



Nanoparticles for topical drug delivery: Potential for skin cancer treatment

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ABSTRACT

Nanoparticles offer new opportunities for the treatment of skin diseases. The barrier function of the skin poses a significant challenge for nanoparticles to permeate into the tissue, although the barrier is partially compromised in case of injury or inflammation, as in the case of skin cancer. This may facilitate the penetration of nanoparticles. Extensive research has gone into developing nanoparticles for topical delivery; however, relatively little progress has been made in translating them to the clinic for treating skin cancers. We summarize the types of skin cancers and practices in current clinical management. The review provides a comprehensive outlook of the various nanoparticle technologies tested for topical therapy of skin cancers and summarizes the obstacles that impede its progress from the bench-to-bedside. The review also aims to provide an understanding of the pathways that govern nanoparticle penetration into the skin and a critical analysis of the approaches used to study nanoparticle interactions within the tissue.

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Contents

1.	Introduction	87
2.	The skin - a formidable barrier	88
3.	Types of skin cancers	88
4.	Clinical management of skin cancers	88
5.	Nanoparticle-based drug delivery for skin cancers	89
6.	The challenge for nanoparticles in topical therapy of skin cancers	93
7.	Nanoparticle technologies for dermal delivery	94
7.1.	Vesicular carriers.	94
7.2.	Lipid Nanoparticles.	95
7.3.	Polymeric micelles and nanoparticles	95
7.4.	Nanoemulsions	96
7.5.	Nanofibers	96
7.6.	Metallic nanoparticles	97
8.	Skin penetration of nanoparticles - a critical analysis	97
9.	Conclusion - challenges for clinical translation.	103
	Acknowledgment	103
	References	103

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1. Introduction

Skin cancers have seen a dramatic rise worldwide [1–5]. They can be broadly classified into (a) melanomas that originate from the melanocytes and (b) non-melanoma skin cancers originating from epidermally-derived cells [6]. Together, they constitute about 95% of skin cancers, with some rare and aggressive cancers making up the remaining. Basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC) account for 99% of all non-melanoma skin cancers (NMSCs). Traditionally, cSCC has accounted for 20% of all skin cancers, with the majority being taken up by BCCs. However, a recent study estimated the ratio of incidence to be at 1:1 between BCC and SCC in the Medicare fee-for-service population [4]. Further, an overall increase of 263% was observed in the incidence rates for cSCC during the periods 1976–1984 and 2000–2010 [7]. This rise is attributed to the growing elderly population and improved diagnostic methods [8]. The less common forms of NMSCs include cutaneous lymphomas, Kaposi's sarcoma, Merkel cell carcinoma, skin carcinosarcoma, and dermatofibrosarcoma [9]. NMSCs are generally curable. However, with increased incidence worldwide and exploding treatment costs, NMSCs have become a matter of great health concern to the public [2,10–12].

The skin is the largest organ of the human body and serves as the first line of defense against microorganisms, UV radiation, or chemicals. Due to its tough barrier properties, the skin also prevents salt and fluid loss while regulating the body temperature. Topical drug delivery via skin makes it possible to localize the drug to the skin for targeted therapies and bypass the first-pass metabolism following oral delivery [13]. Topical products also provide an advantage in treating clinical and sub-clinical lesions over a large surface area of the body [14,15].

The advent of nanotechnology has revolutionized drug delivery for oral, topical, and parenteral medications. Nanoparticles as drug carriers can potentially enhance drugs' specificity, bioavailability, and therapeutic efficacy while improving patient compliance during therapy [16–21]. Besides, nanoparticle-based drug delivery can enhance drug retention with tunable release kinetics at the disease site inside the skin. The skin, however, makes it virtually impossible for nanoparticles to pass through. Skin entry of nanoparticles through the transappendageal route, which includes the hair follicles, sweat glands, the sebaceous and the pilosebaceous glands has been reported [22–25]. This enables nanoparticles to penetrate the superficial layers of the stratum corneum i.e. the outermost protective layer of the skin. However, the transappendageal route covers only 0.1% of the total skin surface [26]. Consequently, it does not contribute significantly towards the penetration of large molecules and nanoparticles into deeper layers of the skin where the disease is primarily localized.

Here, we review the barrier properties of the skin with respect to nanoparticle penetration. We outline the current clinical management of skin cancers with an emphasis on non-surgical field therapy. The review also gives a comprehensive outlook of the different types of nanoparticles tested in topical delivery, especially with a focus on treating skin cancers. Further, we describe the possible interactions of nanoparticles inside the skin; the pathways involved during topical delivery, and provide a critical analysis of this dermal penetration. The review aims to present some of the obstacles that nanoparticles face in topical therapy of skin cancers and provide researchers with the guideline for topical nanoparticle formulation development.

2. The skin - a formidable barrier

The skin is the largest organ of the human body. It has a surface area of approximately 1.8m² and occupies 16% of the total body mass. The epidermis, i.e. the outer surface of the skin, is comprised of the stratum corneum (SC) and the viable epidermis. (Fig. 1). The SC is a 10–20 µm thick matrix of terminally differentiated keratinocytes interspersed with lipids. The SC of healthy skin provides a barrier to molecules both hydrophilic and large in size. The molecular weight is classically

considered to be 500 MW for transdermal drug delivery [27] although a higher molecular weight cut off is seen for topical products, for example, 800Da. Further, the role of molecular weight in topical products could depend entirely on the state of the skin's barrier [28].

The SC is immediately followed by the epidermis composed of viable keratinocytes followed by the dermis comprising of fibroblasts and connective tissue. The dermis serves the host for the sweat glands, sebaceous glands, hair follicles, lymphatic vessels, blood vessels, and nerve fibers. Nanoparticles that penetrate into the epidermis have access to viable and immunologically active cells and could transit into the lymph nodes.

3. Types of skin cancers

The primary risk factor of developing cutaneous cancers is chronic exposure of the skin to sources of UV radiation - both natural (solar) and artificial (tanning beds) [29–31]. Genetic predisposition, compromised immune system or exposures to viral infections (human papillomavirus) and/or chemicals (aromatic hydrocarbons, arsenic) are also potential risks for cancers. This review focuses on NMSCs since melanoma has a high propensity for metastasis, and hence it is more of a systemic disease as far as treatment options are considered.

Basal Cell Carcinomas (BCCs) are the most common of all malignant skin cancers. They originate from basal cells of the epidermis and occur primarily in the head and face. The prevalence of BCC has risen by 33% over the past two decades [32,33]. Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer. It originates from rapidly proliferating malignant cells in the epidermis, which then invades the dermis, and potentially, metastasize to distant sites or lymph nodes. When tumor proliferation is restricted to the epidermis, the disease is termed as SCC in situ or Bowen's disease. A precancerous condition of cSCC, actinic keratosis (AK), is caused by chronic exposure of the skin to ultraviolet (UV) radiations, x-irradiation, or polycyclic aromatic hydrocarbons. Clinically, AK presents itself as a scaly, flesh-colored, or erythematous papule or plaque that ranges in size from 1–2mm. The lesions could advance into squamous cell carcinoma (SCC) in situ or into its invasive form [34–37].

4. Clinical management of skin cancers

The greatest impact on the prognosis of any type of skin cancer is its early diagnosis and immediate treatment. Surgical and non-surgical procedures exist for treating precancerous and cancerous skin lesions [38,39]. The type of treatment for NMSCs generally depends on the number, thickness, and distribution of lesions, past history of its treatment, and recurrence. Patient preference is also taken into account especially with regards to convenience, tolerance, and treatment cost [40]. Surgical procedures such as cryosurgery, laser therapy, curettage, and desiccation, dermabrasion, radiation therapy, and Moh's micrographic surgery are effective for single and visible lesions. However, this could cause complications that often arise due to pain, serious disfigurement, edema, gastrointestinal hemorrhage, secondary infection, chronic ulcer formation, blister formation, hypopigmentation, scarring, hair loss, and radiodermatitis with nonhealing ulcerations, among others [39,41–43].

When lesions are spread all over the body and the field of treatment is large, non-surgical procedures are generally considered for patients who are not candidates for surgery. In such cases, field-directed therapies can eliminate clinically visible and subclinical lesions and prevent its progression to distant sites [40]. This includes the use of radiation therapy, photodynamic therapy, and topical chemotherapeutic or immunomodulator drugs, which may be used alone or in combination with other procedures [43]. Photodynamic Therapy (PDT) involves the topical application of a photosensitizing agent (aminolevulinic acid (ALA) and methyl aminolevulinate (MAL)), followed by the use of a specific wavelength of light to activate the compound and generate cytotoxic reactive

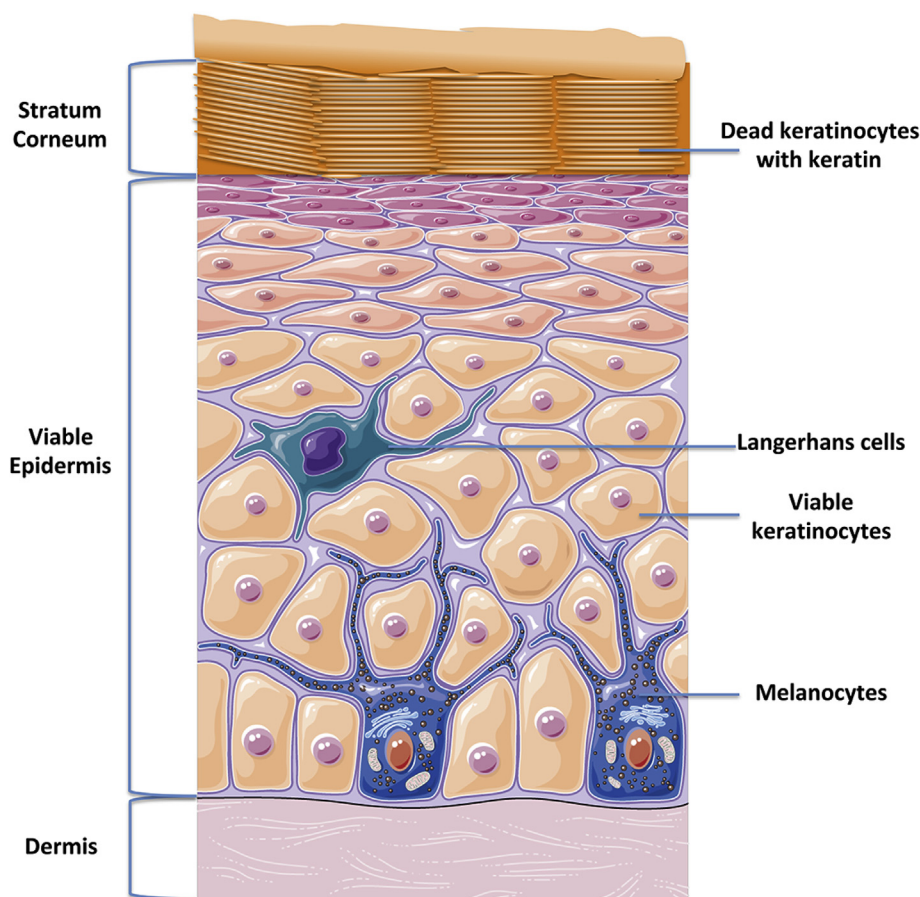


Fig. 1. Structure of the Skin Barrier. The epidermis, which forms the skin's outer surface or barrier, is made of the hydrophobic stratum corneum (SC), the viable epidermal layer, followed by the dermis.

oxygen species that can oxidize the subcellular organelles to kill the tumor. The treatment and cosmetic benefits are far superior for PDT when compared to excision procedures for skin cancers [44,45].

Topical therapies are primarily used when there are multiple lesions or the treatment area is large, or for lesions that take time to heal. Topical therapies are generally adopted for patients who are not candidates for surgery. They could avoid the formation of post-surgical scars. However, the cure rates associated with topical drugs are much lower compared to surgical removal of cancers. Besides, current topical therapies do not penetrate well and require frequent, prolonged applications. Consequently, patient adherence is inconsistent and the risk of developing severe inflammation and/or systemic toxicity is high. Commonly used topical medications include corticosteroids, 5-fluorouracil (5-FU, Efudex), Imiquimod, Resiquimod, Ingenol mebutate, Diclofenac, retinoids, Mechlorethamine, Carmustine, Bexarotene, Tazarotene, and photodynamic therapy, etc. [40,46–51] Among the drugs listed, 5-FU or Efudex, Imiquimod, and Ingenol mebutate are known to cause cytokine release that results in severe skin irritation/inflammation when applied over large areas. When applied on larger surface areas, Imiquimod is known to induce systemic symptoms such as headache, fatigue, myalgia, and influenza-like symptoms [52]. Pain, scarring, irritation, inflammation, and a host of other side effects including contact dermatitis, pruritus, skin erythema, edema, and ulceration currently limit topical therapies [40,47,48,50].

5. Nanoparticle-based drug delivery for skin cancers

Over the years, nanoparticle-based systems have been tested *in vitro* and *in vivo* for topical therapy of skin cancers. The premise for using

nanoparticles (NPs) is primarily based on its ability to improve skin and tumor penetration of bioactive molecules. This enhances drug retention in the skin and tumor, resulting in reduced dosage, minimal toxicity, and improved patient compliance. Despite the advent of several such systems at a preclinical level, nanoparticle-based topical therapies are yet to be approved in a commercial context. Here, we highlight various systems evaluated for the topical therapy of skin cancers and aim to understand the parameters that possibly govern the engineering of an ideal topical formulation.

5-FU is one of the most commonly used drugs in treating superficial NMSCs and its toxic side effects make it a challenge for patients to strictly follow the regimen. Furthermore, the drug's hydrophilicity with a log octanol–water partition coefficient of -0.89 causes it to permeate the skin poorly [53–55]. In an attempt to enhance the local therapeutic effect and identify the dosage limits, 5-FU was loaded into polybutyl cyanoacrylate nanoparticles and applied topically on a daily basis for 35–40 days to treat patients (mean age 74 years, range 56–90) with superficial BCC. Although 31 of the 32 patients treated for achieved complete tumor resolution, the treated skin became red and irritated with sores and crusts. The system did not advance further into clinical development [56]. Safwat et al incorporated 5-FU loaded gold nanoparticles with a mean size of 16.02 ± 0.22 nm and positive zeta potential ($+47.81 \pm 0.43$ mV) into Pluronic F127 gel or vanishing cream bases for topical application on SCC tumor xenografts [54]. *Ex vivo* skin permeation studies performed on a full-thickness mouse dorsal skin demonstrated increased drug flux within the epidermis and dermis. The NP-based treatment reduced the tumor growth by 18-fold when compared with untreated and free 5-FU gel. The cream base was expected to provide high spreadability and increased surface

contact of the nanoparticles applied on the skin. Subsequently, this enables the particles to strongly interact with the SC, fluidize the barrier, and navigate the lipid lamellae domains. Dendrimers incorporating 5-FU, or non-steroidal anti-inflammatory drugs (NSAIDs) including ketoprofen and peptides have also been investigated for topical applications. Venuganti and Perumal demonstrated that amine-terminated dendrimers could potentially act as polymeric skin enhancers for hydrophilic drugs such as 5-FU [57]. Pre-treatment with dendrimers in a vehicle such as isopropyl myristate (IPM) altered the skin barrier to enhance 5-FU diffusion into the tissue. On the other hand, when applied simultaneously, the dendrimer interacts with 5-FU to increase its permeability coefficient via reduced drug solubility in IPM. [57] Elsewhere, topically applied 5-FU loaded chitin nanogels with a mean size of 125–140 nm, and charge +31.9 mV was used to enhance drug retention within the skin [58]. The authors suggest that the positively charged chitin strongly interacts with the stratum corneum to loosen the keratin and achieve drug accumulation within the deeper layers of the skin. In a different study, 5-FU when incorporated into Pheroid™ – a stable colloidal system composed of lipid-based submicron and micron-sized structures – enhanced drug delivery deep within the skin [59]. The mean size and zeta potential of 5-fluorouracil Pheroid™ droplets were 251 ± 0.077 nm and -28.95 ± 2.45 mV. The authors hypothesize that the lipophilic Pheroid™ vesicles fluidize the stratum corneum and reduce its barrier properties to transport 5-FU into the skin. In a broad-spectrum study, 5-FU carrying transfersomes (size 153.2 ± 10.3 nm, zeta potential -39.6 ± 0.3 mV), liposomes (size 120.3 ± 9.8 nm, zeta potential -36.9 ± 0.4 mV), and niosomes (250.4 ± 8.6 nm, zeta potential -33.4 ± 0.4 mV) demonstrated significantly high topical delivery and enhanced drug retention within the skin compared to the drug's nonvesiculated form [60]. A higher lipid content and deformable behavior of the vesicles may have ensured 5-FU to partition within the lipophilic domains of the stratum corneum for enhanced penetration. In addition to the NP composition itself, vehicles carrying the particles themselves could also facilitate skin delivery. Water-in-oil (w/o) nanoemulsions of 5-FU with a mean size of ~100 nm and charge ~-15 mV were formulated from Capryol (propylene glycol monocaprylate) as the oil, Transcutol (purified diethylene glycol monoethyl ether) – a skin permeation enhancer as the surfactant and polyethylene glycol (PEG) 400 as the co-surfactant to enhance topical delivery [61]. The composition used is known to interact with the stratum corneum and change its barrier properties for increased skin penetration. Similarly, 100 nm-sized w/o emulsions formulated using sorbitan monooleate (Span 80), sorbitan trioleate (Span 85), polysorbate 80 (Tween 80), and isopropyl alcohol (IPA) with different oils such as oleic acid, triacetin and isopropyl myristate (IPM) also improved the topical delivery of 5-FU [62]. Recently, nanoparticles fabricated from plant-based extracts such as orange juice, have also been used to deliver 5-FU and treat skin cancers topically [63]. The size and zeta potential of these orange-juice 5-FU NPs (OJ-NPs) were 25 nm and -39.6 ± 2.10 mV. When applied topically, the 5-FU OJ-NPs in a cream base enhanced the survival of mice with cancers by more than 68%. Khallaf et al showed that 5-FU loaded solid lipid NPs (mean size of 137 ± 5.5 nm and zeta potential of -19.70 ± 0.40 mV) formulated from lecithin and poloxamer188 could deliver the drug topically and treat tumors when applied with a negatively charged hydrogel vehicle such as sodium carboxymethylcellulose [64]. The authors hypothesize that a charge-based repulsion between the particles and the vehicle may have supported its increased diffusion into the skin.

The emergence of NP technologies has also made it possible to evaluate new classes of drugs in the topical therapy of skin cancers. Highly elastic and deformable ultra-flexible nanovesicles (UltraFLEX-Nano) with a mean size of 110.50 ± 0.71 nm and zeta potential of $+16.02 \pm 0.86$ mV enhanced the skin deposition of an antioxidant diindolylmethane derivative (DIM-D) for chemoprevention of UV-induced skin cancers in mice [65]. The positively charged nanovesicles interact with the stratum corneum's negatively charged lipid lamellae

domain and squeeze through its paracellular regions via a transdermal water gradient. The authors also hypothesized that incorporating nanovesicles in a hydrogel vehicle such as Hydroxypropyl methylcellulose (HPMC) may contribute to its increased deposition within the skin. The hydrogel vehicle could hydrate and disrupt the stratum corneum forming hydrophilic channels within the lipid lamellae domain. The nanovesicles could then navigate these channels to penetrate the skin. Menezes et al explored the ability to use lipid vesicles (size 212 nm and zeta potential 3.3mV) containing DMSO to improve the skin penetration of 1-(1-naphthyl) piperazine (1-NPZ), a serotonergic derivative of quipazine [66]. DMSO, a polar aprotic solvent is known to interact with the stratum corneum and fluidize the barrier, enabling the vesicles to traverse easily. The 1-NPZ loaded vesicles prevented UVB-induced tumorigenesis by blocking acute inflammation and induced cancer cell apoptosis via oxidative stress. Sapino et al proposed the ability of amine-terminated mesoporous silica nanoparticles with a mean diameter of 250 ± 50 nm and zeta potential $+13.6 \pm 0.16$ mV to improve the dermal deposition of quercetin (an antioxidant and chemopreventive agent) and prevent skin cancers [67]. The authors hypothesized that the positively charged mesoporous NPs accumulated and retained on the skin surface via strong electrostatic interactions. The depot acts as a sustained-release system to achieve greater drug accumulation within the skin. Mesoporous silica NPs have also been used to topically deliver siRNA and silence TGF β R-1 expression by more than 2-fold to reduce SCC tumor growth in vivo [68]. On account of its large MW, hydrophilicity, and negative charge, topical delivery of genetic material is a challenge. The study showed the feasibility of using NPs in topical gene therapies. Researchers have also functionalized NPs with tissue-penetrating peptides to enhance topical delivery of genes. Niu et al incorporated plasmid DNA (pDNA) into cationic gold nanoparticles (AuPT, mean diameter of 19.9 ± 7.76 nm, and zeta potential 16.81 ± 0.56) conjugated with HIV-1 twinarginine translocation peptides (TAT) for improved penetration across the SC and treatment of cutaneous melanoma (Fig. 2) [69]. The authors suggested that gold NPs have the ability to interact with lipids and alter the SC barrier properties to penetrate an intact skin. By surface functionalizing the gold NPs with HIV-1 twinarginine translocation peptide (TAT), the authors took additional advantage of the amphipathic peptides' strong electrostatic interaction with the anionic skin surface and its ability to bind at the lipid bilayer-water interface to perturb the skin. TAT is known to be a skin penetrating peptide and has also been used in transcutaneous immunization [69]. In a different study, Zheng et al. demonstrated that freely permeating spherical nucleic-acid gold nanoparticle conjugates could deliver siRNA and silence the epidermal growth factor receptor (EGFR) in keratinocytes and human skin equivalents. Penetration was achieved without the need for cationic charges [70].

In a further advancement based on the use of nanoparticles to prevent UV-induced skin cancers, self-assembled nanoparticles (size 216 nm and zeta potential -53.3 mV) composed of amphiphilic poly (d,l-lactic acid)-hyperbranched polyglycerol (PLA-HPG) block-co-polymer were engineered to prolong their residence on the skin. The particles were loaded with a combination of UV filters – avobenzone and octocrylene – to prevent skin damage due to UV exposure. The bioadhesive particles were surface-functionalized with aldehyde-end groups that covalently bonded with the amines on the extracellular proteins at the skin surface. This is a classic example of applying chemistry to enhance the topical retention of NPs on a living tissue [71]. Elsewhere, Das et al. showed that nanoparticles loaded with dietary antioxidants such as apigenin (Ap) were used to prevent skin cancer. Ap-loaded PLGA NPs with a mean diameter of 101.3 ± 0.004 nm and zeta potential of -12.1 ± 0.001 mV could prevent skin cancers in mice (Fig. 3.) [72]. In contrast to the free drug, Ap-loaded PLGA NPs with a mean diameter of 101.3 ± 0.004 nm and zeta potential of -12.1 ± 0.001 mV displayed higher efficacy with reduced normal tissue damage in preventing skin cancers. The particles may have adopted the follicular pathway to achieve skin penetration. NPs have also been evaluated for

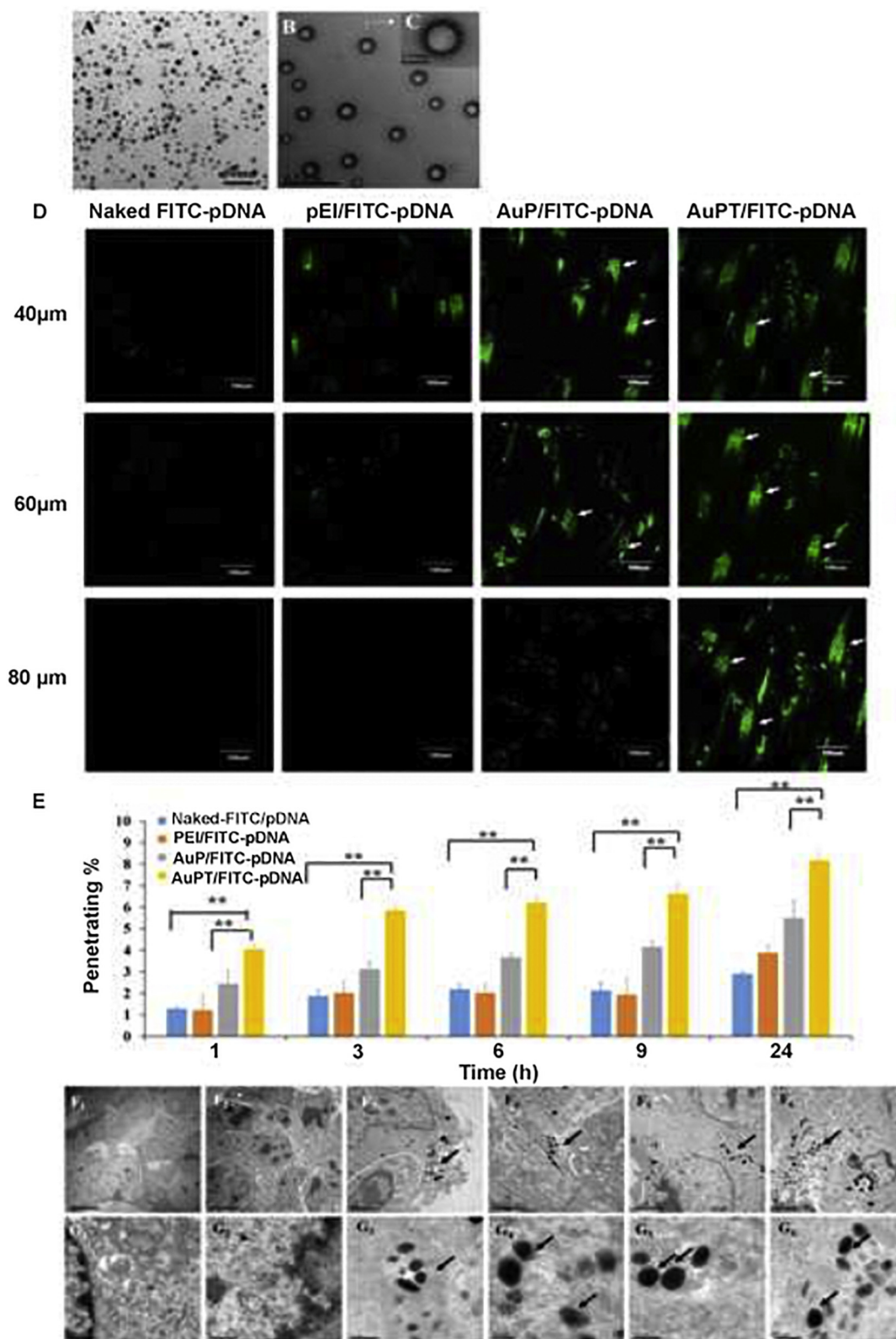


Fig. 2. In vitro and in vivo skin and tumor accumulation of peptide conjugated Au nanocomplexes (AuPT) carrying plasmid DNA (pDNA) for treatment of early-stage and advanced melanoma. Panels A and B represent the TEM images of AuPT nanoparticles, and AuPT/pDNAs respectively, and a higher magnification of the same (insert C). Scale bar = 100 μm. Panel D represents CLSM images collected at different depths inside the skin to record the green fluorescence emission and its quantitative analysis (Panel E) from free FITC labeled pDNA (FITC-pDNA) or FITC-pDNA incorporated into different types of NPs (polyethyleneimine – PEI/FITC-pDNA; naked AuP NPs (AuP/FITC-pDNA) and AuPT/FITC-pDNA NPs). Panels F and G represent the TEM images showing the tissue distribution of AuPT nanoparticles in melanoma following its topical exposure (Scale bars for panels F – 2 μm and panels G – 200 μm) (Adapted with permission from Niu, J., Chu Y., Huang Y.F., Chong Y.S., Jiang Z.H., Mao Z.W., Peng L.H., Gao J.Q., Transdermal gene delivery by functional peptide-conjugated cationic gold nanoparticle reverses the progression and metastasis of cutaneous melanoma, ACS applied materials & interfaces, 22 March 2017; 9(11): 9388–9401. Copyright (2017) American Chemical Society.)

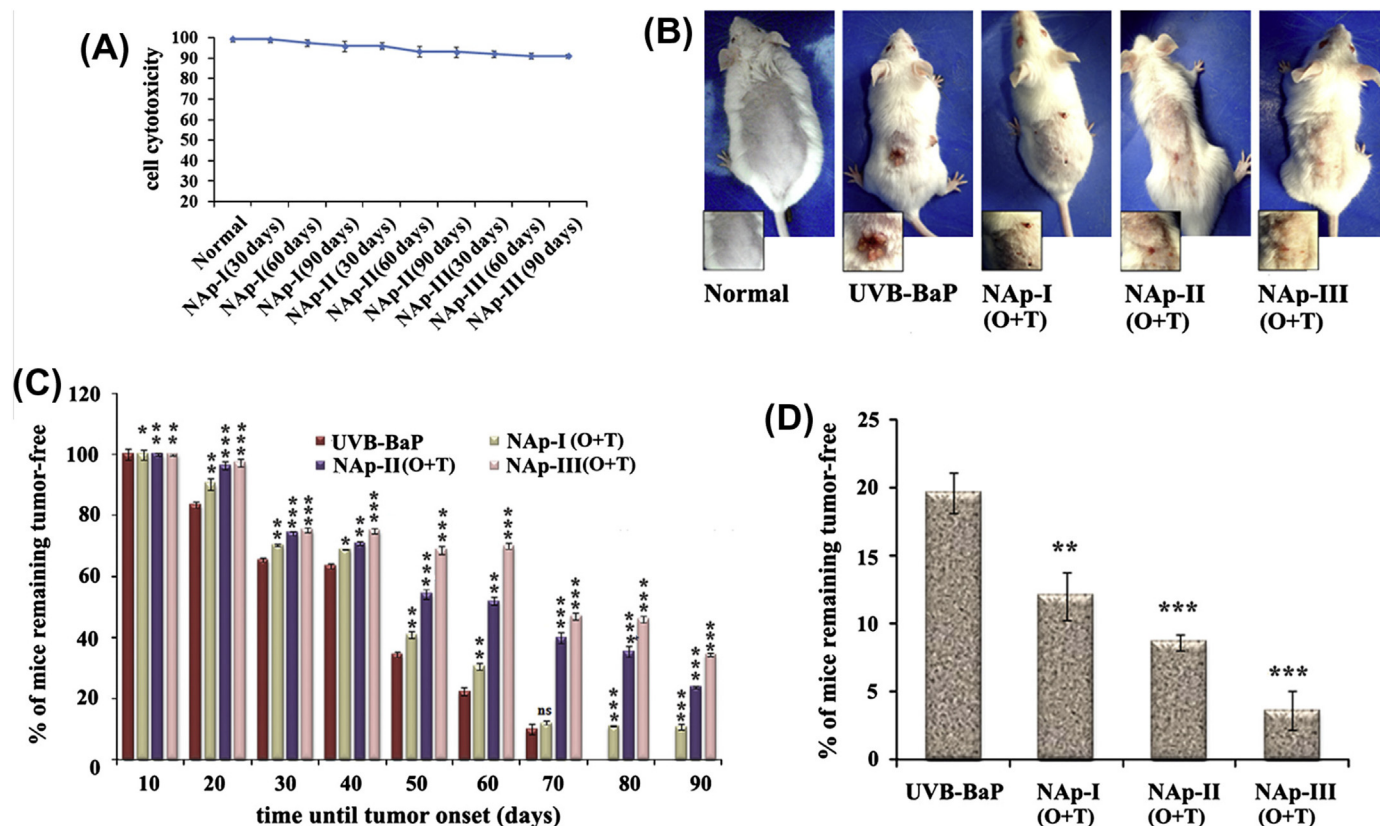


Fig. 3. Nanoencapsulated apigenin-mediated chemoprevention of skin cancers. Panel A shows the in vivo tolerability nano-apigenin (NAP) encapsulated NPs. A combined treatment of oral and topical application of nano-apigenin (O+T NAP) significantly delayed the onset of tumors (Panel C) and formed fewer tumors in mice compared to the carcinogen treated control (UVB-BaP) group (Panels B & D). (Adapted with permission from Das, S., Das J., Samadder A., Paul A., Khuda-Bukhsh A.R., Efficacy of PLGA-loaded apigenin nanoparticles in Benzo[a]pyrene and ultraviolet-B induced skin cancer of mice: mitochondria mediated apoptotic signalling cascades, Food and Chemical Toxicology, 2013 December; 62: 670–80. Copyright (2013) American Chemical Society.)

use in PDT of skin cancers. Photosensitizers such as aminolevulinic acid (ALA) are hydrophilic in nature. This limits their tissue penetration and uptake by neoplastic cells. On this account, liposomes carrying ALA have been tested as a strategic means to improve delivery deep into the epidermis [73].

Hafeez and Kazmi developed a lipid NP based topical cream incorporated with a new drug Dacarbazine (DTIC) to treat cutaneous melanoma [74]. The mean size and zeta potential of the DTIC-loaded NP cream were 16.9 ± 7.8 nm and -5.63 ± 1.67 mV respectively. While the size of the lipid NPs used in this study is small, the authors also proposed the use of a non-Newtonian and highly spreadable cream base to carry the particles and achieve increased surface contact. The lipid NPs would then release the lipophilic drugs on the surface to penetrate the stratum corneum and go deep into the skin [74]. In a non-nanoparticle based study, the drug DTIC was shown to induce an effective T-cell-dependent host-immune response against cutaneous melanoma when administered topically with an immunostimulatory CpG oligodeoxynucleotide [75]. This resulted in enhanced tumor cell killing and local immune activation against further relapse of melanoma. Thus, NPs could potentially offer the advantage of integrating such combinatorial therapies or chemotherapies for a synergistic effect.

In contrast to topically applying a NP-based formulation, radiotherapeutic bandages engineered from nanofibers have also been used to treat skin cancers. Electrospun polyacrylonitrile (PAN) nanofibers were incorporated with iron garnet nanoparticles that contained non-radioactive holmium-165 (^{165}Ho). The particles, when activated by neutrons, formed radioactive ^{166}Ho , which reduced cSCC tumors significantly (Fig. 4.) in mice [76,77]. The advantage of using such bandages are high selectivity to the skin cancer lesions, ease of application,

and minimal risk of radiation exposure to both the patient and the medical personnel on the account of its low half-life. This makes radiotherapeutic bandages easy to store, dispose of, and a commercially viable entity. Table 1 summarizes the examples of nanoparticle technologies evaluated for the topical therapy of NMSCs *in vivo*.

While methods such as iontophoresis, ultrasound, microneedles, and laser irradiation, etc. can enhance NP penetration into the skin, they require the use of specialized types of equipment, and may not be feasible for field therapy. The studies highlighted here focus on systems that deliver drugs topically without the aid of physical methods. And finally, the overall theme centers around the ability of NPs or the vehicle they are present in to strongly interact with the stratum corneum and manipulate its barrier properties, permitting the particles themselves or the released drugs to navigate the lipid domains for deeper skin accumulation.

Nanoparticle-based systems are yet to be approved for topical therapy of skin cancers. In 2007, the FDA fast-tracked the approval for using a liposomal lotion containing T4N5 (a bacterial DNA repair enzyme) to treat photosensitivity in patients with xeroderma pigmentosum. In clinical trials, the lotion prevented the occurrence of new AK lesions when applied topically for a year [78]. NB-001 (Nanobio Corporation) is a novel topical antiviral emulsion that is currently being evaluated in the clinic for recurrent cold sores and Herpes Labialis [79]. BF-200 ALA (Ameluz®), a nanosized lipid vesicle formulation of 5-aminolevulinic acid (5-ALA) is currently in clinical trials for preventing actinic keratosis and treating superficial basal cell carcinoma when combined with PDT [80]. There is also an ongoing clinical trial evaluating the safety, tolerability, and efficacy of a NP-based Paclitaxel formulation (NanoPac; Nanotax; SOR 007) for topical therapy of cutaneous metastases from nonmelanoma skin cancer [81].

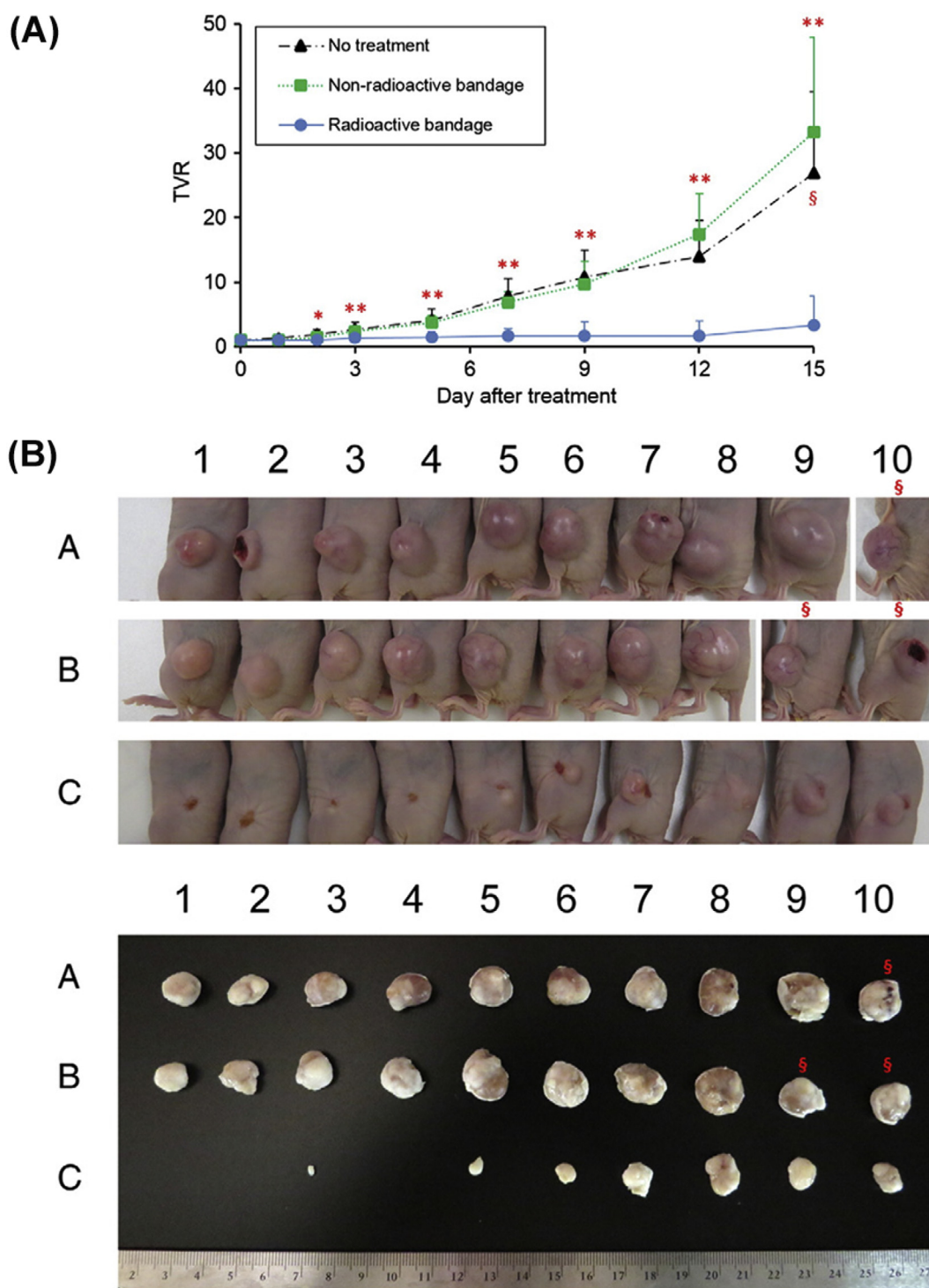


Fig. 4. In vivo tumor volume ratio (A) and size (B, C-upper and lower photograph panels) was significantly low after 12–15 days for mice treated with radiotherapeutic bandages in contrast to groups that received no treatment (A, black trace and B, A-upper photograph panel and A-lower photograph panel) or non-radioactive bandages (A, green trace and B, B-upper photograph panel and B-lower photograph panel). (Adapted from Koneru B., Shi Y., Munaweera I., Wight-Carter M., Kadara H., Yuan H., Di Pasqua A.J., Balkus K.J. Jr., Radiotherapeutic bandage for the treatment of squamous cell carcinoma of the skin, *Nucl. Med. Biol.* 43 (2016) 333–338 with permission from Elsevier.)

6. The challenge for nanoparticles in topical therapy of skin cancers

Clinically, AK and NMSCs present themselves as hyperkeratotic or SC thickened plaques, nodules, or lesions. The scaly thickened SC could limit the penetration of topically applied NPs through the lesion, thereby reducing treatment efficacy. A negative correlation was established previously between the thickness of SC and the local uptake

of ALA in AK [82–85]. While physicochemical properties of the NP-formulations do play a role in initiating interactions required to cross the SC barrier, the actual biological or clinical responses to the payload occur at a cellular level following their exposure to the skin-tumor microenvironment. Besides, differences in skin and tumor permeability exist with respect to its anatomical site (the SC is thick on palmar regions of hands and feet), hair follicle density, skin hydration, pH,

Table 1
Nanoparticle technologies tested for topical therapy of non-melanoma skin cancers (NMSCs).

Nanoparticle technology	Size & ZP	Drug	In vivo xenograft model (animal)	Administration route	Reference
Gold NPs	16.02 nm, +47.81 mV	5-FU	SubQ tumor (mouse)	Topical	[54]
Orange juice extracts	25 nm, −39.6 mV	5-FU	DMBA-induced tumor (mouse)	Topical	[63]
Solid Lipid NPs	137 nm, −19.7 mV	5-FU	SubQ tumor (mouse)	Topical	[64]
Ultra-flexible nanocarriers (vesicles)	110.5 nm, +16.02 mV	DIM-D*	UV-induced tumor (mouse)	Topical	[65]
Lipid Vesicles	212 nm, +3.3 mV	1-NPZ**	UV-induced tumor (mouse)	Topical	[66]
Mesoporous Silica NPs	200–250 nm, +30 mV	siRNA	SubQ tumor (mouse)	Topical	[68]
TAT-functionalized Gold NPs	19.9 nm, +16.81 mV	Plasmid DNA	SubQ tumor (mouse)	Topical	[69]
PLGA NPs	101.3 nm, −12.1 mV	Apigenin	UVB–BaP induced tumor (mouse)	Topical + Oral	[72]
PAN Nanofibers loaded with 166HoIG-NPs	174–208 nm Fibers 52 nm NPs, −	166Ho	SubQ tumor (mouse)	Localized radiation	[77]

* DIM-D: 1,1-bis(3-indolyl)-1-(p-chlorophenyl) methane (DIM-D).

** 1-NPZ: 1-(1-Naphthyl)piperazine).

sebum production, and more importantly, inter-individual responses [86,87]. The ability of NPs to accumulate within the cutaneous tumor core is further hampered by its diffusion rate across the dense ECM, and the interstitial fluid pressure [88]. In addition, the payload must surmount challenges posed by its inherent physicochemical characteristics (MW, hydrophilicity, charge) to be taken up by the target cells and induce therapeutic activity.

A majority of studies pertaining to the evaluation of nanoparticles in topical therapy show their therapeutic effect on mouse models of NMSCs (Table 1). There exists a fundamental difference between the mouse and human skin, including the origin of neoplasms from an anatomical, physiological, and molecular point of view [89]. Compared to the human skin, the mouse skin is loose and has a higher hair follicle density with a very thin epidermis. The disparity further extends to interactions between the tumor cells and its epidermal-dermal environment [90]. Thus, from a dermatological and clinical perspective, conclusions drawn from studies with murine models do not necessarily predict the human response to treatments. To accurately simulate the tumor and stromal component of the human skin, it would be ideal to evaluate nanoparticle-based topical therapies on live patient skin explants or bioengineered human skin equivalents of NMSCs [91,92].

7. Nanoparticle technologies for dermal delivery

Nature by design has shown the way to fabricate biologically inspired materials for improved dermal delivery [93]. For instance, the Human papillomavirus (HPV) is known to invade the skin and cause rapid cell proliferation on its outer layer resulting in warts [93]. Several

nanoparticle (NP) technologies have been designed, engineered, and evaluated for applications in dermatology. These include vesicular systems, lipid nanoparticles, polymeric nanoparticles, nanoemulsions, and metallic nanoparticles, among others (Fig. 5.). Several publications describe these technologies extensively [94–100]. Here, we provide a brief overview of some of the most commonly tested systems in topical delivery.

7.1. Vesicular carriers

Vesicular carriers such as liposomes, niosomes, ethosomes and transfersomes have been among the most studied for topical therapies.

Liposomes - Liposomes are primarily composed of cholesterol and phospholipids resulting in a hollow lipid bilayer allowing encapsulation of hydrophobic entities with an aqueous core for solubilization of hydrophilic agents [101–103]. Liposomes have the ability to improve the drug's pharmacokinetics, specificity, and enhance efficacy with reduced toxicity [16,19]. Mezei and Gulasekharan first proposed the idea of using liposomes for skin diseases in 1980 [104]. Subsequently, numerous studies have been performed to develop and advance liposomes in union with other techniques for dermal delivery [105,106]. Commonly used phospholipids include phosphatidylcholine extracted from egg yolk, soybean, or other synthetic and hydrogenated forms. The lipoidal composition of liposomes permits their adsorption on to the skin surface and fusion with SC lipids thus initiating drug release into the tissue [107,108]. Conflicting views exist with regards to the effect of liposomal surface charge on skin permeation. Popular belief strongly supports the hypothesis that strong electrostatic interaction

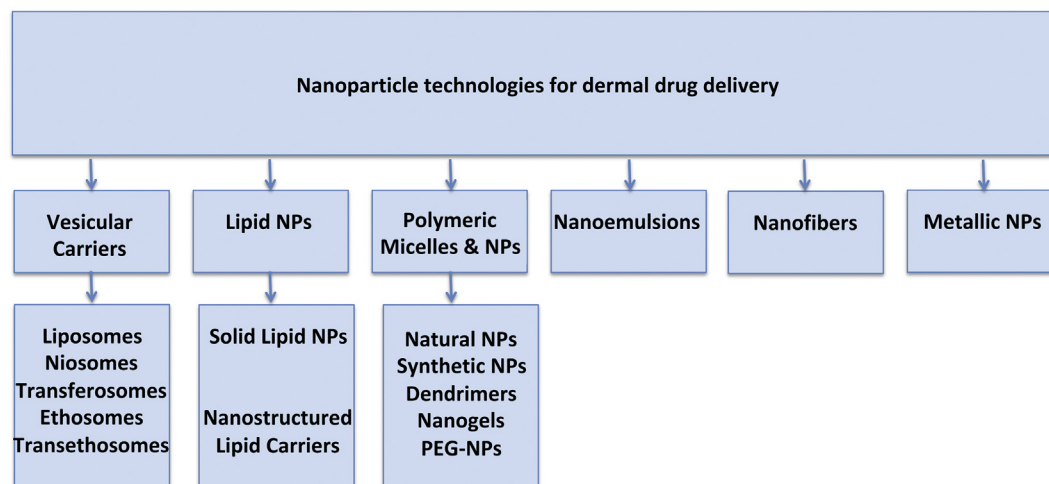


Fig. 5. Nanoparticle technologies for dermal drug delivery

between the positively charged liposomes and the negatively charged SC favors its increased adsorption onto the skin and subsequent penetration compared to the anionic liposomes [109,110]. However, some studies demonstrate that negatively charged liposomes cross the stratum corneum (SC) or accumulate within the hair follicles at a higher rate compared to positive or neutral liposomes [111–113]. Liposomes can also be engineered to achieve drug accumulation in various layers of the skin compared to the free drug. Liposomes with a mean diameter of less than 50 nm have been shown to accumulate within the deeper layers of the tissue in contrast to larger particles that remain adsorbed on the surface [114,115].

Niosomes - Niosomes are liposomes made with nonionic surfactants, which are hydrated in the presence or absence of cholesterol. When compared to conventional liposomes, niosomes are highly stable, less expensive, and more economical to manufacture [116–118]. Niosomes can modify the SC barrier by fusing with the lipids. The particles can also increase the smoothness of the SC by recovering the lost lipids and reducing the transepidermal water loss [119,120]. The aforesaid also depends on the physicochemical properties of the drug, the vesicle, and the lipids used to fabricate the niosomes [117,118]. The effect of charge, cholesterol, and surfactants such as Span 40, Span 60, and Brij 72 in enhancing the skin penetration and retention of niosomes have been studied extensively [121]. Niosomes have been widely evaluated for enhanced dermal delivery and extended retention of cosmetic actives and drugs such as fluconazole, naftifine hydrochloride, and ciclopirox olamine [121–124].

Transferosomes - When applied topically, liposomes accumulate mostly within the SC and do not penetrate further into the skin [120,125–128]. Consequently, its use for topical or transdermal drug delivery is limited to an extent. This led to the evolution of highly elastic, ultra-deformable vesicles called Transferosomes, invented by Cevc and Blume in 1992 [129]. Transferosomes have an aqueous core surrounded by a phospholipid bilayer and an edge activator [130]. Commonly used phospholipids for making these vesicles include Soyabean phosphatidylcholine, Egg-phosphatidylcholine dipalmitoyl, or Distearoyl phosphatidylcholine, among others. The edge activators are single-chain surfactants that provide flexibility by destabilizing the vesicle's lipid bilayer, which in turn lowers the interfacial tension and augments its structural deformability. In addition, ethanol is commonly used as a solvent activator during the formulation of Transferosomes to impart soft flexibility and enhanced skin penetration. Thus, the soft flexible nature of transferosomes and the presence of solvent/edge activators generate a trans-epidermal osmotic gradient achieved by the evaporation of water. This promotes an enhanced SC penetration of transferosomes via the intercellular route to deliver drugs deep inside the skin [131,132].

Ethosomes - These are elastic vesicles composed of phospholipids, cholesterol, water, and large amounts of ethanol [130]. First introduced by Tavitou et al the elasticity of ethosomes is primarily due to the effect of cholesterol and ethanol on the phase transition temperature of phosphatidylcholine [133]. Besides, ethanol helps in improving the solubility of lipophilic drugs and aids in disrupting the SC [134]. Ethosomes can, therefore, deliver drugs deep into the dermal layers or possibly into the systemic circulation [133,134]. In a direct comparison with liposomal and hydro-ethanolic gels-based formulations of econazole nitrate, ethosomes were more stable and achieved superior antifungal activity with controlled-release attributes in the clinic [135]. In a different study, Fang et al. demonstrated a higher rate of skin penetration for 5-aminolevulinic acid (ALA) encapsulated in ethosomes compared to its liposomal formulation during photodynamic therapy (PDT) [136].

Transethosomes - Transethosomes, originally introduced by Song CK et al are lipid vesicles that combine features of both transferosomes and ethosomes [137]. The presence of large amounts of ethanol (approx. 30%) along with the edge activators creates a synergy that enables transethosomes to penetrate and distribute deep inside the skin. Besides, the vesicles are highly flexible and have an irregular shape on account of its lipid bilayer rearrangement. In addition, DMSO has been used to

formulate transethosomes on account of its skin permeation enhancing property, and chemopreventive activity against skin diseases [66].

7.2. Lipid Nanoparticles

Lipid nanoparticles are highly stable, tolerable, and protect drugs from degradation while maintaining a steady release for extended periods. Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLC) constitute the family of lipid nanoparticles [138,139].

Solid Lipid Nanoparticles (SLN) - Solid lipid nanoparticles (SLNs) are colloidal systems that range from less than 50 nm to 1000 nm in size [140]. They are fabricated from a blend of biodegradable and biocompatible solid lipids, emulsifiers and water via a high-pressure homogenization process. Typically used lipids include triglycerides, glycerides, fatty acids, and waxes [141]. SLNs can be used to incorporate highly insoluble drugs within its lipid matrix. The solid lipophilic matrix where the drug is incorporated prevents its premature release and rapid degradation and also provides a highly flexible structure [142,143]. SLNs adhere to the skin to form a monolayer, which creates an occlusive effect to enhance water retention inside the skin. This could facilitate its increased penetration inside the skin [100,144]. They are relatively easy to scale-up during manufacture and the sterilization process while being cost-effective and reproducible [141]. Disadvantages associated with this system include poor drug loading due to a compact lipid matrix network; drug-lipid melt interaction; drug dissolution/dispersion rates in the lipid matrix and/or possible drug loss on account of polymorphic transition during storage [141]. Studies have shown the ability of SLNs to reduce edema or deliver mRNA, and antifungal medications such as terbinafine, econazole nitrate, miconazole nitrate and itraconazole deep into the skin with enhanced retention and sustained release capabilities. [145–148]

Nanostructured Lipid Carriers (NLCs) - Nanostructured Lipid Carriers (NLCs) are fabricated from a blend of solid and liquid lipids that do not possess the ideal crystalline structure. The lipid is either enclosed within the solid lipid matrix or localized on the surfactant layer [149]. Thus, the liquid phase with a reduced water content provides high drug loading and the solid lipid part imparts attributes for controlled drug release [150,151]. In addition, an increase in the distance between the fatty acid chains and the unstructured crystal supports an increased drug loading in NLCs [152]. Thus, they are far more suitable for formulating drugs compared to SLNs. NLCs are easy to manufacture in their final dosage form. They are generally produced via pressure homogenization methods, nanoemulsion techniques, or aqueous dispersion methods. In studies pertaining to dermal delivery, NLCs significantly improved the bioavailability of the triptolide deep inside the skin and boosted its anti-inflammatory activity at a reduced dose with no irritation [153]. NLCs have also been used to formulate TiO₂ as an inorganic UV blocker [154]. NLCs have been functionalized with cell-penetrating peptides for specific delivery and targeting to the skin. Incorporating the transactivating transcriptional activator (TAT) peptide ensured a greater deposition of NLCs into the epidermis compared to the control NLCs following its topical application [155–157].

7.3. Polymeric micelles and nanoparticles

Polymer chains can self-assemble to form micellar like particles with a hydrophobic core and a hydrophilic shell. The inner core can be used to incorporate poorly soluble drugs for increased bioavailability and the outer hydrophilic corona stabilizes the core in contact with its aqueous surroundings [158]. The corona also shields the drug from affecting healthy cells and rapid degradation. Typically, polymeric micelles range in sizes from 10–80 nm for drug delivery. Micelles can be engineered for improved specificity and efficacy by functionalizing it with ligands (e.g. antibodies, peptides, aptamers, carbohydrates, and small molecules, etc.) or by using block copolymers that release the drug in response to chemical and/or physical cues (pH-, thermo-, ultrasound-, or light,

etc.). Polymeric nanoparticles are known to penetrate the skin via a follicular pathway i.e. by accumulating within the hair follicles [25]. Bachhav *et al* examined the skin deposition rates of polymeric micelles carrying azole antifungal drugs such as clotrimazole, econazole nitrate, and fluconazole following topical delivery [159].

Natural Polymeric Nanoparticle - Among natural polymers, chitosan-based NPs have been examined widely for dermal drug delivery [160–163]. Chitosan, the N-deacetylated derivative of chitin is biodegradable and cationic in nature. The positive charge makes it possible for the polymer to strongly interact with the negatively charged skin surface, change the barrier, and deliver drugs [99]. Besides, the polymer has anti-oxidant, anti-inflammatory, and anti-microbial properties. This makes it extremely useful for treating skin diseases [164]. Chitosan-based NPs have been shown to increase the solubility and delivery of retinol for treating acne and wrinkles [99,165]. In a different study, Sahu *et al.* demonstrated the ability of ethylcellulose NPs to improve skin penetration and enhance retention of quercetin with sustained-release rates [166].

Synthetic Polymeric Nanoparticle - Among synthetic biodegradable polymers, poly (lactide-co-glycolide) copolymers (PLGA), polylactic acid (PLA), and poly(ϵ -caprolactone) (PCL) have been tested for dermatological applications. Sun *et al* demonstrated a superior treatment effect for curcumin loaded PLGA (50 nm to 150 nm) when compared to curcumin hydrogel in an imiquimod-induced psoriasis-like mouse model [167]. Ivaréz-Roman *et al.* demonstrated epidermal delivery of the sunscreen octyl methoxycinnamate when loaded in poly (ϵ -caprolactone) nanoparticles, indicating improved sun protection and partial prevention of erythema [168]. Nanospheres fabricated from tyrosine-derived polymers (TyroSpheres™) with a hydrodynamic size of 70 nm have also been widely examined for improved dermal delivery of lipophilic drugs. The particles are primarily made of an ABA-type triblock copolymer. Here, block A depicts the hydrophilic poly (ethylene glycol), and block B represents the hydrophobic oligomers of suberic acid and tyrosine-derived diol [169]. TyroSpheres can easily incorporate hydrophobic drugs such as Paclitaxel or Cholecalciferol for treating diseases like skin cancer and psoriasis or for shielding the drug from photodegradation [169,170]. On account of its small size, polymeric particles have the ability to form depots by accumulating within the hair follicle and on the skin surface. The polymers may also be incorporated with paramagnetic metals, such as gadolinium (Gd) or manganese (Mn), as image contrast agents for topical diagnosis.

Dendrimers - Dendrimers are monodispersed NPs made of synthetic polymers. The particles possess a central core that gives rise to symmetrically arranged repeating units, resulting in a layered architecture. The repeating units have functional groups that grow at an exponential rate. Dendrimers are highly monodispersed, multivalent, and have a well-defined size and offer easy of scale-up [171]. The high degree of branching in dendrimers results in a large number of surface functional groups and available internal cavities. In addition, the core-shell architecture of dendrimers facilitates the incorporation of lipophilic and hydrophilic drugs, image contrast agents, and nucleic acids [95]. The unique traits pertaining to its surface charge, the hydrodynamic size, its molecular weight, generation size, composition, and concentration may enable dendrimers to penetrate the skin [172,173]. Dendrimers commonly used in drug delivery include Polyamidoamines (PAMAM), poly(L-lysine) (PLL) scaffold dendrimers, polyesters (PGLSA-OH), and polypropylimines (PPI) [96]. Studies have shown that low generation PAMAM dendrimers (G0–G4), with hydrodynamic radii of <5 nm can traverse the intercellular lipid matrix [174,175]. When compared with neutral or anionic dendrimers, the cationic dendrimers can alter skin permeability by interacting with its lipids. Cationic dendrimers have been evaluated as permeation enhancers following skin pretreatment or co-administration with drugs [57,174,175]. Studies have shown the feasibility of using dendrimers for effective dermal delivery of NSAIDs, antimicrobial, antiviral, anticancer, antihypertensive drugs, alpha-blockers, and peptides [176,177].

Nanogels and 'PEG'-NPs - Dendrimers composed of dendritic polyglycerol (dPG) have been used to engineer hydrophilic and thermoresponsive three-dimensional crosslinked nanogels that can increase penetration of small and large molecules across the SC and accumulate within the hair follicles [178–184]. Such nanogels can be engineered to undergo a physical transition for increased dermal penetration and payload release in response to the ionic strength, temperature, or the skin's pH gradient following interaction with the SC [182,185]. For instance, pH-responsive and biodegradable nanogels made of chitosan or PLGA-chitosan have shown the ability to release 5-FU in response to the tumor's acidic environment to treat melanoma [186,187]. Chitin-based nanogels have also shown the ability to deliver drugs deep inside the skin for treating inflammatory diseases such as psoriasis [188]. Further, Abu and Heard *et al* demonstrated the ability of poly (N-isopropylacrylamide-co-acrylic acid)-based nanogels to increase the skin penetration of caffeine by a factor of 3.5-fold in response to both pH and temperature when compared with a saturated solution of the active [189]. Stimuli-responsive nanogels have also been fabricated from biopolymers such as heparin, hyaluronic acid, alginate, mannan, poly-L-lysine, poly(γ -glutamic acid) (γ -PGA), dextran and dextrin, etc. to deliver vaccines. Among biocompatible and biodegradable synthetic polymers, PLA, poly(glycolic acid) (PGA), poly(D,L-lactic-co-glycolic acid) (PLGA), poly(methyl methacrylate) (PMMA) and poly(ϵ -caprolactone) (PCL) has been used for topical delivery of vaccines, often in combination with physical enhancement techniques such as iontophoresis [190]. PEG-functionalized NPs or nanogels are also known to possess increased skin penetration properties. On account of its solubilizing properties, PEG can interact with keratin and solvate it, thereby extracting the lipid content and disruption of the SC [191,192]. For instance, PEG and PEG-oleylamine (OAm) functionalized Au NPs were able to penetrate the SC and accumulate deep within the subcutaneous adipose tissue *in vivo* [193]. In a different study, Mahmoud *et al* demonstrated the ability of Au nanorods functionalized with Phospholipid-PEG and Cholesterol-PEG to preferentially accumulate within different layers of the skin and its photothermal induced antibacterial activity [194].

7.4. Nanoemulsions

Nanoemulsions (NEs) are nanosized thermodynamically stable dispersions of oil in water (o/w) or water in oil (w/o) stabilized by an interfacial film of surfactant molecules [195,196]. The mean droplet diameters range from 20 - 200 nm and it typically has a low percentage of surfactant, making them ideal for topical drug delivery with reduced skin irritation [197,198]. They are primarily produced by high-energy (e.g., high-pressure homogenization) or low-energy (based on physicochemical properties of components) emulsification techniques [199,200]. Based on the composition of the dispersed phase and continuous phase, NEs can exist in three variants - (1) Oil-in-water NEs where oil droplets are dispersed in the continuous aqueous phase; (2) Water-in-oil NEs where water droplets are dispersed in the continuous oil phase; and (3) Bi-continuous NEs where microdomains of oil and water are interspersed within the system [201]. NEs can solubilize lipophilic drugs and carry them along with hydrophilic drugs at a high loading capacity. Its large surface area makes it possible to create a close occlusive contact with the stratum corneum thereby helping it to permeate and deliver drugs deep inside the skin. Skin permeation of NEs is further enhanced by the presence of oil and surfactants (e. g., oleic acid or eucalyptol) that may change the lipid structure of the stratum corneum [198,202].

7.5. Nanofibers

Electrospun nanofibers have shown great potential in topical drug delivery, especially as meshes for wound healing and antimicrobial activity. Although nanofibers belong to a class separate from

nanoparticles, the structures could be engineered to possess diameters less than 100 nm with a tunable pore size and high surface-to-volume ratio for hydrophilic or hydrophobic drugs [203]. Over the years, a variety of both natural and synthetic polymers have been used to fabricate fiber mats for topical drug delivery. These include nanofibers based of hyaluronic acid, silk fibroin, chitosan, gelatin, fibronectin, collagen, ethylcellulose, polycaprolactone, PLA, PLGA, PGA, poly (vinyl pyrrolidone), polyurethane, poly (vinyl alcohol), tyrosine-derived polycarbonates, etc. [204] Electrospun fiber mats can be assembled to deliver drugs at a sustained rate by modulating the drug to polymer ratio, the fiber diameter, its morphology, porosity or via surface-functionalization. This would enhance patient compliance by reducing the frequency of topical application [205–207]. Nanofibers have been evaluated extensively as systems for dermal drug delivery. The technology has thus been used to demonstrate the feasibility to topically deliver antifungal, antioxidant, antiproliferative, wound healing, and local anesthetic drugs both in a sustained and controlled manner [96].

7.6. Metallic nanoparticles

Metallic NPs are typically made of gold, silver, and metallic oxides. These particles have been used extensively in various skin products. Drugs are either incorporated within the core or bound to the surface of metallic NPs. In section 6, we described how Niu et al functionalized gold NPs (AuNPs) with skin tissue penetrating peptides to perturb the SC barrier [69]. Further, Chen et al demonstrated the safety and ability of gold nanoparticles (AuNPs), surface-functionalized with vascular endothelial growth factor (VEGF) to promote wound repair [208]. AuNPs and quantum dots (QDs) have also been used extensively for topical diagnostic and imaging applications. When compared between an aqueous solution and an oil-in-water emulsion vehicle, neutral and positively charged AgNPs in aqueous solution penetrated the human skin more compared to the emulsion vehicle [209]. NPs made from metallic oxides of iron; titanium; zinc etc. are being currently examined for dermal delivery of cosmetic actives or drugs. In a different study, 150 nm-sized gold nanoshells were engineered to absorb near-IR light and near-IR laser irradiation to induce thermolysis of overactive sebaceous glands that cause acne [210]. With regards to metal oxides, titanium dioxide (TiO₂), and zinc oxide (ZnO) have been used as inorganic UV filters in sunscreens. However, concerns have been raised over the toxicological impact of using metal oxides in sunscreens, especially in cases where an impaired SC barrier, such as those on lesions or sunburnt areas, could provide access to the particles to go deeper and cause local toxicity. Roberts and his colleagues addressed these concerns by proving the safety of using ZnO in sunscreens both on the intact and impaired skin barrier. The particles just accumulated on the surface and within the furrows without causing epidermal toxicity [211,212].

A variety of nanoparticle technologies have been fabricated and tested for topical drug delivery. While some systems are capable of interacting with the stratum corneum and change its barrier properties

to deliver drugs inside the skin, others accumulate to form a depot on the surface or within the hair follicles. The topical delivery of drugs using NPs is a multifaceted, complex issue. NPs of non-metallic and metallic nature behave in different ways when applied on the skin. Each material is unique and different from one another. Non-metallic NPs such as vesicles, lipid NPs, nanoemulsions are flexible and often incorporate surfactants or edge activators that increase permeation [66,100,119,120,123,131–134,137,142–144,155–157,198,202]. These particles tend to interact with the SC barrier via an occlusive effect or fluidize the barrier for increased skin penetration. Their high deformability or flexibility enables the particles to squeeze through the pores. Polymeric NPs, on the other hand, tend to accumulate deep within the SC or adopt the transfollicular pathway to build a depot inside the skin [23,25,99,159,169,170]. In addition, polymeric NPs such as cationic dendrimers and nanogels have shown the ability to change the barrier properties and improve drug delivery deep into the skin [57,174,175,181–183,185–187]. Metallic NPs have been shown to accumulate superficially or within the skin depending on the material's surface properties. Functionalizing these technologies with skin-penetrating proteins could be an added advantage [54,69,70,194,208,211]. While assessing the interactions of NPs within the SC, it would be prudent to identify strategies that take advantage of the natural furrows and appendages of the skin itself. Understanding the accumulation and elimination kinetics of NP-based reservoirs built within the skin would be critical in developing the ideal system for topical and cellular delivery of drugs. NPs could also agglomerate with a change in surface charge when it dries on the skin or is exposed to its varying polarities and sweat. It would be ideal to study the effect of such parameters as well. While physical aspects of the NP themselves play a vital role in manipulating their interactions with the dermal barrier, external factors such as the formulation vehicle, viscosity, temperature and pH could also affect dermal delivery. The formulation could be a cream or gel, an aqueous or organic dispersion. Thus, choosing the right vehicle should determine the change in barrier properties, thereby affecting the interaction of NPs with the skin.

8. Skin penetration of nanoparticles - a critical analysis

Depending on the indication, nanoparticle-based drug formulations may be designed for (i) drug retention on the skin surface with no penetration beyond; (ii) drug accumulation inside the different layers of the skin where the disease is localized e.g. skin neoplasias. Here, the formulation and drug would be retained for extended periods with no further penetration; and (iii) transdermal delivery for systemic circulation where, the formulation and drug have to permeate much deeper and pass into the bloodstream for treating diseases at distant sites. [213,214]. Thus, understanding dermal penetration and a critical evaluation of its underlying concept are essential in designing and developing the ideal system.

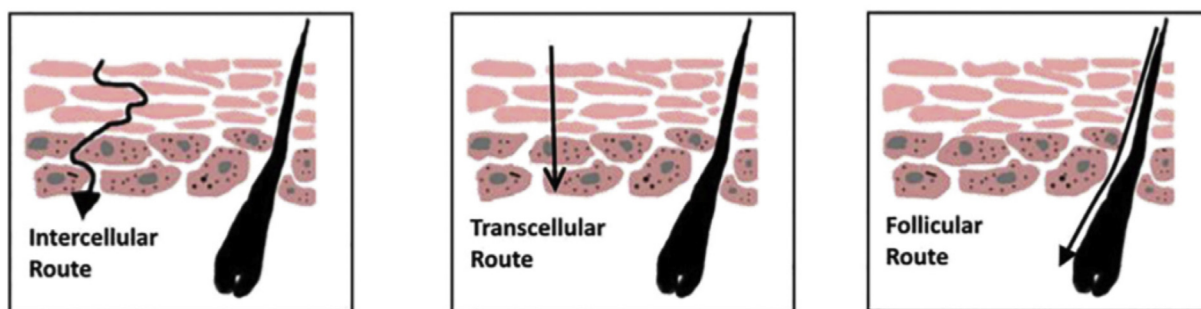


Fig. 6. Dermal routes proposed for nanoparticle penetration (Adapted from Carter P., Narasimhan B., Wang Q., Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for various skin diseases, *Int. J. Pharm.* 555 (2019) 49–62 with permission from Elsevier.)

Even though permeation of nanoparticles across intact SC through classical pathways is challenging [215,216], additional pathways may exist for the particles to be absorbed through the skin including diffusion through perturbed skin or appendages (Fig. 6.). The route selected depends on the physicochemical properties of the particle, characteristics of the final formulation, and condition of the skin itself. Dermal absorption can occur directly through the SC into the underlying layers or through the various appendages present on the skin [217–219]. Follicles can potentially form a large depot for nanoparticles. [23,220] They may enable movement of particles from the skin surface via the ducts of various appendages. This potentially facilitates an uninterrupted route across the SC to deposit deep within the skin. The appendages cover

only 0.1% of the total body surface. However, their contribution to nanoparticle transport can be significant, especially in terms of the depth achieved due to the absence of SC in the lower third of the follicles [23,220–223]. In addition, transfollicular delivery can eventually build a depot at the follicles [220]. Lademann et al. demonstrated long-term retention of dye-incorporated nanoparticles in contrast to the rapid clearance observed for the free dye itself in the hair follicles [23]. Thus, the transfollicular route has been widely shown to promote permeation of nanoparticles, microparticles, and macromolecules [224–227].

The transcellular pathway involves the movement of nanoparticles through the SC via the hydrated keratin of corneocytes. The hydrophilic nature of this pathway entails formulations to undergo a series of

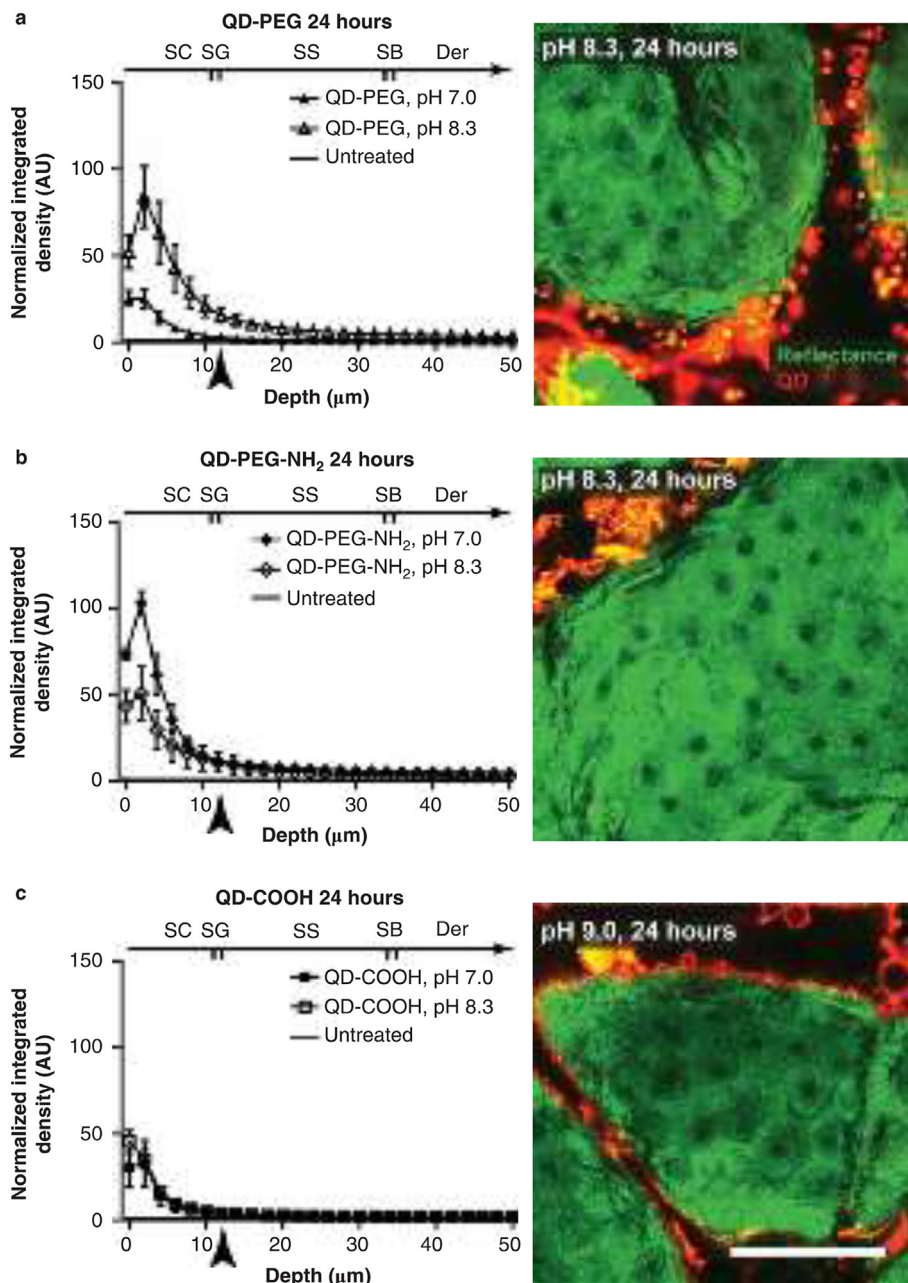


Fig. 7. Effect of pH of vehicle or buffer on QD penetration through an intact human skin after applying for 24 h. Normalized penetration profiles (left, Panels a, b, c) reveal the penetration depths (arrow heads, X-axis) of QDs at which the LSCM images (right, Panels a, b, c) were recorded at the stratum granulosum level. (Adapted from Prow T.W., Monteiro-Riviere N.A., Inman A.O., Grice J.E., Chen X., Zhao X., Sanchez W.H., Gierden A., Kendall M.A., Zvyagin A.V., Erdmann D., Riviere J.E., Roberts M.S., Quantum dot penetration into viable human skin, *Nanotoxicology* 6 (2012) 173–185 with permission from Elsevier.)

partitioning and diffusion steps through the cell-lipid matrix. Hence, nanoparticles having an amphiphilic nature could potentially move along this route [228]. In contrast, the intercellular pathway involves the movement of nanoparticles through the hydrophobic lipid domain between the corneocytes. Thus, nanoparticles engineered with uncharged lipophilic domains could potentially be suitable for this route [228].

Studies have shown that molecules over 500 Da have poor penetration into normal healthy skin [28,229,230]. Nanoparticles generally have MW orders of magnitude greater than this, and their movement across an intact barrier is severely restricted. In terms of the size, it has been shown that rigid nanoparticles as small as 10 nm penetrate the skin passively via the stratum corneum's lipid matrix and the hair follicles and particles around 600 nm penetrate deep into the skin via the hair follicles [223,231]. Regarding shape, rod-shaped (length = 50

nm, diameter = 20 nm) silver nanoparticles displayed the most penetration and maximum accumulation inside the skin compared to its spherical (diameter = 50 nm), and triangular (side length = 50 nm) counterparts [232]. The surface charge on the skin is negative due to the presence of sulfated proteoglycans. Consequently, studies have shown that cationic particles penetrate the skin without comprising its barrier properties and anionic NPs pass through temporary channels created due to the repulsive force [233,234]. In terms of surface properties, hydrophobic molecules exhibit greater penetration into the skin compared to their hydrophilic counterparts [229,230]. The same principle is also expected to be applicable for nanoparticles. With regards to rigidity, studies have shown increased skin permeation of a lipophilic active with nanoemulsions in contrast to levels achieved by a rigid nanoparticle composed of cellulose acetate phthalate [235]. The effect of rigidity has also been studied by demonstrating enhanced

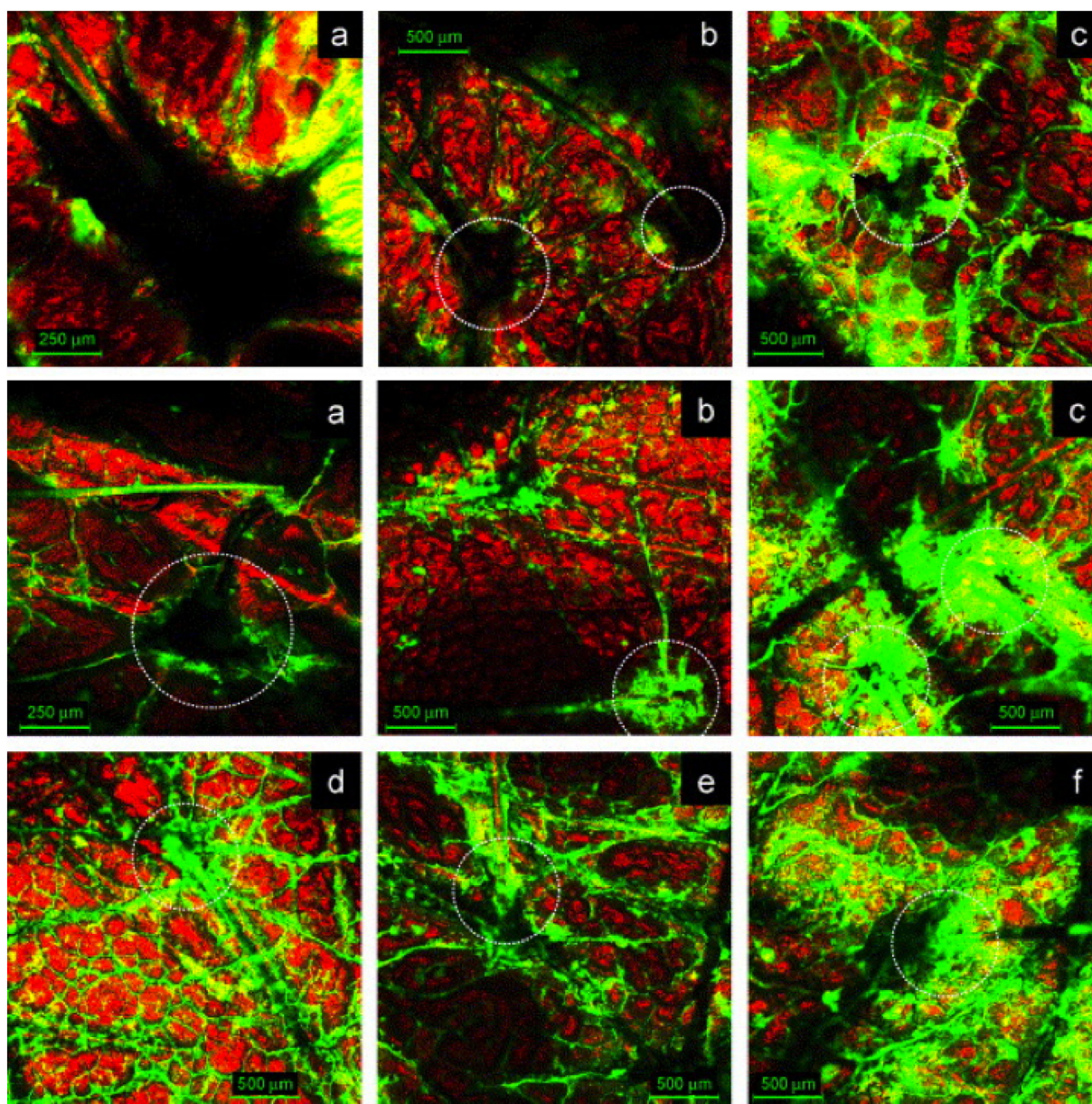


Fig. 8. Effect of size on the penetration depth of nanoparticles. (Top Panel) x-y images reveal FITC distribution (green fluorescence) of the free dye at (a) 30 min, (b) 1 h, and (c) 2 h on the skin surface and not within the hair follicles (white circles). (Central Panel) x-y images reveal FITC distribution (green fluorescence) of 20 nm fluorescent nanoparticles at (a) 30 min, (b) 1 h, and (c) 2 h within the hair follicles (white circles). (Bottom Panel) x-y images reveal FITC distribution (green fluorescence) of 200 nm fluorescent nanoparticles at (a) 30 min, (b) 1 h, and (c) 2 h within the hair follicles (white circles). Note the time-dependent accumulation of nanoparticles in hair follicles (white circles) (Bottom Panel d, e, and f). (Adapted from Alvarez-Roman R., Naik A., Kalra Y.N., Guy R.H., Fessi H., Skin penetration and distribution of polymeric nanoparticles, *J. Control. Release* 99 (2004) 53–62 with permission from Elsevier.)

accumulation of actives or the intact elastic vesicles deep inside the stratum corneum when compared with rigid vesicles [236,237]. Absorption of nanoparticles into the skin and their diffusion is also governed by the concentration gradient. Further, the range of NP penetration into the skin is affected by the pH of its vehicle or buffer. For instance, Prow et al showed that quantum dots (QDs) with a hydrodynamic size of 35 nm could cross the stratum corneum barrier into the viable epidermis of pigskin at pH 8.3 and none at pH 7.0 (Fig. 7.) [238]. Further, QDs surface-functionalized with NH_2 or COOH groups could not cross the barrier at both pH values. The observation here is primarily attributed to the decreased skin impedance or reduced barrier function of the skin to unionized molecules at $\text{pH} > 7.0$ [239,240].

While skin penetration of nanoparticles by themselves is expected to low, various enhancement techniques can be adopted to increase nanoparticle penetration. Several methods, either chemical or physical have been developed over the years to overcome the stratum corneum barrier [224,241,242]. Chemical methods include the use of formulation strategies (colloids, dendrimers, etc.), modifications (prodrugs), and permeation enhancers (alcohols, fatty acids, surfactants, phospholipids, amines, esters, amides, hydrocarbons, terpenes, sulfoxides, urea, urea-based derivatives, cyclodextrin, etc.) [226,243]. Physical methods are energy driven (iontophoresis, electroporation, ultrasound, laser, thermal or radiofrequency ablation, etc.) or mechanical (microneedles, microdermabrasion, needleless injections, etc.) [244–246]. Although relatively easy to use, chemical permeation enhancers could cause skin irritation by disrupting the lipid organization of the stratum corneum or the viability of the epidermis [247–249]. Recently, ionic liquids have also been advanced for topical drug delivery [250]. Other factors that promote NP penetration and diffusivity into the skin include the level of skin hydration, the skin's surface temperature, rate of blood flow to the skin, age, and pathology of the skin.

A significant amount of work has gone into studying and understanding the skin penetration of nanoparticles and their tissue depth. However, conflicting views still exist in this area of interest [251,252]. Metallic nanoparticles such as zinc oxide and titanium dioxide at diameters ≤ 30 nm were found not to penetrate the stratum corneum based on visualization studies with transmission electron microscopy and atomic force microscopy [253–256]. Fluorescent polystyrene nanoparticles at two different sizes (20 nm, 200 nm) either remained on the skin surface or preferentially clustered around the hair follicles in time and size-dependent manner (Fig. 8.) [22]. Fluorescent PLGA nanoparticles with a diameter of ~ 300 nm depicted no penetration across the stratum corneum of human skin [257]. Contrary to these findings, skin penetration was reported for particles ranging in diameters such as 5 nm (iron); 25 nm (silver); quantum dots; 1 μm (dextran) and at 1–10 μm (PLGA), etc. [231,238,258–260]. Such uncertainty or opposing results could be attributed to the approaches used for studying and analyzing the extent of particle penetration. While mechanical skin tissue sectioning could often result in the accidental transfer of particles, the cryofixing could modify the skin's basic lipid organization. This affects the overall distribution of nanoparticles [261]. On this account, an objective evaluation of nanoparticle deposition inside the skin is essential.

A classic example of this approach is a study performed by the Guy group to determine the penetration depth of two types of polymeric nanoparticles (Polystyrene and poly(methyl methacrylate)) inside porcine skin in vitro (Fig. 9.) [262]. For visualizing a clear separation in the fate of the nanoparticles, its payload, and the skin itself, the researchers covalently labeled the particles with fluorescein methacrylate and loaded it with Nile Red – a “model lipophilic active”. For tracking the fluorophores following its topical application, laser scanning confocal microscopy (LSCM) was used. The mean diameter of nanoparticles used in this study varied from 30 nm for polystyrene to 100 nm for

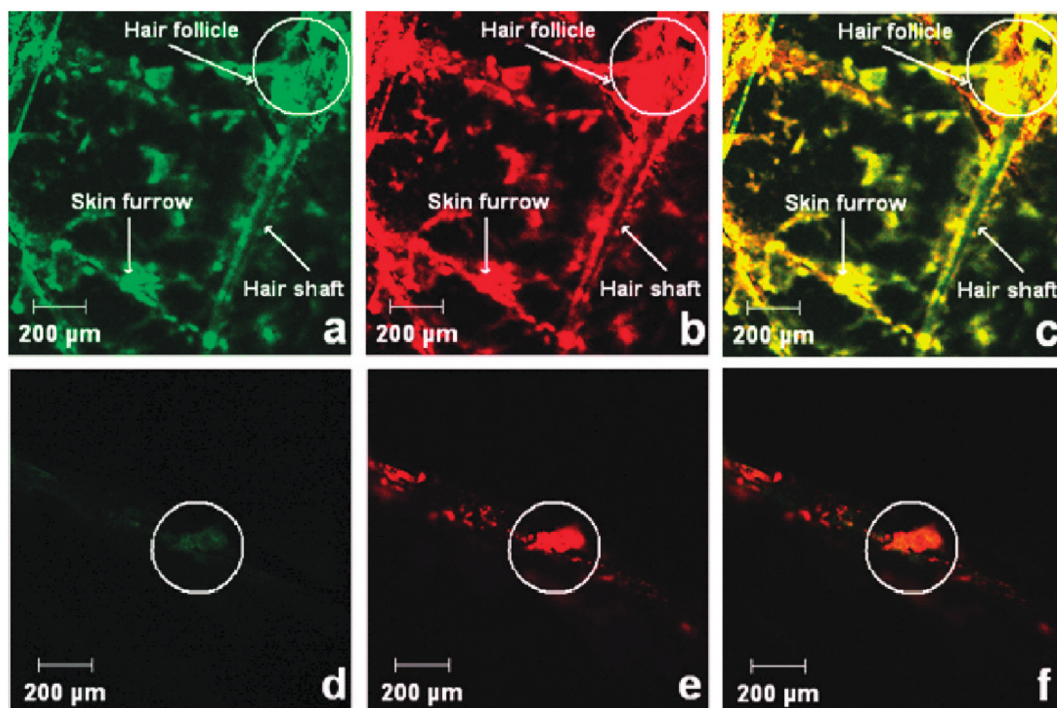


Fig. 9. Penetration analysis of polymeric nanoparticles. Panels a and b depict the individual fluorescent emissions from polystyrene NPs (green) incorporated with the model active NR (red) when observed to accumulate on the skin surface. Panel c depicts the colocalization of both signals (yellow) on the skin surface. Panels d and e show the individual fluorescent emissions from polystyrene NPs (green) incorporated with the model active NR (red) from a cross-sectional image of the skin that identifies with its inner layers. Panel f represents the co-localization of both signals inside the skin. The NR signal is stronger compared to the NP signals suggesting the permeation of the released dye deep into the skin. (Adapted with permissions from Wu, X., Price G.J., Guy R.H., Disposition of nanoparticles and an associated lipophilic permeant following topical application to the skin, *Molecular Pharmaceutics*, 2009, 6(5), pp.1441–1448. Copyright (2009) American Chemical Society)

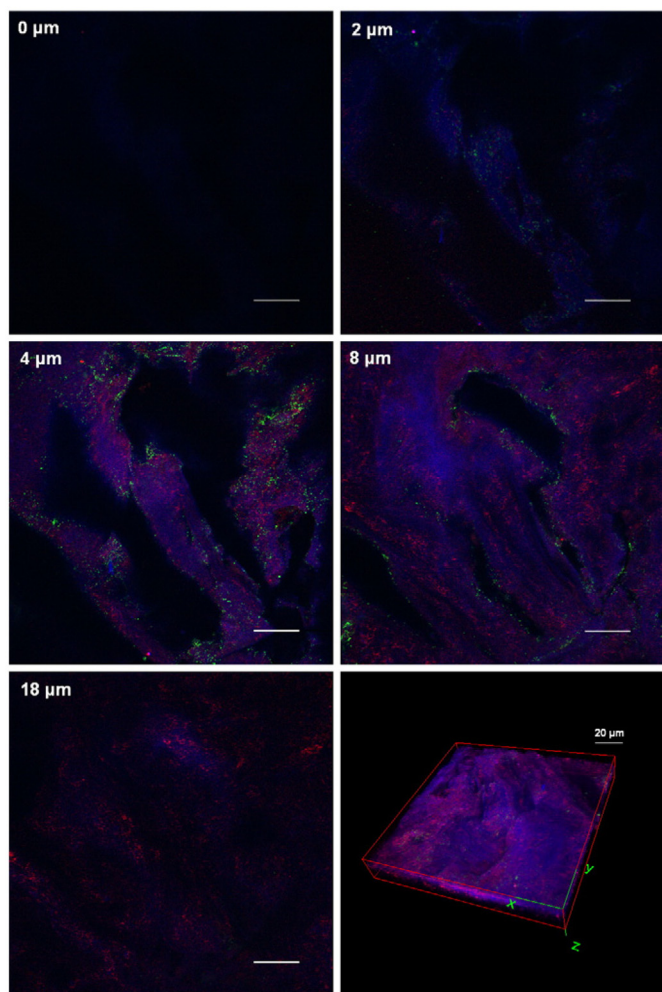


Fig. 10. Quantitative analysis of the spatial distribution of 200 nm Fluospheres® inside the pigskin. Following a 16 h exposure, X-Y images were collected from the skin surface (0 μm) and at depths of 2, 4, 8, and 18 μm within the sample in the z-direction to gather the green fluorescence emission from the particles. Note the intensity of the green signal in each panel that identifies with its corresponding depth. The last panel represents a composite stack of all images collected at a step size of 1 μm . (Adapted from Campbell C.S., Contreras-Rojas L.R., Delgado-Charro M.B., Guy R.H., Objective assessment of nanoparticle disposition in mammalian skin after topical exposure, *J. Control. Release* 162 (2012) 201–207 with permission from Elsevier.)

poly(methyl methacrylate). Nanoparticles appeared to accumulate more within the skin furrows and the hair follicles without permeating beyond the stratum corneum. While co-localization of both fluorophores was evident at the skin surface, Nile red (the lipophilic component) was observed to diffuse and accumulate independently on its own inside the skin following its release from nanoparticles [262]. In a follow-up study, the same group once again used confocal microscopy to perform quantitative analysis of the spatial distribution of fluorescent polystyrene nanoparticles (diameters – 20 nm, 100 nm, and 200 nm) inside the skin (Fig. 10.). The aim here was to determine whether the particles could cross the stratum corneum barrier, and perform an objective assessment of its depth of skin penetration [263]. The study did not take into account the effect of physical parameters such as shape and charge in dermal penetration. However, it was meticulously designed to minimize the uncertainties due to autofluorescence, sample variation, random imperfections in barrier properties, and instrument variability, etc. The particles were observed to penetrate only the surface layers of the stratum corneum up to an approximate depth of 2–3 μm and this was accounted for permeation along the cracks in the stratum disjunctum. Further, no time-dependent and size-dependent

penetration of nanoparticles was observed when the barrier was intact or compromised. The authors further argued against the clustering of nanoparticles in and around the hair follicles. They attributed this to the follicular contraction observed when the skin is dermatomed [264]. In a different study, Raber et al developed an *in vitro* model to systematically quantify the transfollicular uptake of nanoparticles [265]. The study was performed by topically applying onto a pig ear *in vitro* and human forearm *in vivo* similarly sized (163–170 nm) fluorescently labeled PLGA nanoparticles with varying surface modifications. A good correlation was observed between the *in vitro* and *in vivo* results indicating that the pig ear skin is an ideal model to quantify follicular uptake of nanoparticles [265].

In a more recent study, Patzelt et al showed how to exploit the transfollicular pathway for drug accumulation inside the skin (Fig. 11.). The group compared three types of BSA-based nanoparticles possessing different release mechanisms for the loaded drug [266]. By manipulating the release kinetics for each system and in response to a trigger, the particles that accumulate at the hair follicles released drugs to achieve penetration depths at different times and locations each distinct from the other. Such intelligent nanoparticles could potentially improve topical delivery and reduce adverse side-effects [266]. The transfollicular route was more pronounced for nanoparticles in forming a depot as opposed to it penetrating all the way into the viable epidermis [23,267,268]. However, on massaging the skin, the moving hair's ratchet effect created an oscillatory motion, which pushed the particles deeper into the follicles and the skin [269]. Thus, to study the ratchet effect of nanoparticle movement inside the hair follicles, Radtke et al proposed a two-dimensional stochastic model that encompassed oscillatory hair motion and the periodic asymmetric hair structure mimicking the effect of skin massage on hair [270]. Movement of nanoparticles into the hair follicles was found to vary with the frequency of radial hair motion perpendicular to the hair axis along with a slight enhancement in diffusivity. The study identified that the hair's oscillatory motion caused two different mechanisms of nanoparticle movement into the follicles – (1) directed motion and (2) enhanced diffusive motion. The radial movement of hair primarily caused the directed motion of nanoparticles into the follicles. Its efficiency depended on the driving frequency and the particle size. It was also found to push the particles deeper into the skin in the presence of effective viscosity. The model further estimated that the initial diffusive motion of nanoparticles changed over to directed transport at a penetration depth of 5 μm inside the skin. The study further took into account the presence of axial oscillatory motion as opposed to having just the radial motion of hair movement and evaluated its ratchet effect on the transport of nanoparticles [270]. Particles were found to move in the opposite direction out of the follicle. A significant rise in particle diffusivity was found in this case. Since a majority of *in vivo* or *in vitro* permeation studies have shown particle transport into the follicles, the conclusion is that radial hair motion possibly dominates the axial motion of hair.

A majority of studies have clearly shown the inability of nanoparticles to penetrate the stratum corneum barrier. This indicates the non-involvement of transcellular and intracellular pathways for nanoparticles to cross the skin barrier and accumulate deep inside the tissue. The transcellular pathway would involve uptake and translocation of the nanoparticles via the corneocytes and this depends entirely on the physicochemical nature of the particles themselves. On the other hand, the width of an intercellular channel is ~100 nm and the intercorneocyte space is filled with multiple layers of bilipids [22]. This suggests the improbability of nanoparticles ranging above 30–50 nm in size being able to cross the intact stratum corneum barrier. Thus, the transfollicular pathway would appear to be the most dominant and feasible route for nanoparticles to permeate deep inside the skin.

Understanding the rate and mechanism of NP penetration within the skin is vital. However, there exists a significant gap in knowledge regarding the safety, toxicity and biological fate of inorganic nanoparticles following dermal absorption. A majority of concerns related to dermal

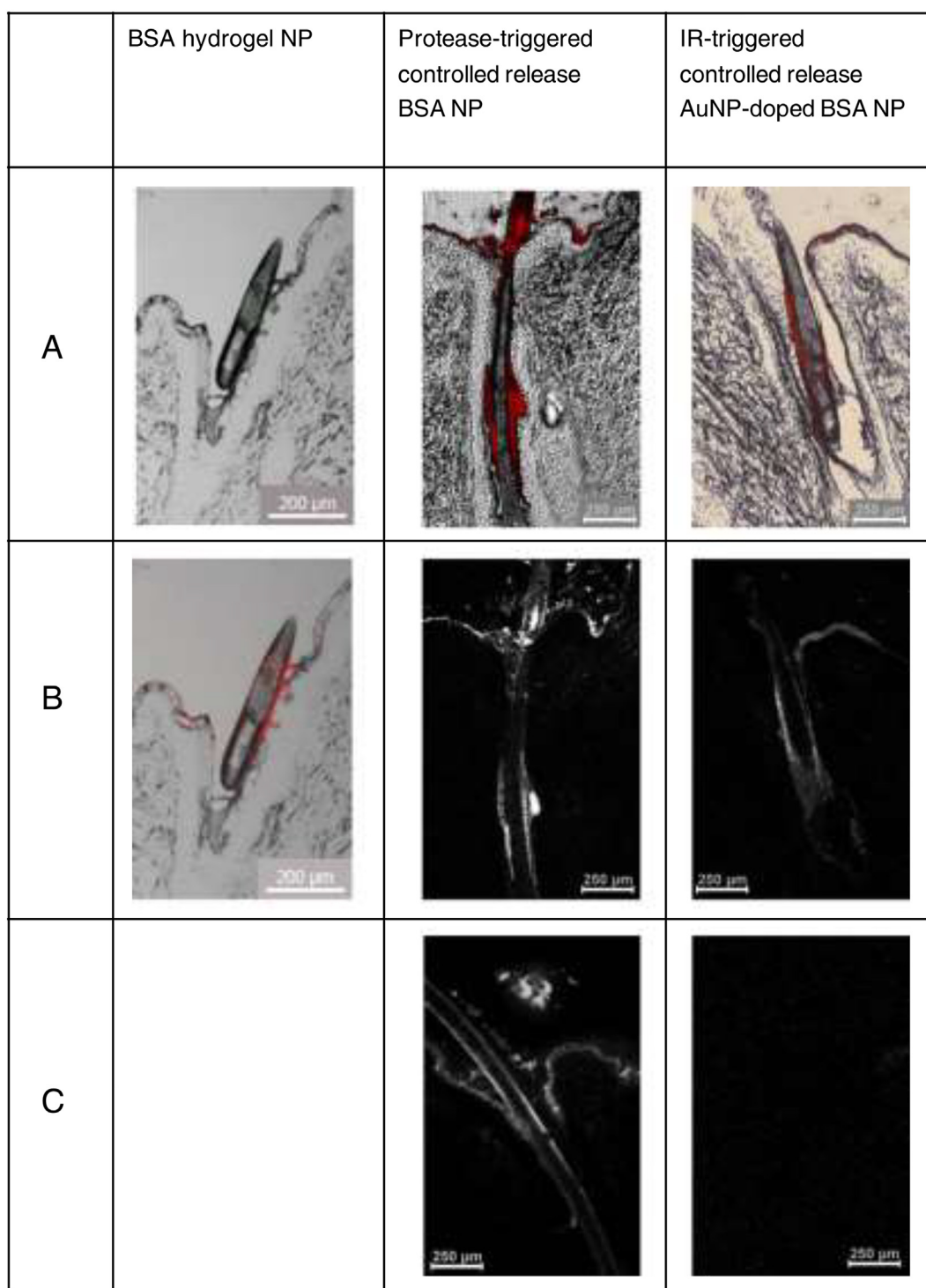


Fig. 11. Follicular penetration of three different nanoparticle systems. BSA hydrogel nanoparticles: Weak fluorescent signal of FITC when bound to the particles is observed in the image in row A and a strong fluorescence signal of TRITC when released with dextran is evident in the image in row B. Protease-triggered controlled release BSA nanoparticles: Image in row A is an overlay of both the fluorescence and transmission channels depicting the follicular presence of FITC released from particles when triggered with proteases. Image in row B is the same recorded in a fluorescence mode and image in row C reveals a weak fluorescent signal of FITC which is not released due to the absence of proteases. Hence the signal is quenched within the particles. IR-triggered controlled release AuNP-doped BSA nanoparticles: Image in row A is an overlay of both the fluorescence and transmission channels depicting the follicular presence of FITC released from particles when triggered with IR. Image in row B is the same recorded in a fluorescence mode and image in row C reveals no fluorescent signal of FITC due to the absence of the IR trigger and hence the signal is quenched within the particles. (Adapted from Patzelt A., Mak W.C., Jung S., Knorr F., Meinke M.C., Richter H., Ruhl E., Cheung K.Y., Tran N., Lademann J., Do nanoparticles have a future in dermal drug delivery?, *J. Control. Release* 246 (2017) 174–182 with permission from Elsevier.).

toxicity stem from the fact that smaller NPs have an increased surface area to volume ratio, which could alter its biological and chemical reactivity within the skin. For instance, Kim et al showed the migration of quantum dots (QDs) from the dermis to the regional lymph nodes,

possibly via skin macrophages and Langerhans cells [271]. This could potentially raise the concern for uncontrolled immunomodulation following skin absorption of nanoparticles. It has also been shown that inorganic nanoparticles such as TiO₂ could potentially form free radicals in

skin cells, thereby damaging the DNA and cause cell death via photocatalytic activity. While toxicity on account of NP accumulation within the SC and the hair follicles could be minimal considering the rate of SC exfoliation, and outward flux of sebum, toxicity studies pertaining to chronic exposure and reservoir buildup of such particles must be performed extensively. Thus, a thorough and comprehensive analysis is required to assess the safety profile of nanoparticles for topical use and treatment of cutaneous cancers.

9. Conclusion - challenges for clinical translation

Nanoparticle based dermal drug delivery is attractive and non-invasive for preventing or treating localized cutaneous cancers. This is especially beneficial for patients that are not viable for surgery or highly intensive non-specific systemic therapies. When designed and engineered optimally, nanoparticle-based drugs can cross the stratum corneum and deliver drugs deep into the different layers of the skin without causing adverse effects to the skin. Considerable effort has gone into studying the barrier properties of the skin and its internal milieu. A variety of skin penetrants and vehicles have also been developed to enhance the permeation of compounds across the skin.

Clearly, NPs offer a highly versatile, and a tremendous opportunity to successfully translate novel therapies, that would otherwise face obstacles in its clinical development and commercialization. However, barrier properties of the hyperkeratotic cSCC lesions, along with its intratumoral barriers limit the amount of NPs that are absorbed. This reduces the effective concentrations of drug molecules within the tumor core. Besides, variability exists with regards to the clinical presentation of lesions for each patient, and the nature of its surrounding skin. Furthermore, differences in inter-individual responses to the treatment and adverse side effects such as irritation and systemic toxicity are detrimental to the development of such therapies. With the field of personalized medicine rapidly evolving; *in vitro* models that authenticate actual clinical and biological manifestations of the disease can reduce the translational gap between the bench and the clinic further. Such models will play an important role in understanding the relationship between cell, tissue, organ, and the tumor microenvironment from a global perspective. A highly interdisciplinary approach is thus required to develop and translate treatments that combine nanoparticles with vehicle and formulation strategies or physical enhancement techniques for enhanced delivery through lesions and its surrounding skin. Knowledge thus gained, would enable the application of such approaches not just in therapeutic, but also for diagnostic and prophylactic purposes. Throughout this review, we have highlighted examples of nanoparticles that have displayed enhanced penetration, retention, and sustained release inside the skin. This review emphasizes the importance of physicochemical parameters for nanoparticles to cross the formidable skin barrier. Obstacles also exist in its large-scale manufacture, batch-to-batch variation (purity, consistency, etc.), clinical performance, and stability as a commercial product. While nanoparticle-based dermal delivery of proteins and peptides could still be a challenge, the knowledge gained from advances in formulation science can be used as guiding principles for the future. It can push boundaries of this rapidly exploding technology beyond imagination. Choosing the right cargo (e.g. the drug), the carrier (e.g. the nanoparticle), and its formulation for dermal delivery according to the indication is critical. The ability of nanoparticles to accumulate inside the hair follicles and the skin can be exploited for treating skin cancer.

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