

NANODERM

Quality of Skin as a Barrier to ultra-fine Particles

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Final Report - Summary

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Summary

Standard methods in dermal penetration research are tape stripping of the stratum corneum and static Franz-diffusion cells on excised skin. Both methods do not visualize penetration pathways and both techniques can over- and underestimate actual penetration. It is unclear to what extent these techniques can be applied to the study of dermal penetration of nanoparticles, e.g. TiO_2 with dimensions in the range of 20 nm which is widely used in sunscreens with physical UV-filters. On the contrary, high resolution transmission electron microscopy (HRTEM) on ultra thin skin cross-sections visualizes individual nanoparticles but suffers from a very limited field of view. There are several preparation steps involved in preparing ultra thin skin cross-sections with the possibility of preparation artefacts. Thus, there are controversial reports on HRTEM studies of dermal penetration of nanoparticles and novel microscopic techniques are required in order to clarify the situation. A relatively new and promising application is confocal laser scanning microscopy which, however, requires a fluorescent label with the associated problem of the stability of the label.

The **objective of the NANODERM project** was to complement **HRTEM** studies with another technique, namely **ion beam analysis** (PIXE: Particle Induced X-Ray Emission; RBS: Rutherford Backscattering Spectrometry; STIM: Scanning Transmission Ion Microscopy), in order to **visualize putative pathways of nanoparticles** in skin cross-sections.

The advantage of these techniques is that very few sample preparation steps are required and thus the risk for preparation artefacts is reduced. A further advantage is that preparation artefacts, should they occur, can be easily identified, contrary to HRTEM. Moreover, larger samples can be analyzed in order to get an overview over large areas; subsequently one can zoom into a region of interest. A disadvantage is that individual nanoparticles cannot be visualized and the measurements are time-consuming. A further disadvantage is that the sample integrity can suffer if too high currents are used for PIXE; therefore the samples have to be analyzed by STIM, a technique which uses currents of about 0.1 fA before and after the PIXE measurement to ensure sample integrity.

A third technique was applied which has not been used thus far for dermal penetration studies: **autoradiography** using skin cross-sections and nuclear microemulsions. For these studies we used TiO_2 and the radiolabel 48-V. This methods has extreme sensitivity.

Biopsies from **porcine skin** and **healthy human skin** from volunteers (male and female, coloured and caucasian, different ages), as well as from **human foreskin transplanted to SCID-mice** were studied. In addition, healthy human **skin ex-**

plants from surgery was used for autoradiography. Biopsies from patients suffering from **psoriasis** were included in the study.

Several pre-treatments of the skin were applied: cleaning with ethanol, excess water exposure, partial tape stripping. **Various dermatological formulations** (mainly carbomergel, polyacrylategel, hydrophobic basisgel, isopropylmyristategel, microemulsion) containing TiO₂ nanoparticles (mainly Eusolex T-2000) and the **commercially available products** Eucerin Micropigment Crème 15 (Beiersdorf), Eucerin Micropigment Lotion 25 (Beiersdorf), Avène 50 (Pierre Fabre), and Anthelios XP SPF60 (Roche Posay) were applied topically to the skin with about 2 mg/cm² under non-occlusive, semi-occlusive, and occlusive conditions. Exposure times varied from 30 minutes to 48 hours.

In all but a few cases Ti was detected **on top of the stratum corneum and in the topmost layers of the *stratum corneum disjunctum*** for healthy skin. Frequently the nanoparticles were aggregated. In most cases Ti-spots in vital tissue could be identified as preparation artefacts. **In none of the roughly 500 images a coherent pathway of nanoparticles was observed, let alone a concentration profile characteristic of diffusive transport.** Hence, we conclude that the TiO₂ nanoparticles are penetrated into the topmost 3-5 corneocyte layers by mechanical action and no diffusive transport takes place. Thus, penetration studies with static Franz-diffusion cells do not seem adequate for nanoparticles. Clearance is expected to proceed via desquamation.

There is **deep penetration into hair follicles, but not into vital tissue.** Clearance is expected to proceed via sebum excretion.

No new species were detected by static Secondary Ion Mass Spectrometry (S-SIMS) and Laser Modulated Mass Spectrometry (LMMS) due to the interaction of the formulations with coated TiO₂ nanoparticles without and with UV-light.

The **interaction of cells** with TiO₂ nanoparticles both coated and uncoated was studied both *in-vitro* and *in-vivo* by **immuno-histochemical methods** and **Atomic Force Microscopy**. The cellular response to TiO₂ nanoparticles was found to depend on the cell type; various endpoints were examined. The elasticity of cells was affected by uncoated TiO₂ nanoparticles and UV-light. Radical scavengers suppressed the change in elasticity. The relevance of these observations on the cellular level is still an open question because the exposure is rather low, if it exists at all. Nevertheless, we conclude that for the sake of safety, direct contact of skin cells with TiO₂ nanoparticles should better be avoided, e.g. application of sunscreens into open wounds is not recommended.

The situation with **psoriatic skin** is less clear. Instead of a *stratum corneum* of about 10 – 15 µm thickness, psoriatic skin has a *stratum corneum* of about 100 µm thickness with corneocytes and vital keratinocytes intermingled. Here, there is no real barrier and TiO₂ nanoparticles can come into direct contact with vital cells. However, we have no evidence that the TiO₂ nanoparticles become systemic.

Summing up, we do not expect any health effects for the topical application of sunscreens containing TiO₂ nanoparticles (especially when coated) on healthy skin which are related to the particulate state.

The **life cycle** of TiO₂ nanoparticles was not examined in the present project and **ecotoxicological aspects** as well as possible subsequent absorption via other ports of entry should be considered in the future.

There are still a few open questions concerning e.g. sunburned skin with skin detachments - a typical misuse of sunscreens - or **atopic skin**. Furthermore, the role of **microlesions** is unclear.

Maybe the largest uncertainty is related to the fact that two recent publications demonstrated that mechanical flexion of skin can greatly enhance the penetration.

A standardized apparatus for mechanical flexion should be developed.

Finally, each method has detection limits and long-term exposure might still lead to appreciable absorption. Thus the **biokinetics**, possible translocation and accumulation into secondary organs, and the excretion should be investigated.

Last but not least, although TiO₂ nanoparticles of much smaller dimensions than 20 nm, say e.g. 2 nm, are not in use in sunscreens, it is conceivable that they might nevertheless be present and adsorbed onto larger particles, a phenomenon well known in aerosol studies. Whether they can be isolated and whether they are more soluble in body fluids than their larger counterparts remains to be investigated.