

Poster: nanotoxicology / nanobiotechnology

## Primary porcine alveolar epithelial cells (pAEpC) as a model for drug permeability assays

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**Subject:** The respiratory tract is considered as an alternative to gastrointestinal or dermal drug delivery systems, due to the fast absorption and the absence of first pass metabolism. Permeability and solubility of candidate compounds are useful data that generally speed up the drug development process. For permeability studies, lung epithelial barrier models are needed. As an alternative for animal testing, epithelial cell cultures would provide a good means for rapidly evaluating the drug delivery process to the lung. While *in vitro* systems that reliably mimic the upper airways epithelia are already available (e.g. Calu-3 cells), there is no such a system (a cell line) that properly reproduces the conditions found in the deeper lung, and researchers still have to rely on primary cell cultures (Steimer et al., 2005).

**Material and Methods:** Pig lungs were minced and bronchioles were removed. Tissue pieces were pre-incubated with a trypsin-elastase combination and then digested. Macrophages and dissociation from blood cells and cell debris were removed and cells were plated at a density of  $8 \times 10^5$  cells/cm<sup>2</sup> on permeable fibronectin/collagen coated Transwell Clear Filter inserts.

TEER was measured with an EVOM and estimated relative to the corresponding surface area. Transport studies were performed in triplicate in ab and ba direction, samples were taken at distinct time points and the Papp was calculated. Sodium fluorescein, 3H-propranolol and rhodamine 123 were used as markers for low, high permeability and efflux transporters, respectively. Triamcinolone acetonide was used as an example of a pulmonary delivered drug compound.

For the inspection with a transmission electron microscope EM10C, cells grown in Transwell filters were fixed in 4% (w/v) paraformaldehyde and 1% (v/v) glutaraldehyde in 0.1 M cacodylate buffer. Then cells were postfixed with 2% (w/v) osmium tetroxide in 0.1 M phosphate buffer and dehydrated in acetone. Filters were embedded in Epon resin and polymerized 60-80 nm thick sections were taken perpendicular to the filter surface, stained with uranyl acetate and lead citrate.

**Results and Discussion: Morphology:** On day 2 after seeding, cells of a different shape with numerous filopodia were adhered onto the filter substrate. By day 8 the monolayer appeared flat and a 100% confluency was observed; multilamellar bodies could be detected, as well as tight junctions and desmosomes. On day 13, two morpho-types could be observed: round cells with multilamellar bodies in the apical cytoplasm and flat, spread cells with no particular ultrastructure. This hinted at a mixed type I/type II pneumocyte phenotype, which was also confirmed by immunofluorescent detection of marker proteins. The paracellular integrity of the monolayers was assessed by transport studies with Flu-Na in the absorptive direction (ab). An inverse correlation between permeability coefficient and TEER was observed. pAEpC had the same behaviour, in terms of permeability of triamcinolone acetonide, as hAEpC or two human bronchial epithelial cell lines (Calu-3 and 16HBE14o-), which are already established models.

**Conclusion:** pAEpC have proven to be a good model for drug permeability assays. They consist of a mixture of type I and type II pneumocyte-like cells, and exhibit good barrier properties (Steimer et al., 2006, 2007).

### References

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