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Study of the toxicity and the antimicrobial activity of different forms of ZnO nanoparticles: ZnO nanoparticles linked to graphene, pristine ZnO nanoparticles and ZnO nanoparticles doped with Mn

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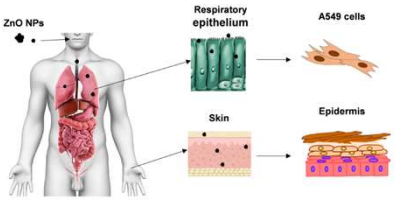
Introduction

Zinc oxide nanoparticles (**ZnO NPs**) present multiple applications due to their special physico-chemical and antimicrobial properties. Moreover, they are inexpensive and less cytotoxic than other metal nanoparticles. The increasing use of the ZnO NPs in different sectors has raised concerns about their potential risks for the workers, consumers and the environment.

The posible toxic effects of different zinc oxide nanomaterials (**G-ZnO**, **ZnO** and **ZnO:Mn**) on the respiratory system and their irritant potential were evaluated.



The antimicrobial activity of **G-ZnO**, **ZnO** and **ZnO:Mn** was analysed against Gram positive (methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecium*) and Gram negative (*A. baumannii* and *P. aeruginosa*) bacteria.

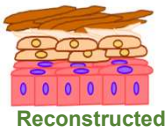
Materials and Methods

Materials

- Graphene-zinc oxide nanomaterial (**G-ZnO**), **ZnO** and **ZnO:Mn NPs**

Biological models

A549 cells



Reconstructed human epidermis (RhE)

Gram positive(**methicillin-resistant *S. aureus*** and **vancomycin-resistant *E. faecium***) and Gram negative (***A. baumannii*** and ***P. aeruginosa***) bacteria

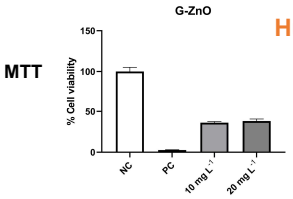
Assays

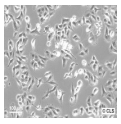
The effect of different concentrations of **G-ZnO NM** on the A549 cell viability was evaluted by the MTT and Neutral Red (NR) assays, as well as the production of reactive oxigen species (ROS). On the other hand, the potential irritant of **ZnO** and **ZnO:Mn NPs** was determined using a RhE model.

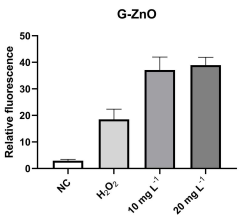
Moreover, the antimicrobial properties of the different **ZnO NPs** were studied in Gram positive and Gram negative bacteria determining the minimun inhibitory concentration (MIC) following the CLSI guidelines.

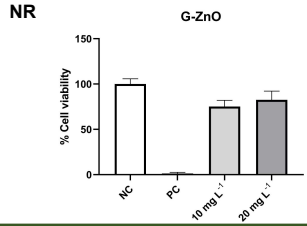
Results

Human cells

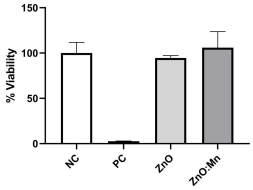








RhE model



Minimum inhibitory concentration (μg mL^{-1})

	S. aureus	*E. faecium*	*A. baumannii*	*P. aeruginosa*
G-ZnO	> 256	> 256	> 256	> 256
ZnO	> 256	> 256	> 256	> 256
ZnO:Mn	> 256	> 256	> 256	> 256

Conclusions

- The results obtained in the MTT assay showed that the **G-ZnO NM** reduced the viability of A549 cells after 24 h of exposure, while this decrease was less marked in those obtained in the NR assay.
- **G-ZnO NM** caused an increase in the production of ROS in the studied exposure conditions.
- According to the EU and GHS classification (Category 2 or Category 1), **ZnO** and **ZnO:Mn NPs** can be considered as non-irritant in the conditions tested (mean relative tissue viability of three individual tissues exposed to the test substance is above 50% of the mean viability of the negative controls).
- None of the different materials showed antimicrobial properties in the selected conditions, being their MICs > 256 μg mL^{-1}.

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