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Skin penetration of silica microparticles

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Knowledge about skin penetration of nano- and microparticles is essential for the development of particle-core drug delivery systems and toxicology. A large number of studies have been devoted to metallic particle penetration. However, little work has been published about the importance of chemical material properties of the particles and the skin penetration effect of the applied formulation. Here, we investigated the penetration of 3 μm silica particles in water and in a 65% ethanolic plant extract on *ex vivo* human skin using scanning electron microscopy. Contrary to most other microsphere skin studies, we observed for the first time that 3 μm silica particles can penetrate the living epidermis. Moreover, when formulated in the ethanolic medium, particles even reach the dermis. The deviating chemical properties of silica compared to previously investigated microparticles (titanium dioxide, zinc oxide) and confounding effect of the formulation in which the silica microparticles are presented, is thus demonstrated.

For the last two decades, skin penetration of nano- and micron-sized metallic particles has been a growing investigation topic, both desired (targeting) as undesired in toxicology. The skin-penetrating properties of nano- and submicron TiO_2 and ZnO particles were intensively investigated (Sadrieh et al. 2010). While some studies demonstrate that microfine TiO_2 particles penetrate the epidermis and dermis, others claim that TiO_2 and ZnO particles remain only in the outermost layer of the stratum corneum (SC) or tend to aggregate into the follicle orifices (Sadrieh et al. 2010; Tan et al. 1996; Toll et al. 2004). Moreover, different parameters (*e.g.* dispersing medium) should be considered (Baroli 2009). Only some fragmentary studies mention that oily suspensions of TiO_2 particles showed increased penetration compared to aqueous suspensions or that the chemical enhancers oleic acid and ethanol enhance the transdermal delivery of ZnO nanoparticles (Landsdown and Taylor 1997; Kuo et al. 2009). Next to TiO_2 and ZnO, other particles were much less investigated, although the importance of chemical material properties has already been highlighted (Baroli 2009). Beryllium particles up to 25 μm were found in the femoral and inguinal lymph nodes of individuals walked bare foot on particles containing soil (Tinkle et al. 2003).

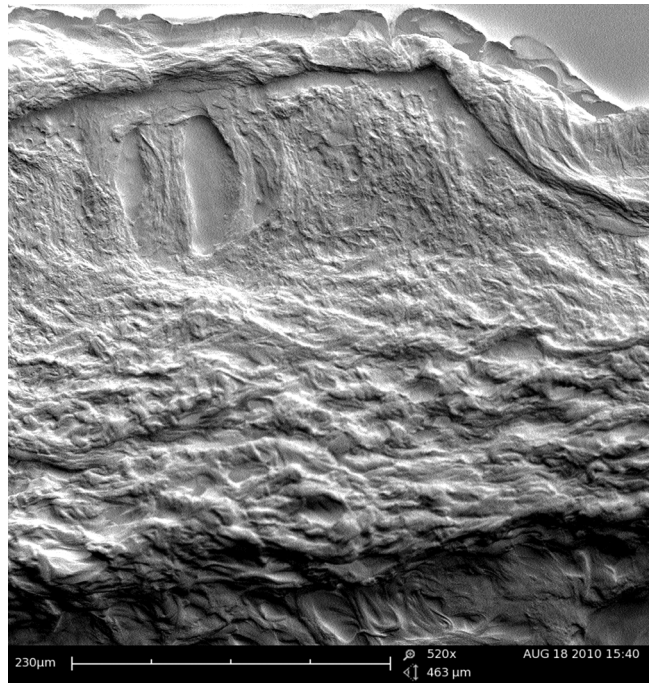

Fig. 1: SEM images of skin control

SiO_2 is an ubiquitous material in plants due to the biomineralisation proces. In function of the plant anatomy, amorphous silica is present as particles with distinct different sizes: microparticles (diameter between 3–7 μm), submicron particles (200 nm–1 μm) and nanoparticles (25–40 nm) (Holzhuter et al. 2003). These plants and their processed materials, like extracts, are often used in topical preparations. As they contain variable quantities of differently sized SiO_2 particles in varying matrices, skin exposure arises toxicity concerns. One of the critical parameters to the skin penetration is the isoelectric point (IEP). Compared to other investigated metal oxide particles (*i.e.* IEP_{TiO2} 5.1–5.4, IEP_{ZnO} 8.1–10.3, IEP_{Fe2O3} 7.6–9.1 and IEP_{MgO} 9.2–11.8), this value is much lower for silica (IEP_{SiO2} 2.3–3.8) (Kosmulski 2009). Indeed, after 6 h exposure, penetration of an aqueous SiO_2 nanoparticle dispersion into the viable epidermis and dermis has been reported (Eskandar et al. 2010). In contrast, Graf et al. (2009) showed that sub-micron silica-coated nanoparticles in PBS spread superficially on the SC or in the hair follicles, without deeper penetration.

In this study, we evaluated the penetration of 3 μm silica particles in water and a 65% ethanolic plant extract on *ex vivo* human skin by means of scanning electron microscopy (SEM).

SEM images reveal no 3 μm particles in any of the control coupes (Fig. 1), indicating neither inherent 3 μm skin particles, nor contamination due to residual particles of previously microtomed skin samples. Moreover, no particles were seen under the dermis in the freezing medium, which confirms the validity of our rinsing method.

Fig. 2 shows SEM images of skin exposed to 3 μm SiO_2 particles in (a) water and (b) the plant extract. Particle localization (SC, epidermis and dermis) was confirmed with light microscopy. For SiO_2 particles in water, the epidermal-dermal junction was the ultimate barrier: no micro-sized particles were found in the dermis. However, SiO_2 microparticles formulated in the ethanolic plant extract penetrate not only in the living epidermal layers, but even into the dermis. This deeper penetration of 3 μm particles applied in the plant extract can be credit to its 65% ethanol content. Ethanol in skin formulation functions as penetration enhancer for sub-micron ZnO nanoparticles (Kuo et al. 2009; Lo et al. 2010). During our studies, we confirmed these findings with

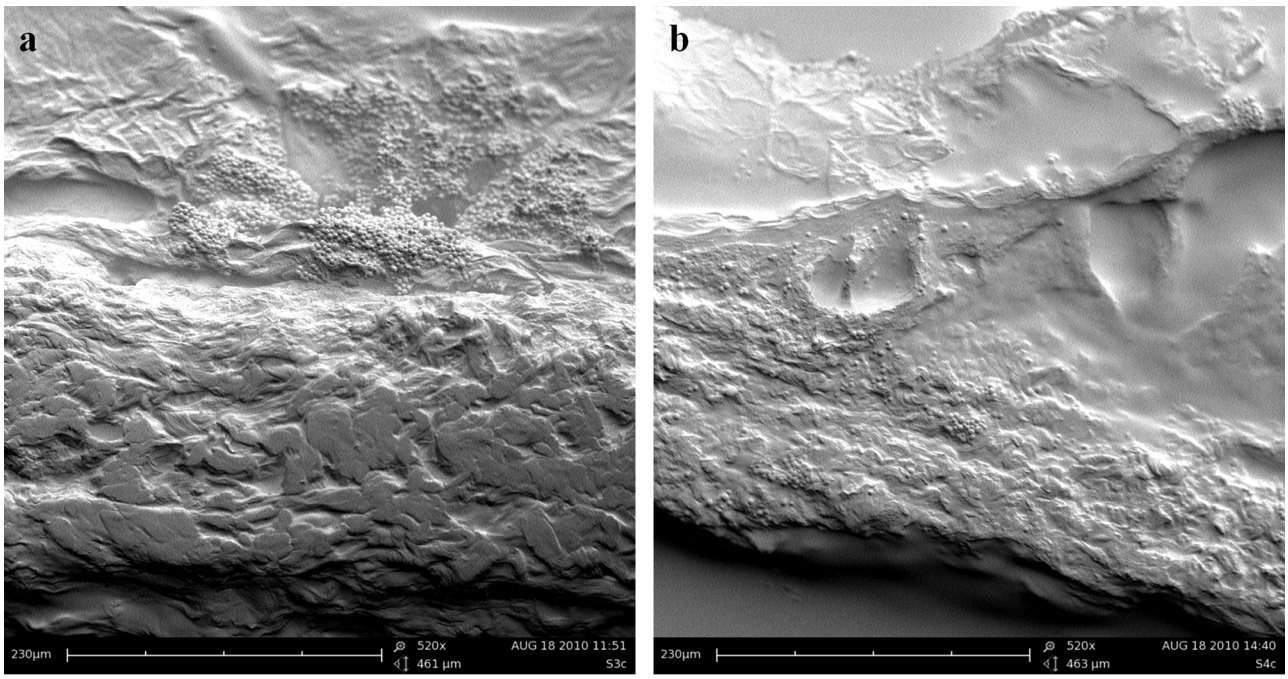

Fig. 2: SEM images of skin exposed to 3 µm microparticles in (a) water and in (b) ethanolic *Spilanthes* extract

300 nm SiO_2 particles (data not shown): in water, penetration was also observed, which increased significantly in the ethanolic medium. Striking is that 3 µm particles also penetrate into the living epidermal layers. It needs to be emphasized that the deviating chemical properties of SiO_2 micron-sized materials might attribute to these observations, as previous researchers generally believed that skin penetration of micro-sized particles was unlikely to happen. Our findings also call for an additional quality attribute for topical plant preparations, *i.e.* particles, which up till now is not included in the quality specifications.

The skin penetration of silica particles in water and in a 65% ethanolic *Spilanthes* extract was characterized by means of SEM. Unlike most other micro-sized particle skin studies, we demonstrated for the first time that 3 µm SiO_2 particles can penetrate into the skin from the SC, over the living epidermal layers, with even some silica deposits in the dermis when formulated in the ethanolic-based plant extract, but not when dispersed in water. A possible explanation can be the deviating chemical properties of SiO_2 compared to the previously investigated microparticles (TiO_2, ZnO). The confounding effects of chemical nature, size and formulation of SiO_2 microparticles on skin penetration is thus demonstrated. Depending on the toxicological qualifications and functionality, our findings call for an additional quality specification, *i.e.* silica particle characterisation, for topically used plant extracts.

Experimental

A 500 µl solution containing 0.1% (w/v) 3 µm silica particles (Micromod, Rostock-Warnemuende, Germany) in water (Sartorius, Göttingen, Germany) and in an ethanolic *Spilanthes acmella* extract (Biohorma, Alken, Belgium) was applied on dermatomed human skin (415 ± 10 µm (mean ± SE) thickness) in a Franz diffusion cell set-up (Logan Instruments Corp., New Jersey) at 32 °C, with 0.01 M phosphate buffered saline (pH 7.4) (Sigma, Bornem, Belgium) as receptor fluid. Skin integrity was measured using a LCR Impedance Bridge (Tinsley, Croydon, U.K.). After 24 h, the solutions were removed. Subsequently, the exposed skin part was microtomed from the dermis upwards the epidermis. After each slice, the microtome knife was cleaned with ethanol (Chem-Lab, Zedelgem, Belgium). To exclude contamination, a control was sectioned between each sample. The samples were gold coated and subjected to SEM (Phenom-World, Eindhoven, The Netherlands). These experiments were repeated in triplicate, using different skin donors.

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