of zinc oxide nanoparticles revealed zinc oxide nanoparticles in the stratum corneum but none in viable epidermis [37]; a similar quantitative analysis was unable to document any noteworthy penetration of the skin by titanium nanoparticles [38]. Therefore, even though aggregates of titanium dioxide nanoparticles and zinc oxide nanoparticles penetrate the skin in some situations, the rate is extremely low or below the limit of detection of most modern methods of quantitation.

6.2.2.2 Silica Nanoparticles

Manufactured synthetic silica nanoparticles are amorphous silica and are divided into two main types: pyrogenic silica and wet-process silica (i.e., precipitated silica and silica gels) [20, 39]. All pyrogenic silica nanoparticles exist as aggregates and agglomerates; some of the wet-process types also are well dispersed. Pyrogenic silica nanoparticles are widely used in cosmetics, but available data regarding the skin penetration of silica nanoparticles largely derive from the wet-process type, which is used for coatings and ink-receptive papers and as a filter aid in food production. In a mouse model involving epicutaneous application ("skin painting") for 3 days or 28 days, TEM analysis revealed well-dispersed silica nanoparticles (diameter, 70 nm) not only in epidermal Langerhans cells but also in the dermis and draining lymph nodes [40, 41], but the images obtained suggested that the penetration rate was minimal, at most. Fluorescence microscopy and flow cytometry showed that fluorescently labeled silica nanoparticles (diameter, 42 nm) enter keratinocytes and Langerhans cells in human tape-stripped epidermis; the authors stated that tape stripping removed part of the stratum corneum and contents of the follicular infundibulum [42]. Although quantitative data regarding skin penetration by silica nanoparticles is currently unavailable, well-dispersed silica nanoparticles likely will enter viable skin at some point during our daily lives.

6.2.2.3 Quantum Dot Nanoparticles

Quantum dot nanoparticles are intrinsically fluorescent, and their dispersion characteristics are easily manipulated by modifying the particle surface; consequently, they are widely used as models to investigate the biodistribution of nanoparticles. In an early study, confocal scanning microscopy suggested that two types of quantum dot nanoparticles with different coatings (PEG [polyethylene glycol]-carboxylic acid and PEG-amine; diameter, 15 to 45 nm) penetrated the epidermis of pigs [43]; however in a follow-up study using TEM and inductively coupled plasma-optical emission spectroscopy, this same group was unable to detect the entry of a similar nanoparticle preparation (40 nm with PEG modification) [44]. In contrast, our TEM analysis revealed that 40-nm quantum dot nanoparticles penetrated slightly into viable skin in mice [40], and confocal microscopy and TEM both demonstrated very low, but qualitatively higher, penetration of quantum dot nanoparticles in UVR-exposed skin compared with intact skin in mice [45]. The findings of another group suggested that, among the preparations and pH conditions they evaluated,

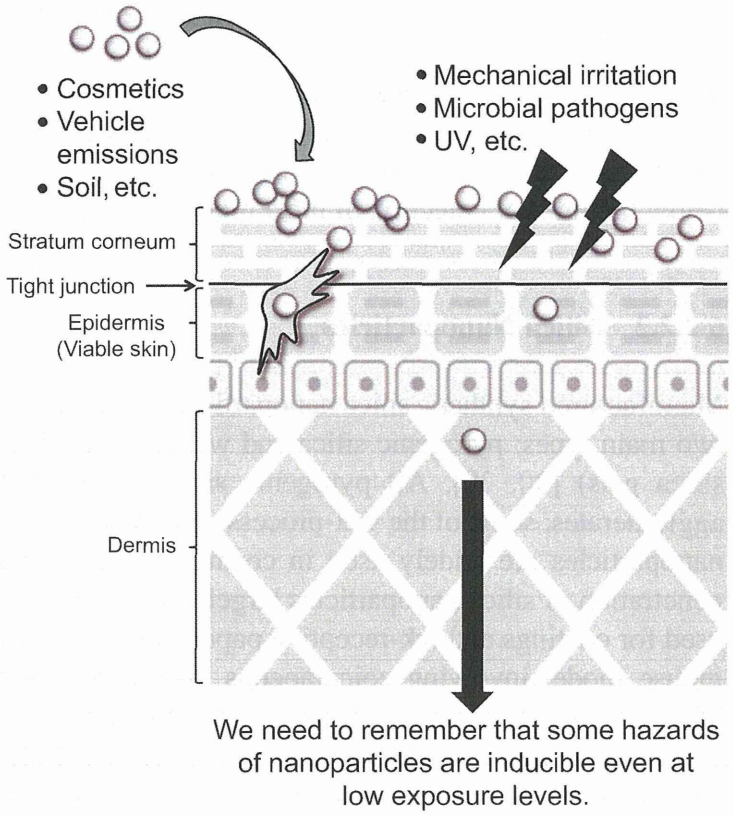

Fig. 6.1 Skin exposure to nanoparticles

only pegylated quantum dot nanoparticles at pH 8.3 penetrated intact human skin [46]. These interesting, but contradictory, observations and quantitative data suggest that, like other nanoparticles, well-dispersed quantum dot nanoparticles can enter our bodies through skin, but the amount that enters is minimal.

The mechanism underlying the penetration of skin by nanoparticles remains unclear. Skin presents two physical barriers: the stratum corneum and tight junctions (TJs). To be absorbed through the skin, a medicine must be less than 500 Da in molecular weight [47]; that is, a compound exceeding 500 Da cannot cross the TJ barrier. Therefore all nanoparticles are thought to be too large to penetrate the TJ barrier, at least when it is fully functional. However, nanoparticles may be able to elude the TJs in hair follicles [48], whose heterogeneously differentiated epithelial cells and their various functions may impart some flexibility in regard to penetration [49]. In addition, Langerhans cells can uptake external antigens despite the presence of an intact TJ barrier [50], thus representing another possible mechanism for the skin penetration of nanoparticles.

The skin is the body's primary defense against the environment and thus is under constant assault from mechanical irritation, microbial pathogens, and chemical insults. Therefore, although the skin barrier is quite strong, it is not always completely protective. For example, tape stripping [51], mechanical flexion [52], and UV exposure [45] all increase nanoparticle penetration in skin. Therefore, in reality, nanoparticles likely gain access into our body through skin that is damaged during the activities of daily living. However, we want to reiterate that the rate of any possible penetration is extremely low, given that state-of-the-art quantitative techniques are unable to detect significant numbers of penetrated particles (Fig. 6.1).

6.3 Potential Hazards of Skin Exposure to Nanoparticles

The potential hazards associated with nanoparticles are divided into two main categories according to the context in which the exposure occurs.

6.3.1 Potential Direct Health Effects of Nanoparticles

Whether nanoparticles are directly inflammatory and cytotoxic currently is the main topic of debate regarding their health risk [53–56]. However, because the rate at which nanoparticles enter through skin is extremely low, we think that they are unlikely to directly cause significant inflammation and cytotoxicity in most situations in skin. Therefore, we now focus on the potential for nanoparticles to lead to mutation and sensitization, adverse effects that are sometimes induced even at relatively low-level skin exposure to a chemical substance.

Many reports suggest that nanoparticles are mutagenic [57–60], but almost all of these studies indicate that the mechanisms underlying the observed mutagenicity were ROS dependent [57–60]. ROS are not "evil" in and of themselves; instead the duration and intensity of exposure determine whether their health effects are harmful or beneficial [61, 62]. For example, high doses of the herbicides, paraquat and juglone, shorten the life span of *Caenorhabditis elegans* by inducing ROS, whereas low doses extend the organism's life span [63, 64]. However, because only very low numbers of nanoparticles enter the body via the skin, the mutagenicity of nanoparticles is unlikely to occur through ROS-dependent mechanisms. Additional research is warranted to address potential mechanisms for the mutagenicity of nanoparticles and the conditions that enhance their entry or accumulation.

Whether nanoparticles act as directly as sensitizing agents has not been determined. A local lymph node assay, which identifies chemical sensitizers by their capacity to induce the proliferation of cells from draining lymph nodes after dermal exposure [65], was unable to detect any sensitizing ability associated with amine-modified polystyrene nanoparticles (diameter, 50 nm) or titanium dioxide primary nanoparticles (diameter, <25 nm) [66]. Another study evaluated the sensitizing ability of two types of wet-process silica nanoparticles, mesoporous silica and colloidal silica, both of which were about 100 nm in diameter [67]. After three consecutive days of skin painting, mesoporous silica but not colloidal silica increased the ear thickness of mice, prompting the authors to conclude that mesoporous silica nanoparticles acted as a chemical sensitizer. However, the changes in ear thickness were slight, and unlike the positive control (2,4-dinitroflourobenzene, a strong chemical sensitizer), the mesoporous silica nanoparticles did not elicit any cell proliferation in the local lymph node assay [67].

To date, the only nanoparticle that is confirmed to be antigenic is fullerene: the immunization of mice with complete Freund's adjuvant and a C_{60} fullerene derivative conjugated to bovine thyroglobulin successfully induced C_{60} fullerene-

specific antibodies [68, 69]. However, evaluating the sensitizing ability based on OECD Guideline 406, Ema et al. however could not detect any sensitizability of C_{60} fullerene [69]. These results suggest that C_{60} fullerene must be haptenized to induce specific antibody. However, such haptenization seems unlikely under natural conditions, implying that C_{60} fullerene would be safe in terms of sensitizing ability, according to our current knowledge [70]. Considering all of these findings together, we conclude that the notion that nanoparticles act as chemical sensitizers after skin exposure has not been confirmed.

In contrast, several epidemiologic studies indicate that airborne particulates containing sensitizing metals contribute to the onset of metal allergy [71–73]. Considering metal nanoparticles release metal ions, a supposed cause of metal allergy, metal nanoparticles may act as sensitizers, not because of their own antigenicity, but because of the antigenicity of their released metal ions. Future safety evaluations of nanoparticles need to address this potential function of nanoparticles as indirect sensitizing agents.

6.3.2 Combined Exposure to Nanoparticles and Other Substances

Exposure to nanoparticles often occurs simultaneously with exposure to other chemical compounds and environmental allergens [30]. However, little is known about the hazards of combined skin exposure to nanoparticles and other substances. As mentioned earlier, exposure to environmental nanoparticles facilitates the onset and severity of allergic diseases. Because the most important exposure route for these effects is unknown, the possibility that concurrent skin exposure to nanoparticles and allergen contributes to the onset of allergy needs to be considered.

We first focus on allergic contact dermatitis, which typically is induced by a chemical sensitizer. In one study, injection of titanium dioxide nanoparticles (diameter, 15–25 nm) was done before 2,4-dinitrochlorobenzene-induced expansion of cells from the draining lymph nodes of mice [74]. Furthermore, 3 consecutive days of skin painting with a mixture of mesoporous silica nanoparticles and 2,4-dinitroflourobenzene induced more severe ear swelling in mice than 2,4-dinitroflourobenzene alone did [67]. Furthermore, the fragrances in cosmetics, which frequently contain nanoparticles as well, are a leading cause of allergic contact dermatitis [75]. Additional studies to reveal the mechanisms of these nanoparticle-associated effects and to identify the threshold amount for an adverse response are needed urgently.

Atopic allergies are IgE-related allergic conditions, such as atopic dermatitis. As mentioned earlier, exposure to environmental nanoparticles is one of the most important factors in the induction or aggravation of atopic allergy. Yanagisawa et al. found that intradermal injection of mixture of mite allergen, which is the

major cause of atopic dermatitis [76], and titanium dioxide nanoparticles or polystyrene nanoparticles aggravated atopic dermatitis-like skin lesions in the NC/Nga mouse model [77, 78]. We confirmed that intradermal injections of silica nanoparticles caused similar adverse effects, and smaller nanoparticles caused more severe reactions [79]. In contrast, skin painting of zinc oxide nanoparticles with ovalbumin and staphylococcal enterotoxin B did not exacerbate atopic dermatitis-like skin lesions [80]. These findings together suggest that although some nanoparticles might aggravate atopic dermatitis directly, this potential might be weaker under non-laboratory exposure conditions. One potential mechanism for the role of nanoparticles in atopic allergy is that the interaction of nanoparticles and allergen changes the skin penetration kinetics of the allergen and induces IgE-biased immune responses, which are a characteristic feature of human atopic allergies [81, 82]. For this effect, skin penetration by nanoparticles is unnecessary. Therefore studies designed to obviate nanoparticles' possible health risks should focus on not only the direct immunomodulating effects of nanoparticles but also on their potential to interfere with a healthy response to coexisting chemical substances or allergens.

6.4 Conclusion

Existing data are inconclusive regarding the health risk of skin exposure to nanoparticles. Although qualitative data suggest that nanoparticles enter the body via the skin in some situations, almost every quantitative study has concluded that the penetrating amount of nanoparticles is less than the detection limit of present technology, at least in the model used. To continue the discussion regarding the adverse effects of skin-acquired nanoparticles, we propose that further quantitative analyses are needed to determine the rate at which nanoparticles penetrate the skin and any potential for their accumulation. We also believe that additional research should be focused on determining whether nanoparticles cause immunologic sensitization, either directly or by promoting the sensitizing effects of co-exposed substances. These future studies will not only reveal ways through which we can accommodate an environment rich in nanoparticles; they also will provide insight into means to improve the safety and efficacy of nanomedicines for skin care.

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