Topical calcineurin and mammalian target of rapamycin inhibitors in inflammatory dermatoses: Current challenges and nanotechnology-based prospects (Review)

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Received April 17, 2024; Accepted June 6, 2024

DOI: 10.3892/ijmm.2024.5409

Abstract. Topical therapy remains a critical component in the management of immune-mediated inflammatory dermatoses such as psoriasis and atopic dermatitis. In this field, macrolactam immunomodulators, including calcineurin and mammalian target of rapamycin inhibitors, can offer steroid-free therapeutic alternatives. Despite their potential for skin-selective treatment compared with topical corticosteroids, the physicochemical properties of these compounds, such as high lipophilicity and large molecular size, do not meet the criteria for efficient penetration into the skin, especially with conventional topical vehicles. Thus, more sophisticated approaches are needed to address the pharmacokinetic limitations of traditional formulations. In this regard, interest has increasingly focused on nanoparticulate systems to optimize penetration kinetics and enhance the efficacy and safety of topical calcineurin and mTOR inhibitors in inflamed skin. Several types of nanovectors have been explored as topical carriers to deliver tacrolimus in both psoriatic and atopic skin, while preclinical data on nanocarrier-based delivery of topical sirolimus in inflamed skin are also emerging. Given the promising preliminary outcomes and the complexities of drug delivery across inflamed skin, further research is required to translate these nanotherapeutics into clinical settings for inflammatory skin diseases. The present review outlined the dermatokinetic profiles of topical calcineurin and mTOR inhibitors, particularly tacrolimus, pimecrolimus and sirolimus, focusing on their penetration kinetics in psoriatic and atopic skin. It also summarizes the potential anti-inflammatory benefits of topical sirolimus and explores novel preclinical studies investigating dermally applied nanovehicles to evaluate and optimize the skin delivery, efficacy and safety of these 'hard-to-formulate' macromolecules in the context of psoriasis and atopic dermatitis.

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Abbreviations: 3D, three-dimensional; AD, atopic dermatitis; CD, contact dermatitis; Chol, cholesterol; CMS, core-multishell; CP, clobetasol propionate; CS, chitosan; CsA, cyclosporine A; CUR, curcumin; DCs, dendritic cells; EE, entrapment efficiency; FKBP12, FK506 binding protein 12; HA, hyaluronic acid; HaCaT, immortalized human keratinocytes; IFN-γ, interferon-γ; IgE, immunoglobulin E; IL, interleukin; LCNPs, liquid crystalline nanoparticles; LCs, Langerhans cells; LN, lipid nanoparticles; ME, microemulsion; MNLC, modified nanolipid carriers; mPEG-hexPLA, methoxy-poly(ethylene glycol)-hexyl-substituted polylactide; mTOR, mammalian target of rapamycin; mTORIs, mammalian target of rapamycin inhibitors; MW, molecular weight; NE, nanoemulsion; NGF, nerve growth factor; NIC, nicotinamide; NLCs, nanostructured lipid carriers; NMFs, natural moisturizing factors; MSNs, mesoporous silica nanoparticles; NPs, nanoparticles; PASI, Psoriasis Area Severity Index; PEG, polyethylene glycol; PIM, pimecrolimus; SA, stearic acid; SC, stratum corneum; siRNA, small interfering RNA; SLNs, solid-lipid nanoparticles; TAC, tacrolimus; TCIs, topical calcineurin inhibitors; TFs, transfersomes; TJ, tight junction; TSLP, thymic stromal lymphopoietin

Key words: topical calcineurin inhibitors, PI3K-Akt-mTOR, atopic dermatitis, topical delivery, skin absorption, dermatopharmacokinetics, skin pharmacokinetics, nanomedicine-based drug delivery systems, nanosystems, nanomaterials

Contents

1. Introduction

Current therapies for inflammatory skin conditions are increasingly directed towards non-steroidal immunomodulatory approaches. Initially used in transplantation medicine, macrolactam immunomodulators have broadened the treatment landscape in this field. Lead compounds in this drug class are calcineurin inhibitors, such as tacrolimus (TAC) and pimecrolimus (PIM), while newer agents, such as sirolimus and its analogs, have recently emerged as mammalian target of rapamycin (mTOR) inhibitors (mTORIs) (1-4).

Given their efficacy in managing skin inflammation when used systemically, macrolactam immunomodulators are currently available or under investigation for topical application on inflamed skin (1,2,4). Topical calcineurin inhibitors (TCIs), known for selectively suppressing cytokine-mediated T-cell activation and proliferation, offer a targeted anti-inflammatory approach without compromising skin immune homeostasis. This mode of action is particularly beneficial in inflammatory dermatoses characterized by immune dysregulation, such as atopic dermatitis (AD) (5-7). Topical mTORIs have also shown inhibitory effects on epidermal and vascular proliferation, opening new perspectives in targeting inflammation-driven proliferative aspects in skin conditions like psoriasis (2,4,8,9).

However, despite their potential to control skin inflammation and hyperproliferation, the topical use of immunomodulatory macrolactams has not always yielded satisfactory results in both experimental and clinical settings, depending on disease states and affected body areas (2,10-12). This therapeutic variability may partly reflect their limited and variable skin absorption due to unfavorable physicochemical properties, such as their hydrophobic nature and large molecular size. Additionally, the barrier alterations of inflamed skin further complicate their absorption (6,10,13,14).

Despite the challenges involved in skin delivery, topical agents for inflammatory dermatoses, such as AD or psoriasis, should ideally overcome the stratum corneum (SC) barrier and maintain therapeutically efficient concentrations at the site of action in the viable epidermis and dermis without entering the bloodstream (15,16). However, the physicochemical features of topical calcineurin and mTOR inhibitors, while offering a skin-selective pharmacological profile, still appear not well-suited for intradermal delivery, especially with conventional vehicles (14,16-18). Since the effect of drug properties on skin permeability is crucial (19), elucidating the skin penetration kinetics of topical macrolactam immunomodulators is therefore important.

To optimize therapeutic outcomes, more sophisticated nanoparticle-based approaches are being extensively explored to improve the absorption and deposition of locally applied therapeutics in inflamed skin. In this regard, nanotechnology has offered a variety of promising carriers capable of delivering macromolecules, such as TAC, in psoriatic and atopic skin, providing an effective, safe and esthetically appealing alternative to traditional topical vehicles (20,21).

The present review aimed to outline the dermatokinetic profiles of topical calcineurin and mTOR inhibitors, with special emphasis on their penetration and biodistribution in psoriatic and atopic skin. In this context, the potential benefits of topical sirolimus are briefly covered, as animal and human skin studies have indicated encouraging results. Finally, it reviewed novel preclinical approaches that utilized topically investigated nanomaterials to explore and optimize the skin pharmacokinetics, efficacy and safety of these 'hard-to-formulate' compounds, focusing on comparisons between nanovectors and conventional vehicles in psoriasis and AD skin models (Fig. 1).

2. The topical route

Skin delivery of bioactive agents remains a favored approach in dermatotherapy (19). As the largest accessible organ, the skin offers a convenient route for direct access to diseased sites, while minimizing systemic exposure and adverse effects. In addition, drug depots formed within the skin tissue may allow for prolonged storage and sustained release, reducing application frequency in favor of patient compliance (22,23).

However, when utilizing the skin for topical drug delivery, both cutaneous biology and the physicochemical properties of penetrants should be aligned, as the structural and functional features of human skin may interact with penetrating compounds (19,22,23). This section provides a brief overview of the key factors influencing the skin absorption of locally applied therapeutics.

Skin barriers. The skin has evolved a complex network of interconnected barriers, i.e., the physical/mechanical, biochemical, immune and microbiome barriers, which protect from external insults while maintaining internal homeostasis (22,24). The outermost lipid-rich layer of the SC, composed of tightly linked dead corneocytes, and the underlying tight junction (TJ)-mediated paracellular sealing form a major physical barrier against penetrating molecules, particularly highly hydrophilic or lipophilic drugs (22,25,26).

Barrier function also involves biochemical elements, including skin pH, natural moisturizing factors (NMFs) and skin enzymes (22-24,27,28). The 'acid mantle' on the skin's surface not only protects against pathogens with its antimicrobial action but also affects drug partitioning from the vehicle into different skin layers. Notably, pH-responsive delivery systems can utilize local pH changes in lesional skin for targeted drug release (27). The NMFs are essential for maintaining skin moisture and SC integrity. Increased skin hydration can enhance drug solubility and permeability by rearranging the lipid matrix to create aqueous pores that facilitate drug transport across the skin (22-24). In addition, cutaneous bioavailability can be further altered since penetrating substances may be trapped and biotransformed by drug-metabolizing enzymes, mostly found in the viable epidermis (28).

Additionally, the skin harbors various immunocompetent cells strategically located in the epidermis and dermis (24). In the context of drug delivery, initial views of the skin's immune surveillance have demonstrated a dynamic immune-epithelial crosstalk. Activated Langerhans cells (LCs) have been shown to extend their dendrites through TJs to uptake invading molecules without compromising TJ integrity (29,30). This interaction may provide useful insights for translating the immune aspects of the skin barrier into clinical implications for topical drug delivery.

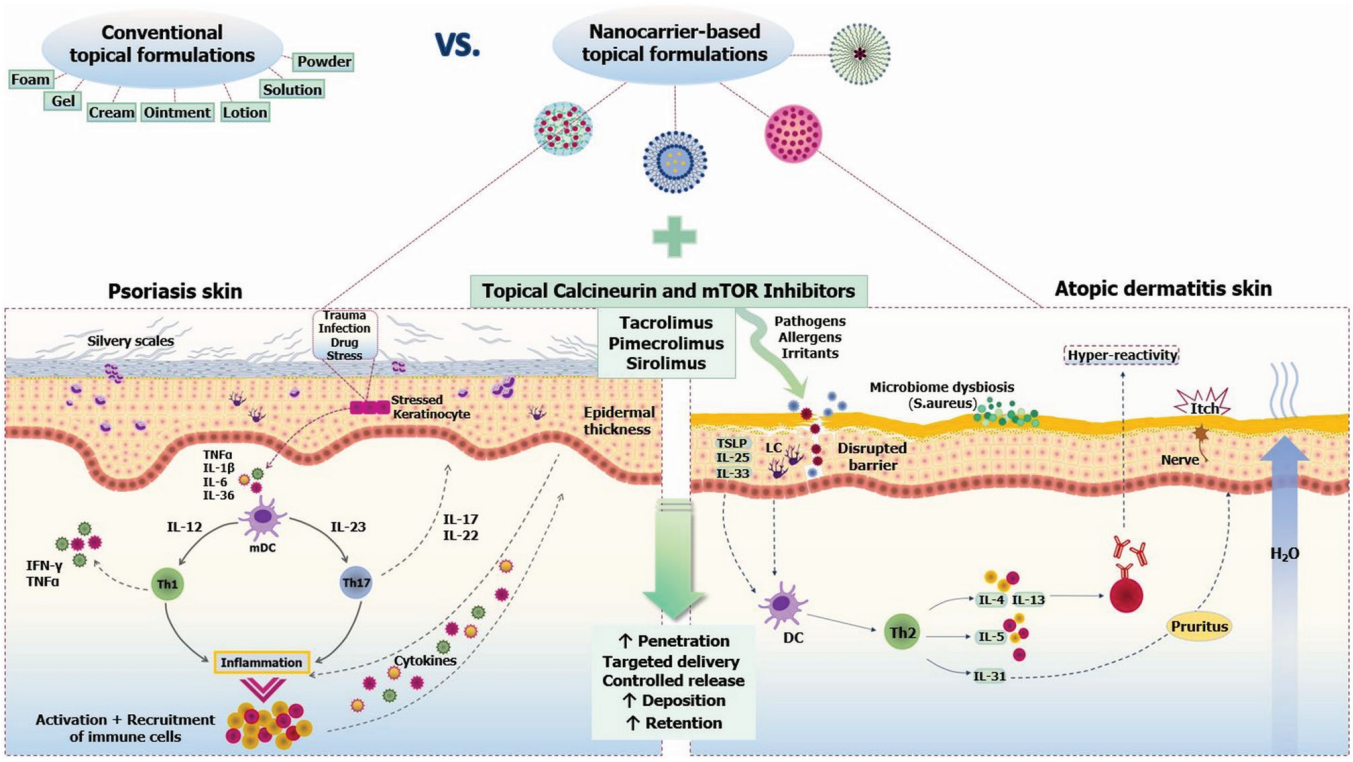


Figure 1. Graphical abstract illustrating the main findings discussed in the article. This review focuses on the dermatokinetic profiles of conventional and nanocarrier-based topical formulations of calcineurin and mTOR inhibitors, with special emphasis on their penetration and biodistribution in psoriatic and atopic skin. TNFα, tumor necrosis factor-α; IFN-γ, interferon-γ; IL, interleukins; mDC, myeloid dendritic cells; mTOR, mammalian target of rapamycin; TSLP, thymic stromal lymphopoietin; DC, dendritic cell.

Furthermore, the microbiome barrier, comprising a diverse ecosystem of commensal microbes and their genetic material, forms an additional barrier covering the entire skin surface (24,31). Although the exact role of skin flora in penetration processes remains elusive, emerging evidence suggests that Staphylococcal strains may affect TJ functionality, indicating an interplay between the physical and microbiome barriers (24,25,31). In this regard, unraveling the enzymatic pathways by which gut commensals metabolize pharmaceuticals may partly decipher the skin microbiota-drug interactions offering a tool for optimizing topical dermatotherapy (31).

However, despite its complex barriers, the skin also offers several opportunities for targeted manipulation of its components in the context of topical drug delivery.

Intradermal penetration pathways. As shown in Fig. 2, drug transport across intact skin involves three main pathways: the transcellular route (traversing corneocytes, preferred by hydrophilic or polar compounds), the intercellular route (across the lipid matrix, mostly used by lipophilic molecules) and the transappendageal route (through hair follicles and sweat glands) (22). While the type of pathway depends on the physicochemical properties of the drug, the intercellular path remains the preferred route for entry into the skin. However, the importance of transfollicular routes in skin penetration, especially for TAC, cannot be ignored (22,32).

Physicochemical drug properties. In addition to skin barriers, it is well known that the physicochemical properties of the permeant agent are equally important in determining its penetration into the skin. Since drug movement across the skin primarily occurs by diffusion via the intercellular lipid matrix, parameters that favor topical delivery include high pharmacological potency, low molecular weight (MW; <500 Da) and moderate lipophilicity (octanol-water partition coefficient-logP-between 1 and 3) (14,22,33). Thus, larger and highly hydrophilic or lipophilic molecules, including topical calcineurin and mTOR inhibitors, can hardly overcome the SC barrier and reach deeper skin compartments due to their size and solubility profiles.

Skin disease. Topical drug absorption is mainly governed by the pathophysiological status of the skin, as specific dermatoses can cause distinct changes in skin barrier elements and permeability (22,30,34). For instance, in conditions such as psoriasis and AD, impaired barrier function due to SC disruptions, increased transepidermal water loss and alterations in lipid composition can enhance drug diffusion in both lesional and nonlesional skin areas (30,35,36). However, the barrier resistance in diseased skin is not always compromised and may allow only modest increases in penetration compared with normal skin. This suggests that an effective barrier function can still be maintained in certain disease stages (22,30,37). Indeed, chronic psoriatic plaques may retain some barrier properties that limit drug absorption, while acute lesions might exhibit enhanced permeability (38,39). This variability underscores the need to exploit disease-specific alterations when designing drug delivery systems tailored to particular skin conditions.

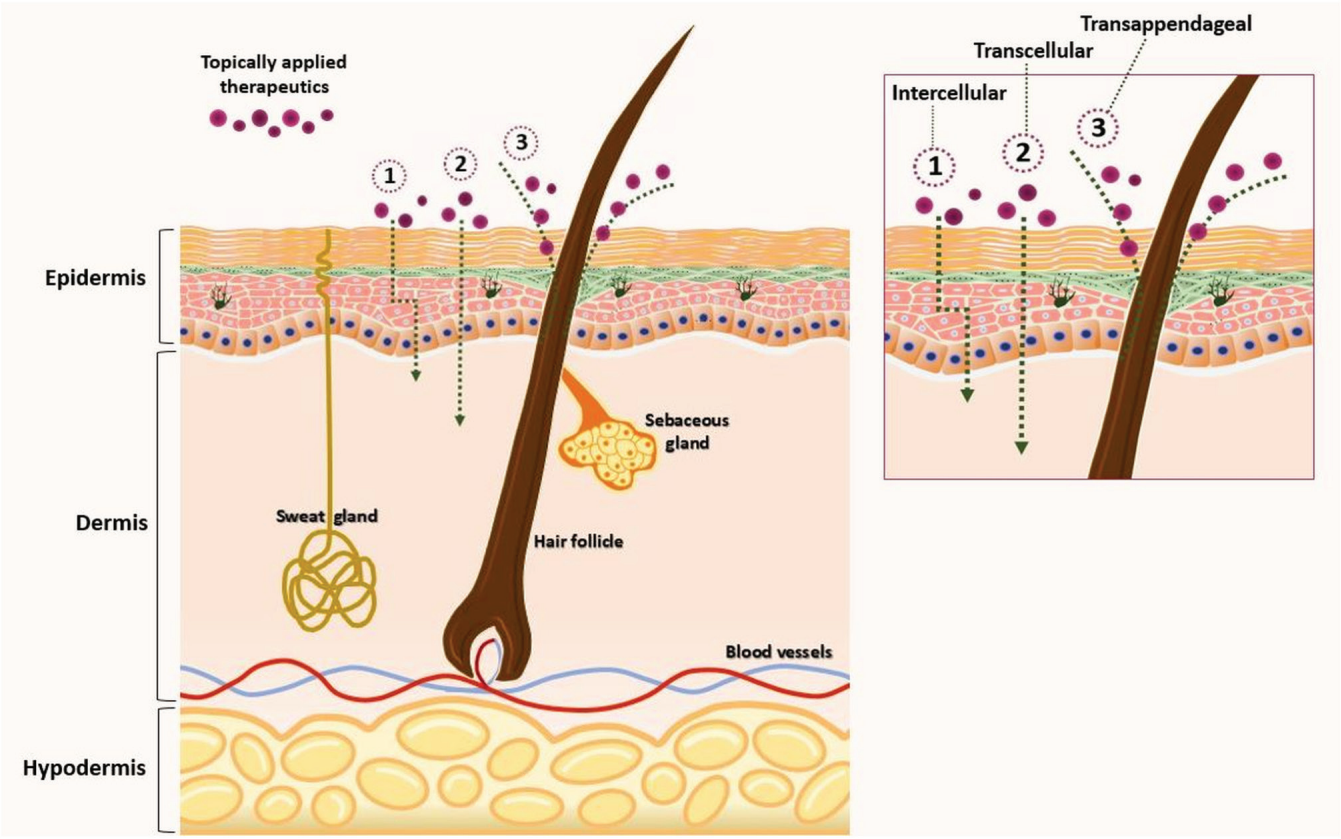


Figure 2. Schematic representation of a skin cross-section illustrating the three main pathways that topical therapeutics use to enter the skin: 1) the intercellular route (across the lipid matrix), 2) the transcellular route (through corneocytes) and 3) the transappendageal route (via hair follicles and sweat glands).

3. Topical calcineurin and mTOR inhibitors in inflammatory dermatoses

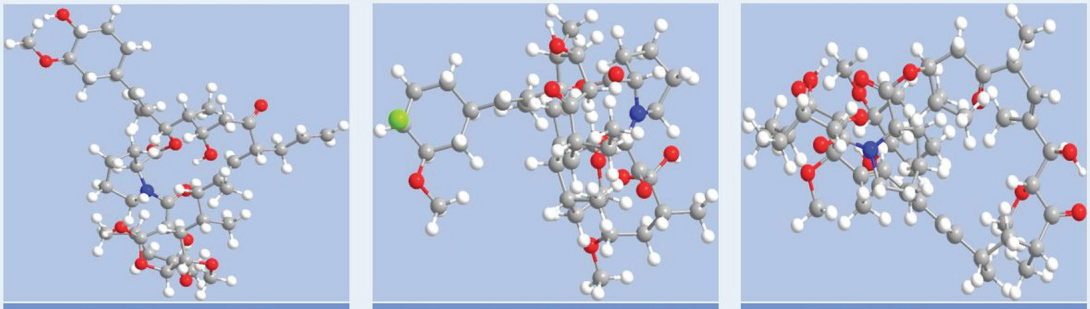
Given the complexities of topical drug delivery, special emphasis should be placed on understanding the dermatokinetic profiles of challenging pharmaceuticals, such as topical calcineurin and mTOR inhibitors. This will provide the rationale for developing advanced topical formulations that can address the limitations of conventional dosage forms and effectively deliver these macromolecules to their target sites in the inflamed skin.

Current challenges and limitations of topical calcineurin inhibitors. TAC and PIM are grouped, along with cyclosporine A (CsA), in the class of calcineurin inhibitors. The two TCIs interact with the cytosolic protein macrophilin-12 (FK506-binding protein; FKBP12) to form inhibitory complexes that block the calcineurin-mediated dephosphorylation of the nuclear factor of activated T-cells, thereby suppressing T-cell activation and cytokine production (6,10,11). In addition, TCIs can inhibit mast cell and neutrophil activation (6,11,40). Notably, PIM prevents mast cell degranulation and release of pro-inflammatory mediators without affecting LCs (6,41).

Topical preparations of TCIs are currently available in two forms in clinical practice: TAC 0.1% or 0.03% ointment for treating moderate to severe AD and PIM 1% cream for mild to moderate atopic eczema (6,11). Of note, the therapeutic success of TCIs in AD, along with their minimal risk of skin atrophy and systemic absorption, have motivated their off-label use as steroid-sparing agents in multiple inflammatory dermatoses, including non-atopic dermatitis, psoriasis and vitiligo (5,11,42-45).

However, while TCIs have shown efficacy in managing AD flares, their topical application faces a number of limitations (46-48). Specifically, the low and variable skin absorption of the commercial formulation of TAC ointment cannot ensure adequate delivery to its target site in the deeper skin layers, which can ultimately limit therapeutic outcomes (46,47). In addition, its greasy nature and sticky sensation, combined with application-site reactions such as irritation, discomfort and/or pruritus, may compromise patient compliance and satisfaction (6,48,49).

Furthermore, therapeutic outcomes with TCIs appear to vary across different body areas and disease states. While facial, genital and inverse psoriasis can respond well to TCIs, refractory cases of the most common plaque-type psoriasis have already been reported, particularly in difficult-to-treat areas such as the scalp (2,11,12,50-52). Additionally, topically applied TAC is often ineffective in thick psoriatic plaques unless used under occlusion (10,53). Indeed, drug absorption in hyperkeratotic psoriatic skin presents challenges. Although key barrier elements, including TJ functionality and lipid composition, are compromised, epidermal hyperplasia and excessive hyperkeratosis can hamper penetration through the follicular routes. This may explain the relative ineffectiveness of TCIs in treating plaque psoriasis (12,38,39).



	Tacrolimus (FK506)	Pimecrolimus (SDZ ASM 981)	Sirolimus (Rapamycin)
Molecular Weight	822 g/mol	810 g/mol	914 g/mol
Aqueous Solubility	Low	Low	Very low
Skin Penetration	Poor	Poor	Poor
Mode of Action	Calcineurin inhibitor	Calcineurin inhibitor	mTOR inhibitor
Routes of Administration	Topical, Oral, Intravenous	Topical	Topical, Oral, Intravenous
Topical Formulation	Ointment 0.03% and 0.1%	Cream 1%	Not marketed
Topical Dermatological Indications	Moderate-to-severe Atopic Dermatitis Off-label: Non-atopic Eczema (Perioral, Seborrheic, Hand, Contact Dermatitis), Facial/Inverse Psoriasis, Vitiligo, Rosacea	Mild-to-moderate Atopic Dermatitis Off-label: Non-atopic Eczema (Perioral, Seborrheic, Hand, Contact Dermatitis), Facial/Inverse Psoriasis, Vitiligo, Rosacea	Off-label: Facial angiofibromas, Psoriasis

Figure 3. Three-dimensional molecular structures and main properties of tacrolimus, pimecrolimus and sirolimus.

Considering the challenges and limitations associated with the topical use of TAC, the skin penetration behavior of TCIs requires special attention. The dermatokinetic profiles of topically applied TAC and PIM are briefly covered in the following section.

Skin pharmacokinetics: Intradermal delivery of topical calcineurin inhibitors. This section focuses on the dermatopharmacokinetic profiles of TCIs by summarizing the evidence from experimental and clinical studies exploring their penetration and biodistribution in normal and inflamed (psoriatic and atopic) skin.

TAC (FK506), the prototype of macrolactam immunomodulators, is a natural metabolite produced by the fungus-like bacterium *Streptomyces tsukubaensis*. Classified as a lipophilic macrolactone (logP=3.2; MW 822 Da), TAC is a crystalline compound that is unstable in alkaline conditions and nearly insoluble in water, but it dissolves in organic solvents such as methanol, ethanol, acetone and chloroform (10,54,55). The newer ascomycin derivative PIM (SDZ ASM 981) is isolated from *Streptomyces hygroscopicus* var. *ascomyceticus*. This macrolide lactone has a molecular weight of 810 Da and is more lipophilic than TAC (logP=6.99) (10,56-58) (Fig. 3).

The large size and high lipophilicity of TCIs indicate a greater affinity for the skin compartment with low potential for percutaneous absorption into the bloodstream, offering a more skin-selective pharmacological profile compared with topical corticosteroids (45). However, these properties remain not ideally suited for topical use (6,14,16,58).

In this respect, previous studies have explored the ability of conventional topical TAC formulations to penetrate into, and permeate through, healthy or inflamed skin using preclinical models and human volunteers. Early *ex vivo* findings demonstrated that TAC penetrates more readily in intact human skin than topical CsA (MW 1202 Da), partly because of its higher potency and 30% smaller molecular size (59-62). However, skin absorption remains highly variable and usually low depending on skin barrier integrity and TAC concentration (10).

Dermally applied TAC has been shown to reside mostly in the SC without entering deeper layers in healthy skin (34,61,63). SC integrity represents a crucial factor limiting TAC transport within the skin. Following a 24-h application on *ex vivo* human skin, ~20-25% of TAC was found in the SC with no drug penetrating deeper skin layers (34). Similarly, in human cadaver skin topically treated with TAC for 24 h, only 13-23 and 0.5-1.1% of the total dose could reach the epidermis (including the SC) and dermis, respectively (61). In a series of *ex vivo* studies, topical TAC could hardly penetrate the intact human skin, whereas flux rates were 7-fold higher after the SC was removed (63). Intradermal TAC delivery enhanced as the formulation strength increased, albeit with no evidence of depot effects (61,63). Notably, occlusion could not affect skin absorption of TAC (60).

While barrier dysfunction in diseased skin may theoretically enhance drug penetration, earlier *ex vivo* studies demonstrated similar amounts of topically applied TAC in the viable epidermis and dermis of both normal and inflamed porcine skin (64). Penetration kinetics of TAC ointment across inflamed skin were further explored in AD patients. In barrier-disrupted human skin, TAC penetrated efficiently into inflamed lesions to control mild to moderate AD relapses. TAC levels beneath the SC increased with prolonged treatment but declined with increasing skin depth. Despite drug depot formation, only a minor proportion (3%) of TAC concentration

was sustained 7 days post-treatment, indicating short-term retention of minimal drug amounts in upper skin layers (65).

Preclinical *ex vivo* and *in vivo* studies on the penetration behavior of PIM in normal skin yielded comparable results. When applied to human and porcine skin under non- and semi-occlusive conditions, >93% of PIM remained unabsorbed creating a depot on the skin surface, with minimal amounts entering the epidermis. PIM levels in the SC of minipig skin were far higher *in vivo* compared with the viable epidermis and dermis (2.2 vs. 0.21 and 0.14% of the applied dose, respectively), while dose proportions of 3.1 and 2.9% accumulated *ex vivo* in the human epidermis (including the SC) and dermis, respectively. Despite drug depot formation in the SC, skin deposition of PIM decreased with increasing treatment duration and was not sustained after repeated dermal application for up to 13 weeks. In fact, only 0.35% of topically applied PIM was retained in the dermis 10 days post-treatment and it was completely eliminated after a wash-out period of 4 weeks (66).

Despite structural and functional similarities, TCIs display distinct differences in skin penetration dynamics. The higher lipophilicity of PIM (logP=6.99) vs. TAC (logP=3.2) results in a greater affinity for the skin tissue during penetration and thus lower systemic exposure, offering a more favorable safety profile (6,56,67). However, preclinical studies on human and animal models showed that both TCIs diffuse similarly across the skin (58,64), corroborating *in vitro* findings (67). In all models studied, both TAC and PIM could cross the SC to a similar degree, achieving comparable levels in the viable epidermis and dermis under normal and inflamed skin conditions (58,64). Notably, permeation of TAC beyond human and animal skin was consistently higher compared with PIM, regardless of the skin origin (animal or human) and formulation composition. Moreover, absorption of both TCIs beyond inflamed skin was up to 6-fold higher compared with healthy skin. Skin inflammation seemed to enhance only the transdermal permeation of TCIs without affecting their skin deposition (64).

Although damaged or inflamed skin may exhibit increased permeability, potentially leading to systemic exposure, accumulated evidence from adult and pediatric pharmacokinetic studies has consistently shown very low or undetectable serum concentrations of both TCIs following dermal application, far below the levels required for systemic immunosuppression (6,45,49,58,65,68,69). Percutaneous permeation of PIM in AD patients remains minimal, even with prolonged or extensive use (58,68). Additionally, systemic absorption of TAC appears to decrease with treatment duration as the skin heals and barrier integrity is restored, indicating a favorable long-term safety profile (45,65,69).

As reported by skin metabolism studies, no evidence supporting the biotransformation of topical TAC and PIM has been observed in human and porcine skin models. This suggests a low potential for intradermal interactions that could influence the *in vivo* skin bioavailability of TCIs (60,63,66).

Emerging prospects of topical mTORIs for psoriasis and atopic dermatitis. Sirolimus (rapamycin) and its derivatives (rapalogs) belong to a novel class of macrolactam immunomodulators. These agents exert potent antiproliferative and immunosuppressive effects by inhibiting mTOR, a multifunctional serine/threonine kinase of the PI3K family. Similar to TAC, mTORIs form initial complexes with the cytosolic FKBP12 protein but then primarily inhibit mTOR complex 1. As a result, mTORIs induce cell cycle arrest at the G_1 to S transition, while calcineurin inhibitors block the cell cycle at an earlier phase (G_0/G_1) (1,5,10,70).

While the newer macrolides, sirolimus and everolimus, have currently no approval for inflammatory dermatoses, the aberrant activation of the PI3K/Akt/mTOR axis has already been involved in the pathogenesis of psoriasis and AD (8,71-76). The upregulated PI3K/Akt/mTOR pathway in psoriatic epidermis appears to play a key role in disease initiation and progression by mediating Th1/Th2/Th17 imbalance, secretion of pro-inflammatory mediators, abnormal keratinocyte proliferation and differentiation and neovascularization (8,74-76). Although less studied in AD, the PI3K/Akt/mTOR cascade has recently been implicated in regulating epidermal barrier formation by influencing filaggrin processing and lipid synthesis (71-73).

Systemic mTORIs, especially everolimus, have shown potential in managing both psoriasis and AD (77-81). Despite limited evidence to support the beneficial effects of topical mTORIs on inflamed skin, cutaneous mTOR blockade appears promising in immune-mediated skin diseases that require modulation or control of inflammation, epidermal and vascular proliferation and keratinocyte differentiation (4).

Indeed, topically applied sirolimus has already demonstrated efficacy in a small clinical trial on psoriasis patients (82). Although preclinical data from contact dermatitis (CD) animal models were initially not promising (83-85), subsequent studies in skin models of AD and psoriasis indicated the broad anti-inflammatory effects of dermally applied sirolimus both *in vitro* and *in vivo* (82,86-92). Traditional topical formulations of sirolimus, investigated in animal and human inflamed skin, have shown potential for addressing several clinical, histological and molecular aspects of cutaneous inflammation (82,86-90). Table I summarizes the key findings from studies exploring the effects of conventional topical sirolimus formulations on inflamed skin.

From a clinical perspective, dermally applied sirolimus, although displaying moderate efficacy in controlling inflammation in irritant CD models, could markedly improve the clinical features of psoriatic and atopic skin lesions while reducing itch and scratching behavior (82,86-88,90).

Laboratory analyses of psoriatic and atopic lesional skin topically treated with sirolimus revealed several biological effects. These include reduced epidermal thickness and neutrophilic microabscesses and decreased dermal neoangiogenesis and inflammatory cell infiltration (comprising T lymphocytes, eosinophils and mast cells). Sirolimus also normalized markers of keratinocyte proliferation and differentiation, such as Ki-67 and keratins. Notably, this compound partially restored skin barrier integrity, as indicated by the increased expression of caspase-14, involucrin and loricrin and further contributed to regulating cellular homeostasis, by modulating cell structure, autophagy and oxidative stress, through the normalization of tropomyosins and related markers (82,86-90).

Furthermore, topical sirolimus could target multiple signaling pathways involved in skin inflammation, including mTOR, ERK 1/2 and NF-κB. It also achieved a broad downregulation of key inflammatory mediators, such as

Table I. Summary of experimental and clinical studies on the biological effects of conventional topical formulations of sirolimus on inflamed skin.

	Topical SIR	Study setup			
Authors, year	Formulation type (strength)	Skin model/disease	Type (species)	Key findings	(Refs.)
Meingassner *et al*, 1992	Conventional (0.13%, 1.2%)	DNFB-induced CD	*In vivo* (Porcine)	No effect on skin erythema and vascular changes.	(83)
Meingassner *et al*, 1992	Conventional (0.4-3.6%)	PMA/Calcimycin-induced ICD OXA/DNFB-induced ACD	*In vivo* (Murine, Porcine)	Moderate decrease in ICD inflammation (↓ edema by 30%). No effect on ACD inflammation.	(84)
Duncan *et al*, 1994	Conventional (0.02%, 2%)	DNFB-induced DTH	*In vitro* (HEK) *In vivo* (Murine)	Stronger keratinocyte growth inhibition than CsA *in vitro*. No effect on skin erythema and T-cell infiltration *in vivo*.	(85)
Ormerod *et al*, 2005	Solution (2.2% followed by 8%)	Plaque Pso	*In vivo* (Human subjects)	Reduced total Pso clinical score; no effect on erythema and thickness of psoriatic plaques. Decreased proliferating (Ki-67) and CD4+ T-helper cells; no effect on CD8+ cytotoxic T cells, macrophages and LCs.	(82)
Yang *et al*, 2014	Ointment (0.2%)	Dfb-induced AD	*In vivo* (Murine)	Improved clinical features (erythema, edema, erosion, scaling, dryness) and reduced itch and scratching behavior. Lesional skin: ↓ epidermal thickness; ↓ dermal inflammatory cell infiltration (including mast and T cells); ↓ IL-4, IL-13, TSLP and NGF; Normalization of total mTOR and p-mTOR levels. Serum: ↓ IgE levels.	(86)
Jung *et al*, 2015	Cream (0.04-4%)	TNCB-induced AD	*In vivo* (Murine)	Improved clinical signs (erythema, edema, erosion, dryness). Lesional skin: ↓ epidermal hyperplasia; ↓ dermal edema and cell infiltration (including eosinophils and mast cells); ↓ IL-4 and IFN-γ. No effect on serum IgE.	(87)
Bürger *et al*, 2017	Ointment (1%)	IMQ-induced Pso	*In vivo* (Murine)	Reduced clinical erythema and scaling. ↓ Epidermal thickness and neutrophilic microabscesses; ↓ immune cell infiltration and neovascularization. Normalization of keratinocyte proliferation and differentiation markers (↓ Ki-67 and KRT6; no effect on KRT10). Partly restored skin barrier markers (↑ caspase-14, involucrin and loricrin). Reduced mTOR activity (↓ Rps6 and p-mTOR). Trend for reduced immune cell migration to lymph nodes. Serum: ↓ Total leukocytes, neutrophils and monocytes.	(88)

Table I. Continued.

Authors, year	Topical SIR	Study setup		Key findings	(Refs.)
	Formulation type (strength)	Skin model/disease	Type (species)		
Gao *et al*, 2018	Conventional (3 mg/ml)	TNF-α-stimulated cell model IMQ-induced Pso	*In vitro* (HEK; HaCaT) *In vivo* (Murine)	Restored cell skeleton and reduced cell proliferation *in vitro*. Decreased epidermal thickness *in vivo*. Upregulation of TPMs *in vitro* and *in vivo*. Reduced ERK1/2 and mTOR activity *in vitro* and *in vivo*.	(89)
Kim *et al*, 2021	Conventional (5 mg/ml)	IMQ + TCDD-induced Pso	*In vivo* (Murine)	Improved clinical features (erythema, thickness, scaling). Decreased epidermal thickness. ↓ TNF-α, IL-6, IL-17A, IL-22 and IL-23. Decreased AHR and increased autophagy-related factors. Normalization of oxidative stress markers (NOX-2/4, Nrf2). Reduced NF-κB signaling (↓ P65 protein).	(90)

ACD, allergic contact dermatitis; AD, atopic dermatitis; AHR, aryl hydrocarbon receptor; CD, contact dermatitis; CsA, cyclosporine A; Dfb, dermatophagoides farinae body; DNFB, 2,4-dinitroflourobenzene; DTH, delayed-type hypersensitivity; ERK, extracellular signal-regulated kinase; HaCaT, immortalized human keratinocytes; HEK, human epidermal keratinocytes; ICD, irritant contact dermatitis; IFN-γ, interferon-γ; IgE, immunoglobulin E; IL, interleukin; IMQ, imiquimod; KRT, keratin; LCs, Langerhans cells; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NGF, nerve growth factor; NOX, NADPH oxidase; Nrf2, nuclear factor-erythroid 2-related factor 2; OXA, oxazolone; PMA, phorbol 12-myristate 13-acetate; p-mTOR, phosphorylated mammalian target of rapamycin; Pso, psoriasis; Rps6, ribosomal protein S6; SIR, sirolimus; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TNCB, 2,4,6-trinitrochlorobenzene; TNF-α, tumor necrosis factor-α; TPMs, tropomyosins; TSLP, thymic stromal lymphopoietin.

TNF-α, interferon-γ (IFN-γ), interleukins (IL-4, IL-6, IL-13, IL-17A, IL-22 and IL-23) and thymic stromal lymphopoietin (TSLP) (86-90). In addition, topical sirolimus showed potential to impact pruritus onset and severity by reducing nerve growth factor (NGF) levels (86).

Beyond local effects, a trend towards reduced migration of immune cells, including lymphocytes, monocytes, neutrophils, LCs, myeloid and plasmacytoid dendritic cells (DCs), to draining lymph nodes has been observed (88). This suggests that sirolimus may counteract the complex feedback loop sustaining chronic inflammation in the atopic and psoriatic skin microenvironment (93,94). Additionally, topical sirolimus appeared to exert extracutaneous effects, as evidenced by decreased serum levels of IgE, total leukocytes, neutrophils and monocytes (86,88). This may indicate its potential to normalize serum biomarkers involved in the so-called 'atopic or psoriatic march', thereby mitigating systemic inflammation and related comorbidities (94).

These findings indicate that locally applied sirolimus may have diverse biological effects beyond its known antiproliferative and immunosuppressive properties. Further research is warranted to elucidate the underlying mechanisms and the precise impact of topical sirolimus on the inflammatory and structural components of the psoriatic and atopic skin microenvironments.

Skin pharmacokinetics: Intradermal delivery of topical mTORIs. Sirolimus is a lipophilic macrolactone initially derived from the soil fungus-like bacteria *Streptomyces hygroscopicus* found in Rapa Nui (Easter Island). In its natural form, sirolimus is a white crystalline solid with a molecular weight of 914 Da, a partition coefficient (logP) of 5.8 and low water solubility (95,96) (Fig. 3). As a result, sirolimus is difficult to formulate into traditional vehicles for dermal application. Currently, topical formulations of sirolimus are not commercially available and are typically prepared from crushed tablets, solution, or powder forms incorporated into ointment, cream, or gel vehicles (4,70,96,97). In addition, the physicochemical properties of sirolimus appear suboptimal for topical use, limiting its capacity to efficiently penetrate the epidermal barrier and reach deeper skin compartments (17).

Thus, understanding the dermatokinetic profile of locally applied sirolimus is important for the development and optimization of topical formulations that ensure maximum skin bioavailability. In this context, an early study by Ormerod *et al* (82) evaluated the penetration of topical sirolimus in *ex vivo* barrier-impaired and *in vivo* normal human skin after a single application of 2.2 and 8% solutions. As the results showed, topical sirolimus could diffuse into human skin with no evidence of systemic absorption. Penetration depth seemed to enhance as the formulation strength increased.

Several topical preparations of sirolimus have also been explored to elucidate factors influencing intradermal penetration, such as formulation composition and skin barrier integrity (98-100). Tanaka *et al* (98) compared the skin absorption of 0.2% sirolimus formulated in gel and ointment forms using an *in vitro* three-dimensional (3D) cultured human skin model. Their findings demonstrated that the hydrogel facilitated greater skin accumulation than the lipophilic ointment (1,360 pg/mg vs. 680 pg/mg, respectively) with lower irritation.

In an *in vivo* murine study, the dermatokinetics of sirolimus were investigated utilizing different formulations (gel, cream and lotion) as delivery vehicles. Overall, superior skin bioavailability was observed with topical application than oral administration of sirolimus. When locally applied, sirolimus displayed dose-dependent skin penetration, showing enhanced absorption and retention when applied as a gel or cream but was less adherent to the skin when incorporated into a lotion (99).

In a series of *in vitro* (on synthetic membranes) and *ex vivo* (on human skin) studies, Le Guyader *et al* (100) also compared the penetration of sirolimus hydrogel, cream and ointment formulations, demonstrating that skin flux and deposition were higher with the hydrogel and enhanced with increased formulation strength. This study emphasized the importance of the formulation type and using sirolimus solubilized (rather than dispersed) at close-to-saturation concentrations to maximize skin bioavailability.

Penetration of topically applied sirolimus was further evaluated in barrier-disrupted *ex vivo* human skin. As expected for a hydrophobic macromolecule such as sirolimus, absorption into the intact SC was minimal after topical application as a solution or gel. However, in barrier-impaired skin, sirolimus could cross the entire SC and reach deeper epidermal layers. The penetration depth increased as the skin barrier damage enhanced (101).

These findings suggest that the skin absorption and deposition of topical sirolimus depend not only on dosage but also on the administration route and the nature of the formulation/vehicle (hydrophilic vs. lipophilic; hydrogel > cream > ointment). The hydrogel appears to be the preferable vehicle in terms of release and penetration profiles. Notably, the integrity of both the SC and TJ barriers seems to have a greater impact on sirolimus penetration into the skin than the formulation vehicles.

Regarding safety, adverse effects of topical sirolimus appear to be rare and mainly consist of mild application-site reactions that are easily managed without requiring treatment withdrawal. Systemic absorption of locally applied sirolimus is minimal and rarely detectable, independent of the formulation strength (4,82,97).

Novel strategies for skin inflammation: Nanocarrier-based topical delivery of calcineurin and mTOR inhibitors. The use of nanoproducts for encapsulating bioactive compounds offers a novel strategy to address unfavorable physicochemical drug properties, such as lipophilicity or hydrophilicity, high molecular weight, stability and bioavailability. This approach can provide enhanced skin penetration with targeted and controlled drug release, ultimately improving the efficacy and safety profiles of topical therapeutics (20,21,46).

As aforementioned, due to the complexities involved in intradermal delivery of challenging pharmaceutics, such as calcineurin and mTOR inhibitors, more advanced nanocarrier-based prospects are being explored to improve the penetration kinetics of these 'hard-to-formulate' macromolecules within the skin. This section summarized the experimental evidence on various nanovectors investigated as prospective carriers for topical TAC, PIM and sirolimus, focusing on research specifically conducted in psoriasis and AD skin models.

Nanocarrier-based skin delivery of topical calcineurin inhibitors. Several types of nanosystems have been explored to overcome the limitations of intradermal penetration and effectively deliver topically applied TAC to its target sites within the viable skin.

Lipid-based nanosystems. Lipid-based nanoformulations have been widely studied for delivering TAC in both normal and inflamed skin. Exploring this concept, Kovačević *et al* (102) fabricated nanostructured lipid carriers (NLCs) for TAC encapsulation after screening 20 selected lipids. The prepared NLCs, composed of mixed lipid cores, displayed favorable physicochemical properties and entrapment efficiency (EE) of nearly 99%. The use of polyethylene glycol (PEG)-free stabilizers ensured optimal particle stability and prevented irritation effects (102). TAC-loaded solid lipid nanoparticles (SLNs) and NLCs, comprising stearic acid (SA) or beeswax as solid lipids, exhibited satisfactory physicochemical features, with beeswax-based nanoparticles (NPs) providing superior loading capacity (3.3 and 2.9% for NLC- and SLN-Beeswax/TAC, respectively vs. 2.7 and 2.3% for NLC- and SLN-SA/TAC, respectively). No incompatibility between TAC and lipid components was observed (103).

In this context, Wang *et al* (104) formulated TAC-loaded SLNs, demonstrating that *in vitro* drug release was fast from free TAC solution (96% at 8 h) but sustained from TAC-SLNs (55 and 86% at 8 h and 72 h, respectively). In *ex vivo* studies, SLNs achieved enhanced skin penetration and deposition of TAC in healthy skin compared with the commercial ointment.

Kang *et al* (18) developed thermosensitive SLNs for the targeted release of TAC in response to thermal variations within the skin. The designed TAC-SLNs could cross the SC (32˚C) as intact particles and release TAC in the dermis (37˚C) without deeper diffusion. *Ex vivo* studies showed temperature-dependent skin penetration; TAC-loaded SLNs failed to traverse the skin at higher temperatures. While TAC was mainly confined to the SC, SLNs delivered greater drug amounts into deeper skin layers (up to 300-450 μm depth) compared with the marketed ointment (undetectable below 150 μm). The TAC-SLN nanoformulation was more tolerated *in vivo*, showing only occasional mild erythema, whereas the commercial ointment induced severe erythema.

Chitosan (CS) has been employed in the design of NPs (105-108). Khan *et al* (105) fabricated TAC-loaded SLNs to investigate the effects of CS coating and gel formulation on skin delivery of TAC. *In vitro* drug release was faster from uncoated SLNs and sustained from both CS-coated SLNs and SLN-gels. *Ex vivo* studies showed that both SLNs and SLN-gels achieved similar intradermal penetration, but the SLN-gel resulted in higher retention of TAC in normal skin. Notably, the CS coating did not markedly affect drug retention in the skin (105).

Furthermore, NLCs, considered successors to SLNs, displayed controlled release patterns *in vitro* (10% of TAC/24 h) and facilitated skin deposition of 61.7% of dermally applied TAC *ex vivo* (109). Minimal toxicity to murine fibroblasts was observed. Notably, this study also explored a novel combination therapy using NLCs for co-delivering TAC and TNFα small interfering RNA (siRNA) in psoriatic skin. This system exhibited synergistic anti-psoriatic effects *in vivo*, preventing the onset of erythematosquamous lesions and achieving greater TNFα reduction (7-fold) than TAC-loaded in NLCs and commercial vehicles (2.5- and 2-fold TNFα reduction, respectively) (109). In addition, emulsions containing TAC-loaded NLCs outperformed the marketed ointment in terms of TAC penetration and deposition in *ex vivo* skin (NLCs: 8.6 mg/cm^2; marketed ointment: 5.4 mg/cm^2) (110).

CS-coated NLCs prepared for co-loading TAC and clobetasol propionate (CP) provided an EE >90% (98% for TAC; 92.8% for CP). *In vitro* analysis demonstrated that co-encapsulated NLCs released TAC more slowly than CP. While CS coating favored TAC retention in the SC, TAC failed to diffuse beneath the SC when loaded alone in NLCs, regardless of the coating. Co-encapsulation of CP, especially in CS-coated NLCs, enhanced the capacity of TAC to penetrate deeper skin layers (106).

Vesicular nanocarriers have also gained attention in this field (111-117). An *in vivo* murine study first reported that a topical liposomal lotion enhanced cutaneous bioavailability, resulting in 9-fold higher TAC skin levels compared with intravenous administration. This formulation also showed superior suppression of skin inflammation than systemic TAC vehicles, either liposomal or traditional, as evidenced both clinically (no erythema or edema) and pathologically (reduced cell infiltration) (111). Similarly, liposomal encapsulation improved the skin flux and deposition of TAC *ex vivo* with respect to free TAC. Liposomal and conventional TAC vehicles both showed similar anti-inflammatory activity *in vivo* in CD skin models (112).

In *ex vivo* comparison studies by Li *et al* (113,114), both ethosomal and traditional liposomal systems achieved higher epidermal accumulation of TAC than the commercial ointment. While classic liposomes facilitated the highest SC deposition, ethosomes delivered greater amounts of TAC to deeper epidermal layers compared with liposomal and conventional vehicles. The *in vivo* capacity to reduce AD-like skin inflammation was in the order of ethosomes > classic liposomes > dexamethasone cream > commercial TAC ointment.

Using transfersomes (TFs), topical TAC was effectively delivered into normal, atopic and psoriatic skin. Transfersomal and liposomal carriers both exhibited improved penetration profiles *ex vivo* and *in vivo* in terms of TAC release, deposition and retention in the skin compared with commercial formulations. TFs displayed enhanced delivery properties, as evidenced by the penetration depth and amount of TAC in both *ex vivo* and *in vivo* viable skin. Notably, TFs were superior to classic liposomes and conventional vehicles *in vivo* in restoring the clinical and pathological features of atopic and psoriatic skin (115-117).

Liquid crystalline nanoparticles (LCNPs) have also been used for topical TAC delivery, providing controlled drug release and enhanced skin penetration and retention. *Ex vivo* studies showed that LCNPs increased the skin concentration and retention of TAC by 6- and ~3-fold, respectively, compared with free TAC. When loaded into LCNPs, TAC exhibited greater anti-psoriatic efficacy *in vivo*, as supported by clinical [Psoriasis Area Severity Index (PASI) score] and pathological (skin thickness and inflammatory infiltration) evaluations. LCNP formulations, with or without oleic acid, were equally effective in repairing psoriatic skin (118,119).

When comparing lipid-based nanoformulations, *in vitro* TAC release at 24 h was 34, 62, 65 and 69% from LCNP, SLN, NLC and liposomes, respectively. LCNP displayed slow, constant release patterns, while SLN, NLC and liposomes showed an initial burst followed by sustained release. LCNP, SLN and NLC increased TAC skin bioavailability *ex vivo* by 2.5-, 2- and ~2-fold, respectively, compared with marketed formulations. Penetration depth decreased in the order of SLN > NLC > LCNP > liposomes, with SLN and NLC reaching deeper skin strata than the commercial ointment. The *in vivo* anti-psoriatic efficacy was as follows: NLC=SLN > LCNP > commercial ointment > liposomes. Unlike conventional vehicles, all nanovectors, especially liposomes, achieved lower transepidermal water loss values without causing skin irritation (120).

Lipid NPs (LN) and modified nanolipid carriers (MNLC) have been exploited to deliver TAC in normal and atopic skin. A series of *in vitro* and *ex vivo* studies showed improved profiles in terms of skin release, penetration and deposition of TAC with all studied nanovehicles compared with traditional vehicles. When delivered by LN or MNLC *in vivo*, TAC achieved higher levels in total skin, especially in the deeper epidermis and dermis, without entering the bloodstream. While nano-based and marketed formulations showed similar occlusive and hydration effects *in vitro* and *in vivo*, the former achieved improved skin moisture restoration *ex vivo*, as well as earlier and superior AD control *in vivo*. Neither skin irritation nor histological changes occurred in intact or inflamed skin with all tested NPs, even after repeated application (48,121-124). Additionally, when TAC was loaded into natural rhamnolipid-based NPs, no cytotoxicity was observed in human dermal fibroblasts (125).

Microemulsions (MEs) and nanoemulsions (NEs) have also been employed as topical vehicles for TAC (32,126-130). *In vitro*, MEs released greater amounts of TAC at higher rates than the commercial ointment (127,128). In *ex vivo* studies, MEs improved the cutaneous bioavailability of TAC, as indicated by the higher drug concentrations in animal and human skin compared with the marketed ointment (126-129). The latter delivered 3-fold lower TAC amounts in the deeper SC than the lecithin-based MEs (128). Although MEs and conventional vehicles carried similar amounts of TAC in the viable epidermis (below the SC), MEs delivered 9-14% of TAC into the dermis, whereas only 6.5% could reach the dermis when applied as a traditional ointment (126). Based on the formulation type, TAC retention in *ex vivo* skin decreased in the order of conventional solution > ME-based cream > commercial ointment (127). MEs were well tolerated *in vitro* and *in vivo*, with no observed toxicity or skin irritation (126-128).

ME systems have already shown therapeutic potential for AD (127,129). As reported by Wang *et al* (129), TAC-loaded MEs achieved enhanced control of AD *in vivo* compared with marketed formulations, as demonstrated by clinical and laboratory investigations, corroborating previous findings reported by Lalan *et al* (127).

Building on the benefits of MEs, a Kalonji oil-based NE system (NE and NE-gel) was developed for the local delivery of TAC to psoriatic plaques. *In vitro* TAC release followed a slower, sustained pattern from NEs compared with free TAC gel. With respect to the latter, NEs displayed improved *ex vivo* penetration kinetics in terms of total delivery and retention of TAC in the SC and viable epidermis. *In vivo*, the Kalonji oil plus TAC-loaded NE gel showed greater anti-psoriatic activity than the commercial ointment, as assessed by clinical and laboratory evaluations. TAC-loaded NEs exhibited stronger antiproliferative effects on epidermal cell lines than the free drug (130).

In a comparative study, Savić *et al* (32) fabricated NLCs and NEs to explore their potential as topical carriers of TAC. *In vitro* TAC release from NLCs was superior to that from NEs and marketed formulations. In *ex vivo* studies, both NLCs and NEs achieved greater TAC deposition in the SC than the commercial ointment, with the highest follicular drug uptake obtained with NLCs followed by NEs and the ointment. The latter resulted in higher transdermal permeation of TAC compared with nanovehicles. Overall, NLCs proved superior to NEs for dermal delivery of TAC.

Natural plant agents combined with TAC have been used as topical antipsoriatic agents. Lipospheres co-loaded with TAC and curcumin (CUR) displayed a slow release of both cargos *in vitro* and compatibility with healthy skin *in vivo*. In *in vivo* studies, the marketed ointment and TAC-CUR lipospheres were equally effective but superior to betamethasone in restoring the clinical features of psoriatic skin, such as scaling and thickness, while the decrease in erythema followed the order of TAC-CUR lipospheres > commercial TAC ointment > betamethasone ointment. Among the studied formulations, only TAC-CUR lipospheres normalized the histological psoriatic changes. Notably, TAC-CUR lipospheres achieved the strongest suppression of TNFα and IL-22, while IL-17 inhibition followed the order of TAC-CUR lipospheres ≈ commercial TAC ointment > TAC lipospheres > betamethasone ointment (47).

Polymer-based nanosystems. Using micellar systems, TAC was successfully delivered into normal and inflamed skin (131,132). While micelles remained largely unabsorbed on the skin surface, they facilitated greater skin deposition of TAC *ex vivo* compared with marketed formulations, without permeating beyond human and porcine skin. Improved penetration profiles were observed in porcine compared with human skin, indicating the importance of transfollicular pathways for micelle-mediated delivery (131).

Using X-ray microscopy, the penetration of topical TAC incorporated into methoxy-poly(ethylene glycol)-hexyl-substituted polylactide (mPEG-hexPLA) micelles was investigated in an *ex vivo* psoriasis murine model. Slightly increased SC deposition was observed when TAC was formulated in micelles rather than in the commercial ointment. SC levels of TAC-loaded micelles gradually increased until saturation and then decreased due to deeper absorption, while amounts in the viable epidermis remained stable. This study, although indicating intercellular delivery routes, also suggested that micelles might penetrate corneocytes (132).

Similar attempts have been made by Gabriel *et al* (133) to design mPEG-hexPLA-based NPs for the *in vivo* evaluation of TAC penetration and anti-psoriatic effects on intact and psoriasiform murine skin. TAC embedded into mPEG-hexPLA NPs showed enhanced absorption in inflamed compared with normal skin, reaching ~2-fold higher levels in psoriatic

lesions than with a commercial ointment. This nanoformulation was superior to CP in lesion clearance and demonstrated comparable antipsoriatic efficacy to the marketed ointment. No toxicity was observed after repeated application on healthy skin.

In a novel approach, Zabihi *et al* (134) exploited polymer-based biodegradable NPs composed of poly(lactide-co-glycerol) (PLG) to deliver TAC into human skin. In *ex vivo* experiments, PLG-NPs facilitated 80, 16 and 4% of encapsulated TAC to reach the SC, viable epidermis and dermis, respectively, resulting in 1.74-, 1.44- and 2-fold higher drug concentrations in the SC, epidermis and dermis, respectively, compared with the marketed ointment. The anti-inflammatory efficacy was assessed *in vitro* on a 3D reconstructed filaggrin-deficient human skin model by measuring pro-inflammatory mediators. While the downregulation of IL-2 was comparable between TAC-PLG-NPs and the marketed product, the NPs were more effective in suppressing TSLP. No evidence of cytotoxicity on primary human keratinocytes and fibroblasts was noted.

Fereig *et al* (107) developed CS-based NPs for topical application of TAC on psoriatic skin. *In vitro* drug release from CS-NPs followed sustained biphasic patterns. When delivered by CS-NPs, 82% of dermally applied TAC was accumulated in *ex vivo* skin, whereas a 34% total deposition was achieved with a conventional ointment. CS-NPs facilitated reduced flux rates into and permeation of TAC beyond the skin compared with a traditional ointment (24 vs. 61% permeated drug, respectively). In *in vivo* experiments, CS-NPs also displayed enhanced skin deposition of TAC (54.6 vs. 13.8% for the ointment) and achieved faster and superior control of the clinical and pathological psoriatic features compared with the ointment. Notably, only TAC-loaded CS-NPs promoted hair growth in the treated areas.

CS-based NPs were further explored in combination with nicotinamide (NIC) or hyaluronic acid (HA) to transfer TAC into atopic skin (108,135). Under normal *ex vivo* and AD-like *in vivo* conditions, the synergistic effect of NIC and CS achieved greater TAC concentrations in total skin compared with the commercial ointment. NIC-CS-NPs showed superior *in vivo* anti-AD activity compared with the marketed formulation, despite containing equal or lower TAC doses, as confirmed by clinical, pathological and molecular analyses (108). HA coating ensured controlled and sustained *in vitro* release patterns, while enhancing TAC deposition and retention beneath the SC in *ex vivo* skin. HA-covered NPs exhibited *in vivo* greater anti-AD efficacy than both the uncovered CS-NPs and the commercial TAC ointment, based on clinical and laboratory evaluations (135).

NPs prepared by HA and cholesterol (Chol) conjugations in a NIC solution (NIC-HA-Chol NPs) synergistically enabled deeper penetration and enhanced deposition of TAC in *ex vivo* and *in vivo* studies on intact skin compared with the commercial ointment. HA-Chol-NPs, with or without NIC, showed improved drug uptake in HaCaT cells (136). Further evaluation showed that the NIC-HA-Chol nanoformulation achieved 2.4- and 2.5-fold higher TAC permeation and retention in *ex vivo* psoriatic skin, respectively, compared with the marketed product. The *in vivo* anti-psoriatic efficacy of the studied NPs, assessed via the PASI score and epidermal thickness, was comparable to CP but superior to the marketed ointment. The NIC-HA-Chol NPs exhibited enhanced cellular uptake and strong anti-proliferative effects in murine macrophage and HaCaT cells (136,137).

In this context, the dermatokinetics and therapeutic efficacy of TAC-loaded polymeric core-multishell (CMS) nanocarriers have been examined *in vivo* using an AD-like murine model. While CMS nanoparticles delivered TAC into all skin layers, they failed to enhance drug deposition compared with the commercial ointment (mean TAC levels in epidermis and dermis: 36 and 77 ng/cm^2 for CMS nanocarriers vs. 93 and 118 ng/cm^2 for the ointment, respectively). However, both CMS nanocarriers and the marketed ointment showed similar efficacy in improving the clinical and pathological features of atopic skin (138,139).

Thermoresponsive nanogels offered a new approach for the topical delivery of TAC in *ex vivo* human skin. Comparative studies showed that the commercial ointment demonstrated superior penetration, particularly in breast vs. abdominal skin and in barrier-impaired vs. intact skin. After barrier disruption, nanogels, although primarily confined to the SC, could enter deeper viable skin. TAC levels increased over time in ointment-treated sites but remained constant in areas treated with nanogels. The increase in IL-6 and IL-8 in damaged skin treated with TAC was attributed to the irritative effects of TAC and/or the vehicles used. Nanogels and the marketed ointment were both equally effective in inhibiting T-cell proliferation (140).

As reported by Limón *et al*, nanostructured hydrogels could entrap TAC both in interstitial spaces and within gel fibers, offering a reservoir for controlled release and protection from degradation. *In vitro* tests revealed moderate rates of biphasic drug release. In *ex vivo* human skin, TAC hydrogel could cross the SC and remain in the epidermis and upper dermis with minimal percutaneous permeation. In *in vivo* psoriasis models, TAC nano-hydrogel showed superior efficacy and tolerability compared with a free TAC solution. The latter achieved only a 9% reduction in skin thickness and failed to prevent local adverse effects, whereas TAC nano-hydrogel achieved a 50% reduction in psoriasiform hyperplasia without causing desquamation or hair loss (141).

Inorganic nanosystems. Recently, mesoporous silica nanoparticles (MSNs) have been employed as carriers for topical TAC, demonstrating potential in managing AD. TAC-loaded MSNs displayed improved *in vitro* release kinetics than a free TAC gel (73 and 55% at 24 h, respectively) without any evidence of cytotoxicity. Although both TAC-MSNs and TAC gel showed low transdermal permeation *ex vivo* (13 vs. 11%, respectively), a markedly higher amount of TAC was deposited in the skin with MSNs than with TAC gel (75 vs. 36%, respectively). In *in vivo* AD-like skin, TAC-MSNs were more effective than TAC gel in restoring both clinical and pathological alterations (142).

Hybrid nanosystems. Hybrid nanosystems, emerging as superior alternatives to conventional nanocarriers, have been used for delivering TAC in psoriatic and atopic skin (143,144). Wan *et al* (143) developed a polymer-based ME as a topical delivery system of TAC. *Ex vivo* and *in vivo* studies on normal and psoriatic skin demonstrated that the studied ME

outperformed the commercial ointment in terms of delivery and deposition of TAC across the entire skin. This system displayed *in vivo* anti-psoriatic activity comparable to CP but superior to the marketed TAC ointment, as evidenced by clinical (PASI score) and pathological (epidermal thickness) outcomes. In addition, this formulation showed enhanced uptake and growth inhibition of HaCaT cells.

Shams *et al* (144) fabricated polymeric nanofibers incorporating TAC-loaded ME for topical application on atopic skin. This system displayed sustained *in vitro* release profiles, with 22% of TAC released over 3 days. The tested ME-nanofibers, applied every two days, proved equally effective as the daily use of commercial TAC ointment in improving the AD histological features.

Nanocarrier-based skin delivery of topical mTORIs. Unlike TAC, only a few studies have investigated the skin penetration and anti-inflammatory effects of topical sirolimus nanoformulations (91,92,145,146). New generations of bioresponsive nanocarriers, capable of exploiting key aspects of the inflamed skin microenvironment, can release their cargos in response to specific stimuli such as pH, temperature, or redox variations within the skin (147). In this regard, redox-sensitive CMS nanocarriers have been explored as topical vehicles for sirolimus in *ex vivo* models of inflamed human skin co-cultured with activated T-cells in comparison with conventional preparations. All tested formulations delivered sirolimus into barrier-disrupted inflamed skin, suppressing T-cell proliferation and release of IL-2 and IL-17A. The strongest inhibition of IL-2 release was observed with sirolimus incorporated in CMS nanocarriers, while no effect on IL-1α, IL-6 and IL-8 pro-inflammatory cytokines was detected. Only the sirolimus-loaded CMS nanocarriers could downregulate mTOR activity and target skin DCs, preventing their activation and migration (91,92).

Furthermore, aqueous formulations of sirolimus-loaded polymeric micelles have been developed for dermal application. Micellar solution and hydrogel both increased the bioavailability of sirolimus in total skin compared with a conventional ointment. Greater amounts of sirolimus were deposited in the SC, viable epidermis and dermis with micelle-based systems. Sirolimus skin levels increased over time, indicating sustained release kinetics. Transdermal drug permeation was undetectable for all tested formulations (145).

Based on these results, Le Guyader *et al* (146) investigated the penetration of sirolimus-loaded polymeric micellar preparations applied topically to *ex vivo* human skin. Although micelle-based and conventional vehicles carried similar amounts of sirolimus to the dermis (700-800 ng/cm^2), a 2.5-fold higher drug deposition in the epidermis was observed with sirolimus formulated in micelles rather than a hydroalcoholic gel (1,900 vs. 700 ng/cm^2, respectively).

Comparative nanocarrier-based skin delivery of topical macrolactam immunomodulators. In a comparative study, Quartier *et al* (54) used polymeric micelles for the topical delivery of TAC, PIM and sirolimus. PIM exhibited a lower linear release from the micelles *in vitro*, while TAC and sirolimus showed higher releases, reaching a plateau before further increase. In *ex vivo* skin, PIM was deposited in higher amounts, especially in the epidermis, due to its greater lipophilicity and stronger binding with skin components. TAC and sirolimus showed lower but similar deposition levels, with greater accumulation in the dermis. The higher water solubility of sirolimus was associated with increased dermal levels. Transdermal drug permeation was not observed. These findings indicate the need for tailored micelles, even for closely related drugs, as minor variations in drug properties, particularly aqueous solubility and lipophilicity, can affect dermatokinetic profiles.

The wide range of nanomaterials discussed in this section underscores their potential to overcome challenges associated with conventional dosage forms. The key outcomes from preclinical studies utilizing nanoparticulate systems to explore the dermatokinetic profiles, efficacy and safety of topical TAC, PIM and sirolimus in normal and inflamed skin models are summarized in Table II.

However, clinical translation still needs to be improved, as only a few studies have progressed to real-world settings. Notably, a recent clinical trial investigating a topically applied TAC-loaded ME on scalp psoriasis patients reported promising results, paving the way for future clinical applications (12).

4. Conclusions

Careful consideration of the administration route is critical in treatment decision-making. While recent drug development has primarily focused on biological and oral small-molecule therapies for managing AD and psoriasis, systemic use of immunosuppressants is often related to significant adverse events. Thus, topical modalities remain the mainstay treatment for the majority of cases with mild-to-moderate disease. Despite its limitations, the dermal application of anti-inflammatory compounds offers an easily accessible route that ensures localized efficacy while reducing the risk of systemic off-target side effects.

In this regard, topical calcineurin and mTOR inhibitors, such as TAC, PIM and sirolimus, provide more skin-selective therapeutic options compared with topical corticosteroids. TAC and PIM have long been associated with a minimal risk of skin atrophy and systemic absorption into the bloodstream, commonly seen with corticosteroid use. This offers a safer long-term approach, especially for sensitive skin areas, such as the facial, flexural and anogenital regions. Regarding newer macrolides, emerging evidence suggests that topical sirolimus may effectively target several immunological, cellular and molecular components of the inflammatory cascades involved in AD and psoriasis (82,86-92).

However, major challenges with conventional topical formulations of these agents include poor skin penetration and local irritation effects, particularly with TCIs, which can compromise therapeutic outcomes and patient adherence. In addition, formulating these lipophilic, poorly water-soluble and unstable compounds into patient-friendly water-based preparations remains difficult with traditional vehicles.

Thus, innovative strategies are needed to optimize skin penetration and enhance the efficacy and safety of these 'hard-to-formulate' macromolecules. In this respect, nanotherapeutics have emerged as suitable carriers to address the limitations of traditional topical dosage forms. Nanotechnology

Table II. Summary of preclinical studies utilizing nanosystems for the topical delivery of tacrolimus, pimecrolimus and sirolimus in normal and inflamed skin models.

A, Lipid-based nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Khan *et al*, 2022	SLN, CS-SLN	TAC	Normal	*In vitro* (Franz-cell setup) *Ex vivo* (murine)	Sustained	SLN ≈ SLN-gel	N/A	N/A	Sustained release from CS-coated SLNs and SLN-gel. Enhanced skin retention with SLN-gel.	(105)
Kang *et al*, 2019	Thermo-sensitive SLNs	TAC	Normal	*In vitro* (Franz-cell setup) *Ex vivo* (murine) *In vivo* (rabbit)	In Dermis	↑	N/A	Mild erythema	Higher skin deposition and penetration depth (300-450 μm) vs. marketed ointment (undetectable <150 μm).	(18)
Wang *et al*, 2012	SLN	TAC	Normal	*In vitro* (dialysis membrane) *Ex vivo* (murine)	Sustained	↑	N/A	N/A	Sustained release vs. conventional solution. Higher skin penetration and retention vs. commercial ointment.	(104)
Viegas *et al*, 2020	NLC	TAC TAC + siRNA	IQM-induced Pso	*In vitro* (dialysis membrane, murine fibroblasts) *Ex vivo* (porcine) *In vivo* (murine)	Sustained	↑	↑	Low cytotoxicity	Co-delivery of TAC and TNFα siRNA prevented psoriasis onset and achieved higher TNFα reduction (7-fold) vs. TAC-loaded NLCs (2.5-fold) and commercial TAC ointment (2-fold).	(109)
Andrade *et al*, 2017	CS-NLC	TAC + CP	Normal	*In vitro* (Franz-cell setup) *Ex vivo* (porcine)	Sustained	↑	N/A	N/A	Co-encapsulation of CP improved TAC skin penetration *ex vivo*.	(106)
Nam *et al*, 2011	NLC	TAC	Normal	*Ex vivo* (murine)	N/A	↑	N/A	N/A	Increased skin penetration and deposition vs. marketed product.	(110)
Patel *et al*, 2010	LP	TAC	DNFB-induced AD	*Ex vivo* (murine) *In vivo* (murine)	↑	↑	Similar to commercial ointment	N/A	Improved skin penetration and accumulation vs. free TAC *ex vivo*.	(112)
Erdogan *et al*, 2002	LP	TAC	OVA-induced DTH	*In vivo* (murine)	N/A	↑	↑	N/A	Superior TAC skin deposition and anti-AD efficacy vs. systemic vehicles.	(111)

Table II. Continued.

A, Lipid-based nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Li *et al*, 2012	ETH, LP	TAC	DNFB-induced AD	*Ex vivo* (murine) *In vivo* (murine)	N/A	↑	↑	N/A	Both vesicular systems enhanced TAC epidermal deposition. Anti-AD effect of ETHs > classic LPs > DXM cream > commercial TAC ointment.	(113, 114),
Ren *et al*, 2024	TFs	TAC	DNCB-induced AD	*In vitro* (dissolution apparatus, HaCaT, HDF) *Ex vivo* (murine) *In vivo* (murine)	Sustained	↑	↑	No cytotoxicity	Increased skin penetration depth, accumulation and retention of TAC with improved clinical and pathological AD features vs. traditional gel. Decreased total serum IgE.	(117)
Parkash *et al*, 2018	TFs, LP	TAC	DPDS-induced Pso	*Ex vivo* (murine) *In vivo* (murine)	N/A	↑	↑	N/A	TFs showed deeper and increased skin deposition and superior antipsoriatic effects vs. classic LPs.	(116)
Lei *et al*, 2013	TFs, LP	TAC	DNFB-induced AD	*Ex vivo* (murine) *In vivo* (murine)	↑	↑	↑	N/A	TFs showed superior skin penetration and AD control over classic LPs and conventional vehicles.	(115)
Thapa *et al*, 2013, 2014	LCNP	TAC	IQM-induced Pso	*In vitro* (dialysis membrane) *Ex vivo* (murine) *In vivo* (murine)	Controlled	↑	↑	N/A	Improved skin deposition and retention and superior anti-psoriatic effects vs. free TAC.	(118, 119)
Jain *et al*, 2019	LCNP, SLN, NLC, LP	TAC	Mouse-tail Pso	*In vitro* (dialysis membrane) *Ex vivo* (porcine) *In vivo* (murine)	LCNP: 34% SLN: 62% NLC: 65% LP: 69%	↑ LCPN: 2.5x; SLN: 2x; NLC: 2x	↑ NLC, SLN, LCNP	No irritation; ↓ TEWL	Improved skin bioavailability with LCPN, SLN and NLC. Penetration to deeper skin: LP < LCNP < NLC < SLN, with SLN and NLC penetrating deeper vs. marketed ointment. *In vivo* anti-psoriatic effects of NLC = SLN > LCNP > commercial ointment > LP.	(120)

Table II. Continued.

A, Lipid-based nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Pople *et al*, 2013	MNLC	TAC	DNFB-induced AD	*In vitro* (microfilters) *Ex vivo* (porcine) *In vivo* (murine)	N/A	↑	↑	No irritation + histological changes; ↓ TEWL	Similar occlusion/hydration effect and enhanced skin deposition and AD control vs. marketed ointment.	(124)
Pople *et al*, 2011	MNLC	TAC	Normal	*In vitro* (Franz-cell setup) *Ex vivo* (porcine) *In vivo* (murine, rabbit)	↑	↑	N/A	No irritation	Improved skin release, penetration and deposition vs. commercial formulation.	(123)
Pople *et al*, 2012	LN	TAC	DNFB-induced AD	*In vivo* (murine)	N/A	↑	↑	No histological changes	Higher skin penetration and retention to deeper skin vs. marketed ointment. Faster and superior control of AD vs. marketed ointment.	(122)
Pople *et al*, 2010	LN	TAC	Normal	*In vitro* (Franz-cell setup, microfilters) *Ex vivo* (porcine) *In vivo* (murine, rabbit)	↑	↑	N/A	No irritation	Improved skin release, penetration and deposition and similar occlusive and hydrating effects vs. marketed product.	(48)
Wang *et al*, 2019	ME	TAC	DNCB-induced AD	*In vitro* (HaCaT) *Ex vivo* (murine) *In vivo* (murine)	N/A	↑	↑	No cytotoxicity; ↓ TEWL	Enhanced skin retention and anti-AD efficacy vs. commercial ointment. Decreased serum IgE.	(129)
Savić *et al*, 2017	ME	TAC	Normal	*In vitro* (Franz-cell setup) *Ex vivo* (porcine) *In vivo* (human)	↑	↑	N/A	No irritation of blank MEs in humans	Higher TAC levels in deeper SC *ex vivo* vs. commercial ointment.	(128)
Lalan *et al*, 2012	ME	TAC	TNCB-induced AD	*In vitro* (dialysis membrane) *Ex vivo* (murine, human) *In vivo* (murine)	↑	↑	↑	No irritation + toxicity	Higher TAC skin penetration depth and retention and superior anti-AD effects vs. commercial ointment. Decreased epidermal thickness and inflammatory cytokines (IL-2/4/10) vs. commercial ointment.	(127)

Table II. Continued.

A, Lipid-based nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Goebel *et al*, 2011	ME	TAC	Normal	*Ex vivo* (human)	N/A	↑	N/A	No irritation + vascular effects	Increased accumulation in dermis vs. conventional TAC ointment.	(126)
Sahu *et al*, 2018	Kalonji oil-based NE	TAC	IQM-induced Pso	*In vitro* (dialysis membrane, A-431 epidermal cells) *Ex vivo* (porcine) *In vivo* (murine)	Sustained	↑	↑	N/A	Increased epidermal retention and stronger antiproliferative effects vs. free TAC. Greater antipsoriatic activity vs. marketed ointment. Reduced serum TNF-α and IL-6.	(130)
Savić *et al*, 2019	NLC, NE	TAC	Normal	*In vitro* (Franz-cell setup) *Ex vivo* (porcine)	↑ NLC > NE	↑	N/A	N/A	Improved SC deposition and follicular uptake with nanovehicles. NLCs were superior to NEs.	(32)
Jain *et al*, 2016	Lipospheres	TAC + CUR	IQM-induced Pso	*In vitro* (dialysis membrane) *In vivo* (murine)	Sustained	↑	↑	No toxicity	Improved psoriatic histology *in vivo*. Higher reduction of TNFα and IL-22.	(47)

B, Polymer-based nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Quartier *et al*, 2023	Micelles	TAC/PIM/SIR	Normal	*In vitro* (dialysis membrane) *Ex vivo* (porcine)	PIM < TAC/SIR	↑	N/A	N/A	Skin deposition: PIM > TAC/SIR. No transdermal permeation.	(54)
Le Guyader *et al*, 2022	Micelles	SIR	Normal	*Ex vivo* (human)	N/A	↑	N/A	N/A	Higher epidermal and similar dermal levels vs. conventional hydrogel.	(146)

Table II. Continued.

B, Polymer-based nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Quartier *et al*, 2021	Micelles	SIR	Normal	*Ex vivo* (porcine)	Sustained	↑	N/A	N/A	Enhanced skin deposition without percutaneous permeation vs. traditional ointment.	(145)
Lapteva *et al*, 2014	Micelles	TAC	Normal	*Ex vivo* (porcine, human)	N/A	↑	N/A	N/A	Improved skin accumulation without transdermal permeation.	(131)
Yamamoto *et al*, 2019	Micelles	TAC	IQM-induced Pso	*Ex vivo* (murine)	Sustained	↑	N/A	N/A	Time-dependent higher SC deposition vs. commercial ointment with steady-state concentrations in viable epidermis.	(132)
Gabriel *et al*, 2016	mPEG-hexPLA NPs	TAC	IQM-induced Pso	*In vivo* (murine)	N/A	↑	↑	No local/ systemic toxicity	Improved penetration in inflamed vs. normal skin. Higher accumulation in psoriatic skin vs. marketed ointment. Antipsoriatic efficacy superior to CP and similar to commercial ointment.	(133)
Zabihi *et al*, 2018	PLG NPs	TAC	3D AD skin model	*In vitro* (PHK, PHF, 3D reconstructed filaggrin-deficient human skin) *Ex vivo* (human)	N/A	↑	↑	No cytotoxicity	Higher skin deposition and greater TSLP reduction vs. marketed product.	(134)
Fereig *et al*, 2021	CS-NPs	TAC	IQM-induced Pso	*In vitro* (dialysis membrane, Franz-cell setup) *Ex vivo* (murine) *In vivo* (murine)	Sustained	↑	↑	N/A	Higher skin deposition and retention, decreased transdermal permeation and superior antipsoriatic activity vs. commercial ointment.	(107)
Yu *et al*, 2017	NIC-CS-NPs	TAC	DNCB-induced AD	*Ex vivo* (murine) *In vivo* (murine)	N/A	↑	↑	N/A	Greater skin accumulation and anti-AD activity vs. commercial ointment.	(108)

Table II. Continued.

B, Polymer-based nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Zhuo *et al* 2018	HA-CS-NPs	TAC	DNFB-induced AD	*In vitro* (Franz-cell setup) *Ex vivo* (murine) *In vivo* (murine)	Controlled + Sustained	↑	↑	↓ TEWL	HA coating enabled controlled and sustained release, improved skin deposition and retention and superior anti-AD effects vs. uncoated CS-NPs.	(135)
Wan *et al*, 2017	NIC-HA-Chol NPs	TAC	IQM-induced Pso	*In vitro* (murine macrophage cells, HaCaT) *Ex vivo* (murine) *In vivo* (murine)	N/A	↑	↑	N/A	Enhanced permeation and retention in psoriatic skin vs. marketed ointment. Antipsoriatic efficacy similar to CP and superior to commercial TAC ointment.	(137)
Pan *et al*, 2016	NIC-HA-Chol NPs	TAC	Normal	*In vitro* (HaCaT) *Ex vivo* (murine) *In vivo* (murine)	N/A	↑	N/A	N/A	Higher skin penetration depth and deposition vs. marketed product.	(136)
Radbruch *et al*, 2022	Polymeric CMS	TAC	OXA-induced AD	*In vivo* (murine)	N/A	↓	Similar to commercial ointment	No systemic toxicity	Reduced skin deposition and transdermal permeation and similar anti-AD efficacy vs. marketed ointment.	(138)
Rancan *et al*, 2021, 2023	Redox-responsive CMS	SIR	Inflamed skin/T-cell set-up	*Ex vivo* (human skin/T-cell co-culture)	N/A	↑	N/A	N/A	Efficient skin penetration. Suppression of T-cell proliferation. Decreased IL-2 and IL-17A; no effect on IL-1α, IL-6, IL-8. Targeting of DCs. Reduced mTOR activity (↓ Rps6).	(91, 92)
Rancan *et al*, 2019	Thermo-sensitive nanogel	TAC	Barrier-disrupted skin	*Ex vivo* (human skin/T-cell co-culture)	Controlled	↓	N/A	↑ IL-6/8	Decreased skin accumulation and similar antiproliferative effect on T-cells vs. marketed formulation.	(140)
Limon *et al*, 2019	Nano-hydrogel	TAC	TPA-induced Pso	*In vitro* (Franz-cell setup) *Ex vivo* (human) *In vivo* (murine)	Controlled	↑	↑	No local desquamation or hair loss	TAC retention in epidermis and upper dermis. Superior anti-psoriatic efficacy and safety vs. free TAC.	(141)

Table II. Continued.

C, Inorganic nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Parekh *et al*, 2021	MSN	TAC	DNFB-induced AD	*In vitro* (Franz-cell setup, HaCaT) *Ex vivo* (murine) *In vivo* (murine)	↑	↑	↑	No cytotoxicity	Higher skin deposition *ex vivo* and improved clinical and pathological AD features *in vivo* vs. free TAC.	(142)

D, Hybrid nanosystems

			Experimental set-up		Results					
Authors, year	Delivery system	Loaded drug	Skin condition	Skin model: Type (species/method)	Release	Penetration	Therapeutic efficacy	Safety	Key findings	(Refs.)
Wan *et al*, 2017	Polymer-based ME	TAC	IQM-induced Pso	*In vitro* (HaCaT) *Ex vivo* (murine) *In vivo* (murine)	N/A	↑	↑	N/A	Improved skin deposition vs. commercial ointment. Antipsoriatic efficacy similar to CP and greater than commercial TAC ointment.	(143)
Shams *et al*, 2021	ME-Nanofibers	TAC	DNCB-induced AD	*In vitro* (dialysis membrane) *In vivo* (murine)	Sustained	N/A	Similar to commercial ointment	N/A	Every 2-day use was equally effective as daily commercial ointment in improving AD histology.	(144)

3D, three-dimensional; AD, atopic dermatitis; Chol, cholesterol; CMS, core-multishell nanocarriers; CP, clobetasol propionate; CS, chitosan; CUR, curcumin; DCs, dendritic cells; DNCB, 1-chloro-2, 4-obenzene; DNFB, 2,4-dinitroflourobenzene; DPDS, dipropyl disulfide; DXM, dexamethasone; ETH, ethosome; HA, hyaluronic acid; HaCaT, immortalized human keratinocytes; HDF, human dermal fibroblasts; IgE, immunoglobulin E; IL, interleukin; IQM, imiquimod; LCNP, lipid-based liquid crystalline nanoparticles; LN, lipid nanoparticles; LP, liposomes; MCE, menthol/camphor eutectic; ME, microemulsion; MNLC, modified nanolipid carrier; mPEG-hexPLA, methoxy-poly(ethylene-glycol)-hexyl substituted polylactide; MSN, mesoporous silica nanoparticles; mTOR, mammalian target of rapamycin; N/A, not applicable; NE, nanoemulsion; NIC, nicotinamide; NLC, nanostructured lipid carrier; NP, nanoparticle; OVA, ovalbumin; OXA, oxazolone; PHF, primary human fibroblasts; PHK, primary human keratinocytes; PIM, pimecrolimus; PLG, poly(lactide-co-glycerol); Pso, psoriasis; Ref, reference; Rps6, ribosomal protein S6; SC, stratum corneum; SIR, sirolimus; siRNA, small interfering RNA; SLN, solid lipid nanoparticles; TAC, tacrolimus; TEWL, transepidermal water loss; TFs, transfersomes; TNCB, 2,4,6-trinitrochlorobenzene; TNFα, tumor necrosis factor-α; TPA, 12-O-tetradecanoylphorbol 13-acetate; TPGS, tocopheryl polyethylene glycol 1000 succinate; TSLP, thymic stromal lymphopoietin; vs, versus.

offers numerous advantages over conventional vehicles, such as modified solubility, greater drug stability, protection from degradation, encapsulation of hydrophobic payloads into hydrophilic carriers and improved therapeutic outcomes resulting from enhanced skin absorption, targeted delivery, controlled or sustained release and retention at the action sites without undesirable or toxic effects.

Based on the literature reviewed, several topically applied nanovectors could effectively deliver TAC in both psoriatic and atopic viable skin, facilitating controlled and/or sustained release with minimal risk of local and off-target side effects. The selective accumulation of specific nanovehicles in the upper epidermis, particularly in the SC, requires special consideration as it could serve as a reservoir from which the dermally applied drug can be gradually released over prolonged periods, penetrating deeper layers of viable skin. This localized retention may also offer additional benefits for the precise targeting of psoriatic and atopic skin, where the epidermal dysfunction appears to play a crucial role in disease initiation and progression.

To maximize therapeutic outcomes, combined or hybrid nanosystems that incorporate multiple therapeutically relevant molecules or different types of nanoparticles could provide multi-target or synergistic effects on various aspects of the inflamed skin microenvironment. For instance, incorporating CS films in nanoformulations offers antimicrobial properties, which are particularly beneficial in skin disorders characterized by microbiome dysbiosis, such as AD. In addition, the combination of nanocarriers with gene therapy for the co-delivery of TAC and siRNA opens new prospects for precise post-transcriptional gene regulation and targeted silencing of disease-specific inflammatory pathways.

While preclinical evidence on nanoparticle-based topical delivery of sirolimus in inflamed skin is still limited, initial findings suggest that nanocarriers could enhance its intradermal penetration and provide foundational data for further investigation of its potential broad effects. However, a number of questions remain regarding the development and optimization of topical sirolimus formulations. Whether topical mTORIs, especially formulated in nanovehicles, could offer a viable steroid-free alternative for AD or psoriasis patients has yet to be determined.

Despite the potential benefits, nanomedicine-based skin delivery of topical calcineurin and mTOR inhibitors largely remains in its preclinical phases, mainly due to the lack of suitable experimental models that closely mimic the *in vivo* complex features of the inflamed human skin. *Ex vivo* studies are often conducted on excised human or porcine skin, where barrier properties may be altered, while *in vivo* studies frequently use murine skin, which does not closely resemble human skin.

In conclusion, as the landscape of anti-inflammatory therapies continues to evolve, ongoing research into optimizing current modalities and developing novel formulations remains essential. Preclinical proof-of-concept studies are important for developing effective drug- and disease-specific nanotherapeutics. In this context, a deeper understanding of disease biology and *in vitro-in vivo* correlations is crucial to overcome translational barriers. Further research on *ex vivo* psoriatic or atopic human skin is needed to refine preclinical models and utilize translational evidence to advance nanomaterials designed for immunomodulatory macrolactams in real-world settings. This may ultimately enrich and optimize the topical management of inflammatory skin diseases in our clinical practice.

Acknowledgements

The authors would like to thank Professor Diomedes Logothetis (Northeastern University, Boston, MA, USA) for his insightful feedback and guidance on the present study.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

ND and PS conceived the present study. PS performed the literature search, designed the figures and wrote the original draft of the manuscript. MK, MS and MP helped to revise the manuscript. PS reviewed and edited the final version of the manuscript under the supervision of ND. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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