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DEVELOPMENT OF AN *IN VITRO* HUMAN ALVEOLAR EPITHELIUM MODEL CANDIDATE TO ALTERNATIVE METHOD FOR TOXICOLOGICAL ANALYSIS OF TiO_2

Marianna Cavalheiro [1]
Maria Isabel Doria Rossi [2]
Tatiana Coelho-Sampaio [3]
Priscila Grion de Miranda [4]
Paulo Emilio Corrêa Leite [5]
Celso Sant'Anna [6]
José Mauro Granjeiro [7]

ABSTRACT

Alternative methods to the use of animals must be reproducible and close to human biology, generating reliable data with a higher predictive result and lower cost. The objective of the study was to develop an *in vitro* model that mimics the human alveolar epithelium for toxicological analysis of titanium dioxide nanoparticles ($NPTiO_2$) of the anatase type. The human adenocarcinoma cell line A549 was grown on a transwell membrane (4 µm pore) or in 96-well plates previously coated with a layer of human laminin 511 polymerized at acid pH (polyLM). After 24 hours, different concentrations (5, 10, 50, and 100 µg/mL) of $NPTiO_2$ were added and the cells were maintained for 72 hours in culture. Morphological analyzes were performed by confocal microscopy, and transmission (TEM) and scanning (SEM) electron microscopy to evaluate the expression and distribution of adhesion complexes (ZO1 and β-catenin), organization of the cytoskeleton (phalloidin) and deposition of laminin. The

[1] Doctor by Biotechnology course of the National Institute of Metrology, Quality and Technology - Inmetro, mariannacavalheiro@gmail.com;
[2] Professor at the Institute of Biomedical Sciences, Federal University of Rio de Janeiro – ICB/UFRJ, idrossi@hucff.ufrj.br;
[3] Professor at the Institute of Biomedical Sciences, Federal University of Rio de Janeiro – ICB/UFRJ, tcsampaio@histo.ufrj.br;
[4] Master by Biotechnology course of the National Institute of Metrology, Quality and Technology - Inmetro, priborchio@gmail.com;
[5] Professor at Biotechnology course of the National Institute of Metrology, Quality and Technology - Inmetro, leitepec@gmail.com;
[6] Professor at Biotechnology course of the National Institute of Metrology, Quality and Technology - Inmetro, cbfilho@inmetro.gov.br;
[7] Advisor at Biotechnology course of the National Institute of Metrology, Quality and Technology - Inmetro, jmgranjeiro@inmetro.gov.br;

permeability of the model was evaluated using the Evans Blue dye assay. The presence of $NPTiO_2$ was investigated by TEM and by energy dispersive spectroscopy (EDS). The cytotoxicity of $NPTiO_2$ was assessed by an apoptosis assay (Annexin V-PI), lactated dehydrogenase (LDH) enzyme activity, and cell senility assay. Multiplex assay was performed to assess the secretion of pro-inflammatory cytokines. Laminin expression was observed in A549 cells grown onto the plastic transwell membrane, but not in those grown onto the polyLM layer. Cells grown on polyLM showed a polyhedral morphology compared to those grown on the transwell membrane, which were more pavement, with cytoplasmic projections that extended under neighboring cells. Permeability across cell layer grown onto transwell membrane was significantly higher compared to cells grown onto polyLM. Accordingly, a linear pattern of cell adhesion complexes and a f-actin cortical network were observed in neighbor cells grown onto the polyLM layer. Although the presence of $NPTiO_2$ was identified by EDS, no induction of cell death or senility was observed. In addition, no significant change in the permeability of the model, nor in the pattern of cytokines secreted by A549 cells after 72 hours of culture with different concentrations of $NPTiO_2$ were observed. The results suggest that polyLM induced organization of the pulmonary adenocarcinoma lineage with basolateral polarization and significantly more efficient epithelial barrier function. $NPTiO_2$ does not seem to affect the A549 cells at the concentrations and conditions studied. The model with basal lamina biomimetic and type II alveolar cell line showed morphological features similar to those found *in vivo*, with the potential to be an alternative method to assess alveolar toxicity.