EVALUATION OF A HUMAN IN VITRO 3D RESPIRATORY EPITHELIUM MODEL IN BIOAVAILABILITY AND SAFETY ASSESSMENT OF PHARMACEUTICAL AND CHEMICAL COMPOUNDS

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INTRODUCTION: The lungs are increasingly used as administration route for pharmaceuticals and are an important entry route for inhalable chemicals. Laboratory animals are mostly used in safety and efficacy evaluation of inhalable test compounds, although animals may not necessarily reflect human physiology or human disease (i.e. allergic asthma and COPD). Hence, there is a need for predictive models mimicking human physiology. Progress has been made in developing three dimensional (3D) models. MucilAir™ is an in vitro model consisting of human basal, goblet and ciliated cells cultured at an air–liquid interface featuring a fully differentiated and functional respiratory epithelium. The model displays in vivo characteristics including tight junctions, metabolic activity, mucus production and beating cilia. Such 3D models may find applications in the predictive in vitro safety and efficacy evaluation of pharmaceutical or chemical test compounds.

OBJECTIVE: To evaluate the MucilAir™ 3D respiratory epithelium model compared to non-primary in vitro models for assessing i) bioavailability of pharmaceutical compounds and ii) safety of nanoparticles.

METHODS: For bioavailability assessment MucilAir™ inserts were exposed to atmospheres containing cerium oxide nanoparticles. Parameters assessed included tissue integrity (transepithelial electric resistance), cell membrane integrity (lactate dehydrogenase [LDH] leakage), oxidative stress (HO-1; heme oxygenase protein activity), genotoxicity ( Comet assay ) and Illumina gene array expression analysis.

STABILITY EVALUATION OF AEROSOLIZED NANO-PARTICLES To evaluate the MucilAir™ model for in vitro air exposure safety testing, we placed the cell-containing inserts in Vitrocell’s modules. MucilAir™ was compared to the non-primary cell lines A549 and BEAS-2B cultured in monolayers. After exposure to aerosols of cerium oxide nanoparticles the MucilAir™ model showed an increased anti-oxidative HO-1 activity. Such response was absent non-primary cell lines A549 and BEAS2B (Figure 3). By contrast, genotoxicity was observed in A549 and BEAS2B cells after nanoparticle exposure (Figure 4), whereas genotoxicity was absent in the MucilAir™ model.

These in vitro data correspond to our previous findings that aerosols of nanoparticle were non-genotoxic in rats in vivo and with recent publication showing that that cerium oxide nanoparticles were non-genotoxic to human lens epithelial cells in vitro. Gene expression profiling revealed a clear distinction between 3D primary-MucilAir™ and 2D non-primary A549 and BEAS2B models (Figure 5). This shows that the cell models used have clear distinct characteristics.

CONCLUSIONS Primary cells appeared more resistant towards experimental air flow compared to non-primary cells, most likely due to the presence of tight junctions, mucus and beating cilia (Figure 6). Our results show that the MucilAir™ respiratory epithelial model allows the assessment of bioavailability and safety of pharmaceutical and chemical compounds with the airways and lungs as intended route of delivery. We optimized the use of Vitrocell modules for air exposure application of test compounds onto the cells mimicking in vivo conditions. The battery of read-out parameters included, but is not limited to (i) oxidative stress, (ii) release of inflammatory markers, (iii) cytotoxicity, (iv) genotoxicity, (v) large-scale gene expression analysis and (vi) cellular transporters. This 3D model of respiratory epithelium may find applications in predictive screening of bioavailability and safety of pharmaceuticals and chemicals for which the airways are the primary route of exposure.