Investigating the influence of nanomaterials metal composite ratios on the toxicity and intracellular uptake in epithelial cell lines

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1. Introduction

The ratio of metals in nano-composite materials significantly influences their efficacy in intended applications as nanoparticle systems. Two such composites, Cobalt-Iron Oxide ($Co_xFe_xO_4$) and Cerium-Zirconium Oxide ($Ce_xZr_xO_2$), have gained attention for their unique properties. Cobalt-Iron Oxide nanomaterials (NMs) are highly attractive due to their moderate magnetization, high coercivity, chemical and thermal stability, catalytic potential, and applications in biomedicine and sensors. In contrast, one of the main properties of Cerium-Zirconium Oxide nanomaterials is their oxygen storage capacity, making them effective environmental catalysts for pollutant removal. They also exhibit high activity, selectivity, and stability, being crucial materials in three-way catalysts.

2. Problem Statement

While intentional modifications to nanomaterials may enhance their function, it may also inadvertently influence their toxicity and interactions with cells. This study investigated the toxicity and intracellular uptake of Metal Oxide NMs: Cobalt (II, III) Oxide (Co_3O_4), Iron Oxide (Fe_3O_4), Cerium (IV) Dioxide (CeO_2), and Zirconium (IV) Dioxide (ZrO_2) as well as nanocomposites Cobalt-Iron oxide ($Co_xFe_xO_4$) and Cerium-Zirconium oxide ($Ce_xZr_xO_2$), with varying metal ratios.

3. Methods

Human bronchial epithelial (BEAS-2B) and human alveolar basal epithelial (A549) cells were exposed to the different NMs, with toxicity assessed using xCELLigence RTCA impedance-based technology system. The BEAS-2B and A549 cells were seeded in 96-well E-plates in 100 µl dispersion medium (i.e., cell culture medium). The cells were incubated for 24 h to allow the cells to adhere and proliferate before treatment. The medium was then replaced with fresh dispersion medium containing NMs at concentrations of 1, 10, 25, 50 and 100 µg/cm^2, with control cells receiving dispersion

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medium only. Data points collected every 15 minutes for 24 hours and every 30 minutes for an additional 24 hours. Data was normalized at the point of treatment.

The intracellular uptake was investigated using the CytoViva dark-field hyperspectral imaging system. Microscope slides were prepared as follows: BEAS-2B and A549 cells were seeded on 8-well EZ-slides in 500 µl dispersion medium. The cells were incubated for 24 h to allow the cells to adhere and proliferate before treatment. The medium was then replaced with fresh dispersion medium containing NMs at sub-lethal concentrations (as determined from the xCELLigence RTCA viability studies) for treatment periods of 12 and 24 h. Control cells received dispersion medium only.

4. Results and Discussion

Results indicated that Metal Oxide NMs increased cytotoxicity with increased concentration, except for Cerium (IV) Dioxide in both cell lines. A549 cells showed greater sensitivity, although interestingly, BEAS-2B cells was more sensitive with Iron Oxide NM in, however recovery of the cell index was seen in the latter part of the experiment. The Cobalt-Iron Oxide composite NMs displayed increased toxicity over the concentration range, with the $Co_{1.5}Fe_{1.5}O_4$ only displaying toxicity at 100 µg/cm^2 in the BEAS-2B cells and from 25 µg/cm^2 in the A549 cells. The Cerium-Zirconium Oxide composite NMs showed relatively little to no toxicity in the BEAS-2B cells, whereas with the A549 cells an increased sensitivity was seen. The increased toxicity could be attributed to the increase in Zirconium ratio in the nanocomposite.

When assessing the intracellular uptake of the Metal Oxide NMs, internalization of the Cobalt Oxide, Iron Oxide and Cerium Dioxide was observed in both cell lines, while Zirconium (IV) Dioxide showed a lack thereof. The Cobalt-Iron Oxide composite NMs were internalized in both BEAS-2B and A549 cell lines and in all cases, internalization of particles appeared to increase from 12 h to 24 h. On the contrary, the only Cerium-Zirconium composite NMs that showed internalization was the Cerium Zirconium Oxide ($Ce_{0.9}Zr_{0.1}O_2$), which interestingly has the lowest Zirconium ratio.

5. Conclusions

In conclusion, Metal Oxide NMs can induce toxicity and when combined to form nanocomposites can decrease the observed toxicity, although this appears to be cell line specific. The effects of metal composite ratio, particularly with Zirconium-based NMs, demonstrate how variations can influence cell response and internalization. Metal composite ratio may therefore be another factor to consider when implementing safe-by-design principles at the synthesis and manufacturing stages of NMs.

6. References

Biabani-Ravandi, A., Rezaei, M. and Fattah, Z., 2013. Study of Fe–Co mixed metal oxide nanoparticles in the catalytic low-temperature CO oxidation. Process Safety and Environmental Protection, 91(6), pp.489-494.

Campos, P.T.A., Lima, J.P.V., de Queiroz Silva, D.R., Oliveira, C.F., Dias, S.C.L. and Dias, J.A., 2020. Cerium–zirconium mixed oxide nanostructures for diesel soot oxidation: synthesis and effect of structure. RSC advances, 10(46), pp.27428-27438.

Dippong, T., Levei, E.A., Borodi, G., Goga, F. and Barbu Tudoran, L., 2015. Influence of Co/Fe ratio on the oxide phases in nanoparticles of Co x Fe 3− x O 4. Journal of Thermal Analysis and Calorimetry, 119, pp.1001-1009.

Fantauzzi, M., Secci, F., Angotzi, M.S., Passiu, C., Cannas, C. and Rossi, A., 2019. Nanostructured spinel cobalt ferrites: Fe and Co chemical state, cation distribution and size effects by X-ray photoelectron spectroscopy. RSC advances, 9(33), pp.19171-19179.

Issa, G., Kormunda, M., Tumurbaatar, O., Szegedi, Á., Kovacheva, D., Karashanova, D. and Popova, M., 2023. Impact of Ce/Zr ratio in the nanostructured ceria and zirconia composites on the selective CO2 adsorption. Nanomaterials, 13(17), p.2428.

Lin, S. and Zhang, T., 2024. Preparation of various Co/Cu and Co/Fe molar ratios and gel-modified nanomaterials as anodes for Li-ion batteries. Materials Chemistry and Physics, 311, p.128547.

Wang, Q., Du, F., Hou, Y., Zhang, Y., Cui, M. and Zhang, Y., 2021. Preparation of a CeO2–ZrO2 based nano-composite with enhanced thermal stability by a novel chelating precipitation method. Ceramics International, 47(23), pp.33057-33063.

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