Light-emitting particles are biocompatible and safe for human cells of the respiratory

Partners involved

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Objectives

We evaluated the safety, in terms of cell biocompatibility, of two light-emitting particles (LEPs) formulations: 1) zirconia oxide-based particles doped with 0.4% titanium and coated on surface with glutamic acid (ZrO2:0.4%Ti@GA; LEP_GA); 2) zirconia oxide-based particles doped with 0.4% titanium and coated on surface with Polyethylene glycol (ZrO2:0.4%Ti@PEG; LEP_PEG) towards human cells of respiratory tract.

The cell lines used as in vitro models of the respiratory epithelium were: Calu-3 (lung adenocarcinoma), HBEC3-KT (normal immortalized bronchial), used as models of trachea and bronchi epithelium), NCI-H441 (lung adenocarcinoma), used as model of bronchiolar epithelium, A549 (lung adenocarcinoma) with alveolar type II characteristics and hAELVi immortalized alveolar type I, used as models of alveoli.

We verified i) the viability of cells incubated for short and long periods with LEPs; ii) the cellular uptake of the two LEP formulations; ii) if LEP formulations are effectively internalised by prone to be phagocyted once in the lung since the eventual clearance of LEPs by macrophages resident in the lung is of outmost importance after bacteria photosensitization to avoid persistent retention of particles in the treated tissues; iv) the genotoxic potential of LEPs once internalized by human cells of respiratory tract.

All these analyses are essential to ensure LEPs suitability for clinical implementation.

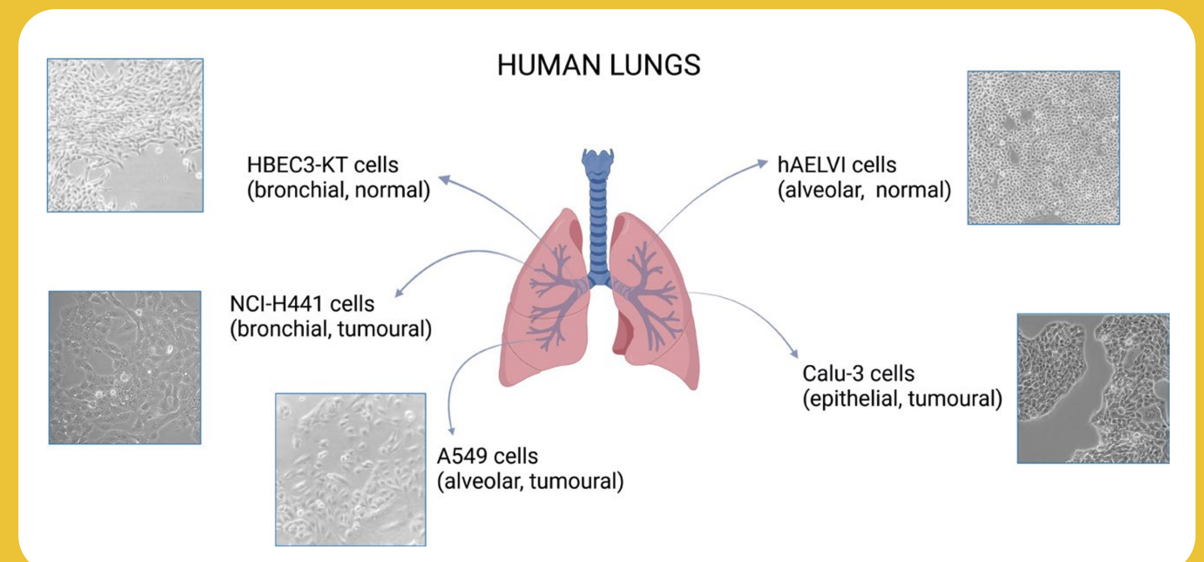

Figure 1
Human cells of the respiratory tract analysed in this study to assess the biocompatibility/toxicity of LEPs. Created with BioRender.com.

Methodology

The biocompatibility of both LEP_GA and LEP_PEG formulations was investigated by assessing cell viability of the selected cell lines of the respiratory tract (Calu-3, NCI-H441, A549, HBEC3-KT, hAELVi) exposed to increasing concentrations of LEPs (in the range 0.1-1000 µg/mL) for short and long periods of time (24 and 72h). Cells exposed to LEPs were then analysed for viability using different assays to obtain a complete assessment of the biocompatibility of the two LEP formulations and to exclude possible cellular damage following exposure to high LEP concentration. In one assay, the cells were stained with Calcein AM (green, live cells) and EthD-1 (red, dead cells) to obtain the fluorescence images showing alive cells in green and dead cells in red (Figure 2). In addition, a metabolic assay Presto blue HS was performed to evaluate the absence of LEP cytotoxicity.

Intracellular uptake of LEPs was analysed by transmission electron microscopy (TEM) in hAELVi and HBEC3-KT cells exposed for 24 h to 100 µg/mL of LEPs (Figure 3).
Human macrophages, derived from monocytes extracted from peripheral blood, were exposed to 100 µg/mL of LEPs for 24 h and then processed for TEM analysis (Figure 4).
Gene expression profiles were carried out by RNA sequencing on total RNA extracted from cells incubated for 24 and 72 h with PEG_LEPs (1 and 100 µg/mL.

Results

Analysis of cells viability: Cells exposed to LEP_GA and LEP_PEG formulations for 24 h did not manifest evidence of cytotoxicity. The same results can be observed after 72 h of LEP exposure, except for the NCI-H441 cell line, that is particularly sensitive to pH alteration, so this could explain the observed cytotoxicity.

Analysis of LEPs uptake: We performed a TEM analysis in normal lung cells (HBEC3-KT and hAELVi lines) incubated with 100 µg/mL ZrO2:0.4%Ti@GA and ZrO2:0.4%Ti@PEG for 24 h. As visible in the representative TEM images of Figure 3, some LEPs were internalized in both types of cell lines, mostly in correspondence of endosomes/lysosomes, confirming that very likely LEPs are internalized by endocytosis. Both LEP formulations are internalized by cells and no morphological alterations or ultrastructural changes were observed in cells exposed to both particle formulations, thus confirming that biocompatibility of both type of particles. Interestingly, ZrO2:0.4%Ti@PEG LEPs are internalized to a lesser extent with respect to ZrO2:0.4%Ti@GA particles (note that a lot of LEPs appeared outside cells, especially in hAELVi cells), confirming the efficacy of PEG coating in reducing interaction with and uptake in normal lung cells.

Analysis of macrophage clearance: We analysed ZrO2:0.4%Ti@GA and ZrO2:0.4%Ti@PEG uptake in human monocytes-derived macrophages isolated from buffy coats. As visible in the representative TEM images of Figure 4, both LEP types were recognized and internalized by monocyte-derived macrophages in a similar extent, high lightening that the PEG coating did not negatively influence ZrO2:0.4%Ti@PEG clearance.

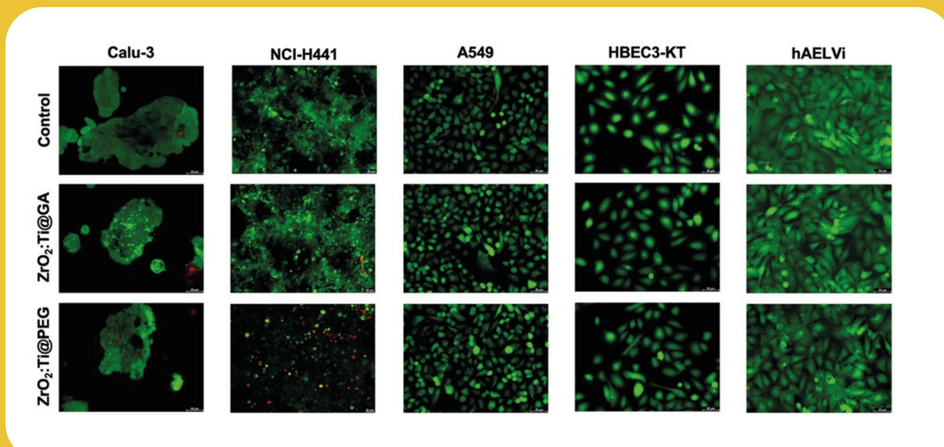

Figure 2
Representative fluorescence microscopy images of cells exposed for 72 h to 100 µg/mL of LEPs and stained with Calcein AM (green, alive cells) and Ethidium homodimer-1 (red, dead cells). Scale bars: 50 µm.

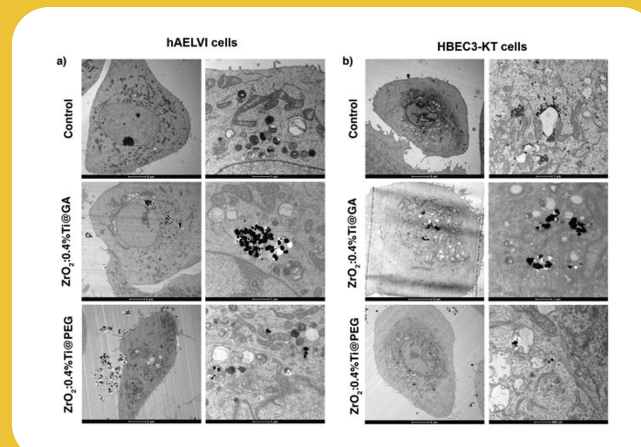

Figure 3
Representative TEM images of human cells of the respiratory tract (hAELVi and HBEC3-KT) incubated for 24 h with LEP_GA and LEP_PEG formulations.

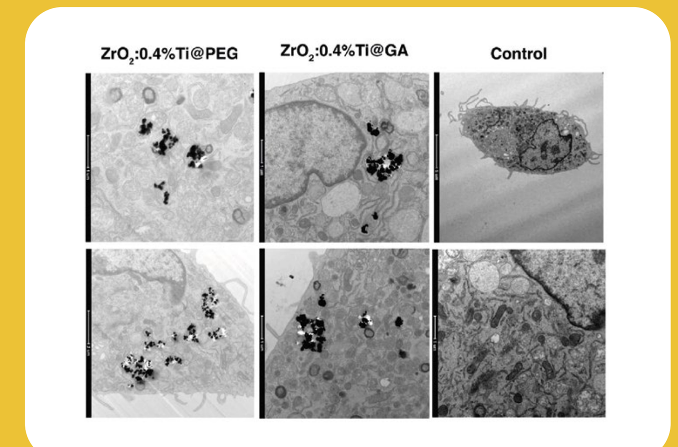

Figure 4
Representative TEM images of human macrophages exposed to 100 µg/mL of LEPs for 24 h.

Analysis of gene expression: : We analysed gene expression profiles in HBEC3-KT, hAELVI, and A549 cells incubated for 24 and 72 h with LEPs (1 and 100 µg/mL). For such kind of analysis, we chose the formulation ZrO2:0.4%Ti@PEG, endowed with lower toxicity and lower uptake in lung cells. Data of RNA sequencing reported overall no evidence of cytotoxicity in cells exposed to LEPs, even after prolonged incubation (72h) at the 100 µg/mL.

Conclusions

The selected human cells well tolerate the exposure to both LEP formulations in terms of cell viability, even at longer incubation times.
PEG_LEP suspensions are internalized to a lesser extent than LEP_GA suspensions, confirming the efficacy of PEG coating in reducing interaction with and uptake in normal lung cells.
Phagocytic cells actively participate in the clearance of both LEP types from the lungs.
Data of gene expression confirmed the absence of toxicity in human cells of the respiratory tract incubated with PEG-LEPs for both short and long times.
These promising aspects underscore the potential for the safe application of LEP_PEG while ensuring their efficient removal and minimizing any potential long-term adverse effects.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 863102