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PERSPECTIVE

Nanoparticles Do Not Penetrate Human Skin—A Theoretical Perspective

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ABSTRACT The penetration of intact particles in the nanometer range (nanoparticles, [NP]) through human skin is a controversial topic, which has attracted much interest from both the pharmaceutical and the personal care industries. Concerns have also been raised about the possible implications that dermal exposure to NP may have for human health, particularly from physical sunblock formulations. Here we use a theoretical approach to determine the feasibility of NP penetration of healthy human skin. The maximum flux of NPs of various dimensions is calculated based on two algorithms that have been developed to model passive diffusion of molecules through skin. The results confirm that NPs are too large to permeate skin by this mechanism. Although components of NPs may dissolve in the skin and measurable amounts have been detected in body fluids, this is not indicative of actual NP transport through the skin. The possible roles for NP formulations in drug permeation enhancement are also considered but are not associated with the penetration of intact NP.

KEY WORDS diffusion · nanoparticles · penetration · physical sunblocks · skin

With the increased interest in nanotechnology over the past decade there have been conflicting reports of the transport of nanoparticles (NP) into and across the epidermis. The skin has evolved to prevent the ingress of xenobiotics and foreign bodies including viruses and bacteria. There is little evidence to suggest entities of micron or submicron size will cross the skin intact. Most recently concerns have been raised about the penetration of sub-micron particles that are used as physical sun blocks (1–3). Titanium dioxide (TiO_2) and zinc oxide (ZnO) NP are the most widely characterised of this group of nanomaterials. However a recent review that examined the disposition of metal oxide particles following application to healthy skin concluded that transdermal penetration of intact particles does not occur (4). Here we set out a theoretical framework that is consistent with the findings of the literature i.e. transdermal penetration of NP through intact human skin *in vivo* is highly unlikely.

The skin is a multilamellar organ and its barrier function is provided largely by the outermost layer, the stratum corneum (SC). Structurally the SC is a thin (~15 μm) and heterogeneous layer (5). It is composed of stacked layers of terminally differentiated and keratinised epidermal cells that are separated by intercellular lipids. Michaels *et al.* (6) proposed a "brick-and-mortar" structure of the SC where the keratinised cells (bricks) are embedded in an intercellular matrix of lipids (mortar). There are a number of possible routes and mechanisms of transport through the SC. The importance of the intercellular route was confirmed by a series of experiments *in vivo* with methyl nicotinate in the 1970's and later confirmed by direct visualisation of molecules in the 'mortar' (7) . The possibility of transcellular transport has also been considered and an aqueous polar/pore pathway has been proposed for skin transport of a number of molecules (8–12). Appendageal transport through hair follicles or pores has been investigated and Scheuplein (13,14) suggested that this pathway

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could act as a shunt for initial rapid transport of molecules across the skin.

Assuming that NPs behave like large molecules, it is possible to estimate their rate of penetration in healthy skin using simple diffusion theory. In order to have confidence in the approach, we first consider a hypothetical molecule of molecular weight 500 and density of 1 g/cm^3, for which the molar volume is 500 cm^3/mole. This can be divided by Avogadro's number to give the volume 8.3×10^{-22} cm^3 for a single molecule, which corresponds to a radius of 0.58 nm if the molecule is roughly spherical. Is this realistic? The molecular structure of adapalene, a retinoid used to treat skin disorders was considered. It has a molecular mass of 412 and the molecular size, as calculated using ACD Chemsketch (ACD Labs, Ontario, Canada) has a radius of approximately 0.5 nm. The two values are similar to the hypothetical case discussed above, and therefore there should be confidence in the permeability calculations for the hypothetical molecule or particle.

Kasting *et al.* (15) described diffusion through the skin as being similar to diffusion through a polymer and indicated that the diffusion coefficient should be an exponential function of the molecular weight. The authors concluded that every increase of 110 Daltons in molecular weight results in an order of magnitude decrease in the predicted cutaneous flux. Similarly when the diffusion coefficient of polystyrene is measured in polymer solutions an exponential function is observed (16). It therefore seems reasonable to conclude that there should be a similar exponential relationship for NP diffusion across the stratum corneum.

Potts and Guy (17,18) also concluded that permeation should be related to an exponential function of molecular weight or molecular volume. They developed a simple relationship to predict permeability coefficients (k_p) across the skin for chemicals dissolved in aqueous solutions (17), using the octanol-water partition coefficient (K) and the molecular weight (M)

$$\log\left(k_p, \left(cm/s\right)\right) = -6.3 + 0.71\log(K) - 0.0061M \qquad (1)$$

Even though this relationship was developed from a limited data set in terms of molecular weight (18–765 Daltons), it is logical to extrapolate to consider NP penetration.

Using this approach, the data in Table I may be generated for spherical NPs of density 1 g/cm^3 and different diameters. It is assumed that the particles partition favourably into the skin with a log K value of 2, which is optimum for a molecule to partition across the heterogeneous structure of the skin and into the systemic circulation (19).

An alternative approach is to use the algorithm of Magnusson *et al.* (20). In this publication the authors relate the maximum flux (J_{max}, mol cm^{-2} h^{-1}) across the skin to molecular weight. For a dataset consisting of compounds ranging from 18 to 765 Daltons,

$$\log J_{max}\left(mol\,cm^{-2}h^{-1}\right) = -4.52 - 0.0141M \qquad (2)$$

From this relationship the data in Table II may be generated. It is easy to see that as the size of the NP increases the amount that can penetrate the skin decreases dramatically. Moreover, the two approaches for estimating the permeation rates concur with each other, which is expected since they were derived from similar data sets. It follows that if deposition in skin of intact NPs occurs then it must be by a mechanism other than diffusion, for example localisation of NP in the hair follicles; i.e. appendageal transport. Lademann *et al.* (21 22) reported penetration of NP into the human epidermis and attributed this finding to the particles being located in the hair shaft. It was also proposed that this occurred because of mechanical pumping as the hair follicle is moved backwards and forwards *in vivo* as a result of formulation being rubbed into the skin (21,22).

There are instances in which nanoparticles have been placed on normal human skin and their component atoms or molecules (Zn) have subsequently been detected in the blood (1,2). In the first study, two sunscreens were tested by these authors, containing either 19 nm particles or particles with dimensions >100 nm. In the more recent paper, NP of ~30 nm were incorporated in a sunblock and evaluated. The reported appearance of Zn in body fluids by these authors cannot be a result of direct transfer of the nanoparticle but must involve dissolution of the component molecules from the particle surface and their diffusion as discrete molecules across the stratum corneum.

Absorption from solid particles has previously been observed for model membranes and skin. Chemical permeation through non-porous silastic membranes from powders of 4-cyanophenol and methyl paraben was only a little smaller than from saturated aqueous solutions of the same chemicals even though these large particles (diameters of about 50 μm) did not penetrate the membrane (23). Penetration of these same chemical from neat powders has also been observed in human skin (24). Thus, appearance of elemental levels of chemical are, in no way, an indication of intact particle penetration. Although there may well be legitimate concerns about molecules contained in NP that can penetrate skin, the

Table I Predicted Permeability Coefficient Values (k_p) for NPs of Varying Dimensions Calculated from Eq. 1

Particles assumed to be spherical with a density of 1 g/cm^3 and log K of 2

Particle diameter (nm)	Particle volume (cm^3)	Molar volume (cm^3/mol)	Molar mass (g/mole)	log (k_p, (cm/s))	k_p(cm/s)
1	5.2×10^{-22}	315	315	−6.80	1.57E-07
2	4.2×10^{-21}	2,520	2,520	−20.3	5.38E-21
5	6.6×10^{-20}	39,400	39,400	−245	0

appearance of these component parts does not show that NP themselves have penetrated.

A separate issue to consider is the suggested penetration of vesicular or particular systems such as liposomes. Early work conducted by Mezei *et al.* (25) reported that such formulations could promote local penetration of actives such as steroids. The claimed enhancement was attributed to the penetration of the intact liposome ("…….the 'capsule' is able to penetrate and cross biological membranes and have some selectivity"). The manner in which the experiment was conducted does not support this statement. Subsequent to that publication it was reported that a particular type of liposome (Transferosome®) could penetrate skin intact because of its "high and self-optimizing deformability" (26). Substantive evidence for the permeation of such vesicles in intact form has not been reported to date.

There are a number of other reports in the literature which demonstrate that liposomes or solid lipid nanoparticles (SLN) give enhanced skin permeation of actives (27–29) compared with simple cream or ointment formulations. The calculations above show that this cannot be as a result of diffusion of the intact particle, and therefore other mechanisms must be inferred. One possibility is that the lipid constituents form an occlusive layer on the skin, which then becomes hydrated. It is well known that hydrated skin is generally more permeable than normal skin; hence, the use of occlusive ointments to improve delivery (30). However, there is another possibility. When a topical formulation is spread on the skin some of the constituents evaporate and some permeate. This can leave the active with a melting point above skin temperature to come out of solution as solid particles on the skin surface (31). Figure 1 shows a schematic of the skin surface. If a lipophilic active crystallises on the surface, those molecules in contact with the lipids of the intercellular channel (designated by solid green) will be able to permeate more easily than those in contact with the corneocytes (designated by red stripes), which must permeate across a corneocyte first. This will lead to poor overall permeation. The lower diagram shows that if a liposomal or SLN suspension is used, the lipids may coat the surface of the corneocytes and allow lateral diffusion of all molecules (coloured green) to the intercellular lipid channels. Enhanced permeation may be seen for these nanoparticulate structures even though they do not permeate as intact particles.

Table II Predicted J_{max} Values for NPs of Various Dimensions Calculated Using Eq. 2

Particle diameter (nm)	Molar mass (g/mole)	log (J_{max}/mol cm^{-2} h^{-1})	J_{max}/mol cm^{-2} h^{-1}	J_{max}/ng cm^{-2} h^{-1}
1	315	−9.0	1×10^{-9}	341
2	2,520	−40	8×10^{-41}	2×10^{-28}
5	39,400	−560	0	0

Particles assumed to be spherical with a density of 1 g/cm^3

It will be evident that the fraction of particles on the skin surface that are in contact with the intercellular lipids may be quite small which serves further to indicate the significant transport barrier faced by NP applied on the skin.

In summary, the calculations outlined here demonstrate that NP are too large to penetrate the healthy skin barrier by passive diffusion. Absorption of components of NP has also been considered but has not been shown to be a result of permeation of intact NP. Although NP formulations have been shown to promote dermal delivery of molecules there is no evidence that this is because of any dependence on permeation of intact NP themselves. The ability of NP to penetrate damaged or diseased human skin is outside the scope of the present commentary but will be the focus of a separate review. We hope that the theoretical analysis discussed here receives the wider discussion that is necessary for a rational and informed debate on the implications of nanotechnology for human health.

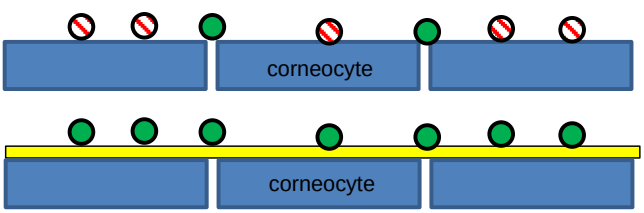

Fig. 1 A schematic of a cross-sectional view of the skin surface showing corneocytes in blue. The upper diagram shows that only molecules from the green shaded particles can diffuse through the intercellular lipids. In contrast, in the lower diagram a film of lipid coats the corneocytes and allows lateral diffusion of molecules from all particles.

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