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Therapeutic Delivery

Skin toxicity of topically applied nanoparticles

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Nowadays, nanoproducts have found numerous applications, allowing them to enter the human body in different ways. Skin is a major body organ that acts as the first-line barrier between the internal organs and external environment. Although the inhalation and ingestion of nanoparticles is more dangerous compared with skin exposure, there are noteworthy information gaps in skin exposure to nanoparticles that need much attention. Despite the few reviews in the literature on the cytotoxic effects of nanoparticles, no research has reviewed the clinical side effects of nanoparticles following topical admonition, including skin inflammation, skin cancer and genetic toxicity.

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Toxicology is a discipline overlapping with biology, chemistry and medicine, dealing with harmful effects of biological, chemical or physical mediators on living organisms [1]. The innovation in chemicals and products cannot be fathomed without suitable and advanced toxicological studies [2]. The importance of toxicity studies lies in setting up a dose–response curve and assuring the safety of new substances for use as pesticides, medicine or food stabilizers before they are recorded for common use in industry or medical clinics. In this regard, studying materials' toxicology and performing standard toxicological experiments would allow for a better assessment of these materials and their effects [3].

Skin-associated toxicology

Works in the field of dermal toxicity are undertaken to enhance the accuracy of predicting dermal responses in humans following the dermal application of chemicals [4]. Dermal toxicity testing evaluates the local and systemic effects of a chemical following dermal exposure. These studies identify substances that enter the skin and produce systemic toxicity; however, the total absorbed chemical cannot be specified via dermal toxicity testing [5]. Dermal penetration commonly occurs through a passive diffusion. Nevertheless, prior to a systemic absorption, the biotransformation of test substance can occur in the deeper viable areas of the skin. The capacities of the stratum corneum (SC) [6], as the outmost layer of the skin, and its bi-lipid layers, regulate the degree of dermal permeation. Certain biological factors affecting absorption process are skin area localization in the body, SC integrity and thickness of the epidermis. Further influencing absorptions are such physiologic determinants as temperature and local blood flow.

Toxicity testing & skin toxicity evaluation methods

Toxicity tests include examining the side effects of materials on laboratory animals through exposing them to high doses. Toxicity testing utilizes a broad range of assessments in various species of animals with long-term administration of medicine via monitoring physiological and biochemical anomalies, and detailed tests. The use of

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animals in toxicity evaluation is most likely to continue in the future due to advantages such as the possibility of the obviously defined genetic constitution and their suitability to controlled exposure and controlled period of exposure, and the possibility of detailed assessment of all tissues following necropsy. Tests evaluating toxicity are performed both *in vivo* (using the whole animal) and *in vitro* (testing on isolated cells or tissues), or through computer simulation [7]. In the past, toxicity evaluation was more common since they were related to testing animals. Such preference was mainly due to the absence of more complicated evaluation techniques and animals. The emergence of animal rights in the 1950s reduced the use of animals for toxicological studies. As a result, the *ex vivo* and *in vitro* methods were replaced to decrease the reliance on live animal testing [8]. Toxicity assessment techniques are altered due to the new vehicles available to formulate test materials, where using one technique to assess the toxicity of the dermal is not satisfactory [4]. A major concern in evaluating skin absorption and toxicity of nanomaterials is how to conduct the experiments. To determine the skin penetration of a substance, both *in vivo* and *in vitro* methods are used, because selecting either of these techniques may result in disparate types of information [9]. The Franz-type diffusion cell is a practical well-established model for dermal and transdermal delivery [10]. Although this method may provide incomplete information on permeability, particularly when synthetic membranes are used, it has been used as an important method for transdermal drug research [11]. The *in vitro* dermal penetration techniques using donated human skin are preferred to *in vivo* tests in animals. It is of note that the skin absorption testing of new cosmetic ingredients in living animals in the EU is no longer legal, hence the need for valid substitutions in the safety analysis of cosmetics. In fact, examining cosmetic components by *in vitro* models has been the standard for several years within the EU [9]. *In vitro* cell culture should be used for diffusion cells or perfuse skin model systems. *In vivo* studies with the skin of rat or pig, since these animals are anatomically, physiologically and biochemically similar to humans – produce better results. In dermal toxicology, hair removal, using repeated clipping for instance, is necessary to ensure cutaneous distribution and sufficient contact between the tested materials with the epidermis. Rapid growth of hair and the continuous use of these methods may entail nominal epidermal hyperplasia and hyperkeratosis [4]. In addition to morphological changes caused by the rapid growth of hair following clipping [12], the physical presence of hair may interfere with the absorption of drugs [13]. This statement was mentioned to warn the researchers that hair removal by repeated clipping routinely carried out in animal experiments before sample administration may cause some skin dysfunctionality, which may confuse the researchers by giving wrong results regarding the toxicity of tested nanoparticles.

Nanotoxicology

Nanotechnology is an important technology of the 21st century and in today's inventive world [14]. Nanoparticles are defined as particles <100 nm, and colloids are a group of particles with a size range of 1–1000 nm. Nanotechnology is a vast multidisciplinary field of applied sciences whose unifying theme is to control, produce and apply nanoparticles for different purposes. Nanoparticles have many benefits over their bigger analogs, owing to their unique physical and chemical characteristics caused by their small size [15]. The current nanomaterial research focuses on the medical applications of nanotechnology, while side effects associated with nanotechnology are not taken into consideration. Nanomedical consumers and developers are to match the related medical and communal benefits and risks with nanotechnology. In assessing the toxicity and hazard associated with exact nanomaterials, the adequacy of available methods such as predictive structure–activity relationships or physiologically based pharmacokinetic modeling is not obvious. The successful growth of future nanomedical tools and pharmaceuticals requires a solid information base to select the optimal nanomaterial in a given situation in order to understand the toxicology and probable side effects related to candidate materials for medical usage [16]. Two decades of nanotoxicology study has revealed that the interactions between nanomaterials, cells, animals, human beings and the environment are extremely complex. Researchers are still attempting to fathom how the physicochemical or other properties of nanomaterials control these interactions and discover the final effect of nanomaterials on health and environment. As new nanomaterials are developed and animal evaluation is reduced, computational methods gradually become more important to emphasize safety studies. Due to the increasing use of nanoparticles in daily life, it is necessary to consider the probable risks in addition to the increased opportunities. Nanotoxicology studies the negative effects of nanoparticles on the human body [17,18], and it is a subdivision of bionanoscience that analyzes the application of nanomaterials toxicity [19]. Safety and toxicity aspects of nanomaterials advance slower than their production, probably due to the researchers' disagreement in determining the test protocols [20]. However, in real life, nanoparticles occur more than other particles, chemicals and biological compounds. It is, therefore, more likely that humans and the environment finally be exposed to these cytotoxic agents. Indeed, nanoparticles have been observed as the carriers of toxic

ions, as in the case of radio nuclei [21]. There is currently no control over nanomaterial products, while scientific investigation has shown the human and environment toxicity potential of a number of nanomaterials [22,23]. In addition to particle-related factors, the administered dose, way of administration and amount of tissue distribution are the main parameters in nanocytotoxicity [24]. To compare the toxicity effect of nanoparticles with the same chemical structure, but different sizes and fit of dose–response relationships, several studies have been conducted on inhaled solid particles. Results have shown that particle surface area is a more appropriate dosimetric compared with mass [25–27]. Surface area is the most significant parameter among other characteristics [28]. This interaction may interrupt normal molecular interactions by changing protein configuration [29].

In vivo & *in vitro* methodology of nanotoxicity tests

In this section, we provide the current state of nanomaterial hazard assessment strategies using *in vitro* and *in vivo* approaches. It is of note that the use of an evaluation method can be extremely cost intensive and time consuming. For *in vitro* and *in vivo* toxicology, one should first define the physicochemical characterization of nanomaterials such as shape (Scanning electron microscope), size, dynamic light scattering techniques, surface charge (ζ-potential measurements), UV-Vis spectroscopy, Brunauer–Emmett–Teller [30] surface area photoluminescence spectroscopy, dielectric spectroscopy, fluorescence spectroscopy [31] and magnetic measurements [30,32], accurately determine chemical structures (NMR), perform spectroscopy to measure chemical composition (using x-ray photoelectron), and chemical and electronic states, and apply MS for mass and elemental analysis and spectroscopy techniques that measure absorption emission, or scattering of either wavelength or frequency [33–35]. Chromatographic techniques such as high-pressure LC or size exclusion chromatography to ensure the purity of nanomaterials in assessing toxicity can indicate the presence of impurities in the sample [32]. The next step in the *in vitro* study is to determine a suitable cell line. Using human primary cells or other cell lines is facile, efficient and cheap [36]. Furthermore, skin penetration of nanoparticles is measured via the following three methods: differential stripping: by removing the SC, it is possible to determine the number of nanoparticles that remain on the skin surface or the upper layers of the SC; diffusion tests using skin membrane: the extension of the interfollicular space during this method may open more holes in the membrane and create an exaggerate in the results [37]; the use of fluorescent dyes in nanoparticles and laser scanning microscopy. Then, the dose (use of analogous and pragmatic dose metrics, and test conditions) and cell response values are checked. In this method, exposure of different cell types to nanoparticles is discussed along with dose–response analysis of oxidative stress nanotoxicological assays. The ferric-reducing ability of serum assay or the electron spin resonance is a useful method for the prediction of *in vitro* approaches to assessing the hazard of nanomaterials. Electron spin resonance technique can detect the surface reactivity of nanomaterials by measuring the free radicals and presenting them through spectroscopy. Ferric-reducing ability of serum also determines the number of free radicals by cytochrome C reduction. Other methods of evaluating free radical formation are electron paramagnetic resonance and 2,3-bis-2-(methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT), which analyze cell viability, and measure the growth of NAD(P)H through glycolysis, associated with metabolic active cells [38,39]. Because of the physicochemical differences between humans and animals, animal testing may impose certain limitations on human prediction about the hazard of nanomaterials [40]. Meanwhile, *in vivo* studies offer advantages such as studying chronic exposure effects, absorption, metabolism and bio-distribution, and understanding the influence of route of exposure on toxicity [41]. The *in vitro* results can work as guidelines for the design of *in vivo* investigations. In *in vivo* studies, it is imperative to ensure that nanomaterials are free of any impurities. In acute examination, penetration of nanoparticles through SC may not be observed, if the nanoparticles are used for prolonged use, they will penetrate through horny layer, and be located in deep layer of epidermis. Also, they can penetrate through the skin, reach different tissues and induce various pathological lesions in several major organs [42]. Reports reflected that the onset of sensitization varies with the type of nanoparticles and exposure duration. In addition, dose-dependent effects have also been taken into consideration. For example, it may be observed skin inflammation by parakeratosis and spongiosis after subchronic exposure but no acute skin irritation induced [43]. When the objective of *in vivo* experiments is acute studies, after determining the accurate dose according to administered per kg of animal weight and surface area per mass of nanomaterials, it is required to control the response to an administered dose, weight change, clinical observation, mortality and clinical pathology. With chronic studies, it is necessary to assess the absorption, distribution, metabolism and excretion of nanomaterials [44]. The most common method for tracing nanoparticle uptake *in vivo* includes radiolabeling via γ-emitters [45].

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Skin toxicity of nanoparticles

Skin penetration of nanoparticles

The main entry routes of nanoparticles into the body include respiratory pathway, digestive tract and/or absorption through the skin. There are four pathways of penetration into the skin: intercellular, transcellular, transappendageal, and through sweat glands and hair follicles. However, due to the limited information on dermal absorption and skin penetration of nanoparticles, more studies are necessary in this regard [46]. Although factors such as skin diseases, contaminate surface and anatomical side can augment dermal uptake [47,48], penetration of nanoparticles into healthy skin entails the generation of free radical, oxidative stress and collagen depletion [49,50]. Although such depletion induces keratinization, atrophy of the dermis and skin wrinkling, the penetration of nanoparticles is doubled when the skin barrier is damaged [51]. Many studies have shown the accumulation of lipid nanoparticles in the follicular openings [52], and the migration of nanoparticles from the dermis to regional lymph nodes through skin macrophages and Langerhans cells [53]. To date, there has been no complete information on whether nanoparticles pass through the SC or through the circulatory system, and accumulate in the dermal tissue. It is noteworthy that the absorption of nanoparticles may be different from chemical absorption due to their unique properties, which influences their passing through the SC [54]. According to certain studies, percutaneous penetration of nanoparticles is limited to hair follicle upper regions or the superficial layers of the SC [55,56]. For instance, iron nanoparticles aggregated in the SC and epidermis [57]. Jianhong *et al.* showed that TiO_2 nanoparticles penetrated SC, but not the dermis; however, after 40 days of dermal exposure in hairless mice, nanoparticles were reported to penetrate the deeper part of the skin, reaching other tissues, which caused some pathological changes in several important organs [58]. Hagar *et al.* studied gold-nanoparticle penetration and metabolic effects of nanoparticles in human skin. They showed that 15-nm gold nanoparticles in aqueous solution aggregated on the surface SC following 24-h exposure; however, 6-nm gold nanoparticles in toluene penetrated the SC and the epidermal layers of human skin. One *in vivo* study assessed the toxicity of Ag nanoparticles on the skin following 14 days of application, where Ag nanoparticles were detected on top of the SC. Transmission electron microscope (TEM) images showed the existence of Ag nanoparticles in the superficial layers of the SC [59]. In another study, the biological interactions of quantum dot (QD) nanoparticles were examined on the skin and in the human epidermal keratinocytes [60] so as to regulate cellular uptake, cytotoxicity and inflammatory probability. In this study, QDs were topically applied to the porcine skin for 24 h. Confocal microscopy exhibited the penetration of QD through the uppermost SC layers of the epidermis. Through this process, fluorescence was found mainly in the SC and near hair follicles. Additionally, transmission electron microscopy [61] showed QDs within the intercellular lipid bilayers of the SC. In human embryonic kidney (HEK) cell line, viability was significantly reduced after 24 and 48 h. There was a notable increase in IL-6 and an increase in IL-8 levels following 24 and 48 h. TEM of HEK treated with QD for 24 h depicted QD in cytoplasmic vacuoles and at the periphery of the cell membranes [62]. Polymeric nanoparticles such as polystyrene nanoparticles are among the systems for topical administration in drug targeting and delivery. Studies in this area reveal the accumulation of polystyrene nanoparticles in the follicular openings, the time-dependent increase in this distribution and the follicular localization preferred by the smaller particle size [63]. There exists a significant knowledge gap in nanotoxicology of the skin, hence the present review focused on this topic. Table 1 summarizes the penetration of nanomaterials in the skin.

Symptoms of skin toxicity associated with nanoparticles

Inflammation

Phototoxicity can be defined as a skin inflammatory response produced from the topical use of chemicals, drugs and consequent exposure to light, particularly ultraviolet radiation. Phototoxicity occurs when a substance is exposed to exogenous materials such as cosmetic products or drugs. Nanoparticles in cosmetics have caused significant health-related risks. Animal models have long been observed as standard tests for the calculation of dermal toxicity. The human skin equivalent model is well known as an attractive model for the evaluation of dermal toxicity. Nevertheless, only a limited number of papers have proposed the usefulness of human skin equivalent model as a screening approach to resolving the dermal irritation potential of nanoparticles [69,81,82]. A majority of nanoparticles, due to their distinctive properties, may be able to stimulate the immune system, and result in inflammatory response through the abnormal secretion of different cytokines and other chemicals [83,84]. Contrary to these nanoparticles, others such as cerium oxide nanoparticles have been shown to antagonize the inflammation in cells [85]. With the application of an irritant to sensitive living skin cells, they show variable degrees of response. Inflammation is the first generalized response aspect of any irritating chemical or physical agent. Redness, pain, heat and swelling

Table 1. Penetrated nanomaterials in skin.

Type of nanoparticle	Dose and size of nanoparticle	*In vivo*/*ex vivo*/*in vitro* study	Outcome	Ref.
Lipid	198 nm, 200 ml	*In vivo* rat skin	Accumulation of SLNs in hair follicles	[52]
(γ-Fe_2O_3)	40 nm	*Ex vivo* excised human skin	Nanoparticles penetrated hair follicle and SC, and reached the viable epidermis. But unable to permeate the skin	[57]
TiO_2	(4, 10, 25, 60 and 90 nm) 400-μg titanium dioxide per cm^2	*In vivo* porcine ear skin	Discovered in the SC, stratum granulosum, prickle cell layer and basal cell layer (4 nm), but not in dermis	[58]
Gold	15 and 6 nm, 90 μg/ml	*Ex vivo* excised human skin	Aggregation of AuNP (15 nm) in aqueous solution on the surface SC but penetration of AuNP (6 nm) in toluene through SC and into epidermal layers of human skin	[64]
Silver	20, 50 and 80 nm, 34.0–0.34 μg/ml	*In vivo* porcine skin	On top of the SC and the superficial layers of SC	[59]
QD	8.40 $\times$ 5.78 nm; 1, 2 and 10 μM for 24 h	*In vivo* porcine skin	Penetration of QD through the uppermost SC layers of the epidermis and near hair follicles. QD were found in the intercellular lipid bilayers of the SC	[62]
Polystyrene	20–200 nm	*In vivo* porcine ear skin	Accumulated in the follicular openings (20 nm), time-dependent distribution and localization of smaller particle size in the follicular. Accumulation of the 200 nm F-NP in skin furrows	[63]
TiO_2 and ZnO		*Ex vivo* porcine skin	Able to penetrate through porcine skin, but not to deeper layer	[65]
ZnO	Uncoated (65.5 $\pm$ 35.6 nm) and coated (74.3 $\pm$ 32.3 nm)	*In vivo* human skin	Penetration only into the superficial layers of the SC, with no penetration to the viable epidermis and no apparent toxicity in the viable epidermis	[66]
Silver	20–50 nm	*In vitro* the mouse skin samples from the Franz diffusion cell system	Investigating dependence of the nanoparticle's shape on penetration. It was observed TNP in the SC region whereas SNP in a viable epidermal layer, which indicated that both TNPs and SNPs could not penetrate through the dermal–epidermal junction into the underlying dermal layers. Rod-shaped nanoparticles were observed with high penetration ability through dermal–epidermal junction	[67]
Silica	43–290 nm, 10 μg/ml	*In vitro* cellular uptake	Positive surface charge of particles enhanced the *in vitro* cellular uptake, also nanoparticles 43 nm were found in disrupted SC independent of their surface charge	[68]
TiO_2 and ZnO	ZnO: 3.0 μmol/g, TiO_2: 0.41 mol/g	*In vivo* skin obtained by biopsy with	Present at the skin surface and in the uppermost SC regions	[69]
ZnO	19 and >100 nm	*In vivo* human	^{68}Zn was not detected in blood and urine, and only trace was tracked after 5 days	[70]
Silver	1.2 mg/ml	*In vivo* porcine ear skin	Maximum penetration depth of AgNPs at $\sim$14 μm penetration depth of AgNPs could exceed the SC thickness	[71]
Cobalt	1.0 mg cm^{-2}	*In vitro* the human skin samples from the Franz diffusion cell system	Found in epidermis and derma after 24 h of application to the skin	[72]
Gold nanorods	100 μl, 500 μg/ml	*In vitro* skin permeation using a Franz-type diffusion cell	Penetrated into the SC	[73]
Gold	2 μl (5–10 nm)	*in vitro* human keratinocytes cell line	Penetration of NPs through the barrier of the SC, epidermis and the dermis. Nanoparticles were found over 500 microns deep into the skin	[74]
SLN	200 μl	*In vivo* porcine skin	Penetrate into the deep layers by reducing the size	[75]
Gold	22–186 nm	*In vivo* rat skin	In epidermal layers just below the SC	[76]
Silver	SNPs: 50 nm, length and diameter of RNPs: 50 and 20 nm, TNPs: 2 nm thick equilateral triangular length of 50 nm	*In vitro* Franz diffusion cell system *In vivo* mice skin	RNPs: most penetration, SNPs: moderate penetration, TNPs: lowest penetration, presence of an SC and a collagen- and muscle-filled dermis. No major differences with differently shaped AgNPs	[77]
Lidocaine-loaded nanoethosomes	200 μl	*In vitro* skin permeation using a Franz-type diffusion cell *In vivo* rat skin	Passed lidocaine through the SC from lidocaine-loaded nanoethosomal	[78]

F-NP: FITC(fluorescein 5-isothiocyanate) nanoparticle; NP: Nanoparticle; RNP: Rod-shaped nanoparticle; SC: Stratum corneum; SLN: Solid lipid nanoparticle; SNP: Spherical nanoparticle; TNP: Triangular nanoparticle; QD: Quantum dot.

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Table 1. Penetrated nanomaterials in skin (cont.).

Type of nanoparticle	Dose and size of nanoparticle	*In vivo*/*ex vivo*/*in vitro* study	Outcome	Ref.
Gold	90 mg/ml, 437 mg/ml	*In vitro* human skin permeation using a Franz-type diffusion cell	Penetration increases with increasing concentration and property of hydrophobicity, also by decreasing the size of NPs	[79]
Gold	109–1011 NPs/ml	*Ex vivo* rat hind-paw skin *In vivo* rat hind-paw skin	Epidermal penetration but in rats exposed to AuNPs demonstrated nanoparticles in blood, and histological analysis revealed	[80]

F-NP: FITC(fluorescein 5-isothiocyanate) nanoparticle; NP: Nanoparticle; RNP: Rod-shaped nanoparticle; SC: Stratum corneum; SLN: Solid lipid nanoparticle; SNP: Spherical nanoparticle; TNP: Triangular nanoparticle; QD: Quantum dot.

are four characteristics of inflammation. A direct result of chemical or physical irritation is various degrees of inflammation [86], which is the main feature of many dermatologic illnesses. It leads to different results in cutaneous physiology, impairing the skin's barrier function being particularly impaired [87]. This interruption can serve as a way of entry for microbes and allergens or other proinflammatory stimuli, causing further inflammation. In this regard, cerium oxide nanoparticles were found effective in reducing reactive oxygen species (ROS) and inflammatory mediators, and possibly useful in curing inflammation [85]. Surface coating of QDs does not affect the uptake by keratinocytes, but their permeation on the skin enhances cytokine creation, irritation and reduction of cell viability [62]. In a study, acute and subchronic dermal toxicity of Ag nanoparticles (sizes <100 nm) was examined. They used different concentrations and evaluated toxic responses by clinical and histopathologic parameters. Dermal histopathologic changes in an acute and subchronic study showed the evidence of inflammation. Further tissue changes were observed in subchronic tests, which can be a reason for the dependence of toxicity on time and dose [88]. The results of dermal exposure to amino acid-derivatized fullerene have also been investigated by evaluating cell viability and proinflammatory potential in human epidermal keratinocytes (concentrations of 0.4–0.00004 mg/ml). The decrease in cell viability and elevated levels of proinflammatory cytokines, such as IL-8, IL-6 and IL-1β indicated that derivatized fullerenes can trigger a toxic response in human epidermal keratinocytes at positive concentrations. In a study, cytokines were analyzed to determine their proinflammatory potential by assessing the release of IL-8, IL-6, TNF-α, IL-10 and IL-1β so as to illustrate the relationship between the concentration and toxicity of nanoparticles. A significant detection increase was observed in IL-1β, IL-6, IL-8 and TNF-α from HEKs exposed to AgNPs (20, 50 and 80 nm) for 24 h. In this study, by reducing the concentration of nanoparticles, a decrease was observed in the secretion of interleukin. Moreover, in porcine skin, a slight intercellular epidermal edema was seen, while with the increase in the concentrations of nanoparticles, an intercellular epidermal edema with focal dermal inflammation was observed [59].

Skin cancer

Skin cancer accounts for a growing share of 8–10% of all cancers. Basal cell carcinoma, as the main cause of skin cancer, is a common malignant tumor. Nevertheless, the highest number of deaths mainly occur in the squamous cell carcinoma and malignant melanoma [89]. Free radical scavenging with nanoparticles is performed by delaying the ROS. Free radicals are molecules comprised of unpaired electron in their outermost shell. ROS is an unstable and extremely reactive compound that bonds the electrons from cellular macromolecules, rendering them dysfunctional (Figure 1) [90]. Chain reactions of self propagating-free radicals mediate lipid peroxidation and cause cell membrane structure damage, thereby prompting cell death [91,92]. Several studies have recommended the biocompatibility of Ag nanoparticles dermal [93], which also leads to the inhibition of keratinocytes proliferation and cell morphology changes in ZnO and TiO_2 nanoparticles utilized in skin care products. These nanoparticles result in the depletion of antioxidants and increased potential for free radicals. TiO_2 nanoparticles induce cytotoxic effects by creating free radicals, thereby producing intracellular damage. In addition, smaller particles are further absorbed, generating more free radicals [15,65,94]. Oxidative injury to the cell membranes by lipid oxidation (Figure 2) was detected in all cases, where fullerene exposure led to cell death. Under ambient conditions in water, fullerenes can produce superoxide anions liable to membrane damage and cell death. Nano-C60 is cytotoxic to human dermal fibroblasts. In certain cases, fullerene materials were able to create superoxide anions that could be responsible for membrane oxidation and cytotoxicity [95].

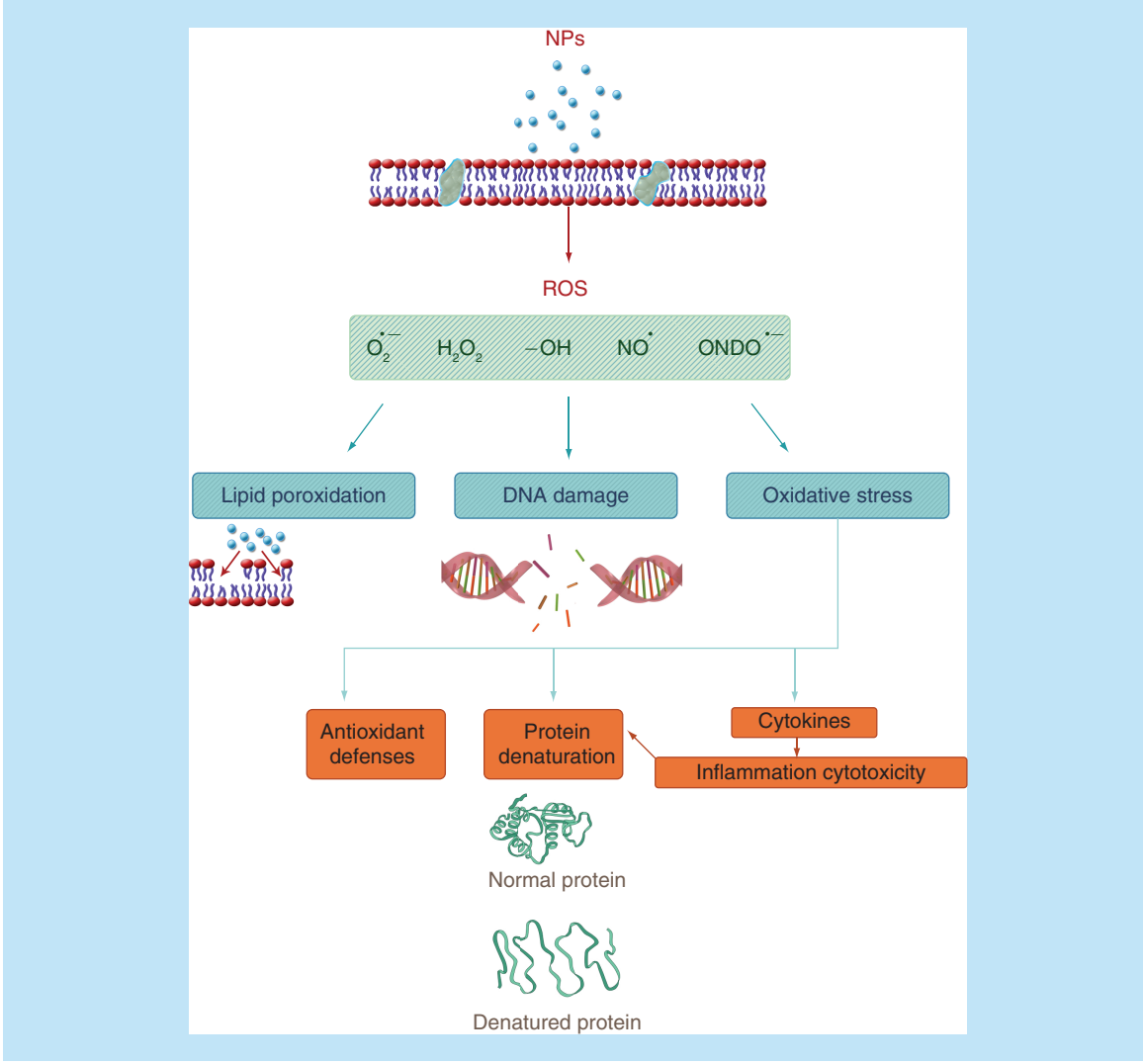

Figure 1. Schematic representation of the effects of reactive oxygen species released by influence of nanoparticles on skin.

Genotoxicity

Genetic toxicity studies identify whether a material can induce genetic harm through a variety of ways leading to cancer. The phase detection toxicologist must be cognizant of the series of examinations used in diagnosis [26] and discover the genetic toxicity risk; therefore, a mere single test is not able to identify all genotoxic styles associated with tumorigenesis [31]. Genotoxicity, as the primary cause of cancer progression, can be probably caused by free radical production [96]. Toxicogenomy is defined as holding the proteins and/or metabolites, as important effector groups, in practical genomics. The aim of the latest approaches is to change toxicology from descriptive to predictive, counting the prediction of *in vivo* results from *in vitro* models and other species [31]. Required further research particulars are data on computational analysis; assessment of the mutagenicity in a bacterial overturn gene mutation testing; and evaluation of genotoxicity in mammalian cells. Understanding how to deal with genetic toxicity hazards (e.g., clastogenic and aneugenicity) is imperative in this regard. There are dissimilar opinions on the safety of gold NPs; however, their uptake by cells and interaction with DNA have been reported [97]. Several methods such as comet assay or HPRT gene mutation test have revealed that metal nanoparticles interrupt DNA and their reproduction processes in all kinds of cells. By studying the effects of nanosilver on DNA, Ag nanoparticles (5–10 nm) were brought into lymphocyte cell DNA and studied for abnormalities [98]. Metal oxides such as copper oxide (10–40 nm) and cobalt oxide have also been observed inducing a considerable stress on the

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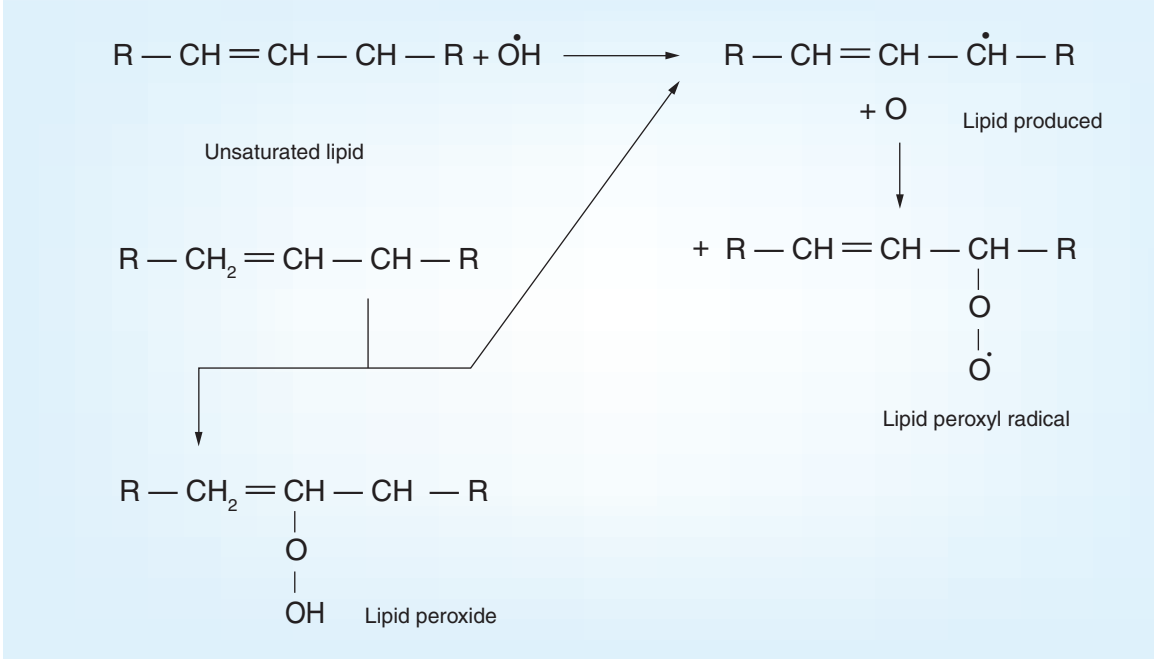

Figure 2. The process of lipid peroxidation caused by reactive oxygen species formed as a result of the nanoparticles.

exposed DNA. These results demonstrate that synthesized copper oxide nanoparticles (6 nm) are toxic to human cells [99]. By studying the effect of carbon nanotube (CNT) on the skin, causes of ultra-structural and morphological changes [100], cell-cycle arrest [101], expression and alterations of several proteins in cultured skin cells [102] have been defined; by sidewall functionalization, on the other hand, SWCNT sample became less cytotoxic [103]. Other studies on ZnO nanoparticles (165 nm) have shown oxidative DNA damaging potential in the human skin epidermal cell line, which can be of genotoxic potential in human epidermal cells by lipid peroxidation and oxidative stress [104]. It has further been revealed that ZnO NPs impair the mitochondrial function in human epidermal keratinocyte HaCaT cells and entail the leakage of lactate dehydrogenase into the medium. ZnO NPs prompt an oxidative damage in cell organelles and membrane lipid peroxidation by generating ROS [105]. Moreover, ZnO NPs significantly affect the expression of genes in human keratinocyte cells associated with apoptosis and oxidative stress, such as superoxide dismutase and glutathione peroxidase. ZnONPs caused a significant growth in the activity of superoxide dismutase [106]. Meyer *et al.* have shown that ZnONPs interfere with mitochondria function in human dermal fibroblasts and induce apoptosis via mitogen-activated protein kinase p38 and protein p53 activation. It had shown that ZnONPs induce genotoxicity in primary human keratinocyte skin cells, which may be related to their potential carcinogenic characteristics. Toxicological tests on sunscreens and cosmetic products, which contain TiO_2 nanoparticles, have shown that although 500 nm TiO_2 particles are only slightly able to affect DNA strand breakage, exposure to 20 nm particles of TiO_2 completely damages supercoiled DNA, even at low doses and in the absence of UV [107].

The genotoxicity of CeO_2 nanoparticles (7 nm) was examined on human dermal fibroblasts. As a result, interactions with organic molecules were observed, especially with protein, leading to the reduction of nano-CeO_2 and decline of Ce4+ atoms, focused at the surface of CeO_2 NPs. These particles induced severe DNA injuries and chromosome break through oxidative stress [108]. Table 2 summarizes the toxicity and influence of nanomaterials in skin.

Conclusion

Influence of nanoparticles on the integrity of the skin and penetration of nanoparticles in the long-term use is not fully investigated. Experimental results on the skin toxicity of nanoparticles are contradictory. For example, a low number of nanoparticles may be able to stimulate the immune system due to their distinctive properties, leading to an inflammatory response. Contrary to these nanoparticles, cerium oxide nanoparticles have been shown

Table 2. Toxicity and influence of nanomaterials in skin.

Type of nanoparticle	Size and dose of nanoparticle	*In vivo*/*ex vivo*/*in vitro* study	Outcome	Ref.
PEGylated gold nano-semicubes	25 × 30 nm, 3.41 μg/ml	*In vitro* (on human skin melanoma Sk-Mel-28 cells)	Using laser-stimulated PEG-GNSCs resulted in inhibited volume of skin tumors by the inflammatory mediators, nitric oxide and cyclooxygenase-2	[6]
CeO_2	150 μM	*In vitro* on cell line human dermal fibroblasts	Effective in reducing reactive oxygen species and inflammatory mediator production, prevent cell death and stimulate proliferation due to the antioxidative property of these particles	[85]
MWCNTs	0.4 mg/ml	*In vitro* [60]	Alters protein expression effective on the expression of cytoskeletal elements and vesicular trafficking components	[47]
Fullerene	10 ng/ml	*In vivo*	Exhibited a potent antioxidant-free radical scavenger activity and inhibit allergic anaphylaxis response *in vivo*	[95]
CeO_2	(7 nm) Dose range 6×10^{-5} - 6×10^{-3} g/l corresponding to a concentration range of 0.22–22 μM	*In vitro* on cell line human dermal fibroblasts	Genotoxic effect of nano-CeO_2, by a clastogenic mechanism. Examination of the oxidative mechanisms in this genotoxic effect by assessing the impact of catalase, a hydrogen peroxide inhibitor, and by measuring lipid peroxidation and glutathione status synthesis in cells	[109]
Cationic liposome	34 nm	*In vitro*	Encapsulation of polyhexamethylene biguanide chloride into nano cationic liposome showed no toxic change skin fibroblast cell lines morphology	[110]
Polystyrene	50 nm, 1000 μg/ml	*In vivo* back skin of pig	Do not induce phototoxicity, acute cutaneous irritation or skin sensitization	[111]
Mesoporous silica	100 mg/ml	*In vivo* mice skin	Did not induce an ear-swelling response in mice or exacerbate allergic contact dermatitis symptoms	[112]
Silver nanolipid	200 nm	*In vivo*	High potential to reduce symptoms of irritated sensitive skin and atopic dermatitis	[113]
Silver	10, 30, 50 μg/ml	*In vitro* human skin keratinocytes (HaCaT)	Observation of apoptosis symptoms, decrease of cell viability and induce production of reactive oxygen species	[108]
Cobalt oxide	0.023–1500 μg/cm^2	*In vitro* human skin keratinocytes (HaCaT)	Penetrate only damaged skin and is cytotoxic for HaCat cells after long-term exposure	[109]

GNSC: Gold nanosemicube; MWCNT: Multiwall carbon nanotube.

to antagonize the inflammation in cells. Moreover, in some cases, different results have been reported in *in vitro* and *in vivo* studies. For instance, in an *in vivo* experiment, fullerene exhibited a potent antioxidant-free radical scavenger activity and inhibited allergic anaphylaxis response. In an *in vitro* examination, fullerenes produced superoxide anions in human dermal fibroblasts. In addition to the well-known effect of size and charge on particle skin toxicity, toxic effects of nanoparticles depend on their shape, because it is effective on particle penetration in the underlying skin layers. For example, rod-shaped nanoparticles have higher penetration ability through dermal–epidermal junction compared with triangular or spherical nanoparticles. Further research is necessary to improve the understanding of whether skin represents a way of entry into the body for nanoparticles. Overall, it is specified that adverse health effects on the topical use of sunscreens containing TiO_2 nanoparticles are not expected for a healthy skin. Nevertheless, numerous studies on carbon-based nanoparticles and QDs confirm the interaction between human dermal cells and nano-sized particles. In addition to several *in vitro* experiments on skin-generated cell lines, a few *in vivo* studies further corroborated the *in vitro* observations. From an experimental perspective, significant knowledge has been produced on the potential toxicity mechanisms of nanoparticle–biological system interactions related to nanotoxicity in the skin. ROS generation, oxidative stress induction and chronic inflammation are all realistic scenarios to be considered.

Future perspective

Nanotechnology is a vast multidisciplinary arena of practical sciences whose unifying theme is to control and produce nanoparticles along with their application for different purposes. The current nanomaterial research focused on the medical applications of nanotechnology, while the clinical side effects of nanoparticles after topical admonition including skin inflammation, skin cancer and genetic toxicity were not taken into consideration. From an experimental perspective, significant information has been produced on the possible toxicity mechanisms of nanoparticle–biological system interactions related to nanotoxicity in the skin.

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Executive summary

- Nanoparticles offer myriad benefits over their bigger analogs, owing to their unique physical and chemical characteristics caused by their small size.
- Although inhalation and ingestion of nanoparticles are more dangerous than skin exposure, there exist considerable information gaps in the skin exposure to nanoparticles, which require further attention.
- Nanotoxicology is a subdivision of bionanoscience that analyzes the application of nanomaterials toxicity.
- Scientific investigation has shown the human and environment toxicity potential of a number of nanomaterials.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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