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Cell response of the human keratinocytes cell line HaCaT to differently produced silver nanoparticles

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New antimicrobial agents based on silver are investigated and improve the treatment of topical bacterial infections. Silver nanoparticles (AgNP) represent a promising achievement of nanotechnology in health care and medicine. Over the last years, AgNP have been employed in several products as for example in antimicrobial gels and wound dressings. First investigations of AgNP showed an increased antibacterial potential caused by a slower but continuous release of silver ions (Ag+). On the other hand, toxic effects due to the AgNP themselves cannot be excluded. However, it has been shown that silver can affect bacterial as well as eukaryotic cells. It could increase the reactive oxygen species (ROS) production which leads to oxidative stress.

The present study investigates influences of AgNP on a human keratinocyte cell line (HaCaT) by means of transmission electron microscopy (TEM), a cell viability assay (XTT) and electron paramagnetic resonance (EPR) spectroscopy. The release of Ag+ ions from AgNP is due to an oxidative reaction on the particle surface and it can be reduced by producing and storing particles under inert atmosphere, e.g. argon (Ar).Thus, to distinguish between particle and ion induced effects, two systems were investigated. One batch of AgNP produced and stored under air (O2) was compared to particles produced under Ar. We have shown the intracellular uptake of AgNP by HaCaT cells using TEM. It could be demonstrated that the particles accumulated in vesicles and did not enter the nuclei. The incubation with silver led to morphological changes of the nuclei and the cell membrane, which indicates cell death. These results could also be confirmed by the XTT assay. We found a silver concentration dependent decrease in the cell viability. To find out if the detected toxicity relates to an increased ROS production, intracellular radicals were measured. Higher ROS levels were detected in cells incubated with AgNP. Moreover, it was also possible to measure significant differences between the two particle types. To our knowledge, EPR spectroscopy was used for the first time to show ROS production induced by AgNP in HaCaT cells.

In conclusion, AgNP made and stored under air induced higher amounts of ROS compared to

the AgNP stored under Ar. This suggests that Ag+ ions released during particle storage are responsible for most of AgNP related cytotoxic effects. Nevertheless, the reduction in cell activity found for AgNP (Ar) suggests that effects caused by the nanoparticles themselves and ions released after particle uptake might also exist. The results highlight the complexity of silver toxicity towards skin cells, which need to be thoroughly evaluated for safe use of these nanomaterials in dermatology and other medical areas.