Functional co-culture models to study nanoparticle-cell interactions

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Abstract

How nanoparticles (NPs) interact with human cells is important not only in environmental medicine but also in modern regenerative medicine concepts which envisage using NPs as a drug- or gene-delivery system. The respiratory tract is a prime example of an organ constantly exposed to the environment as well as being a possible therapeutic target to gain access to the systemic circulation. In addition to the use of suitable models *in vivo*, cell culture systems can be invaluable in helping to understand mechanisms of NP uptake, intracellular transport and modulation of cellular function. This presentation will describe complex co-culture systems for the upper and lower (alveolo-capillary barrier; ACB) respiratory tract and demonstrate how they can be used to delineate molecular mechanisms.

A Transwell® system for 24 multiwell tissue culture plates is employed in which two different cell types are grown on opposite sides of a finely porous (pore size : 0.4 µm diameter) polycarbonate membrane. Human bronchial epithelial cells (HBEC) are isolated from explant culture of bronchial biopsies and these outgrowing basal progenitor cells cocultivated with human primary fibroblasts (fib) or a foetal lung cell line (Wi-38)[1]. For the AC barrier human alveolocytes type II or the cell line NCI-H441 are co-cultured with human pulmonary microvascular endothelial cells (HPMEC) or the endothelial cell line ISO-HAS[2] [3]. For both co-culture systems the upper (epithelial) layer must contact the air, so-called "air-lift" cultures. In both models, immunocytochemistry, confocal laser scanning microscopy, scanning and transmission electron microscopy (SEM, TEM), and protein analysis are performed on the upper and lower compartments of the system (cell layer & supernatant). In addition, functionality of the barriers can be monitored by transcellular resistance measurements.

Both models make use of paracrine cellular crosstalk and show some remarkable features. Thus, for the upper respiratory tract in co-culture, but not in monoculture, the epithelial layer differentiated into a complete respiratory mucosa, with basal, ciliated columnar and mucous cells. A true physiological barrier is formed with marked cell adhesion molecule expression and beating cilia (16-20 Hz). For NP uptake across the AC barrier, various NPs based on silica have been studied in comparison with polyorganosiloxane NPs[4]. The influence of NP diameter and chemistry has been demonstrated, and cellular uptake mechanisms involve clathrin- and caveolin-independent pathways with clear evidence for the involvement of flotillins[5] [6]. The role of surfactants, which are produced in the alveoli, as well as macrophages still have to be studied, the latter in triple culture models. A triple culture has also been established for the bronchial model by adding dendritic cells (DCs), which are important in immunological monitoring.

A cellular and molecular toolbox for human cells is now available to study how NPs interact with the epithelium in a monoculture system[7], and how the reactions change when the higher complexity is introduced by using the co-culture systems.

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