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Ultrastructural view on cellular uptake of metal oxide nanoparticles

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Nanoparticles (NPs) can efficiently enter in living cells with different mechanisms because they have a similar size to the main cellular components. A detailed understanding of the molecular processes involved in the cellular uptake is very important to develop efficient NPs in drug delivery for conveying chemotherapeutic only in specific cells. However, is not to be underestimated the importance of knowing the potential harmful effects due to an incorrect penetration into live normal cells. However the uptake of NPs is not still clear and sufficiently characterized. Besides few data are available on the relationship between NPs internalisation and their physico-chemical properties. Recent works have shown that depending on the cell histotype, the uptake occurs either by passive diffusion or energy-dependent endocytosis mediated through clathrin pits, caveolae or macropinocytosis.

In our work we have studied the uptake of two different types of metal nanoparticles of zinc oxide, ZnO and titanium dioxide, TiO_2 on colon cancer cell lines (LoVo WT and HT29). ZnO and TiO_2 nanoparticles are widely applied in personal care products, paints, textiles foods and food packaging. Recent studies showed that these NPs are versatile plataforms for biomedical applications in cancer diagnosis and therapy. NPs were characterized by analytical electron microscopy and primary size, size distribution and chemical composition were determined. The primary size ranged between 45-170 nm and 20-60 nm for ZnO and TiO_2 respectively. The size distributions of NPs showed an average diameter equal to 196 nm for ZnO and 289 nm for TiO_2. 34% of ZnO NPs possessed dimensions below 100 nm, whereas for TiO_2 NPs large agglomerates were observed and only 11% of particles possessed sizes below 100 nm.

LoVo WT cells treated with ZnO NP (5 $\mu g/cm^2$ for 48h) and observed by SEM showed huge ultrastructural alterations at the plasma membrane level (Figure 1e) compared to the treated cells for 24h (Figure 1c) and control untreated cells (Figure 1a). These morphological alterations (holes in the cell membrane indicated by the arrows) should correspond to the presence of ZnO NPs inside the cell cytoplasm, instead no NPs were found by TEM inside the cell cytoplasm (Figure1b, control cell, Figure 1d, ZnO NP 5$\mu g/cm^2$ for 24h, Figure 1f, ZnO NP 5$\mu g/cm^2$ for 48h). TEM observation showed evident alterations at mitochondrial level suggesting an NP-cell interaction mediated by ion release. This evidence has led to hypothesize that only the zinc ions release may determine the observed cytotoxic effect.

The other colon carcinoma cell line, HT29, treated with TiO_2 (2.5 $\mu g/cm^2$ for 24h), showed the presence of metallic nanoparticles leaning to the cell membrane, wrapped by cell protrusion (Figure 2b, arrow). In the cytoplasm of HT29 cells, TiO_2 NPs were free in the cytoplasm, not surrounded by vacuoles. TiO_2 NPs were well visible in some altered mitochondria demonstrating a direct cytotoxic organelle damage (Figure 2a). These results suggest that nanoparticles may widely permeate or to only release the ionic component through the cell in a variety of biological mechanisms.

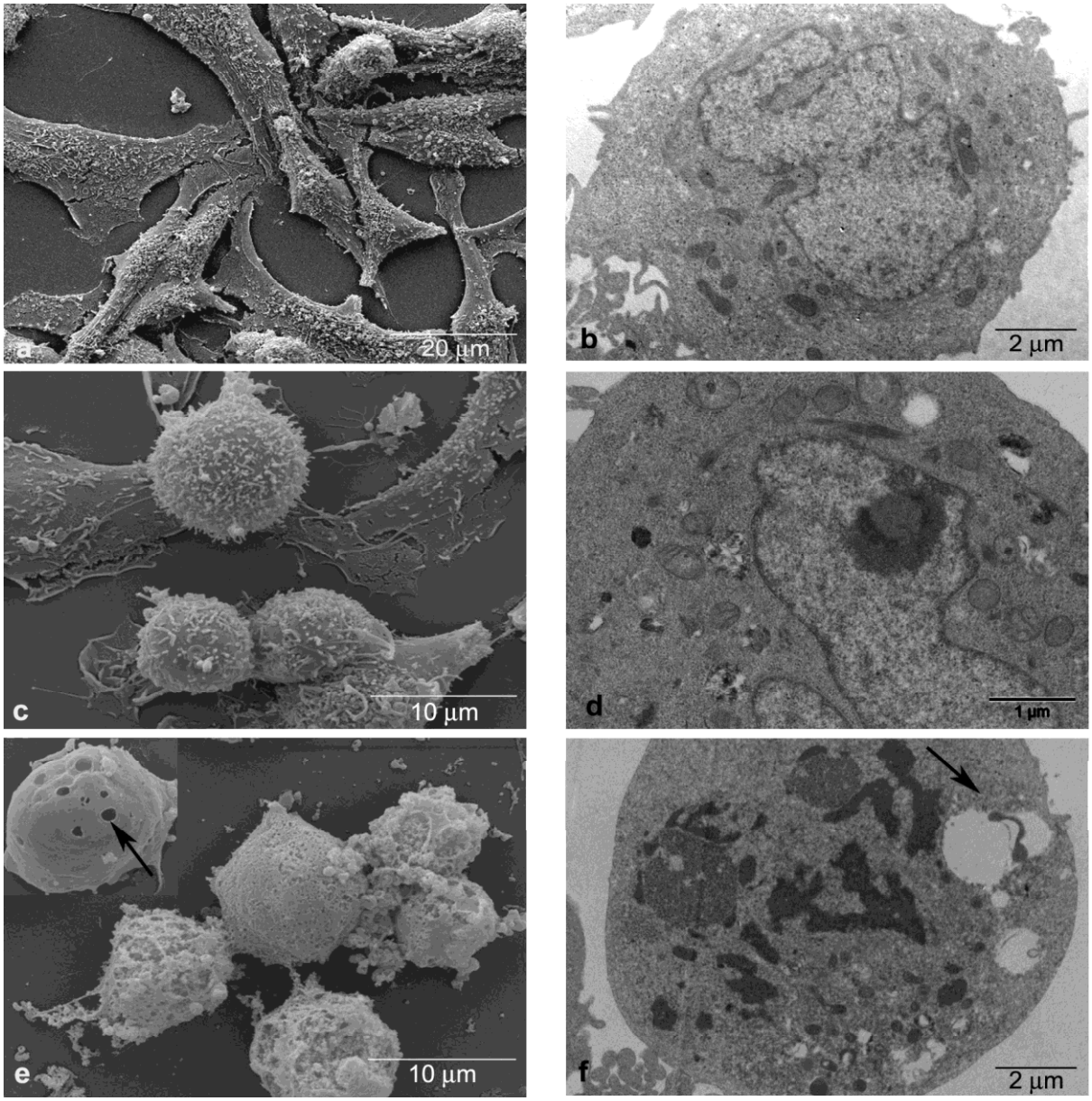

Figure 1. Morphological and ultrastructural modifications induced by ZnO on LoVo WT cells.

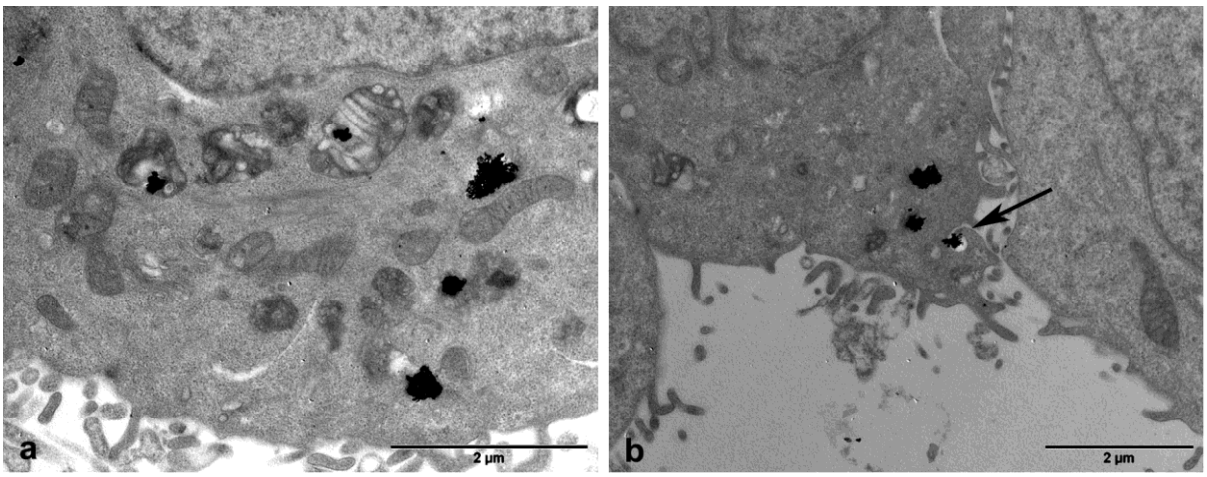

Figure 2. Ultrastructural modifications induced by TiO_2 on HT29 cells.