30 *xeroderma pigmentosa* patients with sun-damaged skin and with the history of skin cancer or actinic keratosis, lowered the rate of new actinic keratosis and basal cell carcinomas significantly [109]. To the best of our knowledge, this formulation reached the Phase III of clinical investigation [112].

Biphasic vesicles

The rationale behind these vesicles was to build the vesicles from excipients that provide synergistic skin permeation enhancement. Biphasic vesicles are structurally related to liposomes and represent the multicompartmental delivery systems. Those vesicles exhibit mixed lipid membrane characteristics, multicompartmental structure and positive charge. Biphasic vesicles appear to interact with the intercellular lipids of *stratum corneoum* and enhance the lipoidal pathway of penetration affecting the molecular rearrangement of intercellular lipids [113]. King and co-workers developed biphasic vesicles-based novel delivery system Biphasix^{TM} as a superior delivery system for transdermal delivery of insulin. Basal levels of insulin were

observed in the serum of diabetic rats treated with Biphasix-insulin up to 3 days after patch application [114]. In consequent experiments, the authors proved that lymphatic transport of insulin after non-invasive topical administration is taking place [115]. Topical application of biphasic vesicles carrying interferon alpha in a guinea pig model resulted in sustained delivery of interferon locally into skin, exhibiting their potential in treatment of anogenital warts. Biphasix™ interferon-alpha-2b cream used in the animal study had a physical appearance of a soft cream [116]. Biphasic vesicles entrapping interferon alpha delivered clinically relevant levels of interferon across intact human skin and elicited marked therapeutic effect in patients [117].

PARTICULATE LIPID-BASED NANOSYSTEMS

Solid lipid nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs) are comprised of lipids (0.1-30%, w/w) that are solid at room and body temperatures and surfactants (0.5-50%) serving as stabilizing agents (Figure 2A). They have been introduced in 1991 as an alternative to vesicular nanosystems, emulsions and polymeric nanoparticles. SLNs contain adhesive and occlusive excipients that form a thin film on the skin surface, thus reducing water evaporation and retaining skin moisture [26, 118,119,]. The occlusive property enhances drugs penetration into the skin and can be increased by decreasing the particle size (at given lipid concentration) or by increasing the lipid nanoparticles concentration (at given particle size) [120]. SLNs are biodegradable and biocompatible, having low toxicity and feasibility for scale up and sterilization. Physicochemical features and drug characteristics influence the drug loading and distribution within particles and dispersions. Generally, lipophilic drugs are better encapsulated than amphiphilic and hydrophilic drugs [121,122]. The lipid composition and drug lipid solubility determine drug distribution within the

particles that consequently reflect to release kinetics [123-125]. The interactions between SLNs and keratinocytes suggest that SLNs in nanosize range may provide advanced skin therapy [126].

SLNs were investigated to improve the treatment of skin diseases such as atopic dermatitis, psoriasis, acne, skin mycosis and inflammations [119]. To facilitate dermal application by achieving the appropriate viscosity of formulation, the fluid dispersions with low lipid content (<10%) can be incorporated into a gel or cream base compatible with nanoparticles, similarly like other vesicular nanosystems [127].

Well documented evidence supports the role of SLNs in improved delivery of antifungals. Recent study by Vaghasiya and co-workers [128] confirmed the ability of SLNs to improve retention of terbinafine hydrochloride inside the skin and to reduce fungal burden in rats in a shorter time than marketed formulation. *In vivo* evaluation of econazole nitrate containing SLNs confirmed a rapid penetration of the drug through *stratum corneum* and its increased diffusion in the deeper skin layers [129].

By the right choice of lipid composition SLNs have been able to overcome limitations of conventional corticosteroid therapy in dermatology [130]. SLNs reduced percutaneous absorption of betamethasone-17-valerate in both the impaired and intact skin. When the barrier was intact, the reservoir effect was more evident and the drug partitioning into the different skin layers was dependent on the lipid properties of the SLNs [125].

Investigations of reseveratrol as a naturally occurring anticarcinogenic compound for the treatment of skin cancers have been received particular attention. Teskač and Kristl [126] confirmed cellular uptake of resveratrol when loaded into SLNs and improved effects of the drug on the cellular fate. SLNs with a size below 180 nm passed rapidly through keratinocyte membranes causing no significant changes in cell morphology, metabolic activity or cell cycle.

SLNs were concentrated around nuclei, releasing resveratrol in a sustained manner to express its cytostatic effect with prominent S-arrest of cell cycle and a large drop of G2/M phase. The same group [126] investigated antioxidative potentials of liposomal resveratrol and found that liposome-mediated uptake of resveratrol was more effective for the improvement of the cell-stress response. Resveratrol loaded into negatively charged oligolamellar liposomes (84 nm-sized) protected cells from free radical damage [131].

Although SLNs have numerous advantages as discussed above, there are some limitations, too. One of them is poor loading capacity which is limited to about 10% of the amount of lipid (leading to about 1% of the final dispersion) to ensure stability of the system. Namely, highly ordered crystalline lipid matrix leave little place for drug incorporation that can expelled out during the storage [127]. To overcome these deficiencies, second generation of lipid nanoparticles, i.e. nanostructured lipid carriers (NLCs) were introduced in the late 90th of the last century [120]. NLCs are able to incorporated lipophilic drugs to a greater extent than SLNs; however the entrapment of hydrophilic drugs is limited.

Nanostructured lipid carriers (NLCs)

NLCs (Figure 2B) are produced using the blends of solid and liquid lipids (mixed in ratios ranging from 70:30 to up to 99.9:0.1) that are stabilized by surfactants as the SLNs. The presence of liquid lipids with different fatty acid chains prevents the formation of arranged lipid matrix, leading to formation of less ordered lipid matrix. Such imperfect matrix permits better accumulation of larger amounts of drug. Drug solubility is often higher in an oily phase contributing to increased loading efficiency [132]. The fluid lipid phase of NLCs can be

embedded into the solid lipid matrix or be localized at the surface of solid platelets and the surfactant layer [127]. NLCs include all advantages of SLNs reported earlier such as biodegradability, occlusion effect, modified drug release, delivery of drug to a specific sites of skin, increased drug stability, etc., and in addition enable improved drug loading and increased shelf life [127].

Specific structure of NLCs can allow biphasic pattern of drug release with initial burst effect followed by a sustained release due to different melting points of solid and liquid lipids [133]. By changing the ratio of solid and liquid lipids in NLCs, the drug release, permeation and pharmacodinamyc activities can be modulated [134]. Physicochemical properties of NLCs such as the lipid composition, size and surface charge influence the delivery of encapsulated drug into the skin [127].

Many attempts were employed to explore the potentials of NLCs in dermatology [119,120]. For example, Gomes and colleagues [135] investigated potentials of finasteride and minoxidil loaded NLCs for the treatment of alopecia *in vitro*. A high loading efficiency was achieved for finasteride (70-90%), while less than 30% was achieved for minoxidil nanoparticles. Both drug formulations were physically stable, provided prolonged release of the drugs and ~~sufficient~~ enabled penetration of drugs into the skin layers.

The potential of NLCs in the therapy of psoriasis has been suggested by Agrawal and collaborators [136]. They have assessed acitretin NLCs-based gel in *ex vivo* and in clinical studies. The formulation showed increased penetration of acitrecin into the human cadaver skin compared with a reference gel. The double-blind clinical study on psoriatic patients reported improvement in a therapeutic response and reduction in the local side effects. However, the low number of patients (n=6) is considered as a limitation.

NLCs containing both calcipotriol and methotrexate in one formulation have been suggested to strengthen the topical therapy of psoriasis [137]. *Ex vivo* studies performed on hyperproliferative mice skin demonstrated increased skin permeation of metrotrexate, while the penetration of calcipotriol was reduced. Good correlation of obtained results has been confirmed *in vivo* by confocal laser scanning microscopy [137].

Modified NLCs for topical delivery of tacrolimus (T-MNLC) in the therapy of atopic dermatitis and other skin inflammation disorders have been shown to enable high entrapment efficiency of the poorly soluble drug, enhanced stability and improved skin deposition [138]. *In vivo* evaluation of T-MNLC based on restoration of skin barrier, therapeutic effectiveness and safety aspects imply the potential of developed formulation in the therapy of atopic dermatitis [139].

Several studies report on the potentials of NLCs in (trans)dermal delivery of NSAIDs for the local treatment of rheumatic diseases (by non-selective COX inhibitors) as well as skin ~~inflammation diseases, e.g.~~ tumors, injuries and wounds (by selective COX-2 inhibitors). For example, penetration of flurbiprofene through the rat skin was found to be 4-5-folds increased after 12 h by using NLCs compared to the solution of free drug [140]. Ricci et al. [141] reported on higher indomethacin transdermal flux and prolonged anti-inflammatory activity by NLC-based gel in comparison to the control gel. In another study Joshi and Patarvale [134] compared NLCs-based celecoxib gel with control micellar gel. *Ex vivo* penetration of the drug from the NLC-gel was less than from the micellar-gel. The *in vivo* evaluation of the percentage edema inhibition produced by NLCs and micellar gel demonstrated a significantly higher inhibition by NLC-based gel up to 24 hours [134].

MISCELLANEOUS

Micro- and nano-emulsions

Literature reports on potential of micro- and nano-emulsions in dermatology often do not discriminate between microemulsions and nanoemulsions in respect to the size of the droplets. Physicochemically, microemulsions are thermodynamically stable colloidal dispersions of water and oil stabilized by a surfactant and, often, a cosurfactant [31]. The active compound (drug) is solubilized inside microemulsion, wherein the formulation ingredients facilitate the penetration into the skin. The selection of cosurfactant is usually based on its penetration enhancing potential. The occlusivity also acts on improving the drug penetration [142]. Nanoemulsions are metastable colloidal systems. The method of preparation is directly affecting their properties and can be manipulated to design and optimize the delivery systems [143]. Compared to the conventional emulsions, microemulsions are characterized by low interfacial tension due to the high ratio of surfactant and co-surfactant responsible for good physical stability of formulation. On the other hand, high ratio of surfactant may affect the system's compatibility of formulation with the skin [144] . Recently, multiple W/O/W nanoemulsions as superior delivery system for acyclovir have been proposed [145].

Cohleates

Cochleates are tubular shaped structures derived from liposomes. They are actually the precipitates obtained through the interaction between phosphatidylserine and calcium, able to

entrap wide variety of drugs [146]. Although designed mostly to target oral and parenteral routes of drug administration, cochleates, may have potentially serve as a topical drug depot system [147].

(PHOSPHO)LIPID-BASED NANOPHARMACEUTICALS WITH POTENTIAL IN (SELECTED) SKIN DISEASES

Antimicrobial skin therapy

Skin represents a unique environment in respect to microbiome. An extensive review on the skin microbiome, including bacteria, fungi, viruses, archaea and small arthropods colonizing the skin surface, has been recently published by Kong and Segre [148]. Antimicrobial agents and antibiotics have been traditionally used topically to treat various skin diseases such as acne (e.g. *Propionibacterium acnes*) [149], persistent bacterial skin infections [150,151], methicillin-resistant *Staphylococcus aureus* (MRSA) infections or infections of prosthetic devices implanted into the skin (*Staphylococcus epidermidis*) [152]. Particularly interesting are fungal skin and nail infections which remain a major reason for patient visits to dermatologists. Antifungal therapy is mostly based on poorly soluble azole agents, which suffer from limited bioavailability due to solubility issues [153]. Additional limitation for effective antimicrobial therapy is the increasing resistance against most potent antimicrobials. It is expected that encapsulation of drug in specific nanosystem will enable its improved delivery to the target sites enabling dose reduction while increasing the specificity[152,154].

The limited success of skin antimicrobial therapy may be attributed to the challenge most of the antimicrobials face when acting against biofilms [151]. Bacterial biofilms are a common cause of

recurring infections that are unresponsive to drug therapy and are recognized as a serious challenge in antimicrobial therapy.

Pevaryl® Lipogel was the first approved antifungal liposome product promoting the advantages of liposomes in topical skin therapy [155]. Encapsulation of vancomycin within cationic liposomes has been shown to lead to enhanced inhibition of *Stapyhylococcus epidermidis*. Cationic liposomal surface enabled adsorption of vesicles onto biofilms and skin-associated bacteria. The adsorbed liposomes were also able release drug over 18 hour regrowth period, assuring prolonged antibacterial action [156].

Benzyl penicillin (penicillin G) was successfully encapsulated in cationic liposomes and its activity against *Staphylococcus aureus* biofilms confirmed [157]. Metronidazole, one of the drugs with very limited solubility, was successfully encapsulated into various types of liposomal systems [15,158]. A very promising approach was proposed by Vogt et al. [159] of using PVP-iodine in hydrosomes, specific type of liposomes in hydrogels, for reduced rejection of skin grafts.

Ethosomes have been shown to improve delivery of various antimicrobials such as for example acyclovir [85] and erythromycin [76]. Godin and Touitou [75] demonstrated penetration of bacitracin into deep skin layers and co-penetration of both the drug and phospholipids into the fibroblasts. *In vivo* experiments have proven efficient healing of *Staphylococcus aureus* deep dermal infections when mice were treated with ethosomal erythromycin. On the contrary, no subdermal healing was observed in infected animals treated with hydroethanolic solution of the

drug [77]. Enhanced *ex vivo* and *in vivo* skin deposition of antifungal drug voriconazole has been confirmed as well [160].

For the topical treatment of fungal infections, Elmoslemany et al. [161] prepared and evaluated miconazole nitrate-loaded PG liposomes. Compared to the conventional liposomes, PG liposomes exhibited stronger antifungal activity and enhanced skin deposition.

SLNs and NLCs were proposed as controlled release delivery systems for topical clotrimazole therapy [162].

ANTIACNE TREATMENT

Acne is a chronic inflammatory dermatosis of the pilosebaceous unit with up to 80 % prevalence among adolescents. The readers are referred to the reviews on novel treatment approaches based on the use of delivery systems by Castro and Ferreira [163] and Date et al. [164]. Delivery of antiacne agents in vesicular and particulate nanosystems represents an innovative alternative to minimize the side effects, while maintaining the efficacy of the treatment, based on the controlled release properties and improved drug penetration into the skin or into the pilosebaceous unit [163]. Although no anti-acne therapy based on nanopharmaceuticals has reached the market yet, several promising approaches are listed below.

The superiority of liposome-encapsulated clindamycin hydrochloride in the treatment of acne has been first proposed by Škalko and co-workers. In clinical trials, the liposome formulation significantly decreased the numbers of pustules as compared to the control formulation [165]. Similar findings have been later demonstrated by Honzak and Šentjurc [166]. Liposomal delivery systems based on antimicrobials for anti-acne treatment have been confirmed to be superior to

non-liposomal systems [31]. Liposomal delivery system for lauric acid, a natural compound from coconut oil, was proposed as innate, safe and effective therapeutic medication for acne treatment. Lauric acid-loaded liposomes have been shown to fuse with the membranes of *Propionebacterium* and release lauric acid directly into the bacterial membranes [149]. Improved treatment of *Propionebacterium* acnes with marked reduction in adverse symptoms has also been demonstrated with liposomes containing benzoyl peroxide [167].

Advantages of using SLNs for the acne treatments have been exhibited by improved photostability of retinoids, enabling lesser drug irritancy and greater skin tolerance [168]. SLNs were shown to enhance skin penetration of cyproterone acetate for 4-folds, compared with nanoemulsion and cream. Incorporation of the drug into the lipid matrix of nanostructured lipid carriers and microspheres resulted in a 2-3-fold increase in cyproterone acetate absorption [169].

Encapsulation of isotretinoin-hydroxypropyl-β-cyclodextrin (HP-β-CD) complex into the deformable liposomes has improved the skin delivery of isotretinoin for the treatment of acne. Transdermal flux was found to be 15-21-folds higher than that obtained from the drug in solution, and 4-5-folds higher than that obtained with the drug-cyclodextrin complex in a solution form. Moreover, the skin irritation study confirmed the significant reduction in irritation potential of isotretinoin elastic liposomal formulation in comparison to the free drug [170].

DERMATO-ONCOLOGY

Most of the advances of nanotechnology can be seen in the field of dermoscopy and confocal microscopy for the early detection of sub-clinical melanoma [2]. In respect to the treatment of

melanoma, several lipid-based delivery systems seems to offer means for improved drug delivery.

Elastic liposomes have been studied for skin delivery of antitumor drugs. *In vitro* data revealed that the LD50 of bleomycin from Bleosome™ (elastic liposomes containing 10% sodium cholate) was 3-fold higher than the free bleomycin solution in the cells of human squamous cell carcinoma (SCC) and nearly 30 times higher in the human cutaneous keratinocytes [171].

Paclitaxel-loaded ethosomes® have been proposed for the treatment of actinic keratosis and squamous cell carcinoma. Improved permeation of paclitaxel through the *stratum corneum*- and isolated epidermis- membrane models have been proven as well as the increased anti-proliferative activity in a squamous cell carcinoma model [172]. Several studies (reported in previous chapters on liposomes and SLNs) emphasize the role of lipid based nanosystems in delivery of enzymes (T4 endonuclease 5) and anti-inflammatory drugs (e.g. celecoxib) in the prevention of skin cancer, i.e. treatment of precancerous actinic keratosis. The high potential of different nanosystems (elastic liposomes, pH sensitive liposomes, nanoemulsions, NLCs) in the treatment and prevention of skin carcinoma has been confirmed in *in vivo* animal and clinical studies (Table 2).

GENE DELIVERY AND PLASMID-BASED VACCINES

Gene therapy is defined as the insertion of a gene into recipient cells [173]. The skin is a very attractive organ for gene therapy, as it is easily accessible and the therapy outcome of skin treatment can be easily monitored.

Lipid-based nanosystems have been tested as means of non-invasive delivery of genetic material into/through the skin to their introduction as possible carriers for non-invasive gene delivery. For extensive review on liposomes as non-viral gene delivery system, the readers should refer to Jeschke et al. [174]. Enhancement of dermal and epidermal regeneration represents a crucial target for the treatment of wounds, including burns. New strategies for the delivery of growth factors were proposed based on gene therapy [174].

Cholesterol containing cationic liposomes encapsulating an expression plasmid vector for cDNA were shown to be able to deliver biologically active proteins to the skin. It is known that keratinocyte growth factor (KGF) stimulates epithelial cell differentiation and proliferation, major steps in successful wound healing. Liposomal cDNA gene complex was proposed as delivery system for KGF therapy. Clear improvements in the epidermal and dermal regeneration were seen in rats with acute wounds [175].

Kim et al. [176] showed that deformable cationic liposomes, prepared using a cationic lipid, 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), and sodium cholate, were able to transfect several cell lines and after a single administration on the intact mice skin transport the genes into several organs for 6 days. In another study, ultradeformable liposomes containing edge activators sodium cholate or sodium deoxycholate could also deliver DNA into mice transdermally [177].

Biphasix, biphasic lipid vesicles, used as a carrier for plasmid DNA were shown to induce the gene expression in the lymph nodes. Interestingly, intradermal injection resulted in expression in the skin and gene gun-delivered genetic material was found expressed both in skin and lymph nodes [178].

VACCINES

Topical vaccines based on the advancements in nanotechnology are one of the most promising pipelines in nanodermatology. Topical vaccination provides an efficient way to activate effector-T-cells and induce immune responses. Currently, the main challenges for cutaneous immunization are to enhance the transport of antigens across the skin barrier and to improve the immunogenicity of topically applied subunit vaccines [179]. Hair follicles were also shown to be a penetration pathway and important target for topical vaccination [2].

Formulation of antigens in carriers of nanosize range for transcutaneous route is gaining more and more popularity [179-181].

Transferosomes® were used a carrier for different antigens, for example gap junction protein [182,183] and tetanus toxoid [184,185] and shown to induce immune response comparable to subcutaneous injection. Mishra and co-workers [186,187] showed the superiority of elastic liposomes with hepatitis B surface antigen. Recently, Chopra and Cevc [188] confirmed that epicutaneous immunization with tetanus toxoid in deformable liposomes protects mice against tetanus.

Baca-Estrada and co-workers [189] proposed biphasic delivery system as a suitable carrier for antigens able to induce antigen-specific immune responses.

More research is needed to determine the predominant route as well as the effect of the nanocarrier characteristics on the penetration and immunization potentials of various nanosystems.

The potentials of different (phospho)lipid-based nanopharmaceuticals in the treatment of various skin diseases and transdermal administration of drugs and biologically potent molecules are confirmed in numerous *in vivo* and clinical studies (Table 2). The success of extensive research

resulted in several registered products, already available on the market. Table 3 summarizes the current state-of-art in the product development of nanosized lipid-based drug delivery systems.

TOXICITY

Nanosized systems carry intrinsic skin toxicity, in respect to their small size and limited ability of the skin to bio-process the exogenous material [190]. In respect to skin, especially the skin with damaged barrier properties such as in atopic dermatitis for example nanotoxicity needs to be addressed [7]. The effects of a long term deposition of nanoparticles in the skin remain to be explored [22]. The advancements in fluorescence microscopy such as the development of superior fluorescent dyes and probes provided a deeper insight on cellular uptake and the trafficking of nanoparticles within the cellular environment [191]. The advanced dyes and accompanying methods provide means to optimize delivery systems in respect to both intracellular localization of nanoparticles and toxicity concerns related to that particular delivery system, with focus on the effect of particle size on both issues [126].

Moreover, very little is known about the cumulative effects of exposure to various nanosized particles, especially cosmetic products. This concern was also addressed by EU through the project: Engineered Nanoparticles: Review of Health and Environmental Safety (ENRHES), funded by EU in 2008. Several initiatives were launched to address the exciting field of nanomedicine in dermatology, such as for example Nanodermatology Society (2010) focused on promoting a greater understanding of both scientific and medical aspects of nanotechnology in dermatology [21].

Phospholipid vesicle-based formulations for (trans)dermal drug delivery, such as Diractin®, have been proven in many cases to be completely safe for continuous use [192]. However, each

formulation needs to be carefully studied during the preclinical and clinical investigation before its recommendation for clinical use. Clinical investigations with soy lecithin/cholesterol liposomes containing econazole, hydrocortisone or local anesthetic indicated no adverse effects. Slight erythema was observed at the site of administration of liposomal tetracaine under the occlusive dressing in a few subjects, but it resolved spontaneously within 3 hours [38]. The clinical studies with liposomal econazole indicated less local irritation than econazole cream; the lipid vesicles minimized the irritation potential of the drug [155].

On the other hand, microemulsions, comprising of the relatively high surfactant concentration may cause toxic effects, especially if applied on the diseased skin with impaired function of the *stratum corneum* [31].

CONCLUDING REMARKS

It can be concluded that opportunities for improved skin therapy based on the advances in nanotechnology and development of nanosized delivery systems are expanding, both in the academia and industrial set up. (Trans)dermal route and recently revived hair follicles targeting [193-199]. Multidisciplinary approaches in nanodermatology are needed to understand the mechanisms of interactions between nanomaterials and the skin, particularly diseased skin. Remarkable number of (phospho)lipid-based nanopharmaceuticals for skin therapy are already on the market or in the late phases of clinical studies (Table 3). The patents in nanotechnology are being issued at a geometric rate indicating a strong focus of various industries, including pharmaceutical, cosmeceutical and biotechnological. However, many unanswered questions and technical challenges remain to be addressed, particularly the long term toxicity issues.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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Figure and Table legends:

Figure 1. Schematic drawings of different types of liposomes commonly studied in the skin delivery of drugs: conventional liposome (A), deformable liposome (B), ethosome (C), penetration enhancer embodying liposome (D, e.g. propylene glycol liposome).

Figure 2. Schematic drawings of solid lipid nanoparticle (SLN, A) having more or less perfect crystalline structure (similar to brick wall) and nanostructured lipid carrier (NLC, B) consisting of a crystalline matrix with many imperfections leading to improved loading efficiency and stability.

Table 1. Potentials and limitations of different (phospho)lipid vesicles for skin delivery of drugs

Table 2. Overview of selected clinical and *in vivo* studies investigating efficiency of lipid-based nanosystems for skin delivery of drugs.

Table 3. Examples of registered lipid-based nanosystems for (trans)dermal drug delivery.