Characterization of Ci-huAEC airway epithelial cells for air-liquid exposure experiments

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Objective

Cell-based lung models of human origin are needed for toxicity and efficacy studies as well as for assessing the toxic potential of environmentally and occupationally relevant airborne substances. Immortalized cell lines often do not adequately reflect the *in vivo* situation in lung tissue, while primary cells, on the other hand, are of limited availability. The CI-SCREEN technology aims to overcome this problem by implementation of expansion genes into freshly isolated primary cells. As a result primary cells can thereby be expanded to cell numbers enabling reliable and reproducible testing protocols while retaining functions of primary cells. In this study, CI-huAEC cells were characterized in a first step to barrier formation, CYP expression and inducibility, expression of surface markers and ALI-exposure.

Methods

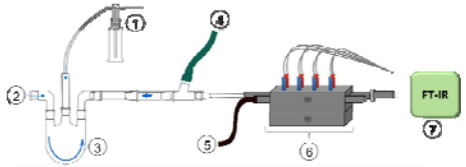

Figure 1: Scheme of the *experimental setup* for exposing CI-huAEC cells to airborne test substances (formaldehyde) using air-liquid interface conditions. The system consists of three parts: A: Generation and transport of the testsubstance atmosphere (1-4) or the clean air (5) control respectively. B: The exposure unit for the target cells, grown under air-liquid interphase conditions (P.R.I.T.® ExpoCube® ;6). C: Analysing unit for testatmosphere (FT-IR Monitor); Position 4 represents the exhaust of excess testatmosphere.

Cell culture: Ci-huAEC Cells (INS-CI-1011) were purchased by InSCREENex Gmbh, Braunschweig and cultivated with serum free medium according to the manufacturer´s instructions. Cells were routinely split twice a week. For air-lifted interface culture cells were grown on PET-membranes (BD Falcon) with 1cm² in diameter and 0.4µm pore size. The transepithelial electrical resistance (TEER) was measured to monitor the barrier properties of the cell monolayers with chopstick electrodes. Viability measurements were performed by using the WST-Assay. Detection of surface markers was performed by FACS whereas expression of xenobiotic metabolizing enzyme genes was shown by RTq-PCR.

Results

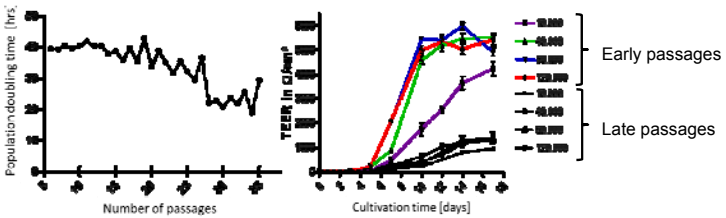

Figure 2: Population doubling time depending on the number of passages (left) and **development of TEER-values** under air/liquid interface conditions depending on seeding density, cultivation time and passage; (early passage ≤ 12, late passages ≥ 20) (right)..

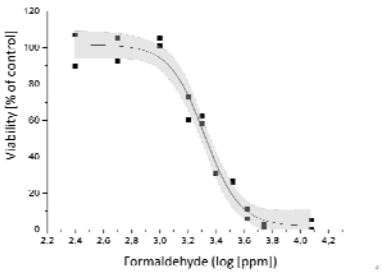

Figure 3: Dose effect curve of CI-huAEC cells exposed to formaldehyde for 1hr followed by a 24hrs post incubation period. Confidence interval of 95% is stained grey. EC_{50} value = 2086 ppm

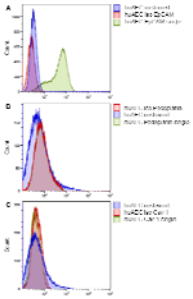

Figure 4: Analysis of Ep-CAM (A) Podoplanin (B) and Caveolin-1 (C) staining. Nearly all cells expressed Ep-CAM in contrast to podoplanin and caveolin -1

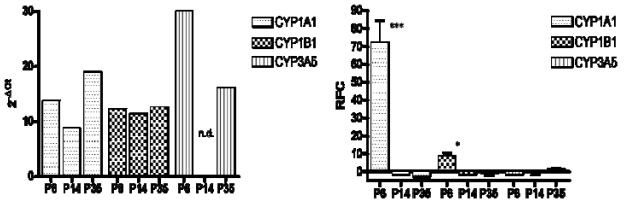

Figure 5: Basal CYP Expression (left) and Inducibility of CYP-genes by Omeprazol dependent on the cell passage (right). All three genes were expressed independently of the cell passage, whereas CYP1A1 and CYP1B1 were induced by Omeprazol in CI-huAEC cells of passage 6 only.

- Ci-huAEC cells showed constant population doubling times up to the 12th passage accompanied by high barrier properties (**Figure 2**).
- Exposure to formaldehyde as a testsubstance for VOCs under ALI conditions resulted in an EC_{50} value which was nearly 20 times higher than those reported with the human lung cell line A549 (**Figure 3**), a result which is in line with primary cells.
- Podoplanin and Caveolin-1 staining was negative, whereas EpCAM was positive supposing that these cells may represent more bronchial than alveolar epithelium characteristics (**Figure 4**).
- Investigation of CYPs revealed expression and inducibility of xenobiotic metabolizing enzyme genes in early passages (**Figure 5**).

Conclusion

The Ci-huAEC cell line may represent a very promising model for inhalation toxicology studies.

COI: Tobias May is shareholder of InSCREENeX GmbH which commercializes the CI-huAEC cell line

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