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Drug Delivery

Nanocarriers for Skin Applications: Where Do We Stand?

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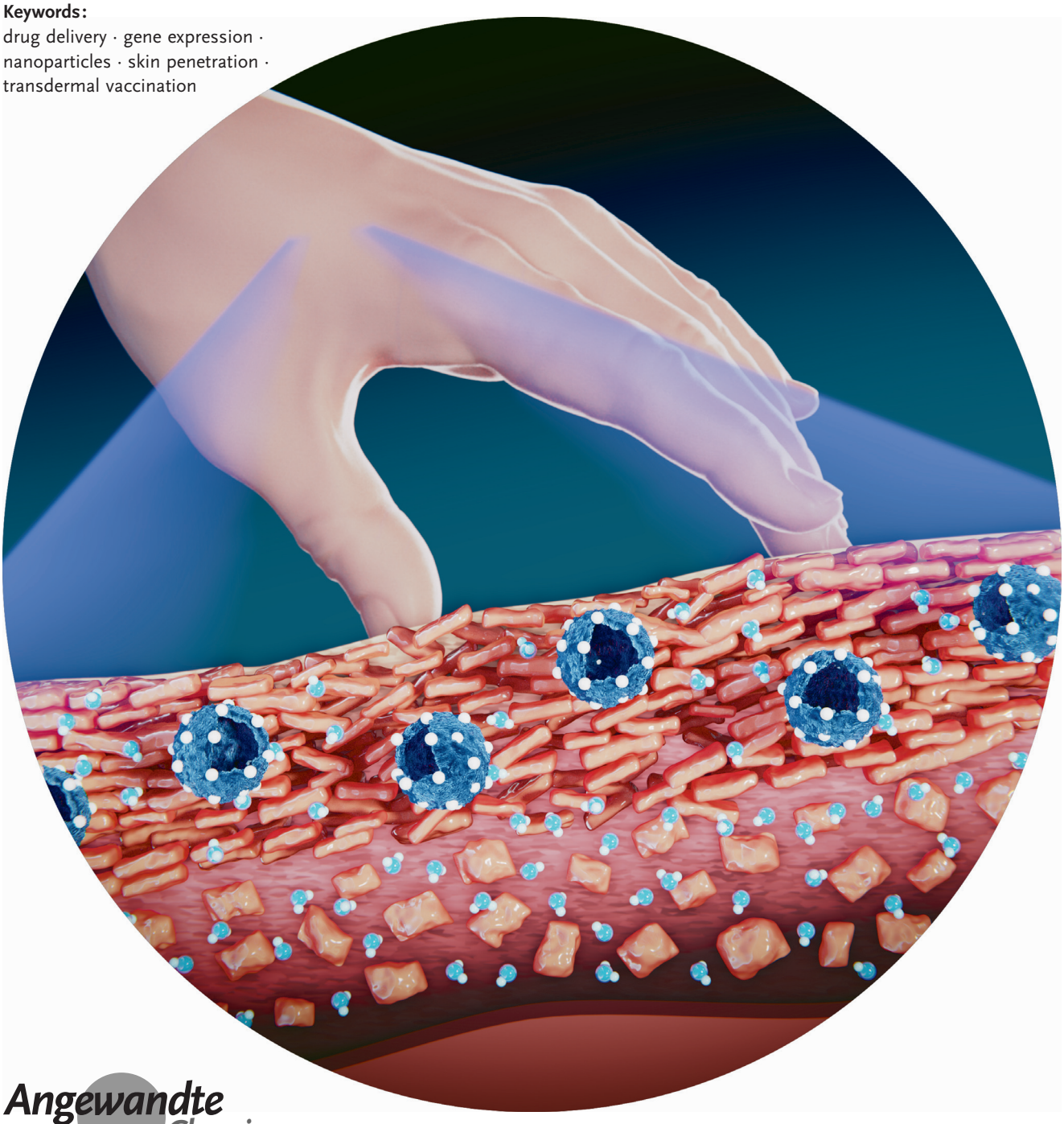

Skin penetration of active molecules for treatment of diverse diseases is a major field of research owing to the advantages associated with the skin like easy accessibility, reduced systemic-derived side effects, and increased therapeutic efficacy. Despite these advantages, dermal drug delivery is generally challenging due to the low skin permeability of therapeutics. Although various methods have been developed to improve skin penetration and permeation of therapeutics, they are usually aggressive and could lead to irreversible damage to the stratum corneum. Nanosized carrier systems represent an alternative approach for current technologies, with minimal damage to the natural barrier function of skin. In this Review, the use of nanoparticles to deliver drug molecules, genetic material, and vaccines into the skin is discussed. In addition, nanotoxicology studies and the recent clinical development of nanoparticles are highlighted to shed light on their potential to undergo market translation.

From the Contents

1. Introduction

The skin is the largest organ of the body fulfilling a wide variety of functions that includes regulating the body temperature, impeding the loss of fluids and salts, and preventing the intrusion of pathogens like viruses, bacteria, allergens, and toxic chemicals.[1] It also offers protection from ultraviolet radiation and particulate materials present in the external environment, acting as a complex physical barrier.[2] The skin is primarily composed of two different layers: epidermis and dermis (Figure 1). The stratum corneum (SC), the outermost layer of the epidermis, acts as a barrier, hindering the penetration of molecules that are typically larger than 500 Da.[3] It mainly comprises corneocytes with surrounding hydrophobic lipid layers (roughly 10–20 μm thick). Another part of the epidermis is the viable epidermis, which is localized beneath the SC and is mainly composed of keratinocytes along with melanocytes, Merkel cells, and Langerhans cells, with a thickness of 50–100 μm.[4] This is the first viable tissue layer of the skin wherein most of the dermatological disorders persist. An important process occurring in the viable epidermis is the production of keratinocytes (in the deepest layer of the viable epidermis, the stratum basale), which during their migration towards the surface of the skin undergo different biological modifications including increasing keratinization.[5] The other layer of the skin is the dermis, which is the largest part of the skin and is located beneath the epidermis. This layer consists of lymphatic vessels, collagen, elastin, sebaceous glands, sensory nerves, and hair follicles and has a thickness of approximately 0.1–0.4 cm. The main role of the dermis layer is to provide nutritional support to the viable epidermis as well as to act as structural support to the skin.[4] Owing to its remarkable barrier properties, the skin is usually seen as one of the most complex and hard-to-overcome barriers in the human body. This feature arises mainly from densely stacked corneocytes packed within the extracellular lipid matrix in SC. The corneal layer consists of keratin-rich lipoprotein envelopes with

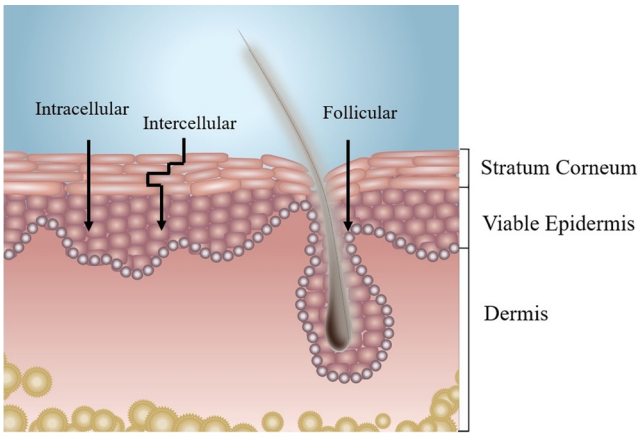

Figure 1. Simplified schematic illustration of the skin, the skin subdivisions, and the three main penetration pathways, i.e., intracellular, intercellular, and follicular.

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hydrophilic regions in between. As a result, the SC barrier allows penetration of lipid-soluble molecules more easily than water-soluble molecules; however, highly lipophilic compounds are hindered by the hydrophilic bilayer region. In the case of water-soluble molecules, the penetration takes place mostly via hair follicles and sweat glands, which constitute 0.1 % and 0.01 %, respectively, of the total skin surface.[3] In addition, the epidermal barrier properties also depend on the various biophysical properties like sebum production, epidermis hydration, pH gradient between the skin and inside of the body, and transepidermal loss of water.[1a] These factors are highly influenced by environmental and individual factors including age, sex, hormonal balance, anatomical area of the skin, and humidity among others.[6]

Although challenging, the skin has attracted a lot of attention for the delivery of a wide spectrum of therapeutics to treat multiple diseases including genetic disorders, infections by pathogens, inflammatory diseases, and skin melanoma.[7] In addition to minimizing pain, delivery of therapeutics via the skin provides multiple advantages over oral and intravenous delivery like low or localized side effects, elimination of the risk of drug digestion in the gastrointestinal tract, prevention of first-pass metabolism, enzymatic degradation, and clearance from the blood circulation.[8] Much effort has been invested in finding methodologies and systems that allow effective treatment via the skin. Among all evaluated systems, in this Review we will focus on the performance of nanoparticles (NPs) in skin applications. Special focus will be given on the role of NPs towards hydration, drug and gene delivery, as well as vaccination. The Review will address the 1) fundamentals of molecular transport across the different skin layers, 2) current stage of NPs development in skin penetration, and 3) challenges for clinical translation of existing NPs in various skin applications.

2. Penetration Pathways

The SC acts as the main barrier towards the permeation of molecules and micro/nanoparticles across the skin. However, permeation of the smallest particles is possible via one of three main pathways: intracellular, intercellular, and follicular (Figure 1).[8] The intracellular route is the most direct and fast pathway for a substance to permeate the skin; however, this pathway is challenging as the particle must overcome both the lipophilic (cell membrane and the lipid matrix) and lipophobic structures (inside the cells) within the skin cells.[9] Nanocarriers with a certain degree of amphiphilicity might be good candidates to circumvent this hurdle. The intercellular route is the most common pathway, wherein the penetrating particle crosses the SC by diffusion between the cells. For this pathway, the size as well as the mechanical properties of the particle need to be taken into consideration, as they need to have the right flexibility.[10] Rigid particles, like metal particles, have been found to scarcely be able to penetrate the SC via the intercellular route. It has been often hypothesized that their lack of flexibility hinders their diffusion between the cells.[11] In contrast, highly flexible polymer-based nanocarriers have been shown to be able to penetrate into the SC. For rigid particles, the most proposed route for penetrating the skin is via the follicular route. This route describes the direct delivery of the therapeutics via hair follicles and glandular ducts. However, this route is strongly limited, as hair follicles and glandular ducts make up only 0.1 and 0.01 % of the total surface area of the skin, respectively.[12]

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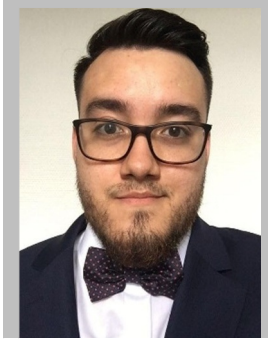

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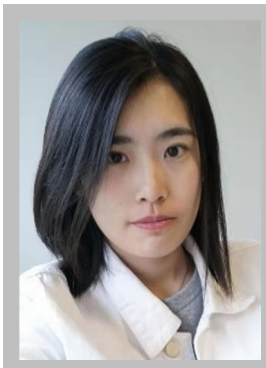
David Esporrín Ubieto obtained his degree in Chemistry and Master of Nanotechnology from the University of Zaragoza in 2017 and 2018, respectively. Since 2018 he has been carrying out his doctoral studies at POLYMAT. His current research deals with the development of gold nanoparticles as nanogel cores to form smart hybrid nanocarriers. He is also exploring the macroscopic scale with the development of physically crosslinked hydrogels that exhibit mucosal adhesion.

In addition, it has also been reported that penetration through the hair follicles is often determined by the size and flexibility of the penetrating substances.[13] As an example, soft NPs with sizes between 300 and 500 nm show good hair follicle penetration.[13] Taken together, each of the penetration mechanisms represents a possibility for particles to overcome the barrier properties of the SC; however, the properties of the nanocarriers must be fine-tuned to fulfil the requirements of the chosen pathways. As far as nanocarriers are concerned, both hair follicle penetration as well as intercellular penetration represents promising opportunities for drug delivery. However, the low hair follicle surface area coverage of the skin and the low diffusion of particles via the intercellular pathway are limiting factors for the penetration of nanocarriers.

In view of the composition of the skin, overcoming the skin barrier is the biggest challenge in both topical and transdermal delivery of therapeutics. Although both therapeutic delivery strategies proceed via the skin, their aims are different. Topical delivery is employed to induce a localized effect, increasing the total amount of therapeutics on the skin along with reducing undesirable side effects and eliminating the need for systemically administered therapies. In contrast, transdermal delivery is utilized to induce a systemic effect. Topical delivery is achieved in the form of creams and ointments, wherein active therapeutics are dissolved, encapsulated, or dispersed in the appropriate vehicles followed by their local application; whereas transdermal delivery is accomplished by using skin patches and active modes of delivery. Regulatory approved and marketed transdermal patches are limited to bioactive molecules, such as nicotine, estradiol, fentanyl, etc. as they have to fulfil a number of requirements such as high lipophilicity, sufficient solubility in water, and low molecular weight (< 500 Da) at pH 6 to 7.4.[14] In order to successfully treat multiple skin diseases, various approaches have been studied in the past. These include both passive and active modes of transport, keeping the composition of the skin in mind. In this perspective, employing NPs has proven to be a promising approach as shown by multiple studies carried out in recent years.

Delivering therapeutics through the skin is a non-invasive alternative to other well-established administration routes, such as intravenous, subcutaneous, intramuscular, genital, or rectal administration, which might be limited by bioavailability and can cause systemic side effects in certain cases.[15] The use of nanocarriers was found to be a suitable strategy to overcome the SC barrier without exerting tissue damage and achieving efficient drug penetration.[10,16] Figure 2 shows some widely used drug nanocarriers, including lipid nanoparticles, carbon nanotubes (CNTs), hybrid (organic–inorganic and lipid–polymer) NPs, dendrimers, micelles, polymeric nanogels, and ionic-liquid-based nanocarriers for dermal delivery. Lipid nanoparticles, such as liposomes and solid–lipid nanoparticles, are among the non-viral agents that have been explored extensively in dermal drug delivery applications.[17,18] However, the stability of liposomes has always been an issue, as they tend to disintegrate during the penetration process.[19] In the case of solid–lipid nanoparticles, low drug loading capacity and uncontrolled release limit their application in dermal drug delivery.[18c,20] Similarly, micelles present poor stability as well as encapsulation efficiency.[21] Recently, ionic liquid nanocarriers have gained attention for delivery of wide range of drugs depending on the type of ion present in the system. However, detailed studies are needed to understand the mechanism to unfold the potential of ionic liquid nanocarriers towards topical drug delivery.[22] As for



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Marcelo Calderón is an Ikerbasque Professor at POLYMAT, the Basque Center for Macromolecular Design and Engineering, in San Sebastian (Spain). He received his Ph.D. in chemistry in 2007 from the National University of Córdoba (Argentina). He then moved to the Freie Universität Berlin (Germany) for postdoctoral work, where he became Assistant Professor in 2013. Since 2019, he leads the Responsive Polymer Therapeutics group in POLYMAT, combining the interests and expertise of researchers working at the interface between polymeric materials and biology. Their focus is the development of drug delivery systems based on environmentally responsive polymers.

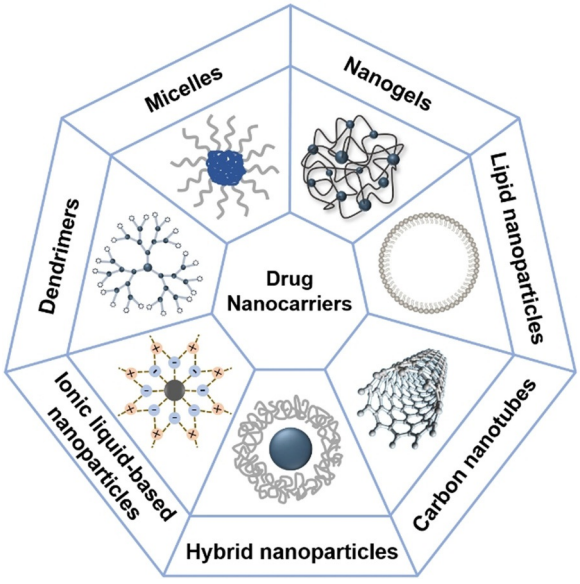


Figure 2. Schematic illustration of the most commonly used drug nanocarriers, including liposomes, carbon nanotubes, hybrid nanoparticles, dendrimers, micelles, ionic-liquid-based nanoparticles, and polymeric nanogels.

carbon nanotubes and dendrimers, toxicity[23] and poor controlled release behavior,[24] respectively, are limiting factors for their application in dermal drug delivery. These insights lead to a shift in the research focus on polymeric NPs as they can be designed strategically, functionalized, and even fabricated to respond towards changes in their external environment.[25] In particular, nanogels (NGs), which can be described as an aqueous dispersion of three-dimensionally crosslinked polymer particles with sizes in the nanometer range (10–1000 nm) and formed by physical or chemical interactions of the polymer chains, are one of the typical polymeric NPs.[25b] They possess interesting properties, such as high water content, stability, softness, and flexibility, biocompatibility, low toxicity, and excellent water dispersibility/solubility.[26] These properties, along with the ability to incorporate great payloads of active pharmaceutical ingredients, including nucleic acids, pose many advantages for their applications on the skin.[27]

NPs have been extensively studied on the subcellular level for various biomedical applications. Particularly, smart NPs that respond to the external environment like temperature and pH (by changing their hydrophilicity/hydrophobicity) have shown promising results for the treatment of multiple skin disorders.[28] However, the high complexity of the skin requires the tailoring of both chemical and physiochemical properties of NPs. Moreover, in order to use NPs for pharmaceutical applications, it is crucial to understand their interactions with different cells and tissues in the skin. Thus, many recent studies have focused on understanding NP–skin interactions.[29] In one such study, the penetration of differently sized citrate-coated gold nanoparticles (AuNPs), with diameters of 15, 102, and 198 nm, were studied through rat skin using Franz cells and characterized using transmission electron microscopy (TEM), inductively coupled plasma-optical emission spectrometry (ICP-OES), and energy-dispersive X-ray spectroscopy (EDS). The AuNPs showed size-dependent permeation through rat skin, wherein smaller particles (15 nm) were found to have higher permeation compared to bigger particles. Furthermore, TEM studies revealed permeation of smaller NPs in deeper regions of the skin (fibrous layer and adipose tissues), whereas the bigger particles were accumulated mainly in the epidermis and dermis.[30] To understand the transdermal delivery of AuNPs in human skin, absorption of citrate-coated AuNPs (ca. 12.6 nm) through full-thickness human skin in an in vitro diffusion cell system was evaluated.[31] The study highlights the dose-dependent penetration of AuNPs in both intact and damaged skin during a period of 24 h. When applied in lower amounts, AuNPs were found to penetrate through both intact and damaged skin in similar amounts. On increasing the dose of AuNPs, it was found that the Au concentration decreases from the superficial layer to the deeper layers of the skin and also, higher amounts of Au were found in damaged skin than in intact skin. This could be explained by the direct interaction between the NPs and skin components like the extracellular matrix and cells that affect the NP migration. Fernandes et al. have investigated the interactions of different colloidal AuNPs in terms of their size, charge, and functionality with human and mice skin in order to understand the parameters affecting their penetration.[29] The elaborative studies involving both qualitative and quantitative analysis showed that positively charged NPs penetrated 2–6 times more in comparison to negatively charged NPs; rod-shaped NPs were found in higher number in skin than spheres, and cell-penetrating peptide-coated nanospheres penetrated the skin in higher amounts (10 times) than polyethylene glycol (PEG)-decorated nanospheres.

In addition to metal NPs, organic systems like dendrimers have also been evaluated to understand the effect of various parameters on their interaction with the skin. In one such study, the interaction of poly(amidoamine) (PAMAM) dendrimers on porcine skin was investigated depending on their size, surface charges, and hydrophobicity. The study showed better penetration of smaller, generation 2 (G2) PAMAM dendrimers compared to larger ones (G4) (MW of PAMAM dendrimers $\approx$ 3000–19000 Da as measured using MALDI-TOF).[32] Furthermore, functionalizing the surface of G2 PAMAM via acetylation or carboxylation resulted in increased skin permeation (likely by diffusion through the extracellular pathway), while amine-modified dendrimers showed enhanced cell internalization and skin retention. As shown in Figure 3A, rhodamine- and amine-functionalized G2-PAMAM (G2-RITC-NH_2) interacted strongly with the epidermal and dermal cells as compared to G2-RITC-COOH and G2-RITC-Ac (Figure 3B,C). In addition, G2 dendrimers conjugated with oleic acid exhibited increased 1-octanol/PBS partition coefficients, and thus increased skin absorption and retention. In a similar study, Kraeling et al. investigated and compared the skin penetration of PAMAM dendrimers (G3 to G6, MW ($g\,mol^{-1}$) $\approx$ 8000–66000) conjugated with amine moieties (positively charged), succinic anhydride (negative surface charge), and glycidol (neutral) in cosmetic formulations on viable pig or human cadaver skin using diffusion cells.[33] Confocal laser scanning micrographs showed greater penetration of G3-NH_2 dendrimer into the dermal layers of human skin compared to greater penetration of G4-OH dendrimer into the dermis of pig skin. The increased penetration of cationic dendrimers could be explained based on their interaction with negatively charged biological membranes, resulting in an increase in permeability.[33]

Another efficient pathway for the penetration of particles through the skin layers is the hair follicle route as explored by Sahle et al.[13] The study revealed that thermoresponsive nanogels (tNGs) with a phase transition temperature of 32–37 °C and particle sizes between 300–500 nm penetrated more effectively than smaller NGs into the hair follicle, with penetration depths proportional to the particle size.[13] The follicular penetration of the NPs can be explained by the "ratchet effect", defined as directed particle motion generated by a non-equilibrium perturbation of a periodic system. Radtke et al. have proposed a simple two-dimensional model that demonstrates the mechanism of nanoparticle transport on a ratchet-shaped hair surface in addition to studying parameters like driving frequency and particle size.[34]

The penetration of NPs loaded with therapeutics into the deeper layers of the skin can be further enhanced by using various penetration enhancers (Figure S1) as discussed in the Supporting Information.

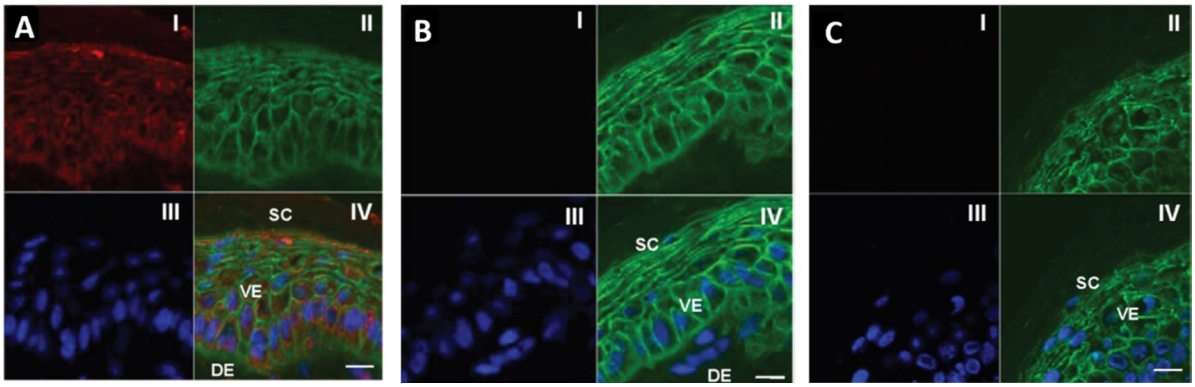


Figure 3. Confocal laser scanning micrographs of porcine skin layers after 1 h incubation with: A) G2-RITC-NH_2, B) G2- RITC-COOH, and C) G2-RITC-Ac. I) The dendrimer conjugates labeled with rhodamine B isothiocyanate (red); II) cell membranes stained by WGA-AF488 (green); III) nuclei stained by DAPI (blue); and IV) merged images. Scale bar: 10 μm. SC: stratum corneum; VE: viable epidermis; DE: dermal layer. Figure adapted with permission from ref. [32].

3. Nanocarriers' Features and Their Role in Skin Penetration of Active Molecules

Nanocarriers are an interesting alternative to overcome the limited penetration of free drug molecules through the skin. They can be designed to interact with skin components in a way to enhance penetration, which opens a broad area of clinical applications. Various physicochemical properties of nanocarriers like size, shape, rigidity, and surface charge can affect skin penetration and the interaction with the biological components. Multiple functionalities can be incorporated in the design of nanocarriers that could be exploited for delivery of wide range of cargos including small drug molecules, genetic materials, and even large antibodies. Various features of nanocarriers that have been exploited till date for the topical delivery of active molecules are discussed in detail in the following sections.

3.1. Effect of Biodegradability on Dermal Drug Delivery

Biodegradable polymeric NPs, which can be degraded in the body by enzymes and/or chemical cues, are an attractive topic of research in the field of drug delivery, due to their biocompatibility and versatility.[35] Generally, biodegradable polymeric materials can be classified as natural or synthetic. Some of them have been approved by regulatory agencies as safe, for instance hydroxypropyl methylcellulose (HPMC), chitosan, alginate, poly(lactic-*co*-glycolic) acid (PLGA), and polycaprolactone (PCL).

Chitosan is a natural, biodegradable polymer with excellent mucoadhesive properties[36] that has attracted much attention in dermal drug delivery. It can be degraded to non-toxic glucosamine derivatives in the body by enzymatic action. The degradation process can take place within a few days or up to several months, depending on the source of the polymer, molecular weight, and degree of deacetylation.[37] Although chitosan exhibits very appealing features, its poor stability is a factor limiting its use in pharmaceutical products.[38] This issue can be overcome, by combining chitosan with more stable anionic polymers as well as the addition of crosslinkers, which often results in NPs with good stability and skin permeation. For example, Takeuchi et al. studied donepezil hydrochloride (DP)-loaded PLGA-chitosan core–shell NPs to treat osteoporosis.[39] In their study, they found that core–shell NPs improved both skin accumulation and delivery of DP into hair follicles as compared to a DP solution.

Compared to natural biodegradable polymers, the biodegradation of synthetic polymers is quite different, and can take several weeks to a few years by enzymolysis or chemical and physical cues. As an example, hydrophobic PLGA NPs, which possess good permeability with respect to the skin, can be degraded in vivo by scission of ester linkages to produce glycolic acid and lactic acid within several weeks.[40] In another study, cotton fabrics were functionalized by melatonin-loaded PCL NPs to fabricate a transdermal drug delivery device.[41] Model skin membrane assays demonstrated that only less than half of the melatonin was released in 14 h. This extremely slow degradation of PCL could be taken into consideration for applications requiring prolonged drug release.[42] Furthermore, Prieto et al. co-encapsulated upconverting NPs and the photosensitizer protoporphyrin IX inside PLGA-PEG NPs to promote skin permeation and reactive oxygen species (ROS) generation. The hybrid NPs exhibited not only higher ROS generation, but also deeper penetration of protoporphyrin IX into epidermal and dermal skin layers, compared to free protoporphyrin IX, which remained on the outer layer of the skin.[43]

On account of the satisfactory biocompatibility and biodegradability, biodegradable polymeric NPs usually possess low immunogenicity, cytotoxicity, and side effects.[35c] Moreover, they endow the payloads with sustained release, resulting from their long-term circulation and degradation.[44] Nevertheless, they still exhibit limitations in dermal drug delivery. For example, as compared to non-degradable nanocarriers, biodegradable NPs present lower stability, difficulty in achieving a specific release of cargoes, and higher production cost.[35c]

Aiming at higher stability and controlled drug release, stimuli-responsive NPs have been developed as attractive candidates for fabricating drug nanocarriers.[45] Stimuli-responsive NPs undergo physicochemical as well as mechanical changes when exposed to changes within the body (e.g. pH, temperature, salt concentration) or external stimuli (e.g. magnetic field, laser irradiation, ultrasound).[7] The responsiveness of certain NPs allows the release of encapsulated cargos in a controlled fashion, reducing undesired side effects and increasing the efficacy of the therapy.[46]

3.2. Thermoresponse and Its Role in Hydration and Triggered Drug Release

NPs able to induce skin hydration have been found to be advantageous in skin delivery applications, as these can enhance the penetration of previously encapsulated cargos. Many clinical preparations and products (e.g. ointments, gels, patches) utilize water to enhance the penetration of formulated drugs, as it is inexpensive and skin can quickly recover from its exposure.[47] Generally, skin hydration seems to increase the transdermal delivery of both hydrophilic and hydrophobic compounds, although in some cases hydrophobic compounds have been found to not penetrate and further induce skin irritation.[47,48] Thermoresponsive soft systems (e.g. NGs and nanocapsules) have been found to induce skin hydration due to the release of water in response to an increase in temperature, which subsequently enhances the penetration of hydrophilic compounds, ranging from dyes to proteins.[49] A recent study by Giulbudagian et al. describes the skin hydration features of thermoresponsive NGs (tNGs).[49] Employing dendritic polyglycerol (dPG) as macro-crosslinker and poly(*N*-isopropylacrylamide) (PNIPAM), poly(glycidyl methyl ether-*co*-ethyl glycidyl ether) (p(GME-*co*-EGE), and poly(di(ethylene glycol) methyl ether methacrylate-*co*-oligo ethylene glycol methacrylate) (p(DEGMA-*co*-OEGMA475)) as thermoresponsive polymers, three types of tNGs were synthesized. These NGs were covalently labeled with indodicarbocyanine (IDCC) and loaded with fluorescein as a model drug, in order to investigate with fluorescence microscopy both the tNG penetration as well as the penetration of released fluorescein in an independent fashion. In addition, the study highlights the power of combining electron microscopy with Raman spectromicroscopy for analyzing the skin hydration effects of the NGs on excised human skin. This elegant mixture of label-based and label-free characterization methods showed that samples treated with NGs were swollen and their SC exhibited a disruption in the structure of its proteins and lipids as opposed to untreated samples. These results were attributed to the hydration effects induced by the tNGs (Figure 4).

In another study, Giulbudagian et al. made use of the thermoresponsive features of p(GME-*co*-EGE), to create a novel thermo-nanoprecipitation synthesis to yield tNGs, using water as both solvent and non-solvent.[26a] The mild conditions of this approach were further exploited to encapsulate delicate cargos like etanercept (ETR), an anti-TNFα fusion protein, during the NG formation. It was found, that the NGs were able to transport ETR into the viable epidermis on inflammatory skin models, regardless of the high molecular weight of the protein (ca. 150 kDa). Moreover, the delivered ETR reduced the inflammation, proving that its therapeutic efficacy and efficiency was not affected by the encapsulation and release process.

Despite numerous publications hinting that thermoresponsive soft nanocarriers transport cargo into the deeper layers of the skin by inducing skin hydration, the direct effects of thermoresponsive soft nanoparticles on the skin have only been elucidated recently. Osorio-Blanco et al. designed novel dPG-based thermoresponsive hollow nanocapsules (tNCs) with a void size of 100 nm. The tNCs were found to encapsulate deuterated water (D_2O) efficiently and release it when a temperature trigger was applied in a controlled fashion. It was found that treating excised human skin with the tNCs resulted in skin hydration. Stimulated Raman spectromicroscopy (SRS) was used to track the D_2O within the skin, showing that the tNCs enhanced the penetration of

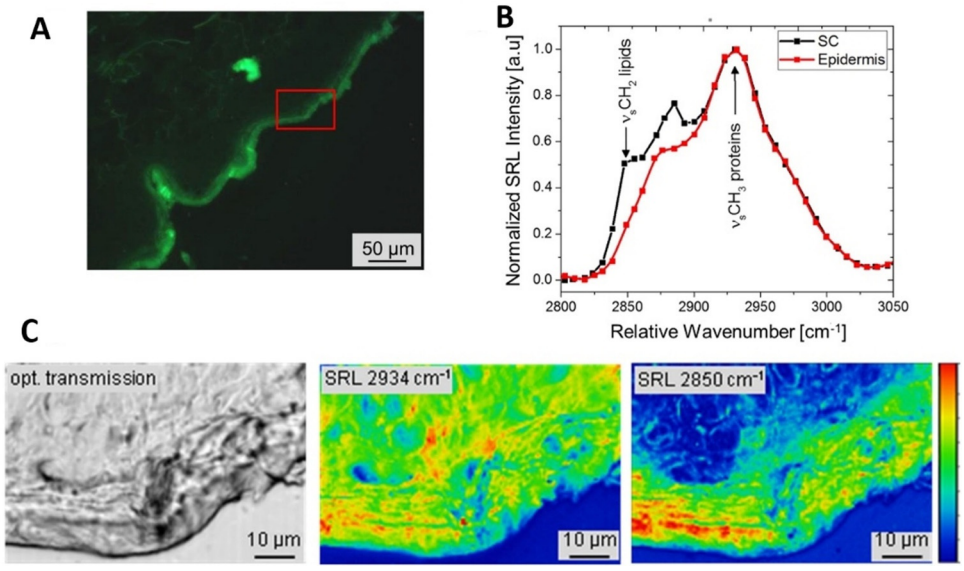


Figure 4. Images of protein and lipid distributions across skin samples that were treated with tNGs. The images were obtained with stimulated Raman spectromicroscopy (SRS), measured in stimulated Raman-loss (SRL) detection mode. A) Fluorescence microscopy image of skin region for SRL (red frame), B) SRL spectra of SC and epidermis (meant is the viable epidermis), and C) optical transmission image (left); distributions of proteins (SRL 2934 cm^{-1}; middle) and lipids (SRL 2850 cm^{-1}; right). Figure adapted with permission from ref. [49].

D_2O into the viable epidermis compared to control samples (Figure 5 A–D). Moreover, it was demonstrated that this effect could be further increased upon application of a thermal trigger, as observed in the fluorescence intensity in Figure 5 E and SRS in Figure 5 F. Finally, skin hydration caused by tNCs was sufficient to significantly enhance the skin penetration of Atto Oxa12 NHS ester, a high-molecular-weight dye, as a model drug.[50]

In addition to inducing skin hydration, thermoresponsive nanocarriers have many attractive features for dermal drug delivery. Due to the natural temperature gradient across the skin and the possibility to control the thermal response, thermoresponsive nanocarriers have been investigated extensively in dermal drug delivery applications.[51] The structure of thermoresponsive nanocarriers undergoes physiochemical changes with environmental temperature variation at a specific range, known as the critical solution temperature. Depending on the changes in miscibility with the temperature, the critical solution temperature can be divided into the lower critical solution temperature (LCST) and the upper critical solution temperature (UCST). Compared to UCST-type thermoresponsive polymers, LCST-type thermoresponsive polymers are more popular especially for the fabrication of tNGs, due to the higher stability of crosslinked networks.[52] Furthermore, their properties can be tuned easily using versatile synthesis methods involving either covalent crosslinking or self-assembly of polymers using physical interactions. Radical polymerization is the most frequently used method for the synthesis of covalently crosslinked tNGs, but plenty of techniques, like click chemistries and Michael addition, have proved to be useful depending on the chosen precursors.

When a thermoresponsive polymer is crosslinked, the thermoresponsive network experiences a volume phase transition and the critical solution temperature translates into a volume phase transition temperature (VPTT). The VPTT is an intrinsic and specific property of a material that can be tailored by co-polymerization of different monomers towards diverse biological applications. Human skin has a temperature gradient from 32 °C (at the skin surface) to 37 °C (at deeper layers of the SC). Upon penetrating into the SC layer, LCST polymer based tNGs whose VPTT is 32–37 °C undergo transition from a swollen state into a shrunken state, triggering the release of encapsulated therapeutics.[51] Asadian-Birjand et al. investigated the advantages of using tNGs for dermal drug delivery. In contrast to non-thermoresponsive NGs, tNGs exhibited a pronounced skin penetration and drug delivery efficiency into the epidermis when the skin temperature is above the VPTT.[53]

PNIPAM is one of the widely studied thermoresponsive polymers with a LCST of around 32 °C. Many reports have shown that on combination with proper crosslinkers, the obtained PNIPAM-based tNGs can exhibit high stability, good biocompatibility, and the ability for controlled drug release.[54] Sahle and Gerecke et al. have explored PNIPAM-based tNGs for the encapsulation and release of therapeutic molecules applied by dermal delivery.[13,55] Meanwhile, dPG is a desirable hydrophilic macromolecular crosslinker that

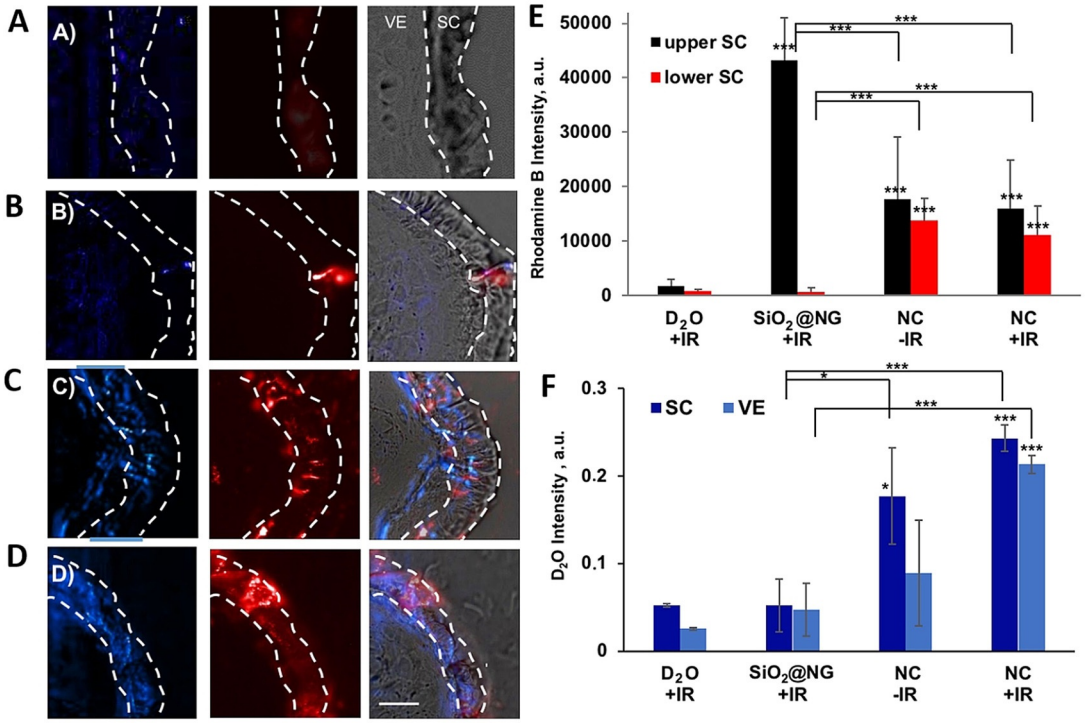


Figure 5. Penetration of D_2O in human skin after 1000 min incubation. A–D) Stimulated Raman spectromicroscopy (SRS) images of D_2O (left), fluorescence image of Rhodamine B labeled nanocarriers (middle), and overlap with optical micrographs (right). SC is illustrated by the dashed lines. A) D_2O, B) SiO_2@NG + IR, C) NC − IR, and D) NC + IR; scale bar represents 10 μm. E) Nanocarrier penetration observed by fluorescence intensity. F) D_2O penetration as average from SRS measurements. Figure adapted with permission from ref. [50].

presents multifunctional properties and protein resistance.[25c,54a] By crosslinking with acrylate-functionalized dPG, PNIPAM-based NGs (tNG_dPG_PNIPAM) are able to load therapeutic cargos, such as the anti-psoriatic drug dexamethasone (Dex) and the anti-inflammatory drug tacrolimus (TAC).[55] With an increase in temperature from 25 °C to 37 °C, the diameter of tNG_dPG_PNIPAM NGs decreased by 50 %, resulting in efficient drug release and cellular uptake. In addition to PNIPAM, there are numerous thermoresponsive polymers that have been explored to synthesize NGs for drug delivery applications. For instance, p(GME-*co*-EGE) NGs were prepared with a VPTT of 30 °C and sizes around 156 nm.[49] Confocal laser scanning microscopy and transmission electron microscopy (TEM) showed that these tNGs exhibited favorable drug delivery properties and skin penetration.

Furthermore, tNGs with a VPTT in the range of 37–42 °C could be utilized in drug delivery by the addition of external infrared stimulus.[56] As a result, the collapse of the NGs with the release of encapsulated cargos takes place in specifically irradiated areas, which endows the drug delivery process with high spatio-temporal accuracy. As the transition behavior of LCST-type tNGs is based on the interactions within polymer molecules as well as the interactions between polymer and solvent molecules, copolymerization with hydrophilic monomers is a good strategy to increase the VPTT.[13,57] For example, the phase transition properties of PNIPAM-*co*-poly(N-isopropylmethacrylamide) (PNIPMAM) copolymeric NGs under different parameters were studied, as shown in Figure 6.[58] With increasing amount of NIPMAM, the VPTT of PNIPAM-co-PNIPMAM NGs increased, as the hydrophilic–hydrophobic ratio within the tNGs changed. When the NIPAM/NIPMAM weight ratio was 1:1, the VPTT of the copolymeric tNGs was around 40 °C. Along the natural skin temperature gradient, these copolymeric tNGs did not show significant release of an albumin-fluorescein isothiocyanate conjugate (BSA-FITC) in the viable epidermis. While in the presence of external infrared irradiation, a significantly improved delivery of BSA-FITC into the viable epidermis was observed. Additionally, the amount of crosslinker is another key parameter to control the nanogel's properties. The influence of varying the acrylate percentage of the crosslinker dPG-Ac and its feeding amount on the VPTT and size of tNGs was investigated.[13,53] It was observed that increasing the hydrophilic dPG-Ac portion raised the VPTT, showing a linear trend with a decrease in size of the NGs. Also, it was observed that with an increase in dPG-Ac functionalization, both VPTT and size decreased.

Besides NGs, hybrid nanocarriers that combine the advantages of NGs and inorganic NPs have gained interest in the current research on dermal drug delivery. Hybrid nanocarrier systems hold great potential in the field of biomedicine due to the combination of properties from different single systems,[59] such as organic–inorganic hybrids[60] and lipid–polymer hybrids.[61] As an illustration, AuNPs, a good candidate in nanomedicine, possess high stability, antibacterial, and photothermal properties.[62] Arafa et al. investigated Pluronic®127-hydroxypropyl methylcellulose thermoresponsive gels conjugated with AuNPs (AuNPs-PF127-HPMC) as antibacterial and wound-healing agents. Compared to a AuNPs suspension, AuNPs-PF127-HPMC showed improved bioavailability, skin permeation, anti-inflammatory, and antibacterial properties as well as prolonged and sustained effects.[60]

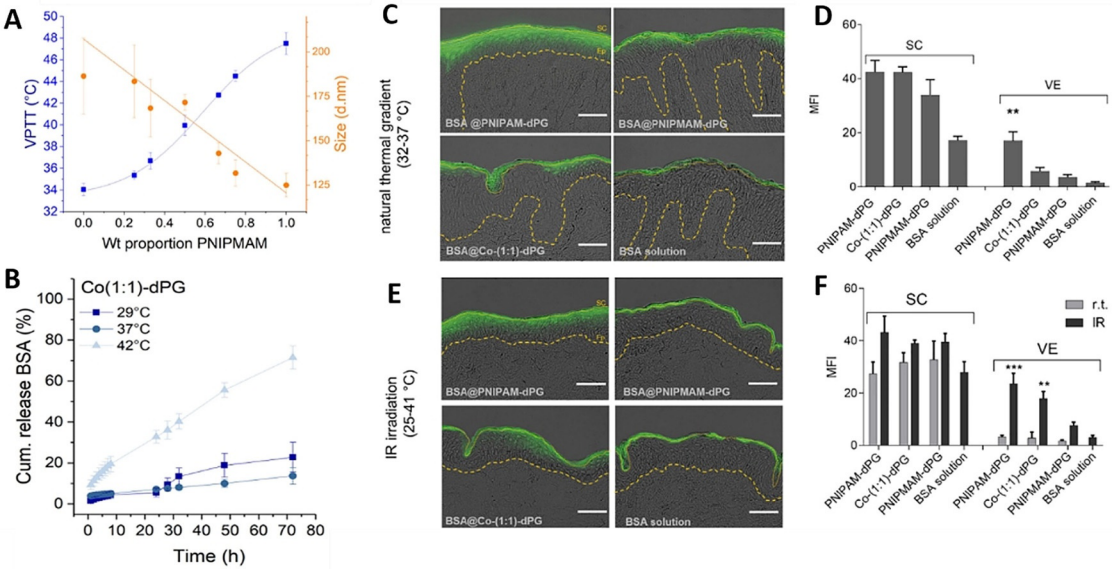


Figure 6. A) The VPTT and hydrodynamic size of PNIPAM-*co*-PNIPMAM NGs with weight proportion of PNIPMAM. B) Release efficiency of BSA-FITC from **P**NIPAM-*co*-**P**NIPMAM? (1:1) NGs at different temperatures. C) Representative fluorescence images of intradermal BSA delivery by means of the natural thermal gradient (32–37 °C) and D) the corresponding analysis of the mean fluorescence intensity (MFI). E) Representative fluorescence images of intradermal BSA delivery using IR irradiation (25–42 °C) and F) their corresponding MFI analysis. Scale bars: 50 μm. Figure adapted with permission from ref. [58].

3.3. pH-Responsive Behavior in Drug Delivery

Together with temperature, pH is another stimulus that has been widely used to trigger drug release from nanocarriers. Generally, the surface of healthy skin is acidic with a pH range of 4.1 to 5.8. Along different skin layers the pH changes to a near neutral environment in deeper skin layers and hair follicles.[63] Furthermore, skin diseases such as inflammations and epidermal lesions can change the pH of the skin.[64] Owing to these pH differences in different skin layers and conditions, pH-responsive nanocarriers can be designed that undergo physicochemical changes in deeper skin layers or pathological regions.[65]

These changes can promote the release of payloads, which can overcome the limitations of topical treatments. Regulatory agencies have approved several pH-responsive polymers that can be applied in pharmaceutical formulations such as cellulose derivatives and Eudragits. Eudragit L100 has been widely selected to formulate drug delivery nanocarriers due to its pH critical point at 6 and good stability.[65,67] Dong et al. investigated the dermal penetration and release of spin-labeled dexamethasone (DxPCA) from pH-responsive Eudragit L100 NPs using EPR studies. In comparison to commercial cream, the in vitro release efficiency and skin penetration of DxPCA was enhanced in both intact and barrier-disrupted skin when the pH was above 6 (Figure 7). Furthermore, it was found that follicular transport led to higher transdermal penetration of NPs than in glabrous skin due to the slightly higher pH value and the weaker barrier in the deeper hair follicle.[66]

As previously mentioned, chitosan is another popular natural pH-responsive polymer that can be used to synthesize pH-responsive nanocarriers. The responsiveness of chitosan arises from its amino groups, which undergo protonation in acidic environment, leading to repulsion of the positively charged groups followed by swelling. For example, Sahu et al. designed pH-responsive biodegradable NGs based on chitosan and loaded with 5-fluorouracil (5-FU). Their study showed that the release was triggered by an acidic pathological environment, and high release efficiency of encapsulated 5-FU (80–85%) and sustained release kinetics were exhibited even at very low drug dosage (0.2% w/v).[68] In another study by Divya et al. a chitin nanogel system loaded with acitretin or aloe-emodin to treat psoriasis was shown to display high skin permeation and drug retention at dermal and epidermal skin layers, as compared to a commercial anti-psoriatic cream.[69]

It is worth noting that NGs consisting of thermoresponsive and pH-responsive moieties, usually present dual pH-/thermoresponsive behavior and improved drug delivery efficiency. For instance, Abu Samah et al. investigated the dual thermo/pH-responsive behavior of NGs by copolymerization of NIPAM (thermoresponsive) and acrylic acid (pH-sensitive) monomers.[70] The nanogel system exhibited shrinking behavior with increased temperature and decreased pH. A pronounced enhancement of the caffeine loading efficiency and skin permeation were observed in an in vitro encapsulation and an ex vivo porcine epidermal membrane permeation assay, respectively. Specifically, temperature acted as the major trigger to release caffeine in the system, whereas the pH-responsive moiety improved the loading capacity as well as the responsive behavior. This multi-responsiveness improved therapeutic efficiency effectively and could be utilized to design NGs with enhanced properties. Table 1 summarizes various polymers that have been utilized to construct responsive NPs for dermal drug delivery in response to stimuli (e.g. biological environment).

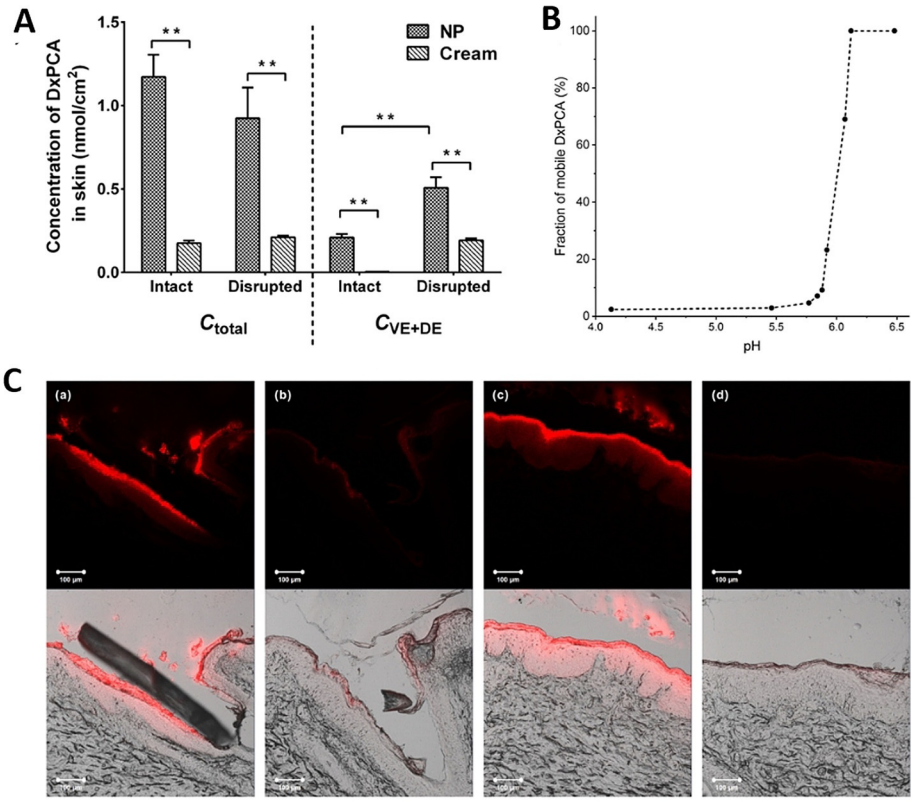


Figure 7. A) Differential permeation of DxPCA from NPs and cream in intact and barrier-disrupted porcine ear skin. B) Release efficiency of DxPCA from NPs dispersion calculated by simulated EPR spectra under different pH conditions. C) Confocal laser scanning microscopy images of Nile red permeation through the hair follicles (a, b) and glabrous skin (c, d) from NPs (a, c) and cream (b, d). Figure adapted with permission from ref. [66].

3.4. Electrostatic Charges towards Gene Delivery

The presence of net charges on the surface of nanocarriers has been shown to play an important role in dermal gene delivery applications. Particularly, positive charges have been demonstrated to facilitate the transport of the nanocarriers into the skin via the inter- or intracellular pathways.[75] Since genetic material possesses a net negative charge, the presence of positive charges on the nanocarriers is a prerequisite for complexation with genetic material and its protection from enzymatic degradation.

Out of 7000 known monogenic diseases, approximately 20 % of them are related to the skin, affecting up to 1 % of the human population. Most of these disorders have no effective treatment that targets the genetic malfunction related to the disease, leading researchers to focus on nucleic acid based therapies.[76] Gene therapy is the modulation of gene expression by addition, deletion, regulation, repair, or replacement of a particular genetic sequence in specific cells.[77] Theoretically, it can be used to target and treat any malfunctionedgenetic sequence responsible for the disease.[78] However, translation of this technology to clinical practice has largely been limited due to the difficulty associated with the delivery of genetic material to the target site. Research indicates that although viral vectors have remarkable transfection efficiency, they could lead to various side effects including immunogenicity.[79] Furthermore, enzymatic degradation in the blood, poor bioavailability, rapid clearance from the system, and poor patient compliance are other major challenges associated with the delivery of genetic material.[5] Topical delivery of genetic material offers several advantages over alternative approaches, as it could avoid the problems associated with systemic administration by controlled delivery.[5] However, the protective layer of the skin acts as a barrier for the entry of topically applied therapeutic genes.[80] To circumvent the barrier properties of skin, various strategies have been exploited, as has been discussed in later sections. The use of NPs as carriers is a promising alternative that ensures high loading capacity, reduced immunogenicity, and reduced premature degradation of genetic material before reaching the target site. Different nanocarrier systems mainly explored in dermal gene delivery applications are liposome based, polymer based, and hybrid nanocarriers as summarized in Table 2.

3.4.1. Liposome-Based Nanocarriers

Among non-viral gene delivery systems, liposome-based NPs (LNPs) have been extensively studied and are the most advanced systems.[78c] The first ever siRNA-based drug, ONPATTRO (delivered via infusion), was approved in 2018, which shows that LNPs have become a clinically validated platform technology.[89] Thus most recently, LNPs have been explored for the encapsulation of RNA encoding the SARS-CoV-2 spike protein as a vaccine against SARS-CoV-2.[90] Li and Hoffman for the first time reported the topical application of liposome/DNA complexes onto mice skin. The transfection mainly occurred via hair follicle cells, as reflected in the conducted studies.[91] Since then, various attempts have been made to use cationic lipid-based nanoparticles for topical gene delivery. Most commonly, cationic lipids like 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) together with helper lipids like cholesterol and phospholipids (lipofectamine) proved to be strong transfection agents for introduction of plasmids in the cells. However, they could not be used clinically due to their carrier-related toxicity.[92] Recently, Blakney et al. have explored the formulation of self-amplifying mRNA (saRNA) with lipid NPs in human skin explants.[81] saRNAs are the next generation of mRNA therapeutics, wherein saRNA self-replicates once inside the cytoplasm, resulting in abundant protein expression. Various formulation studies showed that lipid structure and concentration are crucial and the optimized formulation successfully delivered saRNA to ≈ 2 % of the resident cells in human skin explants.[81] Multiple studies concluded that non-viral gene delivery systems have low efficiency towards topical delivery of plasmids due to their limited efficiency to cross the SC barrier. To address the poor penetrating ability of "classical" lipid-based nanocarriers, new class of lipid vesicles have been designed by introduction of permeation enhancers (like ethanol, surfactants etc.) into the lipid composition.[93] In one such example, Dorrani et al. developed liposomal formulations using 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) loaded with sodium cholate (NaChol) as an edge-activator.[83] NaChol is a known surfactant with an ability to open pores in the SC, thus increasing the NP permeation. The study focused on the topical administration of BRAF-siRNA (v-Raf murine sarcoma viral oncogene homolog B) using liposome formulations. The fluorescently labeled liposome–siRNA complexes were evaluated for their diffusion in human cadaver skin and the highest rate of permeation was observed in DOTAP:NaChol liposomes with a 8:1 ratio and in complexation with siRNA at liposome:siRNA ratios of 8:1, 12:1, and 16:1 (Figure 8A, B). Furthermore, the formulated liposomes were able to knockdown the expression of BRAF protein (over-expressed due to mutation in melanocytes) and induce cell death in melanoma cells.

Although only few studies have been reported, LNP-based dermal gene delivery holds great promise as a therapeutic approach for skin diseases. Future advances in using LNPs in gene delivery requires screening of the lipid library and structure–activity analyses to tailor their properties towards skin applications.

3.4.2. Polymeric Nanocarriers

Another non-viral vector explored extensively in genetic engineering is polymeric NPs. The surface functionalization of NPs with various cell-penetrating peptides (CPPs) and short cationic peptides (up to 30 amino acids) is known to be an interesting strategy to endow cell-membrane-penetrating ability. Although delivery of peptides and biomolecules across the skin using CPPs was reported by multiple research groups,[94] very few reports are available for gene delivery using this approach. This could be due to various factors including neutralization of the charges on the CPPs by the genetic material which could affect its penetration ability. To

Table 1: Examples of polymers applicable for dermal drug delivery applications.

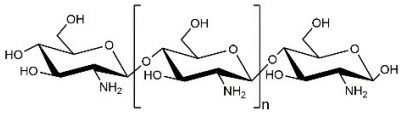
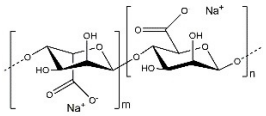
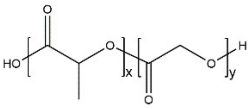
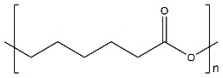
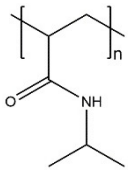
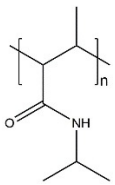
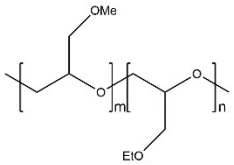
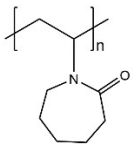
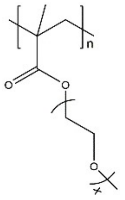
Responsive class	Examples	Chemical structure	Ref.
Biodegradable polymers	Chitosan		[68]
	Sodium alginate		[71]
	Poly(lactic-*co*-glycolic acid) (PLGA)		[72]
	Polycaprolactone (PCL)		[41]
Thermo-responsive polymers	Poly(*N*-isopropylacrylamide) (pNIPAM)		[13, 25c]
	Poly(*N*-isopropylmethacrylamide) (pNIPMAM)		[58]
	Poly(glycidyl methyl ether-*co*-ethyl glycidyl ether) (p(GME-*co*-EGE))		[49]
	Poly(*N*-vinylcaprolactam) (PVC)		[73]
	Poly(oligoethylene glycol methacrylate) (POEGMA)		[53]

Table 1: (Continued)

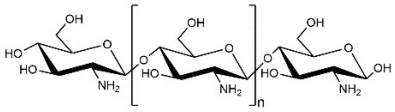
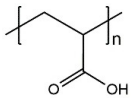
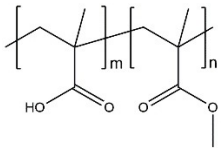
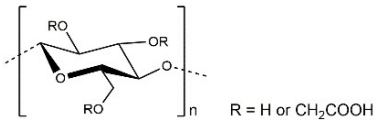
Responsive class	Examples	Chemical structure	Ref.
pH-responsive polymers	Chitosan		[68]
	Poly(acrylic acid) (PAA)		[70]
	Eudragit L100		[66]
	Carboxymethyl cellulose (CMC)		[74]

Table 2: Different nanocarrier-based systems for gene delivery via the skin.

Nanoparticles	Formulation	Genetic material	Application	Ref.
Liposome-based nanocarriers	C12-200, cephalin, dimethyldioctadecylammonium bromide (DDA), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)	Self-amplifying mRNA (saRNA)	Luciferase expression	[81]
	DOTAP + sodium cholate (NaChol)	Keap1 siRNA	Wound healing	[82]
	DOTAP + sodium cholate (NaChol)	BRAF-siRNA	Melanoma	[83]
Polymeric nanocarriers	Protamine + polyethylenimine (PEI)/DNA + poly(γ-glutamic acid) (PGA)	EGFP DNA	Gene expression	[84]
	Skin-penetrating peptide + HA + poly(β-amino esters)	siRNA	Skin melanoma	[85]
	PLGA + chitosan	pEGFP-N2 DNA	Gene expression	[27]
Inorganic and carbon-based nanomaterials	Twin arginine translocation (TAT) peptide-decorated gold nanoparticles	pDNA encoded with miRNA-221 inhibitor gene	Cutaneous melanoma	[86]
	Carbon nanotubes	BRAF siRNA	Melanoma	[87]
	Mesoporous silica nanoparticles	siRNA targeting TGFβR-1	Skin squamous cell carcinoma	[88]

overcome this problem, Yang et al. explored a novel approach for the delivery of genetic material using skin-permeable quaternary NPs modified with low-molecular-weight protamine (LMWP), a skin penetrating peptide.[84] The quaternary NPs were synthesized with polyethylenimine (PEI)/DNA complex as the cationic nanocore and layer-by-layer (LbL) deposition of a ternary layer of anionic poly(γ-glutamic acid) (PGA) and a quaternary layer of cationic LMWP sequentially coated on the nanocore. The LbL approach ensures the maximum availability of LMWP on the surface, which helps in the penetration of the NPs along with the DNA present in the core. NPs containing a DNA model drug, pEGFP, showed enhanced cellular uptake and transfection efficiency in melanoma cells. Recently, Wang et al. have designed a polymer system (SCP-HA-PAE in short SHP) based on skin-penetrating peptide (SCP), hyaluronic acid (HA), and the amphiphilic polymer poly(β-amino ester) (PAE), to fabricate nanocarriers (SHP) showing good penetration ability and delivery of siRNA through the SC and targeting mice melanoma.[85] From Figure 9A, it is evident that the topical application of SHP/siRNA to the melanoma site resulted in significant inhibition of tumor growth and thus the highest survival rate of model mice (Figure 9B) compared to the control groups. Also, Hematoxylin-eosin (H&E) stained sections of the tumor explanted from the skin showed clear necrotic and apoptotic regions only in the groups subjected to SHP/siRNA treatment (Figure 9C).

3.4.3. *Inorganic and Carbon-Based Nanomaterials*

In addition to polymeric NPs, hybrids of inorganic materials like Au, Ag, or CNTs in combination with various polymers have been explored extensively for their skin transfection efficiency.[95] Along with incorporating additional chemical and thermal stability in the delivery systems, inorganic NPs can be exploited for simultaneous imaging and treatment. In recent studies, Niu et al. synthesized Au NPs conjugated with twin arginine translocation peptide

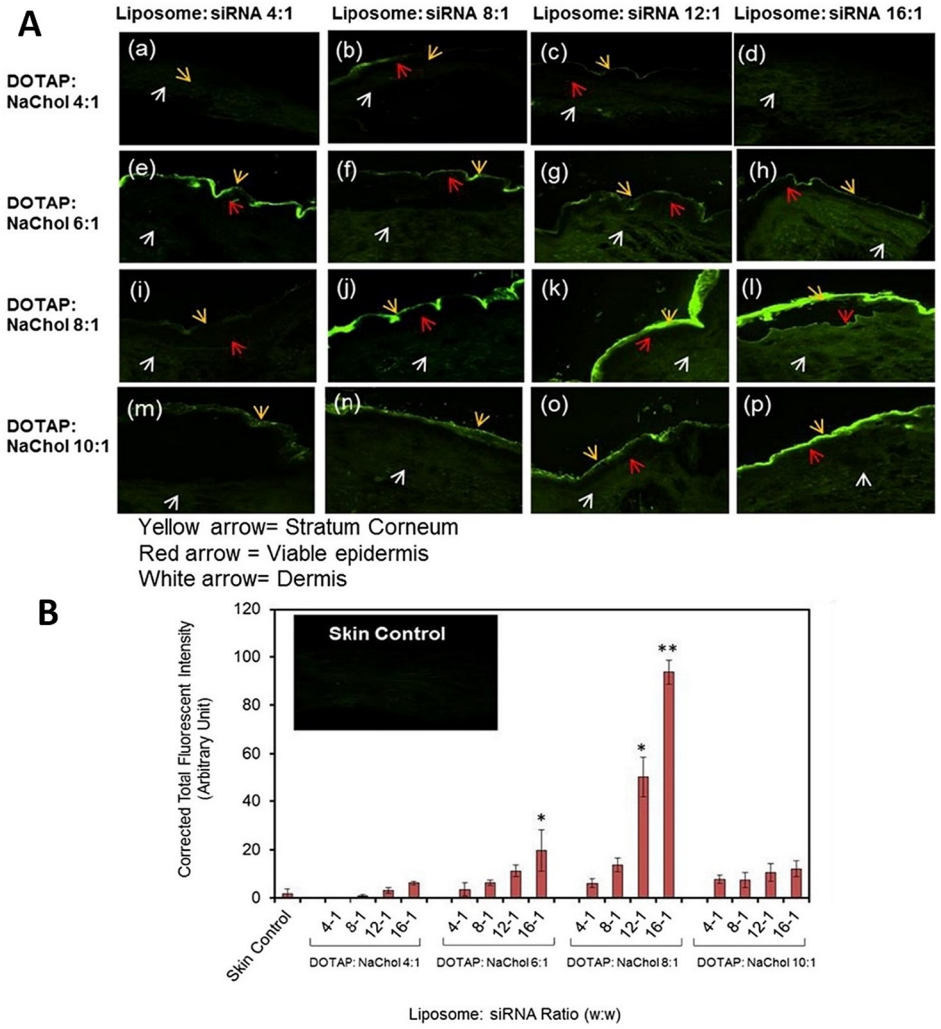


Figure 8. A) Fluorescent microscopy images of siRNA complexed green fluorescently labelled liposomes permeation through the skin layers, a–d) liposome:siRNA complexes at different w/w ratios of siRNA and B) Fluorescent intensity quantification of lipoplexes in the skin. Data are reported as mean values ± SD ($n = 3$). Figure adapted with permission from ref. [83].

(TAT) (AuPT) and complexed them with pDNA encoding the miRNA-221 inhibitor gene (abnormally expressed in malignant melanoma cells) (Figure 10). The NPs were shown to penetrate through the SC in the absence of any additional penetration enhancers. The penetration and the transfection efficiency of the NPs were studied in melanoma cells and melanoma xenograft models that showed reversal both in the progression and metastasis of advanced melanoma.[86] In another example, Siu et al. demonstrated the effective delivery of siBraf (siRNA specific to Braf, v-raf murine sarcoma viral oncogene homolog B) using single-walled CNTs functionalized with PEI in a mouse melanoma model. Significant inhibition of tumor growth was observed in a C57BL/6 mice melanoma model over 25 days due to the gene-silencing effect of the siRNA used in the study.[87] Furthermore, Zheng et al. reported simultaneous transfection and gene regulation using spherical nucleic acid nanoparticle conjugates (SNA-NCs), wherein gold cores were surrounded by highly oriented and covalently bound siRNA. The conjugates were shown to successfully penetrate keratinocytes, mouse skin, and human epidermis within a few hours of application in the absence of any disruption agents like liposomes, viruses, or penetrating peptides.[96]

In addition to the aforementioned NPs, MSNs have been shown to be potential candidates for the encapsulation of sensitive genetic material, ensuring its safe passage through cell membranes. In one such example, Lio et al. successfully utilized the pores of MSNs to encapsulate TGFβR-1 siRNA and then coated the MSN surface with poly(L-lysine) to improve transdermal delivery of the particles. The studies revealed that MSNs containing TGFβR-1 siRNA exhibited a 2-fold suppression of TGFβR-1 followed by 2.5-fold suppression of tumor growth as compared to PBS-treated control or scrambled siRNA on the mouse xenograft model.[88]

Although the non-viral gene transfection efficiency of NPs holds great potential, the literature revealed that they are mostly effective when used in combination with other physical penetration enhancers (Table S2 discussed in the Supporting Information).[97] The physical methods help in the internalization of the NPs loaded with genetic material into the deeper skin layers, and the NPs themselves help prevent the degradation of nucleic acids. Furthermore, thorough knowledge of the interactions and toxicological effects is needed to understand the intracellular fate of both NPs and genetic material in the skin in order to translate the technology to clinical research.

3.5. Effect of Mechanical Properties for Vaccination

The flexibility of nanocarriers is found to play a key role in the penetration of therapeutics and thus must be carefully considered. This is particularly true in the context of big cargoes like in the case of vaccinations, where large hydrophilic molecules must be delivered through the skin. Barrier disruption methods, which will be discussed in detail in subsequent sections, are very interesting in nanoparticle-based topical vaccination, because they not only facilitate

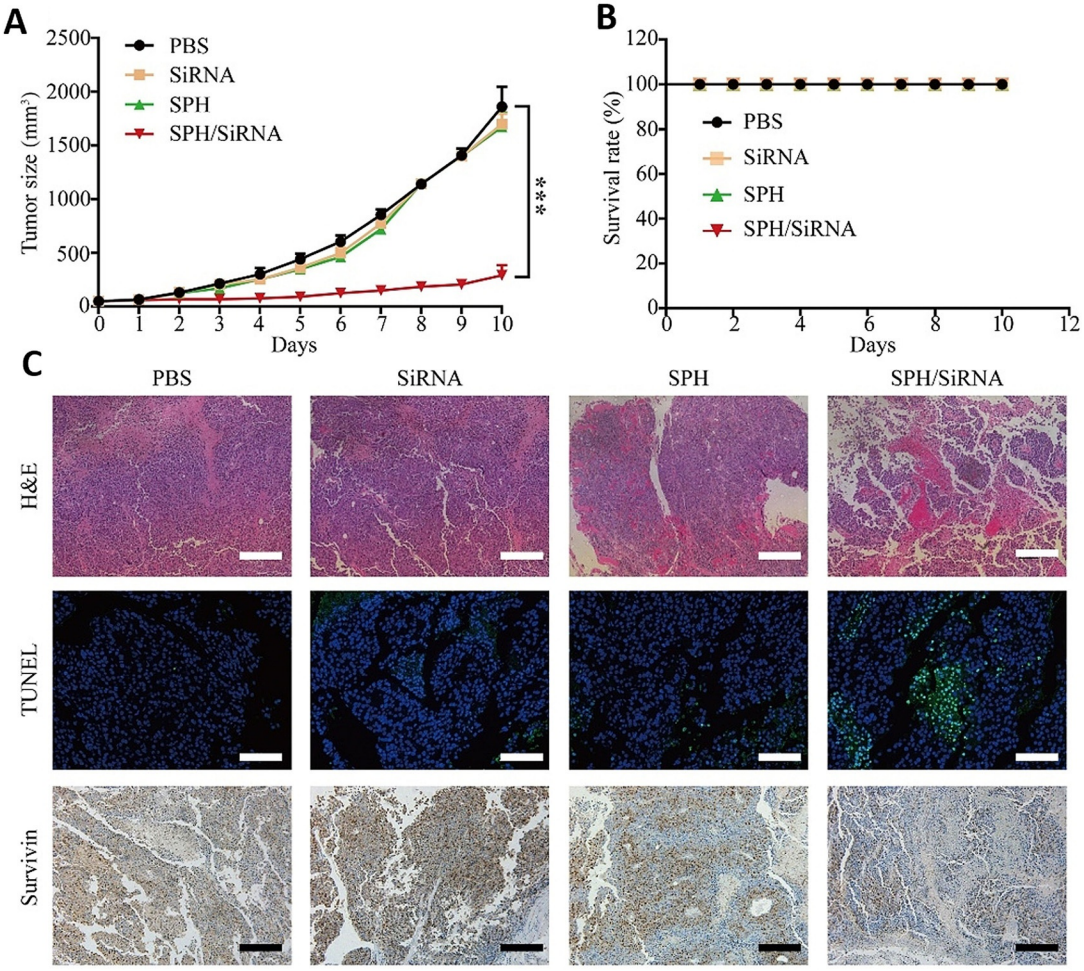


Figure 9. SHP/SiRNA nanocomplexes on topical application in melanoma xenograft-bearing mice. A) Tumor size, B) survival rates of mice, C) H&E, Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL) and survivin staining images of mice tumor tissues. Scale bar: 100 μm. Figure adapted with permission from ref. [85].

vaccine and NP permeation but also stimulate non-specific immune responses.[98] These responses include the secretion of pro-inflammatory signals that improve antigen-specific responses. Nevertheless, these methods could lead to long-term or even irreparable damage of the SC structure and therefore, non-barrier-compromising methods are preferred. Nanoparticle systems that have been explored for vaccinations could be generally divided into soft and rigid systems depending on their mechanical properties. These nanoparticle systems, with difference in their rigidity, follow different penetration mechanisms into the skin.

The viable epidermis hosts a large number of antigen-presenting cells (APCs), such as Langerhans cells (LCs) and dermal dendritic cells (dDCs), which permanently fight against innumerable pathogens that lurk in and on the skin as a very exposed area.[1b] These cells have a key role in the immune response as potent APCs against pathogens. LCs and DCs capture antigens, migrate to the peripheral draining lymph nodes, and then process and present the antigen to the naive T-cells to initiate immune responses. Their activation induces very specific and efficient responses, including not only systemic but also mucosal and dermal responses.[99] This powerful cell team present in the skin is usually bypassed by injection, the conventional vaccination method. Even with the most external injection technique (intradermal injection), needles are not able to activate LCs, and the vaccine is released in tissues where immune cells are present in smaller amounts (Figure 11).[98] In order to reach these APCs, the skin has been investigated as an alternative site of vaccination. Transdermal vaccination involves the topical application of antigens and adjuvants to induce systemic and local immune responses. In this sense, transdermal immunization (as a non-invasive and easy-to-use method) emerges as a potent alternative to injection vaccination. In addition, it can also address other typical disadvantages of commonly used injectable vaccines, such as pain, stress, need for trained personnel, and a significant record of contagion.

Although transdermal vaccination has enormous advantages, it has not been exploited yet due to the great obstacle that the SC represents. The ideal vaccines for transdermal vaccination are subunit vaccines, which consist of an antigen—a substance capable of producing a specific immune response against a certain pathogen—and an adjuvant—a substance added to enhance the immunogenicity of the

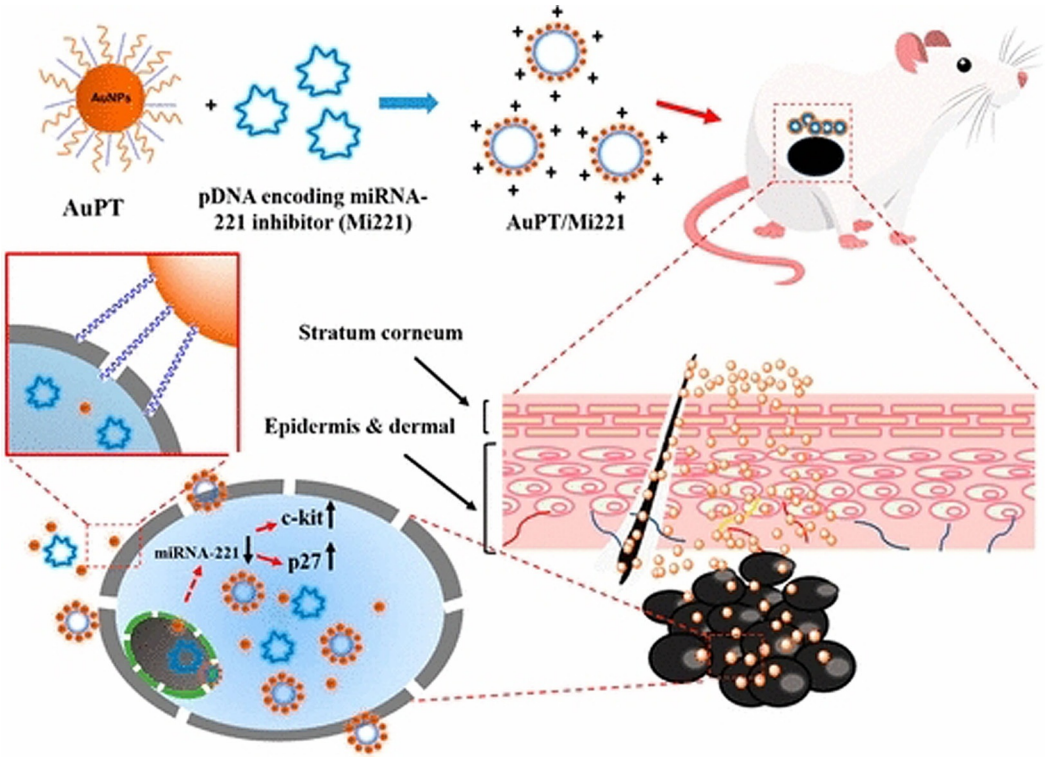


Figure 10. Pictorial presentation of transdermal delivery of pDNAs encoding the microRNA-221 inhibitor gene (Mi221) using AuPT NPs towards treatment of cutaneous melanoma. Four different steps shown are: A) AuPT/Mi221 complex synthesis; B) AuPT/Mi221 topical application; C) penetration into melanoma, and D) gene transfection by AuPT/Mi221 into melanoma cells. Figure adapted with permission from ref. [86].

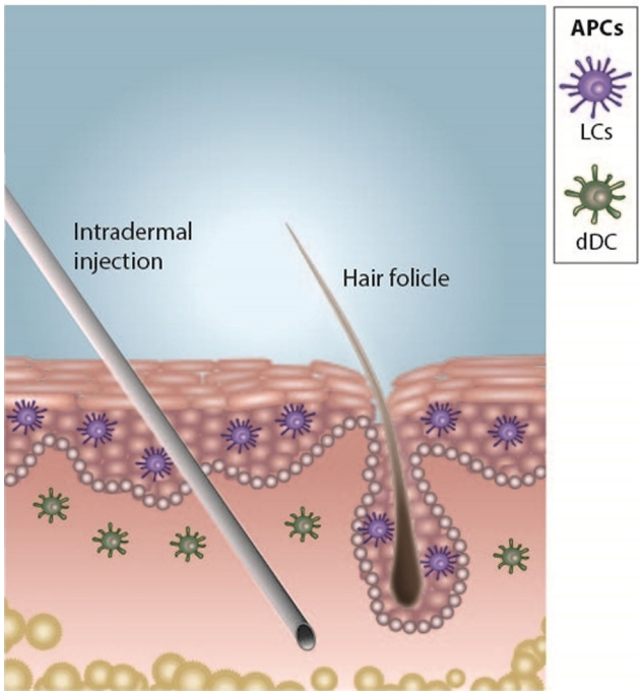


Figure 11. Distribution of antigen-presenting cells (APCs) in skin layers and intradermal injection releasing a vaccine far from the Langerhans cells (LCs).

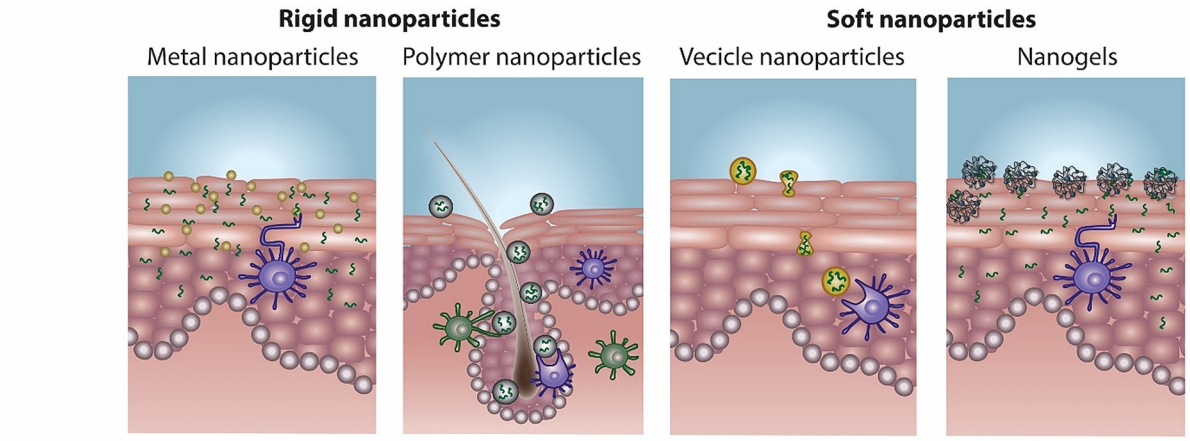
antigen or redirect its action to a specific response profile, increasing its effectiveness. Antigens are usually large, hydrophilic molecules like peptides or proteins, which cannot passively cross the SC. In addition, adjuvants can be a wide range of compounds and systems, which also have problems crossing the SC. Numerous systems have been developed to overcome the SC and activate APCs, acting as carriers and as adjuvants sometimes. Nanocarriers are protagonists in this field, since they have several advantages in transdermal vaccination systems:

1. They are similar in size to the pathogens.
2. They can encapsulate the antigen and release it in a controlled manner. Some nanocarriers can even be loaded with both antigen and adjuvant.
3. They can be functionalized to increase APC–particle interactions.
4. NPs with sizes between 20 and 200 nm can be internalized by APCs, protecting the antigen until it reaches the inside of the targeted cell and playing a double role of delivery system and adjuvant at the same time.[100]
5. Particles between 0.5 and 5 μm are taken up by macrophages. This difference between NPs and microparticles suggests that NPs produce better immune responses than microparticles.[101]
6. NPs smaller than 10 nm are able to passively penetrate the SC, and NPs bigger than 20 nm penetrate via hair follicles, an area with an abundance of APCs.[102]

In this section, nanocarriers used for transdermal vaccination are reviewed and classified as rigid and soft NPs, according to the different paths they follow to enter the skin and find the APCs. Principal characteristics of different nanoparticle systems applied to intact skin for transdermal vaccination are summarized in Figure 12.

3.5.1. *Rigid Nanoparticles*

Unlike soft NPs, rigid NPs with sizes smaller than 10 nm have been shown to be able to improve penetration of co-administered antigens through healthy skin, resulting in the



	Metal nanoparticles	Polymer nanoparticles	Vecicle nanoparticles	Nanogels
Delivery pathway	Intercellular and transcellular	Transfollicular	Intercellular and transfollicular	Intercellular and transcellular
Stimulated APC	LCs	LCs and dDCs	LCs and dDCs	LCs
Adjuvant power	High	High	High	Low

Figure 12. Characteristics of vaccination with different nanoparticle types.

generation of a specific immune response. This is the case for quantum dots[103] and gold and carbon NPs,[104] which the literature also describes as adjuvants since they promote DC maturation.[105] In the case of polymer NPs, the preferred place for their application is the hair follicle. This vaccination route is very attractive due to the large amount of APCs present around hair follicles.[106] For example, Mittal et al. investigated the potential of PLGA NPs and chitosan-coated PLGA NPs as transfollicular vaccination systems.[100] The two NPs have similar sizes and size distribution but opposite surface charges. To evaluate their delivery efficacy, ovalbumin (OVA) was used as a model antigenic protein. In the study, no significant differences in either follicular penetration of NPs or in OVA penetration could be found between the two NPs. However, 2 to 3 times higher OVA penetration could be achieved by using either nanoparticle compared with the pure OVA solution. These findings emphasize the potential of the transfollicular route for transdermal immunization, since it is possible to deliver antigens near to APCs by this pathway. Similarly, two chitosan-based NPs were investigated for topical genetic vaccination: plasmid DNA condensed chitosan NPs and plasmid DNA coated on preformed cationic chitosan/carboxymethylcellulose NPs.[107] The NPs were applied topically on the skin of shaved mice and a significant increase of antigen-specific IgG titer was detected after three inoculations separated by one week.

Although further evidence for successful transfollicular immunization using NPs can be found in literature,[108] there are some aspects to consider about this immunization route. For example, there are studies indicating that the humoral immune response (antibody-based response) obtained by intradermal and intramuscular immunization is superior to that obtained by the transfollicular route.[109] However, transfollicular vaccination can induce mucosal response, characterized by the presence of IgA antibodies.[110] Mittal et al. evidenced the need for incorporating adjuvants in vaccine formulations in order to generate both efficient antigen-specific humoral and cellular responses to modulate such responses according to the specific clinical needs.[111] Transfollicular antigen delivery induces CD8 + T-cell responses, via LC targeting,[112] but formulations including adjuvants as c-di-AMP stimulate antigen-specific multifunctional CD4 + T-cells, allowing the development of a more balanced response. Also, the effect of adjuvants is widely visible in humoral response.[111] Figure 13A,B shows IgG titer present in mice sera after four topical vaccinations. Although the presence of NPs in the formulation increased IgG titer in stripped skin, the more efficient immunization is achieved with the presence of c-di-AMP adjuvant, which generously increased total IgG titer and produced a more balanced response with the presence of the IgG2a subclass. Also, in Figure 13C it can be seen that the use of adjuvant induced an early immune response, presenting levels of specific OVA antibodies after 41 days that are comparable to the observed levels in the other groups after 56 days.

Finally, the greatest challenge for transfollicular vaccination is the loss of dose on the skin surface and within wrinkles without entering the hair follicles where it can contribute to APC activation. Consequently, transfollicular immunization requires the application of much higher amounts of vaccine than what is finally going to reach the target site.

3.5.2. *Soft Nanoparticles*

Among soft particles, liposomes are the protagonists as vaccine delivery nanocarriers. Liposomes are non-immuno-

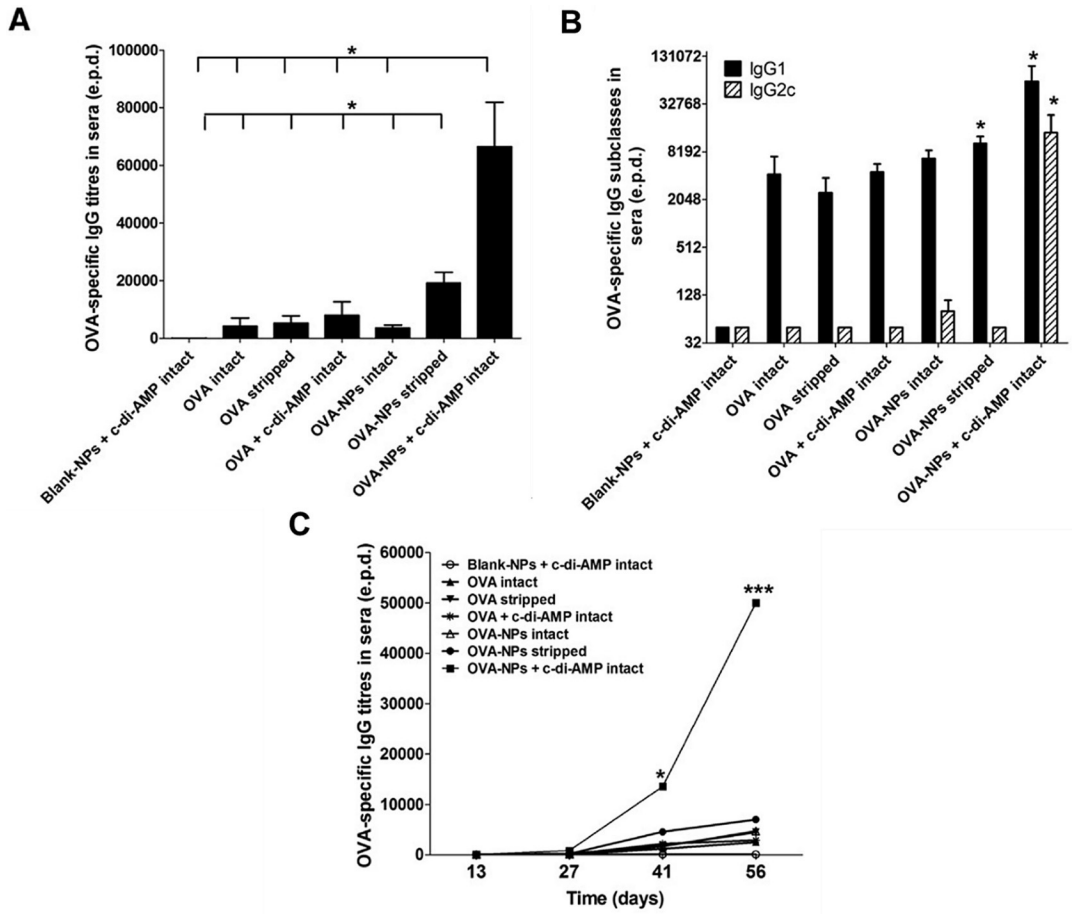


Figure 13. Systemic humoral immune responses after 4 vaccinations in C57BL/6 mice ($n=5$). A) OVA-specific IgG titer in sera after immunization. B) OVA-specific IgG subclasses 12 days after the last immunization. C) Kinetic analysis of OVA-specific IgG titer in sera of immunized mice on days 13, 27, 41, and 56. Figure adapted with permission from ref. [111].

genic vesicle carriers made of glycerophospholipids and sometimes cholesterol.[113] Although these structures do not have immunogenic properties, it is possible to incorporate molecules into their structure extending their properties not only as vehicles, but also as adjuvants to increase the immunogenicity of antigens.[114] This is the case for virosomes, vesicles composed of membrane lipids and integrated into viral envelope proteins.[115] Virosomes are a commercially validated vaccine platform with an excellent safety and efficacy track record.

Liposomes for transdermal vaccination, especially cationic liposomes, have been extensively explored as carriers for protein and DNA vaccines as they can carry both membrane-associated and water-soluble antigens.[116] In particular, elastic carriers have the ability to penetrate through the SC into the deep layers of the skin and even reach the blood circulation.[98] This is the case for transferosomes, a variation of liposomes especially designed for transdermal and/or topical delivery of a wide variety of molecules. Transferosomes are called ultra-deformable carrier systems because they have a high capacity for changing their shape via deformation and reformation mechanisms, and can pass through the natural pores in the SC. These nanocarriers were the first reported flexible liposomes for transdermal vaccination,[117] and it was claimed that their topical application induced IgG and IgA responses comparable to subcutaneous vaccination. Many studies were performed using elastic vesicle systems, demonstrating the enormous capacity of these systems for transdermal vaccination.[118]

Finally, the potential of NGs in transdermal vaccination was studied by Sonzogni et al.[119] For this propose, the authors compared the performance of three poly(*N*-vinylcaprolactam) (PVCL)-based thermoresponsive assemblies (NGs, hydrogels, and film-forming NGs) as dermal antigen-delivery systems. Platforms were loaded with OVA as antigen-model protein and topically applied onto ex vivo human skin. Besides the fact that the three systems enhanced OVA penetration in comparison to OVA solution alone (Figure 14), the application of NPs-based systems resulted in the delivery of OVA into the deeper layers of skin. Furthermore, PVCL NGs only showed improved protein penetration in tape-stripped skin, while film-forming NGs allowed deeper OVA penetration into intact skin, due to the addition of occlusion effects (Figure 14D,E). In addition, Toyoda et al. used mild electric currents to deliver cancer antigen gp-100 peptide KVPRNQDWL-loaded NGs for vaccination.[120] They achieved the accumulation of gp-100 peptide and NGs in the

epidermis, and subsequent increase in the number of LCs in this layer, by applying iontophoresis.

Although NPs have an enormous future as transdermal delivery systems because of all their advantages, plenty of knowledge is still missing. Having APCs as targets for vaccine delivery requires a proximity between these cells and the NPs. Moreover, maturation, antigen processing, and homing of APCs and induction of T-cell differentiation are essential for a well-oriented and effective immune response, and the presence of NPs can affect these processes.[105] In this sense, better understanding of how NPs affect the APC functions is crucial.

4. Nanotoxicology

Translating nanomedicines to the market involves considering the underlying hazardous characteristics that a nanomaterial can exhibit in a biological environment. In light of the growing interest in using NPs towards skin applications, it is imperative to fully investigate their nanotoxicological profile. Nanotoxicology is the study of the undesired effects of nanomaterials on the human body.[121] For example, when foreign components penetrate into an eukaryotic cell, an automatic mechanism is activated wherein lysosomes release free radicals inducing oxidative stress. These free radicals can oxidize lipids, proteins, and DNA, causing damage to different cell organelles.[122] Thus, information obtained from toxicological studies is crucial for the development of novel and safe products for biomedical applications.

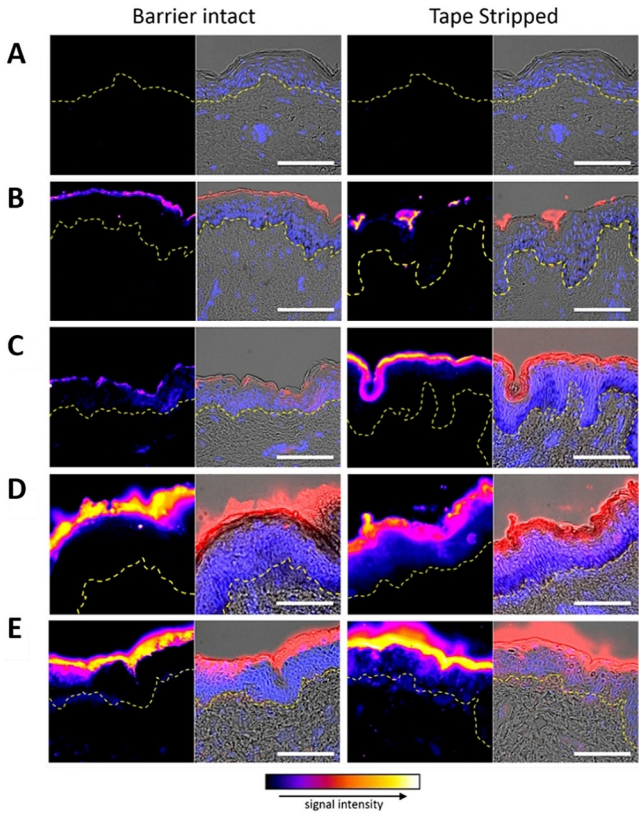


Figure 14. Representative OVA immunohistochemistry (temperature map or red staining) of A) untreated skin biopsies or skin biopsies treated with OVA in B) phosphate-buffered saline (PBS), C) hydrogels, D) PVCL NGs, or E) film-forming NGs. Dermal–epidermal boundary is marked by dashed yellow lines. Overlays show OVA staining (red), DAPI staining (blue), and bright field images (gray). Scale bars = 50 μm. Figure adapted with permission from ref. [119].

Nanomaterials with metal nuclei tend to enter directly into the mitochondria, inducing the generation of reactive oxygen species (ROS). Regarding inorganic NPs, a great deal of interest has emerged in understanding the effect of NP size on toxicity. To recognize the correlation of size and shape of gold nanomaterials on cytotoxicity in skin cells, Wang et al. studied the behavior of gold nanospheres and nanorods in a human skin cell line, HaCaT keratinocyte.[123] After several cellular uptake and cell viability assays, their results showed that gold nanospheres of different sizes (5–70 nm) were non-cytotoxic, whereas gold nanorods inhibited the cell proliferation at 0.01 $\mu mol\,mL^{-1}$. They concluded that this toxicity was due to the presence of cetrimonium bromide (CTAB) ligands used to stabilize gold nanorods. CTAB with a concentration of < 0.1 μM was used for gold nanorod stabilization; however, the results showed toxicity even with this small amount of CTAB whereas free CTAB was found to be non-toxic at a 10-fold higher concentration. The toxicity of the gold nanorods could be explained by the aggregation of the nanorods in the presence of CTAB. After an exchange of the CTAB ligand with polystyrene sulfonate (PSS), the gold nanorods did not stop the cell proliferation with the same ligand concentration, indicating the importance of biocompatible stabilizing agents when AuNPs are used in living cells.[123]

In another study, Labouta et al. investigated the topical exposure of two different AuNPs formulations to understand the effect of size, charge, surface chemistry, and solvent towards penetration and metabolic changes in excised human skin using multiple techniques like reflectance confocal microscopy (RCM), dermoscopy, TEM, and multiphoton tomography (MPT) with fluorescence lifetime imaging microscopy (FLIM).[124] They compared the penetration and metabolic effects of ionically stabilized polar 15 nm AuNPs in water and sterically stabilized non-polar 6 nm AuNPs in toluene on excised human skin. After a thorough investigation, it was observed that after 24 h exposure, 15 nm AuNPs in aqueous solution tend to aggregate on the surface of SC, whereas, 6 nm AuNPs in toluene showed penetration into the epidermal layers of human skin. The penetration of the 6 nm AuNPs could be explained by the disruption of the lipid layers in the presence of toluene. The total NAD(P)H autofluorescence signal was quantified as a function of skin depth and the results revealed that after the skin had been exposed to 90 $\mu g\,mL^{-1}$ of 6 nm AuNPs in toluene for 4 h, the NAD(P)H value decreased to 0 in the epidermal layer (30–40 μm of depth), whereas for the bigger particles (15 nm AuNPs) NAD(P)H values were stable. The studies indicated that toluene-treated skin modified the metabolism towards toxic values in the cells after 4 h of exposure, while 15 nm AuNPs in water with the same concentration exhibited no cytotoxicity.

Silver is well-known for its antimicrobial properties, which resulted in numerous investigations for biomedical purposes.[125] NPs with a silver core have been explored extensively for skin treatment; however, it is known that silver NPs

(AgNPs) can cause skin irritation and gray discolouration on the skin surface at high exposure times.[126] Several studies have employed colloidal suspensions of AgNPs on skin cells to determine their toxicity levels. NPs of different sizes have been tested on human cells (from 20 nm to 80 nm) for 48 h of exposure at a fixed concentration of 100 $\mu g\,mL^{-1}$. Surprisingly, no toxicity was observed for AgNPs considering that the $AgNO_3$ salt (used as starting material) is highly toxic at a concentration of 10 $\mu g\,mL^{-1}$. Comet assay performed on silver nanomaterials revealed no changes in the cells, indicating that silver nanomaterials are non-mutagenic at 100 $\mu g\,mL^{-1}$, whereas $AgNO_3$ salt solutions at the same concentration can damage genetic material due to the release of Ag^+ ions. A continuous ion release could increase the risk of allergic dermatitis from NPs based on transitions metals like Ni, Pd, and Co. In addition, epidermis inflammation was observed when TiO_2 NPs were applied on the skin due to the generation of free radicals.[127] Impurities that can be present in the final material, as a consequence of the synthetic process (metals, toxic chemicals, etc.), can cause cytotoxicity triggered, for instance, by oxidative damage.[128]

The phototoxic effect analysis becomes important for light-sensitive metals like silver.[126] Two different shapes of colloidal silver NPs, nanospheres and nanoprisms (100 $\mu g\,mL^{-1}$) were exposed to sunlight in order to determine the influence of shape on toxicity.[129] Spherical AgNPs (100 $\mu g\,mL^{-1}$) were exposed up to 1–3 weeks under sunlight before carrying out the colorimetric assay MTT, based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. After 3 weeks, silver nanomaterials did not show any toxicity for HaCaT keratinocytes after 24 h, but aggregation was observed, which means that after increased exposure time, toxicity can be expected with the same concentration. In contrast, $AgNO_3$ (10 $\mu g\,mL^{-1}$) showed 98 % of cell death after 3 weeks of similar exposure. Unexpected results were displayed by silver nanoprisms (100 $\mu g\,mL^{-1}$), where no aggregation was observed after 4 weeks of sun irradiation and drying. The key to the different toxicity behaviors was related to the ligand that stabilized the NPs. When a biocompatible stabilizing agent (like polyvinylpyrrolidone, PVP, used during the synthesis of silver nanoprisms) was used to cover the surface of the AgNPs, toxicity was not observed even after sun exposure with a concentration of 100 $\mu g\,mL^{-1}$. Consequently, the importance of the PVP stabilizing agent was demonstrated.[129] This effect has been established for different kinds of NPs. For example, SiO_2 NPs covered with silanes show less cytotoxicity in vitro and in vivo than the same NPs without functionalization.[130]

In addition to inorganic NPs, the biocompatibility of organic NPs has also been examined through assays like MTT, comet assay, ROS determination, and eye irritation potential. Edlich and co-workers investigated polyglycerol-based NGs to determine their inhibition of cell proliferation capability.[131] No toxicological effect was observed on skin for concentrations in the range 50 to 500 $\mu g\,mL^{-1}$. Polyglycerol-based NGs were compared to a true positive control and the results indicated that the NGs did not increase ROS production levels at a concentration of 500 $\mu g\,mL^{-1}$. Furthermore, to evaluate their potential to modify genetic material, comet assay was performed which showed no mutagenic effect in Langerhans cells. Similarly, chitin NGs loaded with curcumin have been found to exhibit low toxicity at 1 $mg\,mL^{-1}$ in HDF and A375 cell lines using similar assays.[132] With the current trend of organic NPs for topical applications, synthetic polymers like PLA or PLGA have been explored and combined with chitosan to improve their biological properties. To analyze the immune response of these materials, in vivo inflammatory models have been developed by Singh et al.[133] Allergic contact dermatitis was quantified by measuring the reduction of ear swelling in inflamed mice ears. The studies revealed that the thickness of ear swelling was reduced after 3 days, from 106.56 μm to 56.23 μm after the application of NGs for three consecutive days. Table 3 summarizes approaches and results from different nanotoxicological studies using different nanomaterials.

One of the major areas of research in toxicology studies is the development of skin models that can mimic the real conditions of human skin.[134] Nowadays, several ex vivo human skin models have been improved to analyze the antimicrobial properties of different nanomaterials. Mouse, guinea pig, rat, and rabbit represent the most widespread models.[135] Although animals are good models, they are not fully comparable to human skin due to the anatomical and physiological differences between the species. In addition, due to ethical considerations, alternatives need to be further developed.[134a] Schaudinn et al. have developed a bacterial wound infection model based on ex vivo human skin to test the skin toxicity of nanomaterials.[135] The model consists of induced wounds on ex vivo human skin and *Pseudomonas aeruginosa* bacteria under the wound that can be used after 20 hours of incubation. The principal advantage associated with the ex vivo wound models is that the use of healthy human skin and quantifiable bacterial infection leads to reproducibility of the results.

5. Clinical Development

Bringing a product from bench to market is a long, but crucial process. Materials that exhibit promising results in in vitro and in vivo models can be considered for further clinical testing. Table 4 gives a general outline of the different phases of clinical development that a new drug delivery system must complete to enter the market. These phases can be adjusted with respect to length, nature of the drug or nanocarrier, disease, and agency that approves it. Here, we discuss the latest clinical advances of soft NPs and hybrid nanocarriers studied in human trials for dermal applications.

Recently, Span 60-Tween 20 (70:3 w/w) vesicles, formulated with 1 % Carbopol, and loaded with retinoic acid (RA) were tested in in vivo models to deal with *Acne vulgaris*.[137] These particles form a nanovesicular carrier with a non-ionic surfactant that consists of an elastic vesicle that can penetrate through the SC. A total of 15 patients were filed for treatment (clinical phase 2) with Span 60-Tween 20 vesicles. After 4 weeks of treatment, the prepared vesicles showed better results than the already commercialized Acretin®. Acretin® contains Tretinoin (0.05 %) as the active ingredient, along

Table 3: Summary of approaches and results from different nanotoxicological studies of different nanomaterials.

Material	Experiment	Result	Ref.
Gold nanospheres, gold nanorods	Cell uptake, cell viability (Keratinocytes cells model)	Gold nanorods inhibit cell proliferation	[123]
Polystyrene nanoparticles	Distribution along the skin layer	Accumulation in the hair follicle	[123]
15 nm gold nanospheres	Penetration study of NPs on ex vivo excised human skin	Aggregation on surface of SC, increases cytotoxicity	[124]
6 nm gold nanospheres	Penetration study of NPs on ex vivo excised human skin	No aggregation, no increase of cytotoxicity	[124]
Silver nanoparticles (20-80 nm)	MTT, comet assay	Non-mutagenic, no cytotoxicity	[126, 129]
Silver nanoparticles	Cytotoxicity after exposure of the material to sunlight (3 weeks)	No cytotoxicity	[126, 129]
Silver nanoparticles	Aggregation due to the ligand	Silver nanoprism, with PVP ligand, no cytotoxicity, no aggregation	[129]
TiO_2 nanoparticles	Skin toxicity	Epidermis inflammation	[127]
SiO_2 nanoparticles	Cytotoxicity in vivo and in vitro as a function of the presence of a silane ligand	Less cytotoxicity when the ligand is present	[130]
Polyglycerol-based nanogels	MTT, comet assay, ROS determination	No cytotoxicity from 50–500 $\mu g\,mL^{-1}$; No mutagenic effect; No increase in ROS levels	[131, 132]
(PLA, PLGA)-chitosan particles	In vivo inflammatory assay	Reduction of swelling of inflamed mice ears	[133]

Table 4: Basics on drug development. Information adapted from ref. [136].

Phase	Years (approx.)	Task description
Preclinical (Phase 0)	5–7	Establish the target and the material that can act there. Optimize the material and determine its effectiveness and safety in animal models.
Clinical Phase I	1–2	Search for the safe dose. Requires up to 100 healthy volunteers
Clinical Phase II	1–2	Evaluate the effectiveness and possible short-term side effects. Requires up to 500 patient volunteers
Clinical Phase III	2–3	Confirm the material effectiveness and possible long-term side effects. Requires up to 5000 patient volunteers
Review and approval	1–2	Agency confirm the results. If they agree, the material is approved to enter the market.

with poloxyl 40 sterate, stearyl alcohol, isopropyl myristate, stearic acid, BHT, sorbic acid, xanthan gum, and water as inactive components. In summary, the number of total lesions decreased with Span 60-Tween 20 vesicles in comparison with Acretin® after the same period of treatment. The main reason is related to the small size of the vesicles, which is shown to enhance penetration of RA through SC.

Another novel system that reports encouraging results is based on solid lipid NPs (SLNs) for the release of the antifungal agent Fluconazole (FLZ) for the treatment of *Pityriasis vesicolor* (PV), a type of fungal infection caused by the *Malassezia* species in SC.[138] Two formulations of FLZ-SLNs topical gels, with a release capacity of 50% and 80% of the encapsulated drug, were investigated. A randomized controlled clinical trial (RCT) was performed on 30 well-diagnosed PV patients and the results were compared with the market product Candistan®. The formulation consists of clotrimazole as active ingredient and isopropyl alcohol and propylene glycol as inactive ingredients. The study was carried out for 4 weeks and the formulations were applied locally on the affected area twice a day. The results demonstrated that after full treatment, the cure from fungal infection was increased up to 99% using FLZ-SLNs formulations. In contrast, Candistan® could only eradicate 80% of the total fungal infection (Figure 15).

Recently, new nanoliposomal particles loaded with amphotericin-B 0.4% (tradename SinaAmpholeish) have been developed in Mashhad University of Medical Sciences, Iran, towards the treatment of cutaneous leishmaniasis, a disease caused by infection with *Leishmania* parasites, which are spread by the bite of infected sand flies. The formulations were applied topically on healthy volunteers in a randomized, double-blind, right-left, placebo-controlled phase 1 clinical trial.[139] In total, seven biophysical parameters were tested before and two weeks after application: temperature, transepidermal water loss, hydration, surface lipid amount, erythema index, melanin index, and pH. No adverse effects were found on volunteers when the formulations were applied twice a day for a week. The nanoliposomal formulations showed promising results and the next step would be to test them in second phase clinical trials.

Currently, hybrid systems based on core–shell materials (metal cores with an organic stabilizer) are in clinical trials as well. Silver NPs (AgNPs) are currently under investigation for antibacterial purposes in different wounds in human probands, like diabetic ulcers, ulcers in burn patients, and

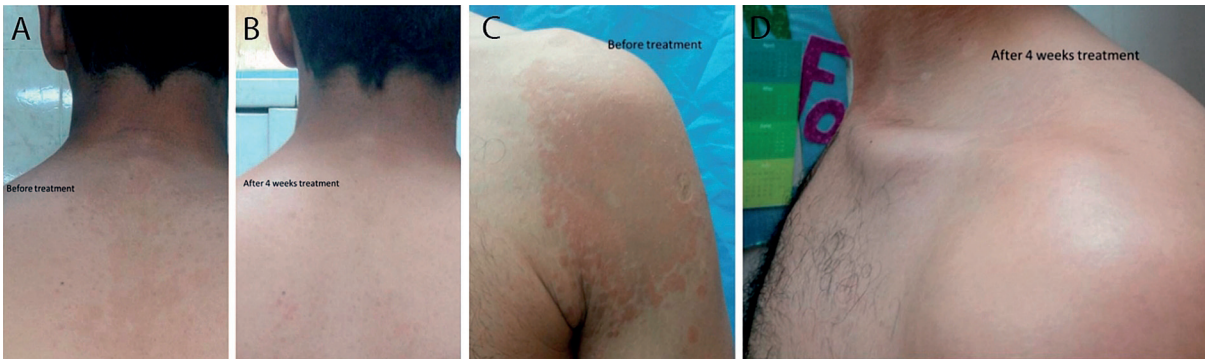


Figure 15. Photographic images of two examples, before and after patient treatment with FLZ-SLNs materials. A) Before treatment, B) after 4 weeks treatment, C) before treatment, and D) after 4 weeks of treatment. Figure adapted with permission from ref. [138].

burn wounds.[140] Wound dressings coated with AgNPs have been applied successfully as a new clinical tool to combat infections encountered after second degree burns.[141] Although the power of AgNPs has been demonstrated to efficiently fight bacterial pathogens, more clinical studies are needed to determine their dermal toxicology. Gold, an alternative to silver, is a widely used metal for dermal treatment and especially used in anti-ageing cosmetics. Previous studies using AuNPs on mercury-exposed mice models showed an increase in collagen quantity and cell proliferation in liver, kidney, and brain.[142] The results indicated the potential of AuNPs in organ wound healing. Recently, creams based on AuNPs have been explored for their skin applications and are currently under clinical study. In one such study, the skin was able to recover from wounds caused by phenol impregnation after 4 weeks of treatment using nanogold formulations as active components.[143] The wound located on the neck was observed every week and the total wound area was found to decrease by a thousand-fold after the whole treatment. The results underline the potential application of AuNPs for dermal treatment.

Organic soft NPs and hybrid NPs have shown fascinating results both in preclinical and clinical trials for dermatological applications. However, only few nanocarrier technologies have successfully made it to the market, including Estrasorb®, a topical micellar-encapsulated emulsion of estradiol for the treatment of vasomotor symptoms in menopause.[144] In addition, a few liposome/Ethosome[145] formulations that have reached the market are: Noicellex™ for cellulite,[146] SEQuaderma® for treatment of acne, Dermo-Expertise®[147] for skin care, Equisomin™[148] for hair root and scalp maintenance, Nanosomin™[148] for skin aging, and Ambisome®,[149] a liposomal nanocarrier, for the encapsulation of Amphotericin B in the treatment of fungal infections. Observing the great potential of NPs, it might be surprising that the list of approved and marketed technologies is not longer than this. The reason for the manageable list is the negligence of key issues during research and development of NP production, the development of nanocarrier systems without following good manufacturing practice regulations, the high cost of production, as well as the lack of consideration of biodegradability and sustainability of the NPs. In addition, in light of the high complexity of the skin, researchers should strive to investigate new nanocarriers in close collaboration between various research areas, e.g., medicine, chemistry, pharmaceutics, physics, and biology. This interdisciplinary work is invaluable for rationally designing novel nanocarrier systems for dermal applications, as well as to unravel their full potential. Another area whose further development is essential for bringing new nanocarrier systems to the market is the development of new skin models. These should allow the study of the nanocarriers under realistic conditions (e.g., disease conditions), therefore giving valuable information about nanocarrier performance. Due to the outstanding potential of nanocarrier systems, we expect to see the clinical approval of more nanocarriers in the near future, especially when the benefits from such technologies outweigh the health and economic aspects of currently established therapies.

6. Summary and Outlook

NP-based dermal delivery of therapeutics is an attractive and non-invasive technique for the treatment of skin diseases arising from multiple conditions like inflammatory diseases, genetic disorders, and infectious diseases. Studies have concluded that the barrier properties of SC have found to be a limiting factor for the penetration of therapeutics in the diseased area. Although various penetration enhancers could deliver therapeutics to the deeper layers of the skin, these methods could often lead to long term damage to SC. Thus, a highly interdisciplinary approach is needed that could combine the potential of NPs as delivery vehicles and physical enhancement techniques for delivery of therapeutics. In this Review, we have highlighted multiple examples of NPs displaying enhanced penetration, retention, and the delivery of therapeutics at the diseased site. Owing to the advancements in dermal drug delivery using NPs, there is a promising outlook for the translation of this technology to the market. Several products have reached the market for the topical/transdermal delivery of therapeutics; however, cosmetic NPs products dominate over pharmaceutical products. This is majorly due to obstacles like batch-to-batch variations, large-scale synthesis, stability, and clinical performance along with more complex regulations that limit the translation of this

technology into clinical development and commercialization for skin diseases. Another barrier for the commercialization of the NPs based formulations are associated with the skin models currently used for research purposes. The current models like pig skin, mice skin, reconstructed skin and ex vivo human skin fail to mimic the real-time conditions of the diseased skin. The research focus on personalized medicine and in vitro models that mimic the actual biological conditions of the skin can reduce the translational time between the bench to the market. With this broad perspective, and multiple layers of complexity, we highlight the potential of the nanocarriers in overcoming the skin protective barrier for the delivery of active therapeutics. With this comprehensive revision, we intend to encourage research on nanoparticle development for skin therapy by a holistic and multidisciplinary approach that considers the challenges in translation of technology. For this, key players from different fields, chemistry, pharmacy, dermatology, toxicology, etc. can bring their respective insights to overcome the current challenges associated with efficient delivery of therapeutics in skin diseases.

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Conflict of Interest

The authors declare no conflict of interest.

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