Chapter 2
Literature Review

Dermal Delivery of Protein/Peptide Based Antimicrobial to Treat Secondary Infection in Psoriasis and Eczema

2.1 Introduction

2.1.1 Psoriasis

Psoriasis is a chronic, immune-mediated, disfiguring, painful, non-communicable, and disabling incurable disease having a great negative impact on quality of life patients [1]. The reported occurrence of psoriasis in developed countries varies between 0.09% to 11.4% [2-4], making psoriasis a severe disease with at least 100 million people affected globally.

Various risk factors influencing psoriasis and its recurrence are genetic changes, infections, allergens, sunlight exposure, alcohol intake, smoking, and endocrine factors [5]. Moreover, psoriasis can be triggered by internal and external triggers, together with infections, mild trauma, stress, and systemic drugs [6]. Randomized clinical trial results have shown that the condition may worsen by skin/gut colonization with microorganisms, i.e., *S.aureus*, *Propionibacterial species*, *candida albicans*, and some other species [7-9]. Specifically, *S.aureus* may induce purulent superinfection and increase inflammation by superantigen-mediated T-cell activation [10]. Also, the recent clinical survey demonstrated that people with psoriasis had a high frequency of getting serious infections (20/1000 person) [11].

2.1.1.1 Pathogenesis of psoriasis

In the normal skin, the dividing cells, epidermal stem cells reside at the lowermost layer of the epidermis. These cells divide randomly to form more stem cells. After a certain degree of cell proliferation, they commit to differentiate. Epidermal stem cells and their differentiated progeny cells constitute the epidermal differentiation unit [12, 13]. Each stage of differentiation is controlled by specific differentiation markers like filaggrin, involucrin, keratin 1,5,10,14, loricin, caspase 14, etc. Depending on the expressed differentiation markers, the cells move supra basally and become part of stratum spinosum, granulosum, lucidum, and finally become stratum corneum cells (corneocytes). Corneocytes are terminally differentiated keratinocytes, which are anuclear and lack other cytoplasmic contents. The corneocytes constitute the stratum corneum (SC) layer of the epidermis, and subsequently, this layer of cells is continuously exchanged with newer ones.

Generally, it takes 28days for complete replacement of the whole epidermis layer of the normal skin [14, 15].

Whereas in psoriasis, the replacement time of epidermal layer of the psoriatic skin is decreased and cellular growth in the epidermis is increased which may lead to non-complition of the differentiation process of the cells. Therefore, skin looks as scaly erythematous cuts [16, 17]. Morover, the histological characteristics of the psoriatic skin consist of the epidermal thickening (acanthosis), extentsion of epidermal layer to the dermis (elongated ridges), and altered cell differentiation in the epidermal layer. Additionally, the incomplete differentiation and altered proliferation of keratinocytes is accountable for scaling while dilation of the blood vessels in the dermal papillae is responsible for the redness in psoriatic skin. Apart from this, the increased infiltration of immune/inflammatory cells in the dermal and epidermal layers is also observed in the psoriatic skin [18, 19].

The pathogenesis of psoriasis is started by numerous interconnected factors under a favorable hereditary background along with distinct environmental conditions [20, 21]. In past, psoriasis was observed as a skin disease that happens by reason of abnormal keratinocytes; but then it is documented as an overactive immune disease. It is likely to define pathogenesis of psoriasis in three stages: activation of T lymphocyte and its subsequent migration into the skin, release of pro-inflammatory cytokines by T cells and immune responses (Fig 2.1) [16].

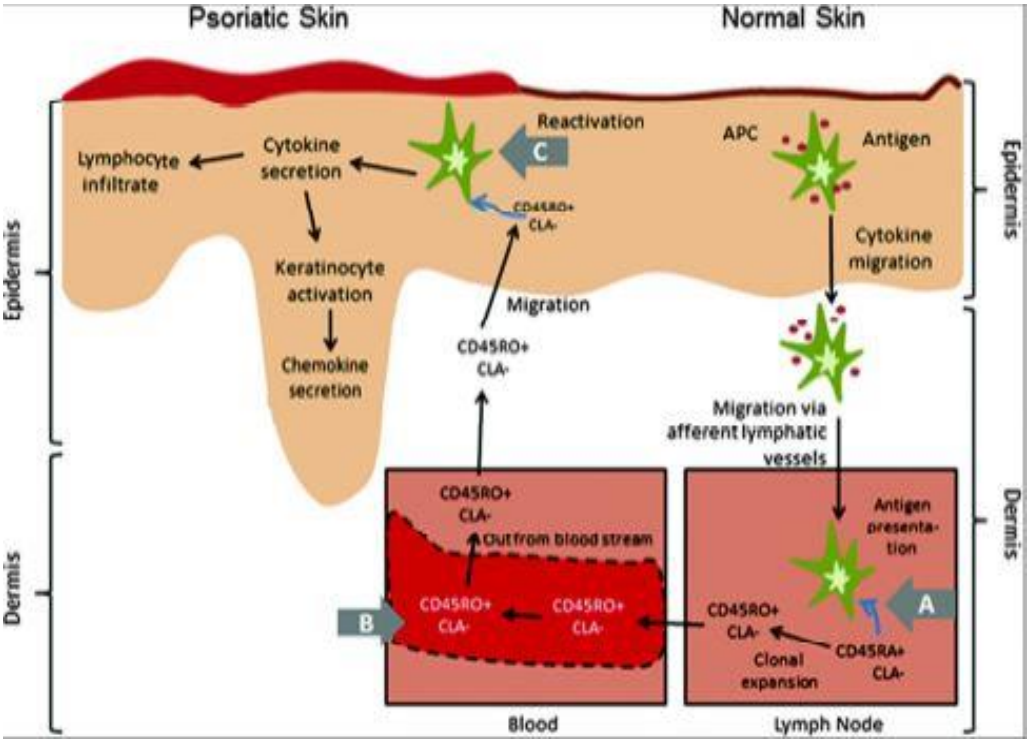

Figure 2.1 Pathogenesis of psoriasis

2.1.1.2 Types of psoriasis

A) Plaque psoriasis

It is also known as psoriasis vulgaris, which generally occurs (almost 80-85 %). It is usually seen on elbows, knees, scalp, and lower back [22]. Symptoms include,

- Spherical lesions range about 0.99 cm to several cm in width and may further grow into patches.
- Red-colored lesions are encrusted with silvery, loose, and shining skin.
- Lesions are generally seen on the elbows, knees, and trunk.

Causes: Infections, skin abrasion, medications, sunlight, strain, smoking, and drinking.

B) Guttate psoriasis

It is also known as rain drop psoriasis and is the second general form (around 10 %) seen in populations with psoriasis [22, 23]. Symptoms include,

- Several minute rain drops sized lesions
- Lesions grow instantly, generally on the trunk, arms, legs, and scalp
- Eruption of lesions may occur along with any upper respiratory infection.

Causes: Streptococcal infection, viral or bacterial contagions, skin wounds and burns, insect bites, etc., sunlight, medicine, etc.

C) Psoriatic arthritis

It is a condition in which swelling occurs that shows an impact on the joints. Generally, 6 % to 40 % of the population has this skin disorder [22, 24]. Symptoms of psoriatic arthritis include:

- Inflated, sore, thick, and painful joints,
- Above signs may be seen earlier, along with or following the development of symptoms of the skin.
- In the hands and feet, joint symptoms are seen.

Causes: Shock or wounds on the skin, medications, agents that irritate the skin, smoking, drinking, etc.

D) Pustular psoriasis

It can occur as patches that are small or wide spread on feet, hands, or fingertips [22, 25]. Seen in 5 % or less population having psoriasis. Symptoms include,

- Fluid-filled lesions are seen on soles and palms—very scaly skin.
- Alterations in the nail.
- Eruptions are seen after the discontinuation of certain medications and creams.

Causes: Pregnancy, overexposure to UV light, systemic steroids, contagions, mental and emotional strain, and sudden withdrawal of certain medications.

E) Erythrodermic/exfoliative psoriasis

It is a very uncommon type that may be damaging or lethal [22]. In the population with this type, along with skin, symptoms are seen on the whole body like,

- Inflation and soreness occur on the entire body's skin. The skin may slough off and is generally itchy and tender.
- Incapable of monitoring temperature of the body and chills.

Causes: Use of steroids, extreme sunburn, strain, drinking alcohol, contagions, sensitivity, etc.

F) Nail psoriasis (5)

Along with the building up of skin cells under the epidermis, in half of the psoriasis population, they develop under nails, which become impenetrable. They mostly split, and

in extreme cases, they fall or collapse. Yellow or brownish-red spots are seen below the nail. Causes of this type are unknown, but generally it is considered a genetic factor [26].

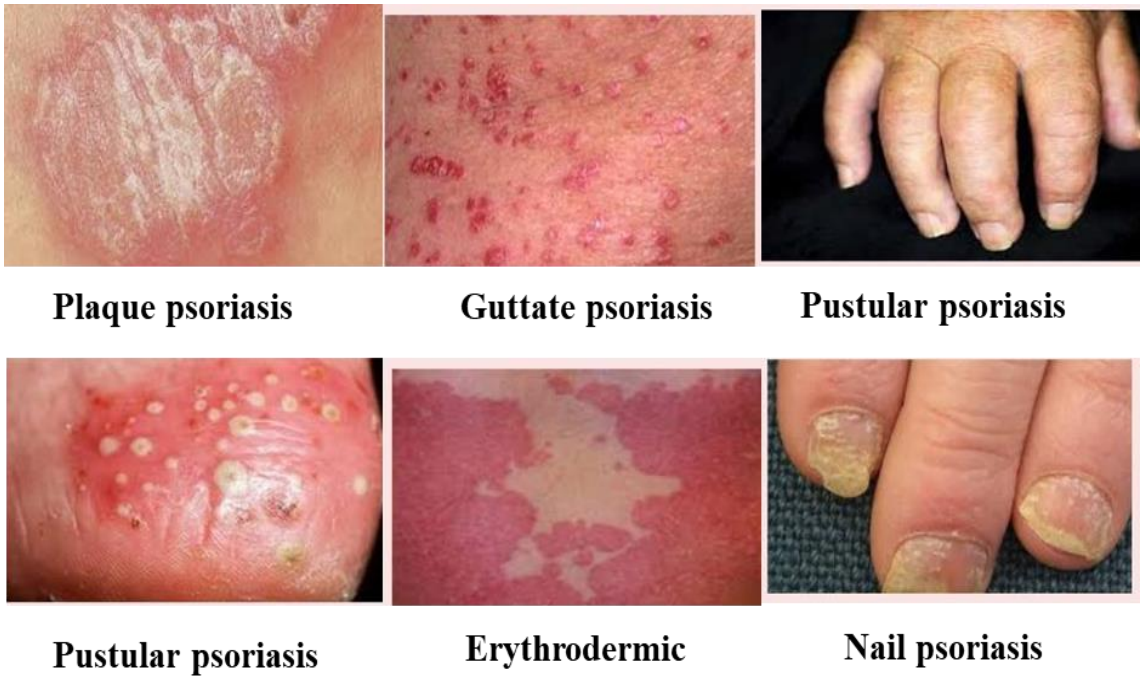

Figure 2.2 Types of psoriasis

2.1.1.3 Treatment of psoriasis

Treatment of psoriasis is still based on controlling the symptoms. Different topical and systemic treatments, along with phototherapy, are available. Actually, a combination of these treatments is frequently used. The topical treatment of psoriasis requires vitamin D_3 analogs, corticosteroids, i.e., betamethasone and hydrocortisone, topical retinoids in the form of a gel, ointments, creams, lotions, and foams. At the same time, phototherapy (UV light therapy) and the use of systemic treatments of methotrexate, cyclosporine, and biological agents (Alefacept, infliximab, adalimumab, ustekinumab) are other treatment approaches [27].

In treating bacterial infections associated with psoriasis and eczema, fusidic acid and some antibacterial were used that had a chance of resistance when applied on a long-term basis [28]. The current treatments though manage inflammatory symptoms but have several limitations, i.e., corticosteroids can cause thinning of the skin, vitamin D_3 analogs irritate the skin, retinoids do not act as quickly as topical corticosteroids.

In contrast, phototherapy can cause severe and long-lasting burns. Systemic and biological treatments also have limitations, i.e., methotrexate can cause liver damage and decrease the production of RBCs, cyclosporine may offer fast relief from symptoms, but the improvement stops when therapy is discontinued, Alefacept can increase the risk of infection, possibly including cancer [28].

2.1.2 Eczema (Atopic Dermatitis)

AD is a chronic inflammatory disease characterized by dry eczematous skin lesions with intense pruritus and itching (Fig. 2.3) [29]. The prevalence of AD in adults is around 2.1-4.9%, and in children, it varies between 2.7-20.1%, presenting a significant influence on the quality life of patients [30, 31].

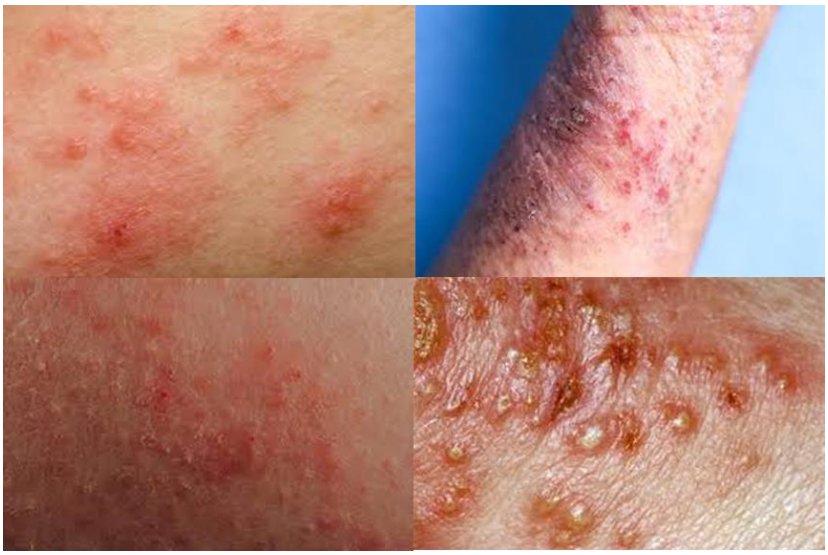

Figure 2.3 Clinical symptoms of Atopic dermatitis

2.1.2.1 Pathogenesis of eczema

The pathophysiology of AD is very complex, with several factors (immunological, genetic, and environmental) contributing to the disease progression [32]. Multiple studies have already demonstrated that AD patients are most likely to be colonized with *S. aureus*, which aggravates and promotes further inflammation [33, 34]. Additionally, the decreased expressions of some of the Antimicrobial peptides (AMPs) i.e., human beta-defensins (hBD-2 and hBD-3), LL-37, dermcidin are often observed in AD pathogenesis [35, 36]. This may be due to the Th2-biased immune responses, which may increase the secretion

of IL-4, IL-5 and IL-13 cytokines [37]. Fig. 2.4 depicts the different factors associated with the pathogenesis of the AD.

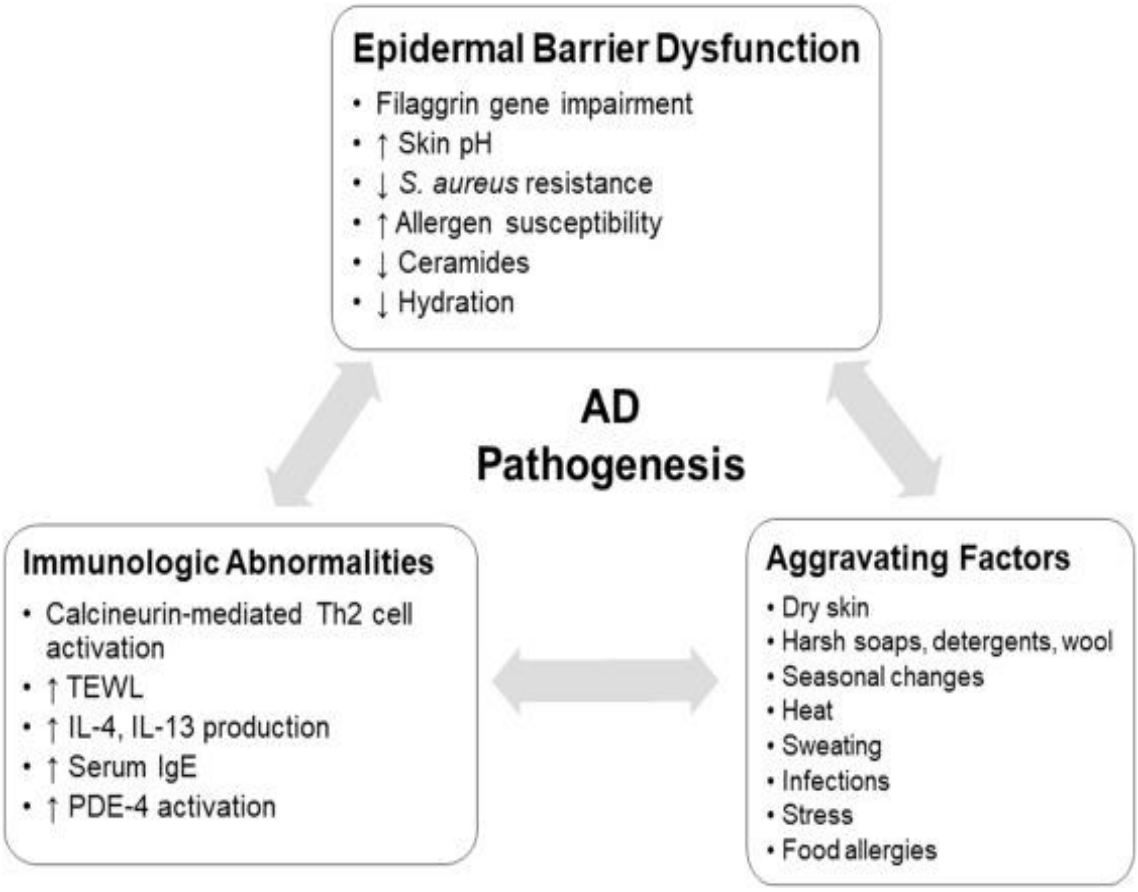

Figure 2.4 Pathophysiology of Atopic Dermatitis

AD, atopic dermatitis; PDE-4, phosphodiesterase-4; IgE, immunoglobulin E; IL, interleukin; TEWL, trans-epidermal water loss; Th2, T helper 2

2.1.2.2 Treatment of eczema

Treatment of eczema is still based on controlling the symptoms. The topical treatment of eczema involves emollients, topical corticosteroids (hydrocortisone, clobetasone, and mometasone) [38, 39]. The detailed information on the current therapies available for the treatment of eczema is depicted in Fig. 2.5.

Potency class*	Formulation (%)	Preparation
Topical Corticosteroids		
1	Clobetasol propionate 0.05	Cream, ointment
	Halobetasol propionate 0.05	Cream, ointment
2	Betamethasone dipropionate 0.05	Ointment
	Desoximetasone 0.25	Cream, ointment
	Fluocinonide 0.05	Cream, ointment, gel, solution
3	Betamethasone dipropionate 0.05	Cream
	Betamethasone valerate 0.1	Ointment
	Fluticasone propionate 0.005	Ointment
	Triamcinolone diacetate 0.5	Cream
4	Hydrocortisone valerate 0.2	Ointment
	Mometasone furoate 0.1	Cream, ointment, lotion
	Triamcinolone acetonide 0.1	Ointment
5	Betamethasone valerate 0.1	Cream
	Fluticasone propionate 0.05	Cream
	Hydrocortisone butyrate 0.1	Cream, ointment, solution
	Hydrocortisone valerate 0.2	Cream
	Triamcinolone acetonide 0.025–0.1	Cream
6	Aclometasone dipropionate 0.05	Cream, ointment
	Desonide 0.05	Cream
	Fluocinolone acetonide 0.01	Cream, solution
7	Hydrocortisone 0.5–2.5	Cream, ointment, lotion
Topical Calcineurin Inhibitors		
	Tacrolimus 0.03	Ointment
	Tacrolimus 0.1	Ointment
	Pimecrolimus 1	Cream

*1 = Most potent;7 = Least potent

Figure 2.5 Conventional topical treatments available for Atopic dermatitis

2.1.3 Antimicrobial peptides (AMPs)

Antimicrobial peptides (AMPs), multifunctional, immunomodulatory peptides with a broad spectrum of activity against the number of microbes, are emerging as novel therapeutics. Additionally, its non-specific mechanism of action confers reduced chances of microbial resistance. Figure 2.6 demonstrated the different mechanisms of action of AMPs.

More than 3000 AMPs have been discovered either from living organisms or synthetically derived [40, 41]. Based upon secondary backbone in their common structures, AMPs are mainly classified into four groups [42]. The first class, the α-Helix structure of AMPs, is linear and cationic. The amphipathic α-helices like temporins, LL-37, magainin, cecropin, etc., on interaction with lipid surfaces, increase concentration-dependent permeabilization of a cell membrane. [43, 44]. While another class of AMPs having a cyclic structure confined by the cyclization of the disulfide bond in aqueous solution may attain β-sheet conformation. Examples of this group of peptides are defensins, protegrins, gramicidins, lactoferricin, which may act by forming channels or discrete pores or by perturbation of lipid bilayers [45, 46].

On the other hand, Indolicidin and tritrpticin are examples of the linear peptides comprised with unusual amino acid sequences, making the third group of a widespread family of AMPs [47, 48]. Furthermore, fourth class, looped peptides like ranalexin, bactenecin-I, Nisin, etc., are proline and arginine-rich AMPs possess helical structure and amphipathic loss nature due to the high proline contents [49]. Table 2.1 demonstrated the role of AMPs in different infections.

Table 2.1 Dermal infections and antimicrobial proteins/peptides used

Infection type	**Examples**	**Infectious species**	**Antimicrobial proteins/peptides [*Clinical Trial*]**
Bacterial	Cellulitis, Erythrasma, Erysipelas, Impetigo, Folliculitis, Furuncles, Carbuncles, Acne Vulgaris, Psoriasis, Atomic dermatitis	*Staphylococcus* and *Streptococcus, Propionibacterium acne*	Pexiganan acetate (MSI 78) ***[NCT00563433, NCT00563394]*** Omiganan (MX226/ MBI226) ***[NCT00211523, NCT00027248],*** PMX30063 (Brilacidin) ***[NCT01211470],***
Viral	Shingles, Herpes, Warts/ Verruca Vulgaris, Condyloma Acuminatum and Simplex, Measles, Chicken pox, Crosti-	*Herpes simplex, Herpes zoster, Molluscum contagiosum,* Echona/Adeno/Epstein bar viruses.	

	Gianotti syndrome, Kaposi's sarcoma		Cathelicidin (hCAP18/LL37) ***[NCT00407979],*** Locilex (Pexiganan acetate -MSI 78) ***[NCT01594762, NCT01590758],*** Lytixar (LTX-109) ***[NCT01223222].***
Fungal	Athlete's foot, Ring worm, Eczema, Candidiasis, Jock itch, Tinea versicolor, Intertrigo	*Tinea pedis, Tinea corporis, Candida albicans, Epidermophyton, Micr osporum, and Trichoph yton species*	

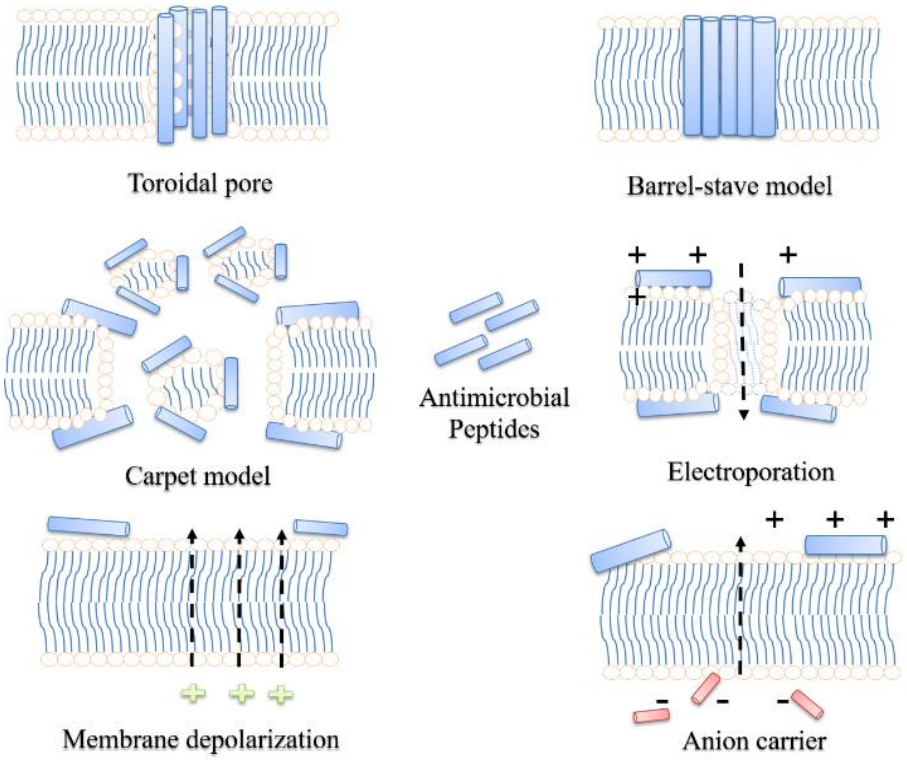

Figure 2.6 Different mechanism of actions of Antimicrobial Peptides; i) Toroidal pore: AMPs bind to the membrane and forces lipids to fold inwards to form a channel lined by lipid headgroups and AMPs at the membrane interface, ii) Barrel-stave model: AMPs reorientate themselves perpendicular to and are proposed to span the lipid bilayer, iii) Carpet model: AMPs forms a carpet that can induce weakness within the lipid bilayer using destroying electrostatics, iv) Electroporation: The binding of AMPs may induce pore formation through electroporation in addition to the existence of mechanical stress, v)

Membrane depolarization: AMPs having the ability to depolarize the lipid bilayer, vi) Anion carrier: Pore formation of AMPs in the lipid bilayer using anion carrier.

Omiganan

Omiganan is a novel cationic peptide (12 amino acid) and an analog of indolicidin. Omiganan has antimicrobial activity against various gram negative and gram positive micro-organism including fungi [50, 51]. Apart from its antimicrobial effect, Omiganan also has anti-inflammatory activity [52]. These antimicrobial and anti-inflammatory properties make Omiganan a promising agent for treating eczema/atopic dermatitis and psoriasis. Furthermore, the positive phase II clinical results were obtained in patients with AD (mild to moderate) with Omiganan 1% gel [53].

- The detailed sequence and structure (Fig. 2.7) of Omiganan is mentioned below:
  - 12- amino acid sequence (ILRWPWWPWRRK-NH2)
  - Molecular weight: 1779.2 gm/mole ($C_{90}H_{127}N_{27}O_{12}$)

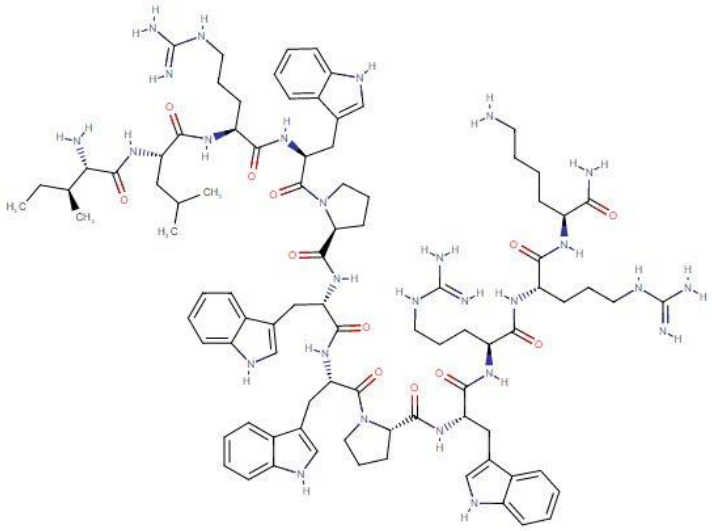

Figure 2.7 Structure of Omiganan

DPK 060

DPK-060 is a synthetic 17 amino acid peptide, structural derivative from the human protein kininogen [54]. DPK-060 mainly acts by membrane disruption mechanism along with immunomodulation, thus demonstrating strong broad-spectrum antimicrobial activity against both gram +ve and -ve bacteria, including *MRSA in-vitro* and *in-vivo* [55].

Moreover, the safety and effectiveness of 1% DPK-060 in a PEG-based ointment has been evaluated in phase II clinical trial (NCT01522391) to treat AD patients. Additionally, the positive results were obtained in these clinical trials but found not statistically conclusive due to the instability of DPK-060 as a drug substance in the formulation [56]. Further, nanotechnology-based formulations of DPK-060 have been developed to improve the functionality, stability, and release profile of DPK-060 [57-60].

- The detailed sequence of DPK-060 is:
    - 17- amino acid sequence (GKHKNKGKKNGKHNGWKWWW)
    - Molecular weight: 2505 gm/mole

2.1.3.1 Formulation strategies for AMPs

Skin is the most prominent barrier to deliver therapeutic protein and peptides. Despite tending large molecular weights and hydrophilicity, the permeation of the therapeutic proteins and peptides through the stratum corneum makes it difficult. Therefore, significant attention should be given to the proper choice of the delivery systems and the encapsulation of therapeutic protein and peptides for better outcomes. Nano-carriers have the advantages of biocompatibility, controlled/sustained release, and the ensurance of *in-vivo* stability of the therapeutic protein and peptide. Nanotechnology-based delivery strategies like nanoparticles, liposomes, nanofibers, nanoemulsions hold great promise in the delivery of AMPs compared to conventional delivery systems such as gels or solutions.

Nanoparticles (NPs) have been extensively explored as the delivery strategy for the AMPs due to their unique advantages like the small size, controlling the release of therapeutics, imparts the stability and ease of any modifications. Several nanoparticulate systems like chitosan NPs, carbohydrate-based NPs, mesoporous silica NPs, and Core-shell nanoparticles have been evaluated as the delivery strategies for the APMs. PLGA NPs incorporated with LL-37 have been prepared by Chereddy et al., and results exhibited more significant wound healing and angiogenesis in the full-thickness wound model [61]. While Temporin-B loaded chitosan, NPs have been prepared by Piras et al. showed extended antimicrobial activity to the staphylococcus epidermis along with the reduction in cytotoxicity against mammalian cells [62]. One of the best approaches for controlling the

release of therapeutics was the layer by layer self-assembled thin-film method comprised of poly (β-amino esters) biodegradable polymer investigated by Shukla et al. presented the controlled release of Ponericin G1 [63].

Moreover, preserving the stability of AMPs and maintaining their efficacy during storage time is one of the vital aspects associated with the delivery system. Lin Bi and et al. incorporated the AMP Nisin into the carbohydrate NPs demonstrated prolong efficacy against *L. monocytogenes*. Authors have also concluded that both hydrophobic and electrostatic interactions accelerated the adsorption of Nisin, and the glycan moiety at the surface of the NPs affects Nisin loading into the NPs and its retention during the storage [64].

Other nanoparticulate system like liposomes, vesicular systems having numerous advantages as delivery of therapeutic protein and peptides. Peptides like epidermal growth factor and interferon-alpha have been effectively incorporated into the liposomal core [65, 66]. In addition, liposomes containing interferon alpha-2b, the anti-fibrogenic factor, showed excellent efficacy on dermal fibroblasts *in-vitro* [67]. Further, lipid-based nanoparticulate systems like nanostructured lipid carriers (NLC) and solid-lipid NPs (SLN) have been investigated for the efficacy of the recombinant human epidermal growth factor (rhEGF) in healing impaired *db/db* mice [68]. Besides, NLC has also been explored for the effective delivery of the LL-37 prepared by melt emulsification technique showed potential antimicrobial effect *in-vitro* [69].

Moreover, nanofibers and nanoemulsions also have been investigated as the delivery strategy for the therapeutic peptides. Sebe et al. evaluated the nanofibers prepared by electrospinning technique to assess the antimicrobial activity of the synthetic peptide A3-APO in the clinically isolated multidrug-resistant strain of *A.baumannii* [70]. Vladislav et al. formulated the nanoemulsion comprised of NB-201 and NB-402 as the curative therapy in burn wounds infected with either *S. aureus* or *P. aeruginosa* [71]. While Zhengyi et al. evaluates the potential of NB-201 as antimicrobial and an anti-inflammatory in a *MRSA* infected porcine model deliver as nanoemulsion [72].

2.1.4 Dermal route for drug delivery for proteins and peptides

Protein and peptides are the essential class of therapeutics currently being investigated for various disorders such as cancer, diabetes, immune diseases, brain disorders, etc. The potential benefits of protein and peptide antimicrobials for dermal use are their no or reduced chances of development of resistance, the broad spectrum of antimicrobial activity with almost no side effects observed in conventional therapy, and may also possess anti-inflammatory activity [73, 74]. Delivery of these classes of drugs through the dermal route remains challenging due to conformational instability, low permeability across stratum corneum, partitioning in a different stage of the subdermal region, enzymatic degradation, etc. [75]. Dermal route for delivery of therapeutics has been continuously investigated to supplement the limitation of oral and other routes of drug delivery for skin disorders. There are limitations of delivering protein and peptide antimicrobial-based therapeutics orally due to the harsh environment of the GIT. Delivery via the intravenous route, intramuscular, intra-peritoneal routes display higher bioavailability, are invasive ones and limit patient compliance, and are of little benefit in treating skin disorders. The dermal route of drug delivery is non-invasive and offers the advantage of ease of application. [75].

Skin infections are the most common form of disease encountered in humans due to the constant exposure to pathogenic micro-organisms invading the human body via topical route. Most of these infections causing pathogens are barred from entry to the skin due to the preventive layer of the SC. Still, in many circumstances, the microbe invades skin leading to infections. Conventionally the therapy for these infections consists of antimicrobials and steroids as a supplementary therapy. Conventional treatment is effective in up to 80% of diseases but fails to treat the remaining 20% [76]. The treatment is to be continued for prolonging the period for complete remission of the infection. During treatment, drug resistance and the associated adverse effects to the therapy make the skin disorders contagious and difficult to treat. This mandates a therapy consisting of combining or alternating antimicrobials, steroids, immunosuppressants, and so on so forth. These prolonged use of such medication is linked with various side effects like rash, atrophy, skin darkening, hypersensitivity, diarrhea, abdominal pain, nausea/vomiting, hypersensitivity

(allergic) reactions, and other more severe side effects include photosensitivity, anaphylactic reactions, ototoxicity, chondrotoxicity, retinopathy, etc. [77]

2.1.4.1 Factors affecting drug absorption across the skin

Active Pharmaceutical Ingredients (APIs) with a log P-octanol/water value of 1-3 show adequate solubility and diffusion within the lipid domain, and appropriate hydrophilic nature to partition into the epidermis. In percutaneous absorption through stratum corneum, diffusion of hydrophilic and amphiphilic penetrants will be the rate-limiting step, whereas lipophilic substances are favorable [78]. However, for highly lipophilic drugs, the epidermis can perform as a rate-limiting factor in drug permeation in and across the skin. Various aspects and formulation perspectives that influence the percutaneous absorption of a topically applied drug into or across the skin are listed in Fig. 2.8.

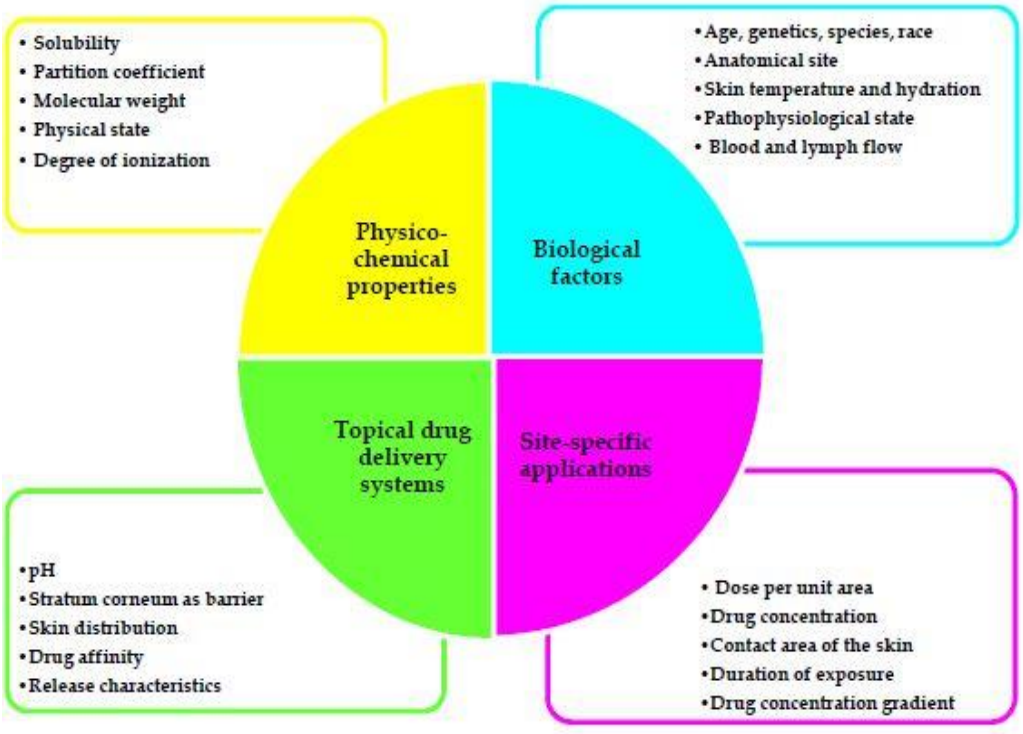

Figure 2.8 Various vital factors which affect the percutaneous absorption of drugs

2.1.4.2 Skin penetration enhancement techniques

Mainly topical drug molecules possess low permeability across the skin and its layers. Therefore, various approaches/enhancement techniques (Fig. 2.9) have been employed for dermal drug delivery, which offer exciting ways to deliver therapeutics at the target site. Physical or active methods, i.e., iontophoresis, sonophoresis, electroporation and temperature, and chemical or passive methods, deliver the drug across the skin at elevated levels [79, 80]. The active techniques are expensive and require expert staff for their handling. However, much research is needed to create long-term safety data and the cost-effectiveness of such methods [81, 82].

Amongst various passive methods, nano-technology based drug delivery strategies offer a targeted drug delivery because of their ability to localize the drug activity at the target site, thus lowering its concentration in other body organs [80].

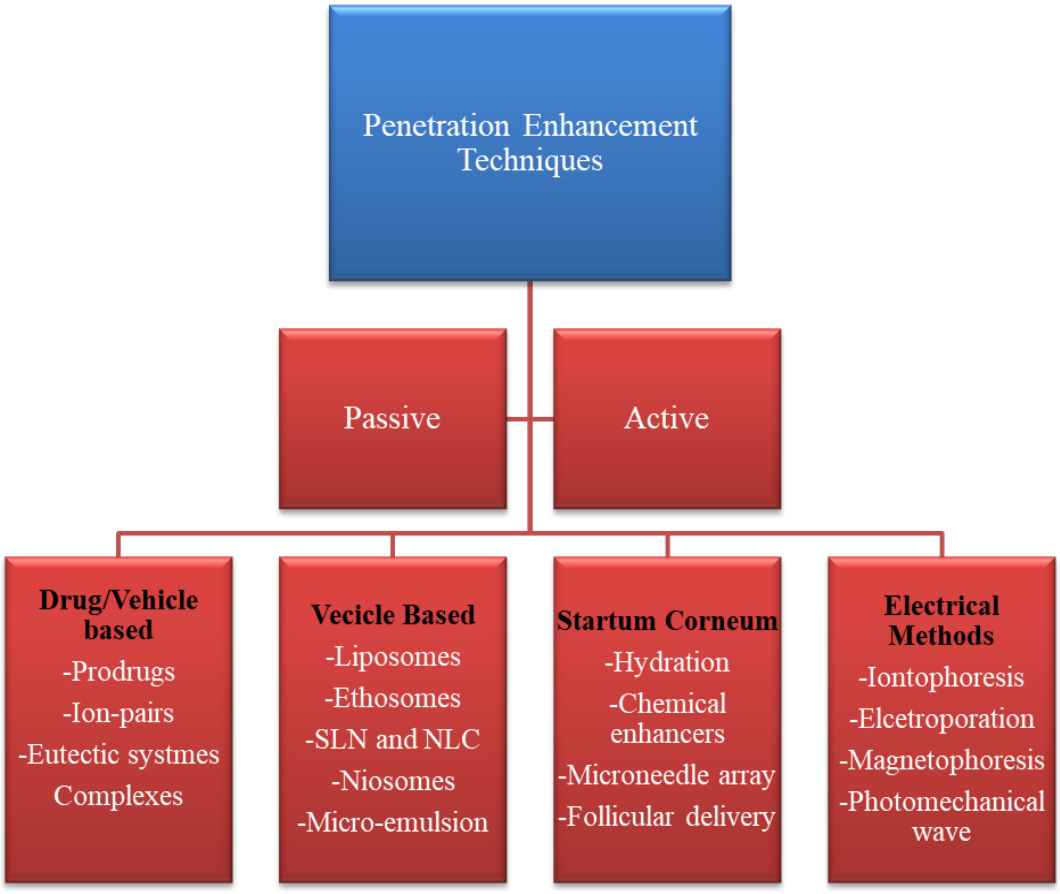

Figure 2.8 Several permeation enhancement techniques employed for drug delivery

2.1.4.3 Conventional drug delivery systems

The conventional drug delivery systems used for topical delivery are illustrated in Fig. 2.10.

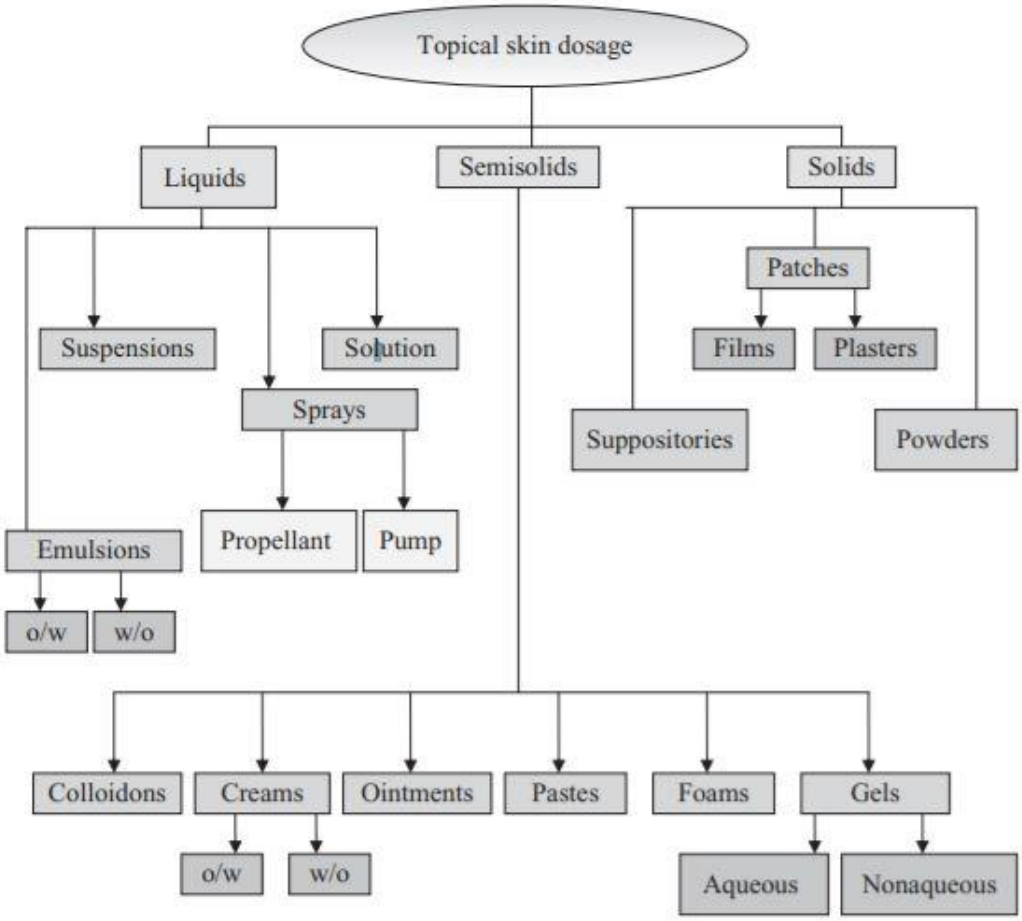

Figure 2.10 Conventional drug delivery systems used for topical delivery

2.1.4.4 Lipid-based nanotechnology drug delivery systems

Lipid-based nano-carriers i.e., liposomes, NLCs and SLNs, microemulsion, etc. have a natural affinity for skin and facilitate the drug transport by enhancing its partitioning from the vehicle to the skin. Moreover, they offer enhanced permeation of drug(s) due to improved contact with skin, sustained-release properties, and an occlusive effect [83]. Also, in water-soluble drugs, the high lipid content can cause an occlusive effect and formation of the film on the skin, increasing skin hydration due to decreased trans-

epidermal water loss, helping the diffusion of drugs into the SC [83, 84]. Nano-carriers used for dermal delivery are typically illustrated in Fig. 2.11.

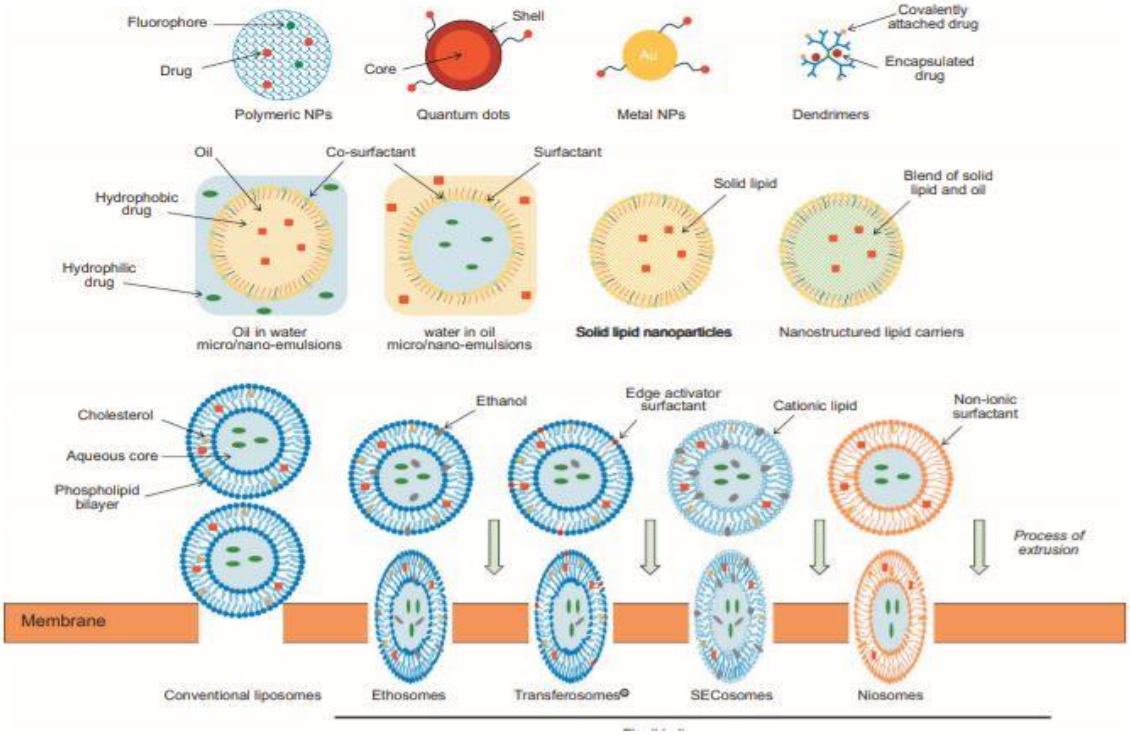

Figure 2.11 Nano-carriers based drug delivery systems for dermal route

i) **Nanoemulsions (NE) and Microemulsions (ME)**

They are composed of Oil, surfactant, co-surfactant and aqueous phase and are monophasic, transparent, and isotropic colloidal dispersion systems having globule size of below 100 nm [85]. ME is thermodynamically stable, while NE is kinetically stable, provides grater surface area and even distribution into the skin. NE typically having relatively low concentration of surfactant and thus less irritative than ME. They improve skin permeation by: (i) higher solubilization potential for both hydrophilic and lipophilic molecules, enhances the loading efficacy; (ii) their occlusive nature confirms surface interaction with the dermal layers; (iii) their oil and surfactant/co-surfactant compounds can offer enhaced permeation across the stratum corneum [86].

ii) Solid Lipid Nanoparticles (SLNs)

They are formulated by using solid lipids and stabilized as a nano-dispersion by a surfactant [87]. They provides enhanced stability of the lipophilic molecules,

increased permeation across the stratum corneum and decreased flux [88, 89]. Greater SC permeation is due to the (i) extended contact time with the skin; (ii) their occlusive nature; and (iii) interaction between skin lipids and formulation lipids, facilitates the permeation of lipophilic molecules [90].

iii) Nanostructured Lipid Carriers (NLC)

NLCs are considered as second-generation SLN, which comprise a blend of solid and liquid lipids and thus have higher drug loading capability and stability [91, 92]. NLC and SLN have a similar mechanism of permeation improvement via occlusion and mixing between the skin lipids and formulation lipids. Though, the liquid lipid of NLCs enhances the solubilization of APIs and thus loading efficacy, demonstrating grater skin deposition [93, 94].

iv) Lipid vesicles

They are composed of ingredients that will aggregate into bilayer assemblies to form sphere-shaped vesicles but can deform in shape. Liposomes are the most extensively studied nanoscale antibiotic delivery systems [95, 96], mainly due to their ability to enahance the bioavailability, permeation, bio-compatibility, and safety of encapsulated antimicrobial agents [97]. Liposomes are lipid vesicles having single or multiple lipid bilayers made up of mixtures of phosphatidylcholines (long/short hydrocarbon chains). They can be designed to suit the need to achieve various morphological states based on the processing conditions such as hydration, temperature, and composition [98, 99]. Numerous vesicle structures have been developed, including ethosomes (phospholipids having a high ethanol content), transferosomes (phospholipids having a surfactant i.e., sodium cholate), niosomes (non-ionic surfactant vesicles) [100-103].

2.1.4.5 Literature survey based on NLCs

Gonullu U et al. [104] formulated and characterized Lornoxicam loaded SLN, NLC and NE for transdermal drug delivery. Compritol 888 ATO, Lanette O, and oleic acid were used as solid and liquid lipids. SLN, NLC, and NE were physically stable up to 6 months at different stability conditions. Fickian drug diffiusion mechanism was observed from the

NPs and NE. The drug permeation across the rat skin was observed as; NE>NLC>SLN>gel, indicated the grater permeation from the nanoformulations.

Shah et al. [105] developed and compared ciprofloxacin-HCl-loaded SLNs, NLCs, and NE by quick solvent diffusion evaporation technique. The *in-vitro* drug release profile of all 3 formulations displayed quick release from NE while controlled release from SLNs and NLCs. Results of stability studies demonstrated that NLCs and SLNs could prevent drug expulsion throughout the storage time. The study proposed that the SLN and NLCs could possibly be exploited for improved drug entrapment and controlled release of hydrophilic molcules.

Ge et al. [106] investigated the percutaneous permeation of Idebenone in guinea pig skin after applying formulations (NE, NLC, or oily solution). Permeation experiments revealed that the NLC formulation releases threefold times that of NE or oil formulations. NLCs have demonstrated better stability than NE. The results demonstrated that NLCs have the abilities to improve chemical stability of Idebenone and improve skin permeation compared to NE and oil solutions.

Dasgupta et al. [107] prepared and characterized NLC loaded Aceclofenac gel to treat inflammation and allied conditions. The anti-inflammatory effect of Aceclofenac loaded NLC gel was evaluated by the rat paw edema procedure and was compared with the marketed gel-based formulation of Aceclofenac. The results demonstrated superior anti-inflammatory effect and rapid onset of action in comparision with the marketed Aceclofenac gel.

Xia et al. [108] developed α-Lipoic acid-loaded NLCs by high-pressure homogenization method and characterized physico-chemically. Polymer-surfactant emulsifying ingredients significantly affetcs the viscoelastic property, particle size, and stability of NLCs. Surfactants having a small and flexible hydrophilic head group may have solid interaction with the polymer and forms strong interfacial film, therefore could enhance the stability of NLCs. These findings will be prolific for a well understanding of the development and stability of NLCs.

Li et al. [109] developed and characterized Flurbiprofen loaded NLC gel (FP-NLC) for topical application. The results of *in-vitro* permeation studies across the rat skin demonstrated higher permeation of FP-NLC gel in comparision to plain drug loaded gel.

No edema and erythema were observed subsequently treatment with FP-NLC gel into the rabbit skin. Additionally, the drug accumulation in the rabbit skin was significantly higher compared to the convenctional formulation.

Carmelo et al. [110] prepared and characterized Lidocaine and Benzocaine loaded NLCs for topical application. The optimized NLCs were subjected to percutaneous absorption across the excised human skin samples. A radiant heat tail-flick test was performed to evaluate the antinociceptive effect of drug loaded NLCs in the mice. Addiotionally, the *in-vivo* study demonstrated the prolonged release profile of drug loaded NLCs, thus providing the prolong anesthetic effect.

Fang et al. [111] prepared and characterized lipophilic Calcipotriol and hydrophilic Methotrexate loaded NLCs for topical application. They have demonstrated the profound effects of Precirol®/squalene ratio on the physico-chemical properties of the NLCs. They have observed good correlation in the *in-vitro* and *in-vivo* results after topical application of drug loaded NLCs. The study demonstrated that two different drugs can successfully be loaded into the NLCs.

❖ **Techniques of NLC Formulation:**

The formulation of NLCs is melt-emulsification, emulsification-solvent diffusion, emulsification-solvent evaporation, solvent injection or solvent displacement, and high-pressure homogenization. The use of natural excipients, with protocols of minimum use of hazardous material in the process and the product and ultimately the environment, is of prime importance. High-pressure homogenization and microemulsion techniques are green since no organic solvents are used and are performed at low temperatures [112-114].

A) High-Pressure Homogenization

Hot Homogenization: Lipids are melted by heating to a temperature 5–10oC above their melting point. Since these temperatures are low, much heating is not required. APIs are dissolved in molten lipids and then dispersed in a hot aqueous surfactant solution to form a primary emulsion, which is after homogenized through a narrow orifice to obtain the desired particle size [115].

Cold Homogenization: APIs are dissolved in molten lipids, subsequently quick cooling to form a solid solution. This solid solution is then ground to microparticles, which are homogenized in the presence of a surfactant. This procedure also avoids temperature-induced drug degradation and is a greener technique because high temperatures are not used [116].

B) High Shear Homogenization

A technique widely used in the production of nano-dispersions. The melted lipid blend and hot surfactant aqueous solution are homogenized in a high shear mixer. Although it is easy to handle, the dispersion quality is often poor. It is most often combined with ultrasonication to obtain better results [117].

C) Solvent Emulsification-Evaporation

In this method, lipophilic drug(s) and lipidic excipients were first dissolved in a water-immiscible organic solvents such as chloroform, dichloromethane, cyclohexane, toluene, etc.). Later, the emulsification was carried out in an aqueous phase by using a homogenizer. Additionally, to enhance the efficiency of emulsification, the coarse emulsion was immediately subjected to passs through the microfluidizer. After that, organic solvent was removed under reduced pressure and at ambient temperature e.g., rota evaporator [118].

D) Solvent injection

In this method, solvents that distributes very quickly in water such as ethanol, DMSO were used. Briefly, the lipidic excipients first dissolved in solvents and then transferred to the soltion of surfactant/water, resulting the rapid migration of solvent molecules into the water which leads to formation of lipid particles. The size of particles depends on the speed of circulation processes; grater the smaller the particle size. Low shear stress and low temperature are the advatnges of this method [119].

E) Double Emulsion Technique

In this method, mostly hydrophilic drugs were first dissolved in an aqueous phase and emulsified in molten lipid phase. Subsequently, the stabilization of this primary emulsion was carried out by using a stabilizing agents such as poloxamer-407, gelatin, etc.). After that, this primary emulsion was further dispersed in an aqueous medium having a hydrophilic emulsifying agent such as Poly-vinyl alchohol and was stirred and isolated through filteration. However, the limitation of this method is the formation of microparticles in a high percentage [113].

2.1.4.6 Literature survey-based liposomes

Saka et al. [120] formulated and optimized Bexarotene loaded liposomes and incorporated into the gel base; characterized for various physico-chemical paramaeters. Optimized Baxarotene loaded liposomes exhibited vesicle size of 67.82 ± 7.15 nm (0.26 ± 0.02 PDI) with better entrapment efficiency (>90%). Liposomal gel demonstrated improved *in-vitro* release profile (1.8 folds) compared to plain drug gel. Additionally, the dermal penetration was better in comparision to plain dye. Optimized Baxarotene loaded liposomes showed marked decrease in psoriatic lesions in an imiquimod induced animal model in BALB/c mice. In addition, significant decrese in cytokines levels and improved hisopathological structure of the skin demonstrated the effectiveness of the liposomal gel.

Wang et al. [121] formulated trans-retinoic acid and Betamethasone loaded liposomes and characterized for different physico-chemical parameters. Optimized liposomes exhibited vesicle size of approx. 70 nm with % EE of >98.0%. Further, optimized liposomes showed sustained release profile and enhanced permeation across the skin (5.4 times) compared to free drug solution. Cell-viability study results demonstrated non-toxic nature of liposomes on HaCaT cells. In addition, *in-vivo* study results showed markerd reduction in epidermal thickness and cytokines levels in animals treated with dual drug loaded liposomal gel.

Fathalla et al. [122] formulated and compared ethosomal and liposomal formulatios of Anthralin to improve its effectiveness in psoriasis animal model. The Ethosomes and liposomes demonstrated % EE of 77.0% and 97.2%, respectively with vesicle size in range of 146-381 nm and 116-199 nm, respectively. FTIR and DSC studies demonstrated the

compatibility between the drug and excipients. HEC, HPMC, and PL-127 were evaluated to prepare gel base for optimized nano-carriers. Nano-carriers embedded gel with PL-127 exhibited excellent sustained release profile compared to other gel base formulations of nano-carriers. Further, the effectiveness of nano-carrier embedded PL-127 gel was evaluated in psoriasis patients. The gel demonstrated imparovement in PASI index (3.6 for Ethosomes and 3.4 for liposomes).

Vanaja et al. [123] prepared liposomes of methotrexate using the thin-film hydration technique. The prepared liposomes were dispersed in carbopol gel (0.25% w/w) compared with marketed methotrexate gel (1% w/w) in psoriatic patients. Fourteen patients were treated with methotrexate liposomal gel, and 11 were treated with methotrexate 1% gel. The mean PASI score for methotrexate liposomal gel (0.25%) was reduced from 5.3 to 2.4, while the conventional methotrexate gel (1%) reduced the PASI score from 6.2 to 2.9. The liposomal therapy was found to be effective as conventional but with a much lesser dose.

Bhatia et al. [124] prepared 3 formulations of tamoxifen and evaluated their anti-psoriatic efficacy in the mouse tail model. A thin-film hydration technique prepared flexible membrane phospholipid-based vesicles of tamoxifen. Pluronic lecithinized organogels of tamoxifen were prepared by mixing oil phase (containing phospholipid, span 80, isopropyl palmitate, and tamoxifen) and aqueous phase (containing pluronic 16%w/v). Tamoxifen was dispersed in carbopol gel which served as a placebo, and normal saline as control. The drug activity with tamoxifen flexible membrane vesicles was found to be highest (35.8%), followed by pluronic lecithinized organogels of tamoxifen (24.6%), and the least activity was encountered with tamoxifen hydrogel (10.2%).

Knudsen et al. [125] prepared the liposomes of calcipotriol to enhance the retention of the drug into the skin. Liposomes were prepared by the thin-film hydration technique. The liposomes were incorporated with lipopolymer, i.e., poly(ethylene glycol)-distearoyl phosphoethanolamine (PEG-DSPE), which stabilized the formulation. The prepared PEGylated liposomes were studies for skin permeation and retention and significantly increased drug accumulation into the stratum corneum of the skin collected from pigs.

Gupta et al. [126] prepared liposomes, niosomes, and emulsomes of capsaicin using the thin-film hydration technique. The prepared vesicles were incorporated into carbopol hydrogel, and the drug dispersed in plain hydrogel served as control. Flux was calculated

for the prepared formulation i.e., emulsomal gel (2.6 μg/cm2 /h), liposomal gel (1.19 μg/cm2 /h), niosomal gel (0.92 μg/cm2 /h) and plain hydrogel (0.65 μg/cm2 /h). The accumulation of drug into the stratum corneum was found to be emulgel (3.95 times), lipo-gel (2.75 times), and niogel (2.16 times), as compared to plain-gel.

Jain et al. [127] prepared thymoquinone-loaded lipospheres using egg lecithin, propylene glycol solution, which melted tween 80, chremophor RH 40, and thymoquinone was added. The prepared lipospheres were evaluated for anti-psoriatic potential using cell line (in-vitro) and imiquimod induced psoriasis in a mouse model (*in-vivo*). The lipospheres and plain thymoquinone solution were found to be reducing the levels of inflammatory cytokines by 7.59 and 1.65 folds compared to positive control. In-vitro analysis of thymoquinone resulted in the reduction of 1.3 folds of IL-2, 1.56 folds of IL1β, 1.83 folds of IL-6, and 1.58 folds of TNF-α levels.

Walunj et al. [128] prepared liposomes containing cyclosporine and DOTAP (cationic phospholipid) using the ethanol injection method (Walunj et al., 2020). Prepared liposomes were incorporated into 1% carbopol gel having cyclosporine (0.45% w/w) and plain hydrogel dispersed with cyclosporine (0.45% w/w). The plain gel and lipogel were evaluated for anti-psoriatic potential in the imiquimod-induced psoriatic plaque mouse model. The ELISA results showed that lipogel reduced the concentration of inflammatory cytokines levels (3.4 folds IL-22; 1.47 folds IL-17; 1.71 folds TNF-α).

❖ **Techniques of Liposomes preparation:**

Numerous techniques can prepare liposomes. The drug loading in liposomes can be achieved by two methods, i.e., active drug loading and passive drug loading. The active drug loading technique is dependent on the lipid-drug interaction. The drug is loaded after the liposome formation, giving 100% drug loading efficiency with active loading. Passive drug loading can be achieved by different techniques of encapsulation [129, 130]. Passive liposome preparation techniques are mainly divided into three categories of encapsulating mechanisms:

a) Lipid thin-film hydration

This method involves forming lipid thin film using a rotatory evaporator and then hydrating with the aqueous solution to be entrapped within the vesicle. The lipid is dissolved in the organic solvent and introduced to a rotatory evaporator under reduced pressure. The solvent is allowed to evaporate, leading to the formation of a thin film of lipid. This thin film of lipid is hydrated with the aqueous phase comprising the drug to be entrapped. The resulting solution leads to the formation of heterogeneous vesicles, which are then sonicated to get SUVs [131].

b) Ethanol injection

The lipid phase having ethanol is readily dispersed in a pre-heated buffer solution. MLV's are formed by this technique with heterogeneous sizes ranging from 30 - 110 nm. The main disadvantage of this technique is the formation of ethanol and water azeotrope, which leads to incomplete evaporation of ethanol which might lead to inactivation of various macromolecules even at a very low amount of ethanol [132].

c) Ether injection

Ether injection technique involves a volatile solvent (such as diethyl ether) and lipid directly introduced to a preheated aqueous solution containing entrapping material at 55 – 65 °C or under reduced pressure. The evaporation of the solvent leads to the formation of liposomes. This technique cannot be used for thermolabile substances [133].

d) Reverse phase evaporation technique

The reverse-phase evaporation technique is based on the phenomena of creating inverted micelles by sonicating both aqueous medium (containing encapsulating material) and organic medium (containing lipid) together. Slow evaporation of organic leads to forming a viscous gel-type solution containing inverted micelles and excess phospholipid. At the critical point, the gel or viscous state collapses, and inverted micelles combine with excess phospholipid to complete the bilayer around the residual micelles and lead to create liposomes [134].

e) Detergent removal method

This method includes the use of separating techniques to remove the untapped components from the liposomes [135].

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