Development and optimisation of an Air-Liquid Interface model using immortalised airway epithelial cells and cellQART® inserts

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Abstract

Traditional cell culture models of the human respiratory tract often fail to resemble normal physiology and biology due to the inability of airway epithelial cells to differentiate properly in submerged cell culture. In contrast, airway epithelial cells cultured at the air-liquid interface on permeable support membranes polarise and form a pseudostratified epithelium which closely resembles the in vivo airway epithel. The availability of primary airway epithelial cells is limited, and donor-donor variability poses a severe challenge, especially in highly regulated research environments such as drug development and toxicology. We therefore generated immortalised airway epithelial cells representing both, the upper (nasal epithelial cells), and the lower (bronchial and alveolar epithelial cells) respiratory tract. Here we describe the culture of immortalised airway cells at the air-liquid interface on cellQART® permeable membranes. All immortalised airway epithelial cells formed a tight barrier and showed markers of polarisation. We further used this cell culture model to develop a differentiation medium for the alveolar cells, which reduced the time needed to form a tight barrier 2-fold. This can be a useful in vitro airway model for infection research, drug development and respiratory toxicology.

Materials

Cell Culture Inserts

- cellQART® clear inserts PET 0.4 µm (SABEU; # 9320412)
- Transwell® clear inserts PET 0.4 µm (Corning; #3470)

Key Equipment

- EVOMX Voltohmmeter (World Precision Instruments)
- Axio Imager.A2 Microscope (Zeiss)
- Zeiss LSM 980 Confocal (Zeiss)

Cells

- CI-hAELVi Alveolar Epithelial Cells (InSCREENeX; #INS-CI-1015)
- CI-huBroBEC Bronchial Basal Epithelial Cells (InSCREENeX; #INS-CI-1025)
- CI-huNaBEC Nasal Basal Epithelial Cells (InSCREENeX; #INS-CI-1026)

Medium and Coating

- huAEC-Medium (InSCREENeX; #INS-ME-1013)
- huBroBEC Medium (InSCREENeX; #INS-ME-1033)
- huNaBEC Medium (InSCREENeX; #INS-ME-1034)
- hAELVi FasTEER Medium (InSCREENeX; INS-ME-1032)

Antibodies

- Polyclonal ZO-1 antibody (ThermoFisher Scientific; #40-2200)
- Secondary goat anti rabbit labelled with Cy3 (Dianova; #111-166-045)

Cell growth and morphology

Primary airway cells were immortalised using the CI-SCREEN technology as previously described.[1] Human Alveolar Epithelial Cells (CI-hAELVi) cells have already been extensively characterised[2,3], whereas

human Bronchial Basal Epithelial Cells (CI-huBroBEC) and Nasal Basal Epithelial Cells (CI-huNaBEC) are both novel developments.

We first evaluated the growth of all three airway cell lines on cellQART® inserts in submerged culture and compared cell morphology to a competitor product (Corning Transwell®). All three cell lines formed a confluent cell layer three days after seeding and no morphological difference was observed between cellQART® and Transwell® inserts (Fig. 1).

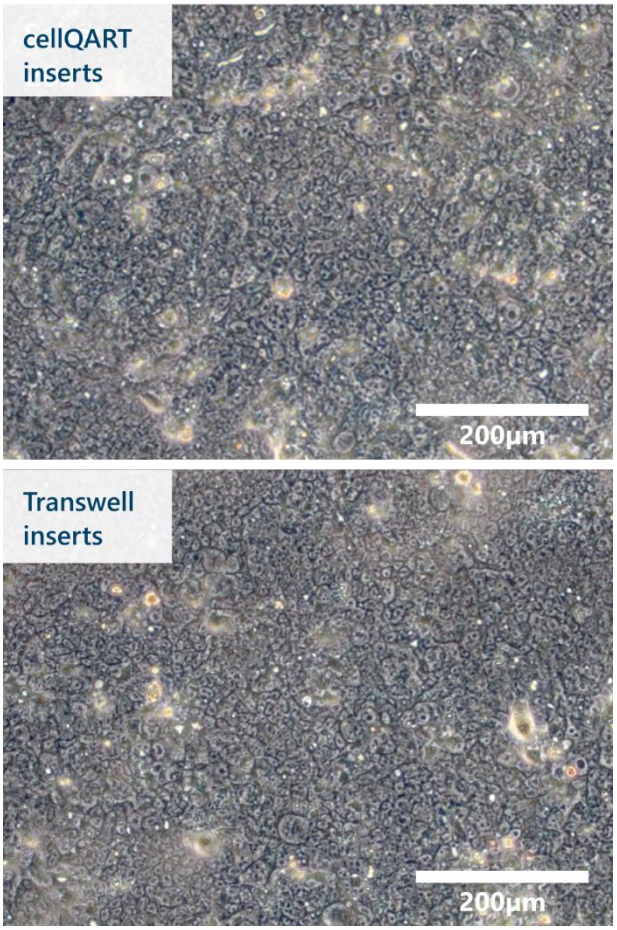

Fig. 1 No apparent morphology difference between CI-huBroBECs grown on cellQART and Transwell® inserts. Phase Contrast microscopic image of CI-huBroBEC cells grown on clear cellQART or Transwell® inserts for 14 days.

Interestingly, clear cellQART® inserts allowed easier routine monitoring of cell growth and morphology by light microscopy, especially at lower cell densities (Fig. 2). Finding the proper focus plane was easy and we hypothesise this might be due to better optical properties of cellQART® inserts, compared to Transwell® inserts.

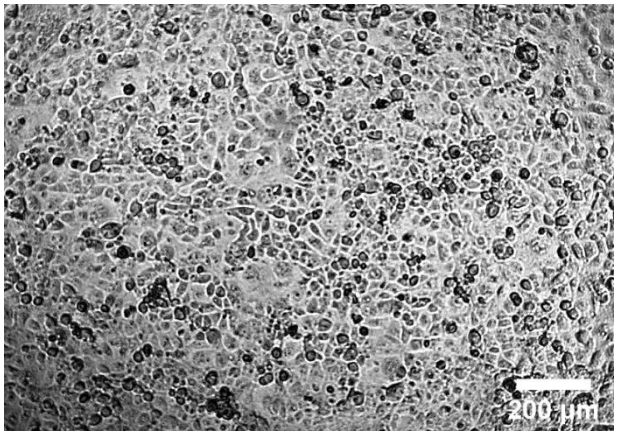

Fig. 2 Clearly visible cells on clear cellQART inserts. Phase Contrast microscopic image of CI-huBroBEC cells grown on clear cellQART inserts for 3 days. Note that cells are clearly in focus and cell borders can be easily distinguished.

GFP-labelled CI-huBroBECs

To allow easier cell tracking and provide a cell line suitable for high throughput assays, we used a GFP-encoding lentiviral construct to introduce a stable fluorescent label into CI-huBroBECs (Fig. 3). The fluorescent label was stable over at least 30 passages and had no influence on the cellular phenotype.

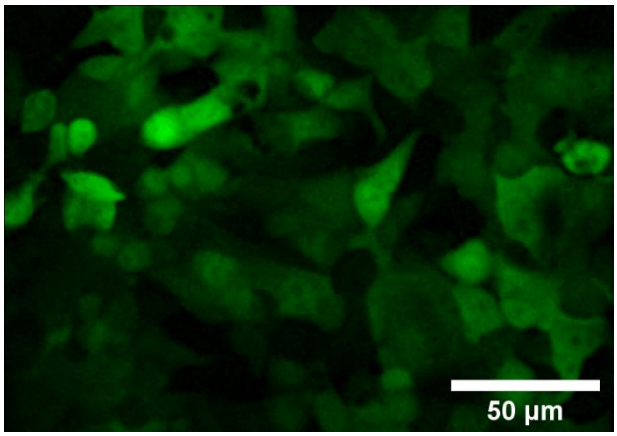

Fig. 3 Development of a GFP-labelled CI-huBroBEC cell line. Fluorescent image of GFP-labelled CI-huBroBEC cells grown on clear cellQART inserts for 3 days.

Barrier Formation

Proper barrier function is a key phenotype of lung in vitro models, still it is missing from commonly used standard lung cell models such as A549 or Calu-3 cells[4], with TEER values failing to reach 1000Ω×cm^2. We therefore evaluated the potential of the immortalised cells to form a tight barrier on cellQART inserts at the Air-Liquid Interface (ALI).

CI-hAELVi, CI-huBroBEC and CI-huNaBEC were seeded on cellQART® inserts cultured in submerged conditions until confluent. When cells formed a confluent cell layer, air-lift was initiated. Medium from the apical (upper) part of the insert was removed and cells were culture in ALI conditions. Prior to transepithelial electrical resistance (TEER) measurements using the chopstick method, enough medium was added to the apical part of the insert to submerge the cells.

All three cell lines formed a barrier to an extent which is consistent with their position and function within the respiratory tract. CI-hAELVi cells reached a maximum resistance of up to 4500 Ω×cm² consistent with previous reports. CI-huBroBECs and huNaBECs reached maximal TEER values of 1500 Ω×cm² and 260 Ω×cm², respectively (Fig. 4).

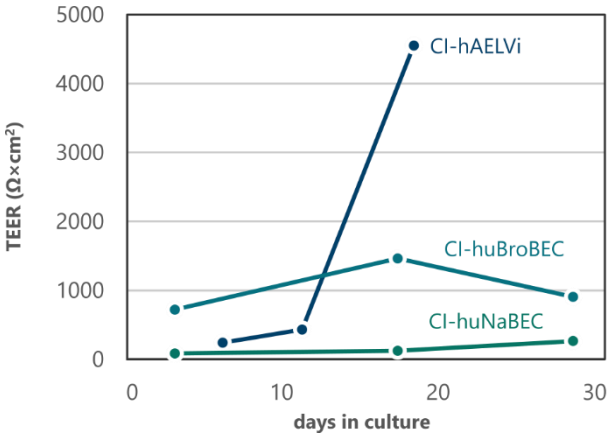

Fig. 4 Barrier formation of airway cells. Transepithelial electrical resistance (TEER) measurements of three immortalised airway cell lines at the ALI. Cells were cultured on clear cellQART inserts for the indicated amount of time and TEER was measured using the chopstick method.

To compare the ability of cellQART® to support barrier formation to a competitor product, we cultured huBroBECs and three different huNaBEC lines (A-C) on both, cellQART® and Transwell® inserts and monitored barrier formation by TEER measurements. Both products showed similar TEER readings across all four tested cell lines (Fig. 5).

Polarisation

Successful polarisation of airway epithelial cells is necessary for their physiological function, i.e. establishing a tight barrier. A hallmark of a tight epithelial barrier is the formation of tight junctions (TJs).

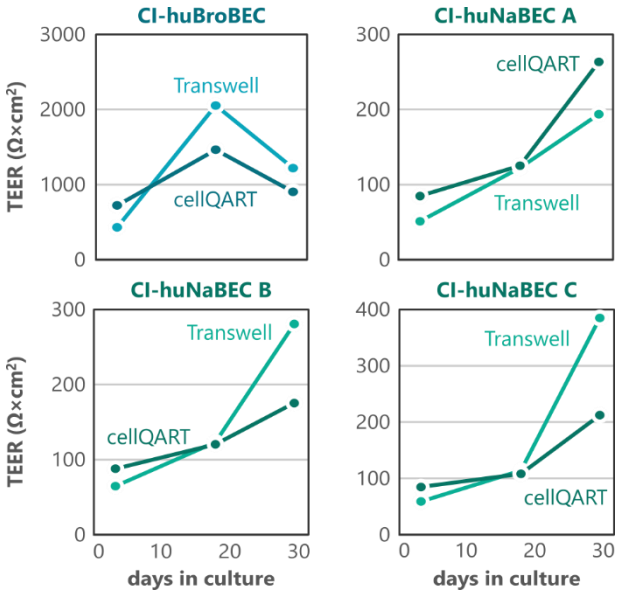

Fig. 5 Similar barrier formation on cellQART® and Transwell® inserts. Transepithelial electrical resistance (TEER) measurements of four immortalised airway cell lines at the ALI. Cells were cultured on clear cellQART® or Transwell® inserts for the indicated amount of time and TEER was measured using the chopstick method.

TJs are multiprotein complexes between neighbouring cells, which prevent leakage of transported solutes and water thereby sealing the paracellular pathway. The key marker for TJs is the protein Zonula occludens-1 (ZO-1).

To demonstrate TJ formation, we detected ZO-1 by indirect immunofluorescence on CI-hAELVi cells cultured in ALI conditions. ZO-1 was detected on almost all cells and the observed net-like staining pattern is consistent with its expected location at cell-cell borders (Fig. 6).

Analysing ZO-1 staining pattern within the z-plane (apical-basal axis) revealed increased expression towards the apical side, which indicates proper polarisation of CI-hAELVi cells cultured in on cellQART® inserts (Fig. 7).

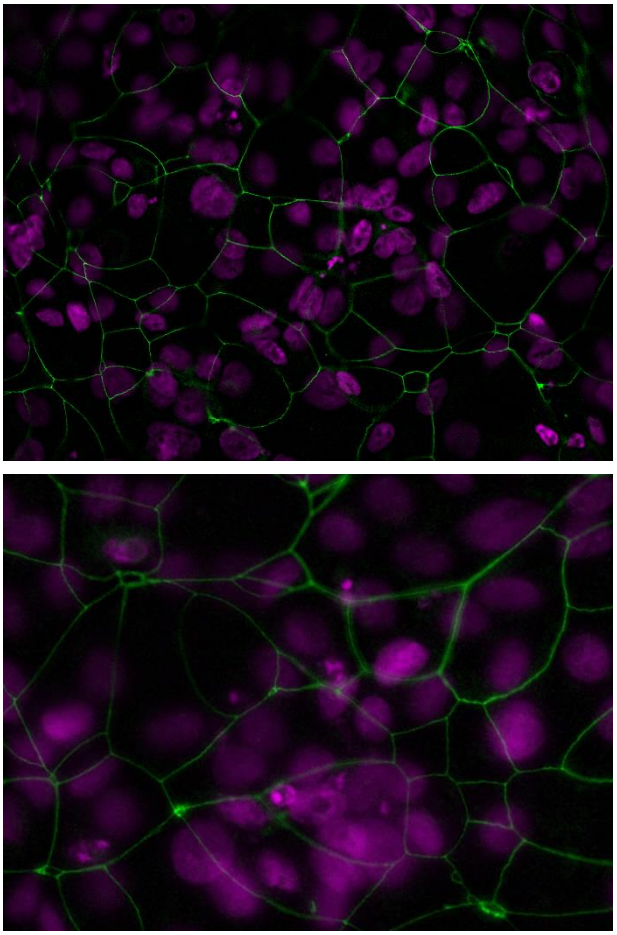

Fig. 6 Tight junction formation on cellQART® inserts. Indirect immunofluorescence staining of ZO-1 tight junction protein in CI-hAELVi cells cultured for 19 days in huAEC Medium on cellQART® inserts at the ALI. Upper panel: 200x magnification; lower panel: 630x magnification. *Magenta: DAPI/Nucleus; Green: ZO-1*

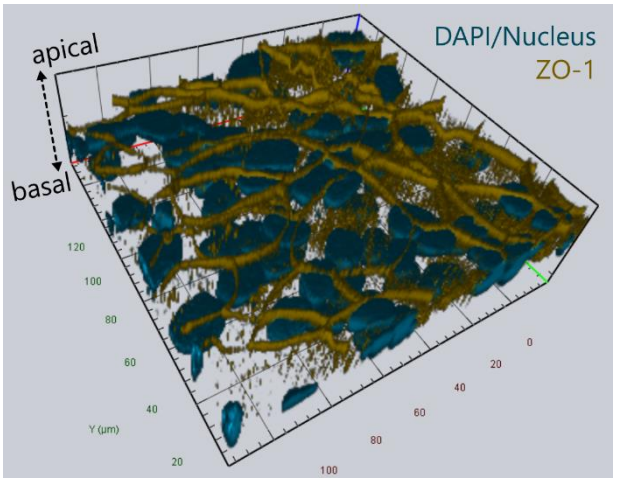

Fig. 7 Polarisation on cellQART® inserts. 3D Z-stack analysis of indirect immunofluorescence staining of ZO-1 tight junction protein in CI-hAELVi cells cultured for 19 days in huAEC Medium on cellQART® inserts at the ALI.

Development of the FasTEER Medium

Measuring TEER as a proxy for proper barrier function of lung cells in vitro is a key method, especially in respiratory toxicology. However, throughput is often limited by relatively long incubation times (at least 10-14 days), which are necessary to allow the cells to form a tight barrier (TEER above 1500 to 2000 Ω×cm^2). We therefore aimed to develop a medium formulation that, in combination with cellQART® inserts, would result in reduced CI-hAELVi barrier formation time.

Cells were seeded and cultured until confluence in standard huAEC Medium, before air-lift was initiated and medium was switched to novel medium formulations. Up to 7 different formulations were evaluated for their ability to support formation of a tight barrier.

We identified a medium formulation, which resulted in formation of a tight barrier (TEER above 1500 to 2000 Ω×cm^2) around day 7 to 8, compared to day 12 to 14 for standard huAEC medium. We named this novel medium hAELVi FasTEER Medium (Fig. 8).

Reducing the time for tight barrier formation by almost 2-fold will increase assay throughput while simultaneously lowering cell maintenance costs. Interestingly, cells cultured in standard 2D conditions in hAELVi FasTEER Medium ceased almost completely to proliferate, in contrast to culture in standard huAEC Medium.

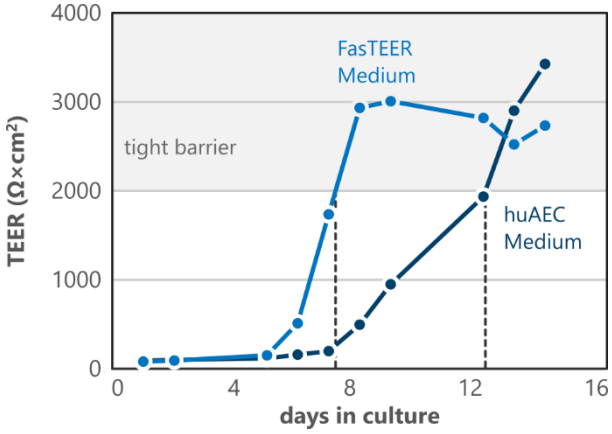

Fig. 8 Development of a rapid barrier building medium on cellQART® inserts. Transepithelial electrical resistance (TEER) measurements of CI-hAELVi cells at the ALI. Cells were cultured on clear cellQART® inserts for the indicated amount of time and TEER was measured using the chopstick method. huAEC Medium is the standard culture medium, FasTEER a new development.

Conclusion

- cellQART® permeable inserts from SABEU can be used to culture immortalised airway cells at the ALI to form polarised epithelial with a tight barrier.
- The clear inserts are especially useful for routine monitoring of cell growth and behaviour using standard light-microscopy.
- No performance difference compared to a competitor product (Corning Transwell®) was observed.
- cellQART® inserts were successfully used to develop an in vitro airway model including optimal medium for barrier formation and immortalised CI-hAELVi cells.

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