

Conference abstract LNT03

## **Does Continuous Exposure to ZnO and TiO<sub>2</sub> NPs Cause any Changes in Keratinocytes?**

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Sci Pharm. 2010; 78: 577

doi:10.3797/scipharm.cespt.8.LNT03

Many current dermatological preparations, sunscreens and other cosmetics, contain nanosized particles (NPs), which were observed to be rapidly internalized by keratinocytes [1]. Besides their ability to enter into cells, NPs can be responsible for higher toxicity compared to bigger particles, mainly due to their larger surface area and enhanced chemical reactivity. Application of NPs increase the risk of cell damage and consequently cause alterations in mitochondria, actin filaments, cell membranes, etc., usually resulting in diminished cell functions or even cell death [2]. Despite the fact that ZnO and/or TiO<sub>2</sub> NPs are frequently used, only few long-term toxicological studies are available. Our research was focused on long-term investigations, where the effects of repeated application of ZnO and TiO<sub>2</sub> NPs on keratinocytes were evaluated. The preliminary short-term experiments showed no adverse effects of ZnO and TiO<sub>2</sub> NPs on viability and morphology of keratinocytes in concentrations up to 15 µg/ml. Furthermore, TiO<sub>2</sub> NPs did not significantly affect cell viability as well as cell growth in concentrations up to 100 µg/ml. Oppositely, ZnO NPs decreased cell viability in concentrations above 20 µg/ml sharply, and caused detachment of keratinocytes. Thus, the keratinocytes can survive short-term exposure to low concentrations of ZnO and TiO<sub>2</sub> NPs, however, question regarding cell ability to maintain essential functions during their life span arises. Therefore, the concentration that did not show any cytotoxicity in short-term experiments was used for long-term investigations. The results after 3 months continuous treatment with 10 µg/ml NPs showed no significant changes in generation of reactive oxygen species (ROS) compared to untreated cells. Microscopical observation of cells continuously exposed to TiO<sub>2</sub> indicated unchanged cell surface and presence of endosomes filled with TiO<sub>2</sub> NPs, which were enlarged compared to endosomes of untreated control cells. Contrary, exposure to ZnO caused significant decrease in cell number as well as appearance of dense nuclei and disappearance of visible actin filaments, elevated ROS levels and decreased mitochondrial activity, indicating oxidative stress as a main cause for cytotoxicity of ZnO NPs. To sum up, the results indicate that the right selection of NPs is crucial for formulation of safe dermal products.

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