How can nanoparticle-based technologies revolutionize the topical therapy in psoriasis?

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Abstract
Psoriasis is one of the most common dermatoses with a heterogeneous pathogenesis which can be successfully exploited therapeutically as it is increasingly well understood. Topical therapy is the gold standard for psoriasis patients with mild disease courses and for complementary and maintenance treatment in moderate and severe forms. However, while new systemic therapies are rapidly implemented in the daily routine as our pathomechanistic understanding of psoriasis evolves, the development of topical psoriasis therapies stagnates. Modern topical treatments though would require not only new active substances but also improved galenics. Due to their unique ability to directly exert biological functions, but also to deliver drugs in optimal concentrations, enabling increased therapeutic efficacy, reduced adverse effects and improved patient compliance, nanoparticles may represent ideal drug carriers for local therapeutics in psoriasis. In recent years, a series of reports added important insights into the biology of skin-nanoparticles interactions and on how they impact the epidermal and dermal inflammatory compartments in vitro and in psoriasis plaques. Furthermore, by targeting anti-inflammatory substances to specific skin compartments, nanotechnological advances offer the exciting opportunity to fine-tune skin inflammation at molecular and cellular levels, paving the road to a high-precision, skin-directed topical therapy in psoriasis. However, nanoparticle-based therapies have not yet found their way into clinical routine in dermatology. We here resume the current advances in the research of nanoparticles and skin inflammation in general and psoriasis in particular and discuss how this promising technology should develop in order to fulfil the requirements of an optimal skin therapy.

Keywords
green technology, inflammation models, nanomaterials, pro-inflammatory cytokines, skin-directed treatment
Psoriasis is a genetically determined model disease for cutaneous and systemic inflammatory conditions driven by T cell–mediated inflammation. With a prevalence of 1%-3%, psoriasis is one of the most frequent skin conditions occurring in all age groups. It frequently associates systemic comorbidities with cardiovascular, respiratory and metabolic dysregulations as well as high psychosocial burden, and profoundly impairs quality of life for most patients, independent of disease severity.\(^1\text{–}4\) The psoriatic plaque is characterized by keratinocyte hyperproliferation and dermal inflammatory infiltration with dendritic cells, macrophages, T-lymphocytes and neutrophils. Pro-inflammatory molecules of the TNFα-IL-12-IL-23 axis released by autoreactive Th1 and Th17 lymphocytes and activation of intracellular pro-inflammatory signalling pathways (NFκB, STAT, MAPK/ERK) in T cells and M1-activated macrophages are crucial mediators of psoriasis pathogenesis and, therefore, first-line targets of effective therapeutic strategies.\(^7\) While a series of antibody-based biological and non-biological systemic strategies have developed to control psoriatic inflammation, this new pathogenetic knowledge has only been used to a little extent to advance topical therapeutic approaches which are still limited to corticosteroids, vitamin-D derivatives and calcineurin inhibitors. However, skin-directed treatment remains indispensable for large patient categories. 80% of psoriasis patients have mild-to-moderate disease and may be controlled with local therapy only.\(^8\) Moreover, for children, pregnant women, elderly or immunosuppressed patients, systemic treatments implicate unacceptable risks; particularly in life-changing circumstances such as the present SARS-COV-19 pandemic, patients’ acceptance for systemic immunosuppressants considerably decreases.\(^9,10\) Furthermore, effective topical therapies are urgently needed for difficult-to-treat areas such as scalp, nails, intertriginous and palmoplantar regions.\(^11\)

Thus, development of new topical therapies enabling the efficient delivery of drugs to psoriatic plaques and minimizing systemic side effects, thereby increasing safety and compliance, is a still unmet need in the management of psoriasis. Modern topical treatments though would require not only new, effective active substances but also improved galenic formulations.

Nanoparticles (NPs)-based technologies are most promising approaches to create modern, efficient and safe disease-specific topical drugs. Compared to conventional therapies, NPs exert their own biological functions and can effectively deliver drugs in close proximity or directly to target cells, increasing the therapeutic benefit while reducing side effects. Furthermore, NPs can improve the drug bioavailability by protecting them from biodegradation, increasing their stability and solubility even for hydrophobic substances, and enable controlled drug-release into the skin at desired concentrations.\(^12,13\) Altogether, NPs-based therapeutic approaches may overcome the current limitations of conventional antipsoriatic drug formulations and improve their therapeutic potential while targeting key pathogenetic molecules. This targeted drug delivery would help psoriasis patients to achieve better therapeutic results by enabling a higher specificity with less adverse effects, an improved drug biodistribution and longer lifetime resulting in less frequent applications, and, most importantly, a higher therapeutic index with enhanced efficacy and increased tolerability.

In spite of convincing evidence for the efficacy and safety of NPs-based skin-directed therapeutics, their breakthrough in the clinic did not occur so far. We and others previously asked if and how NPs could be employed to translate current pathogenetic knowledge into modern topical therapeutics in psoriasis and other inflammatory dermatoses.\(^14\text{–}17\) Here, we aimed at discussing most recent insights on NPs-based technologies regarding modulation of skin inflammation, in order to identify which critical issues remain to be addressed.

2 | TOPICAL NPS COMPLEXED WITH POLYPHENOLS SUPPRESS INFLAMMATION IN HUMAN PSORIASIS PLAQUES

The anti-inflammatory role of silver and gold NPs (Ag-NPs, Au-NPs) functionalized with polyphenol-rich extracts was previously demonstrated in keratinocytes in vitro and in several models of inflammation, including psoriasis.\(^17,18\) David et al reported for the first time that Ag-NPs conditioned with European black elderberry significantly decreased pro-inflammatory IL-1α and IL-6 release by HaCaT keratinocytes exposed to UVB, as well as the levels of IL-1α, IL-1β and IL-6 cytokines in carrageenan-induced rat model of acute paw inflammation. Furthermore, topical treatment of human psoriasis plaques also resulted in a significant clinical improvement of the lesions, as assessed by ultrasound, confirming the reduction of psoriasis plaque thickness in NPs-treated plaques when compared to cortisone.\(^19\) Later on, application of Au-NPs conditioned with polyphenolic compounds of Cornus mas (CM) in HAcat and A431 cell lines showed low toxicity, producing low amounts of reactive oxygen species (ROS) and virtually no DNA damage; following UVB radiation, NPs application displayed an anti-inflammatory effect at the cellular level, modulating the secretion of specific cytokines, as confirmed by the decrease in TNFα.\(^20\)

Besides keratinocytes, the sustained T cell–mediated dermal inflammation is causal in the pathogenesis of psoriasis. We and others have previously shown that macrophages essentially drive inflammation in psoriatic skin in animal models and humans.\(^21\text{–}26\) They perpetuate inflammation in psoriatic plaques by activating inflammatory signalling pathways NFκB, MAPK, STAT, releasing pro-inflammatory cytokines such as TNFα and IL-12 and growth factors, which in consequence promote neoangiogenesis, keratinocyte proliferation and epidermal thickening. Therefore, direct targeting of activated macrophages in psoriatic plaques may represent a promising therapeutic approach.

Recently, we identified so far unreported features of Ag-NPs-CM and Au-NPs-CM, showing that they specifically and selectively inhibited pro-inflammatory-activated M1 macrophages in vitro and in human psoriatic plaques. Clinical and ultrasonographic examinations of psoriatic lesions revealed an almost complete
depletion of the inflammatory infiltrate after 6 weeks of daily topical treatment with NPs-based ointments. To investigate whether macrophages were directly targeted and specifically depleted by NPs in this setting, we performed in vitro co-incubation experiments which clearly demonstrated an inhibitory effect of NPs on nitric oxide (NO), TNFα and IL-12 release by pro-inflammatory activated murine macrophages. Immunofluorescence studies on human psoriatic skin before and after treatment revealed that Au-NPs-CM and Ag-NPs-CM integrated in ointments significantly reduced the numbers of CD68-positive infiltrating macrophages and their release of IL-12 and TNFα after 6 days of daily application. Moreover, NPs significantly reduced the numbers of CD68-positive activated macrophages expressing phosphorylated IκBα. These data suggest a novel inhibitory effect of Au-NPs-CM and Ag-NPs-CM on NFKB activation in macrophages and on their production of pro-inflammatory cytokines and imply a causal role in the pathogenesis of psoriasis. Accordingly, treatment with NPs-CM resulted in a significant reduction of erythema and scaling of psoriatic lesions.34

Our study added an important mechanistic insight into the impact of NPs complexed with polyphenolic compounds on psoriatic inflammation, showing for the first time that locally applied NPs exert complex anti-inflammatory effects not only on keratinocytes but also on macrophages in psoriatic lesions, most likely enforced by direct NFKB inhibition in activated macrophages. Based on these findings, it is reasonable to presume that NPs can be used as versatile modifiers of inflammation and disease activity by targeted delivery of anti-inflammatory natural or synthetic compounds to key cellular compartments in the skin, while diminishing systemic side effects.

3 | NANOPARTICLE-BASED TECHNOLOGIES IN SKIN INFLAMMATION—A RAPIDLY GROWING FIELD OF RESEARCH

There have been several exciting developments in NPs-based anti-inflammatory strategies in recent years. Great efforts have been made to conjugate NPs with other known anti-inflammatory or immunosuppressive substances including vitamin-D derivatives, methotrexate or calcineurin inhibitors such as cyclosporine and tacrolimus for topical application.

A comparative study of different nanoformulations with the calcineurin inhibitor tacrolimus showed a significantly increased entrapment into HaCaT keratinocytes, and a higher skin penetration and increased dermal bioavailability of tacrolimus for all formulations when compared to control marketed cream formulation, in a mouse model of toxic contact dermatitis.30 Even dithranol which is less and less used by dermatologists due to its toxic dermatitis reactions and staining of clothes could be successfully loaded in a liposomal gel, showing a significant decrease in disease activity by PASI score and led to profound reduction of IL-17, IL-22 and IL-23 cytokine levels in skin homogenates of imiquimod-treated psoriasis mice.31

Another novel approach consists in loading NPs with known systemic immunosuppressive agents. As opposed to NPs alone, methotrexate-loaded Au-NPs administered topically significantly reduced keratinocyte proliferation, epidermal thickness and infiltration with cytotoxic CD8 lymphocytes in the skin of the imiquimod-induced psoriasis-like mouse model.32 In the same mouse model, a gel formulation of cyclosporine conjugated to liposomal nanocarriers significantly improved erythema and scaling as assessed by a modified PASI score and histology, and decreased TNFα, IL-17 and IL-22 levels in lesional skin homogenates.33 Likewise, loading the PDE4-inhibitor apremilast in nanocapsules significantly increased its bioavailability facilitating long-term retention in in vitro release studies and in vivo pharmacokinetic evaluations in rats.34 Taken together, these recently published approaches suggest that nanoparticles can be easily engineered to deliver novel psoriasis therapies with high precision to psoriatic skin lesions instead of using systemic application.

Even though metal NPs alone exhibit significant anti-inflammatory properties, they potentially exert toxic effects, especially via ROS production, DNA damage and cellular apoptosis.35 Currently, the “green” method of nanomaterial synthesis by conditioning NPs with natural materials such as polyphenolic preparations, exploits the reductive capacity of herbal compounds to diminish toxicity of NPs, thus making them stable and safe for biomedical use.36 However, NPs produced with plant extracts by “green” synthesis have only been tested in a small number of disease models so far.

Just recently, application of ethanolic extracts of Woodfordia fruticosa flowers complexed with Au-NPs in a 1% ointment, resulted in significantly decreased keratinocyte proliferation rate, parakeratosis and epidermal thickness in the imiquimod-induced psoriasis-like model. Remarkably, NPs also reduced the serum levels of pro-inflammatory TNF-α, IL-22 and IL-23 suggesting a systemic effect of topically applied NPs, most probably secondary to the suppression of skin inflammation.37

Psoralen-containing babchi oil extracted from Psoralea corylifolia, incorporated into a nanogel applied topically, decreased hyperkeratosis in a mouse tail psoriasis-like model. Histopathological and pharmacologic assessment revealed an antioxidant effect of the babchi oil-nanogel which increased the expression of the antioxidant superoxide dismutase (SOD) and reduced the oxidation stress markers like malondialdehyde and NO.38 A new topical formulation based on celastrol, a triterpenoid extracted from Tripterygium loaded onto niosomes also showed a significant clinical improvement of erythema and scaling on the dorsal skin of an imiquimod-induced psoriasis-like mouse model as well as a significant decrease of IL-22, IL-23 and IL-17 levels.39 In another interesting approach, curcumin-loaded chitosan/alginates NPs and blue LED light irradiation synergistically suppressed HaCaT cell proliferation in a TNFα-induced psoriasis-like keratinocyte proliferation model. This experimental in vitro setting provides an innovative approach combining nanotechnology and photodynamic therapy for the treatment of psoriatic lesions.40

Employing other inflammation models, Baldea et al investigated NPs-CM effects on human gingival fibroblasts and human dysplastic oral keratinocyte cell lines. Of note, NPs-CM exerted a selective toxic...
effect on dysplastic cells and induced cell death by activation of p53/ BAX/BCL2 apoptotic signalling pathway and inhibition of the PI3K/ AKT survival pathway. Moreover, NPs-CM significantly reduced lipid peroxidation as assessed by malondialdehyde concentrations, and TNFα levels in dysplastic oral keratinocytes proving antioxidant and anti-inflammatory effects with possible therapeutic application in oral epithelial dysplasia.[41] The group further investigated the antioxidant and anti-inflammatory effects of Ag-NPs complexed with extracts from Cornus sanguinea (CS) in vivo in the carrageenan-induced acute inflammation rat model. Ag-NPs-CS significantly reduced COX-2 expression and oxidative stress levels while increasing the antioxidant defense and inhibiting NFκB activation.[42] Interestingly, in the same model, kinetic analyses unravelled an early antioxidant function of topically applied Ag-NPs-CM and Au-NPs-CM followed by late anti-inflammatory and apoptotic effects, most probably driven by activation of the MAP/ERK pathway.[43]

4 | NEW NANOTECHNOLOGY APPROACHES OF INTEREST FOR SKIN-DIRECTED THERAPY

An important step forward was achieved by the development of PEGylated nanoparticles containing the vitamin-D derivative calcitriol modified with antibodies against the macrophage-specific receptor CD163 to directly target calcitriol to macrophages. The group reported that the in vitro uptake of CD163-bound calcitriol-NPs-complexes by human and murine macrophages was specific and significantly higher when compared to control calcitriol. NPs-bound calcitriol diminished mRNA expression levels of TNFα, NF-κB, MCP-1 and IL-6, while upregulating the expression of anti-inflammatory IL-10. The enhanced TNFα release by LPS-activated macrophages was significantly reduced in the presence of NPs-calcitriol compared to the control free calcitriol. When administered intravenously, the CD163-expressing PEGylated NPs specifically accumulated in splenic and hepatic macrophages, suggesting this technology as a highly attractive candidate for targeting skin inflammatory macrophages as well.[43]

The use of NPs for cell-specific gene manipulation constitutes a novel development of potential clinical relevance. Intravenous injection of NPs loaded with miRNA-21 in a mouse model of experimental myocardial infarction switched the pro-inflammatory, TNFα-producing M1 macrophages with unrestrained activation in the infarct zone towards wound-healing macrophages which, in consequence, promoted angiogenesis, decreased fibrosis and apoptosis while improving cardiac healing.[44]

In a similar approach, intravenously injected lipid-NPs were loaded with modified IL-10 mRNA and coated with anti-Ly6G antibodies to target myeloid cells. Indeed, they specifically targeted inflammatory leukocytes in the gut mucosa and expressed IL-10 to dampen inflammation in a dextran-sulphate colitis mouse model.[45] This model could apply for many other models of autoimmune diseases including psoriasis.

5 | OPEN QUESTIONS IN THE FIELD OF NANOTECHNOLOGY IN MEDICINE

In the last decade, cumulated evidence suggests that the interface between nanomaterials and living systems is very complex, as nanoparticles interact with cells at multiple levels and may induce toxic, immunogenic or even teratogenic responses. Understanding these multifaceted interactions and learning to control the side effects are essential for the future development of biomedical applications in nanotechnology.[46-48] Several studies unravelled that size, shape, concentration, agglomeration or aggregation essentially contribute to the toxicity of NPs.[49-51] Therefore, fine-tuning of these factors will be critical for the production of future NPs.

Skin penetration studies showed that the highest amount of topically applied solid NPs is restricted to the stratum corneum. However, especially at sites of skin barrier dysfunction as occurring in psoriasis or atopic inflammation, NPs may penetrate to and enrich in deeper epidermal layers at biologically relevant concentrations.[52-54] Furthermore, there is convincing evidence that skin penetration critically depends on NPs size. Accordingly, particles smaller than 4 nm can penetrate the intact skin, those between 4 and 20 nm potentially permeate intact and damaged skin, NPs between 21 and 40 nm only permeate damaged skin while NPs larger than 45 nm apparently cannot permeate skin at all.[55] The extent of aggregation of NPs in the tissue represents another independent risk factor for toxicity. The denser NPs aggregates, the easier they penetrate into cells and their subcellular compartments including mitochondria and nuclei.[56] NPs can accumulate in mitochondrial membranes, where they induce oxidative stress, activate apoptosis and cellular death pathways.[49,57] In combination with hydrogen peroxide, metallic NPs may form toxic hydroxyl radicals which induce irreversible protein, lipid and nuclear DNA damage eventually leading to cell death.[58,59]

There is only scant information on the interaction of nanoparticles with the different target cells such as epithelial cells, immune cells or stem cells for therapeutic purposes. Advancing the better understanding of binding, internalization and processing of the nanoparticles in these cells is mandatory to employ nanodevices for successful drug delivery with high cellular precision, thus protecting the surrounding microenvironment from side effects.[56,60]

The current lack of knowledge on interactions of NPs with cells and cellular compartments along with their environmental toxicity decisively limits the implementation of NPs in clinical routine.

The generation of NPs using green techniques to overcome toxicity is currently a very attractive and rapidly developing research field. As previously shown, the green method of NPs synthesis is endowed with the potential to reduce NPs toxicity by conditioning them with plant metabolites, natural substances or microorganisms. Further nanomolecular formulations such as nanopolymers or lipid-NPs hold great promise for biodegradable non-toxic and eco-friendly alternatives.[56] However, data on long-term toxicity profiles and their impact on biological systems still have to be generated to successfully translate these nanotherapeutics into a meaningful clinical context.[62]
A comprehensive understanding of how nanoparticles act in cutaneous inflammation at molecular and cellular mechanistic level is essential to forward the implementation of nanotechnologies in inflammatory skin diseases and particularly in psoriatic inflammation. Employment of NPs-based therapies in the currently available, very well-characterized animal models which address distinctive pathogenesis-relevant genetic, cellular and immunologic aspects of psoriasis represents an important strategy to provide some missing answers in the near future.

As summarized here, NPs have anti-inflammatory properties and ameliorate psoriatic features in human skin and the imiquimod-induced mouse model mainly by inhibiting the pro-inflammatory NFκB pathway. As this pathway is activated in both epidermal and dermal inflammatory compartments in psoriasis, it is not clear which cellular compartment is required or sufficient to be targeted by NPs for inflammation clearance. This could be addressed in experiments with application of NPs in transgenic mice with epidermis-specific NFκB activation, with psoriasis-like inflammation such as transgenic epidermal TGFβ overexpressing mice, mice with constitutive activation of Stat3 in keratinocytes, or the imiquimod-induced psoriasis murine model with keratinocyte-specific deletion of IL-17RA.[63-66]

A highly interesting model to study the effects of NPs in different cell compartments represents the CD18 hypomorphic PL/J mice with a psoriasis phenotype driven by high numbers of TNFα-producing macrophages as previously described by our group.[21] Either depletion of macrophages or T-lymphocytes, but not of other inflammatory cells in this murine model resulted in a fundamental improvement of the psoriasis phenotype. This model may serve to elucidate the effect of topically applied NPs on these two causal cell populations and by assessing disease severity. Using fluorescent NPs would even allow for tracking NPs in the tissue.

In a complementary approach, NPs coated with skin-homing receptors, adhesion molecules or with antibodies against cell-specific markers could be engineered by novel nanotechnologies as newly described for anti-Ly6G-coated NPs to target gut macrophages in the aforementioned experimental colitis model.[46] Virtually, every skin and cellular compartment could be specifically targeted with NPs and separately analysed, thus eliminating the biasing “off-target” effect induced by unspecific, innate immune activation.

CONFLICTS OF INTEREST
None.

AUTHOR CONTRIBUTIONS
AS and DC performed research work, designed the research study and wrote the manuscript. AF and K. S-K. performed research work. AS and DC performed research work, designed the research study and wrote the manuscript. All authors have read and approved the final manuscript.

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