CELL CULTURE

1. **CFBE/ HBE cells**

16HBE14o- cells

Human bronchial epithelial cells, have the ability to polarize immortalised cell line generated from a one-year-old heart-lung transplant patient non-secreting phenotype

CFBE41o- cells

Cystic fibrosis bronchial epithelial cell line, generated by transformation of tracheo-bronchial cells with SV40 from cystic fibrosis, homozygous for ΔF508 mutation, form electrically tight and polarised monolayers with functional cell-cell contacts

Coating

For growth of cells obtained from the Grünert laboratory culture flasks were coated with a fibronectin/collagen/BSA coating solution made up with the following components;
100ml LHC-8 basal medium (Gibco, 12678-017)
10ml Bovine serum albumin (BSA, 1mg/ml) (Sigma, A9647)
1ml Collagen I, bovine (2.9mg/ml) (BD Laboratories, 40231)
1ml Human fibronectin (1mg/ml) (Calbiochem, 341635)

Approximately 3-4ml of coating solution was transferred aseptically into 75cm^2 flasks and swirled around to ensure complete coverage of the flask base. Flasks were allowed to sit for a minimum of 2h in the incubator, following which excess solution was removed. Flasks were then stored at room temperature for up to one month in their original packaging.

Medium

All *in vitro* cell lines were routinely grown in minimum essential media (MEM,
+ 10% FBS
+ 1% L-Glutamine
+ 1% Penicillin-Streptomycin
in collagen-coated 75cm^2 flasks and maintained in a 37 °C humidified incubator containing 5% CO_2. Cultures were renewed from frozen stocks every 4-6 weeks. For experiments, cells were sub-cultured once 60-90% confluency was achieved.

Polarized cell culture

Air-interfaced condition

Cells are seeded at a density of 500.000 cells/1.12 cm^2 on filter inserts and after 48 hours the apical fluid is removed. CFBE41o- cells grown to grown to confluent monolayers on filter inserts under air-interfaced culture conditions for 12 days. Transepithelial electrical resistance (TEER) measurements were taken every 48h (with the medium change, CAVE for measurement: fill up the apical side) using the EVOM epithelial voltohmmeter (World Precision Instruments; EVOM2, Epithelial Voltohmmeter for TEER) according to manufacturer´s instructions and converted to $\Omega.cm^2$ based on surface area of cell culture inserts.

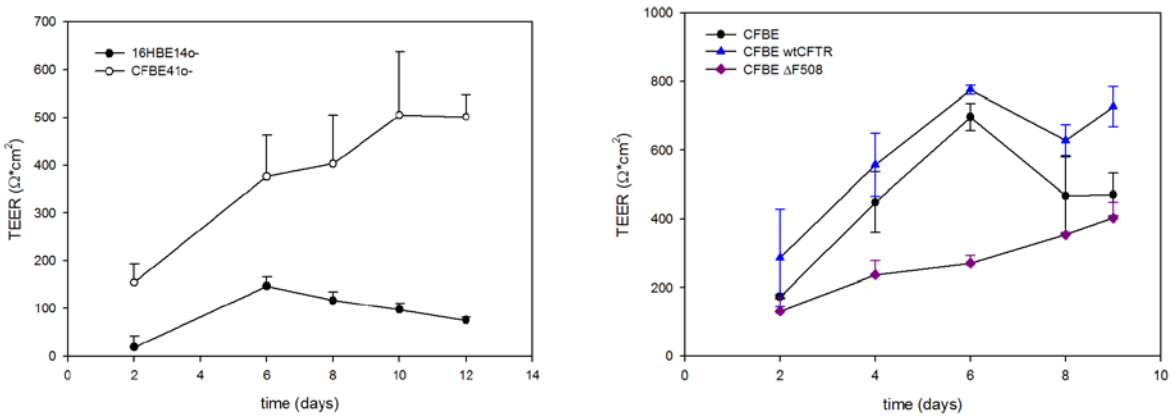

Figure 1.Transepithelial resistances were monitored in 16HBE14o- and CFBE41o- cells grown on Snapwell inserts under **air-interfaced culture conditions**. Cells were seeded at a density of 5×10^5 cells/cm^2**.** Each data point represents means ± SD (*n* = 3) from one passage (Johanna Salomon, Heidelberg).

Liquid-liquid condition

CFBE41o- cells were grown as polarized cultures at a liquid-liquid interface. Cells (8×10^4) were seeded onto 1.0µm Polyethylene therephthalate (PET) transparent Millicell hanging cell culture inserts (Millipore) and maintained in MEM+GlutaMax supplemented with 10% fetal calf serum and 1% penicillin-streptomycin. Apical (0.2 ml) and basolateral (1.25 ml) media were changed every alternate day. Once TEER>1000Ω.cm2 was achieved (indicating confluent monolayer formation -approximately 14-21 days- LCC), cells were used for experiments.

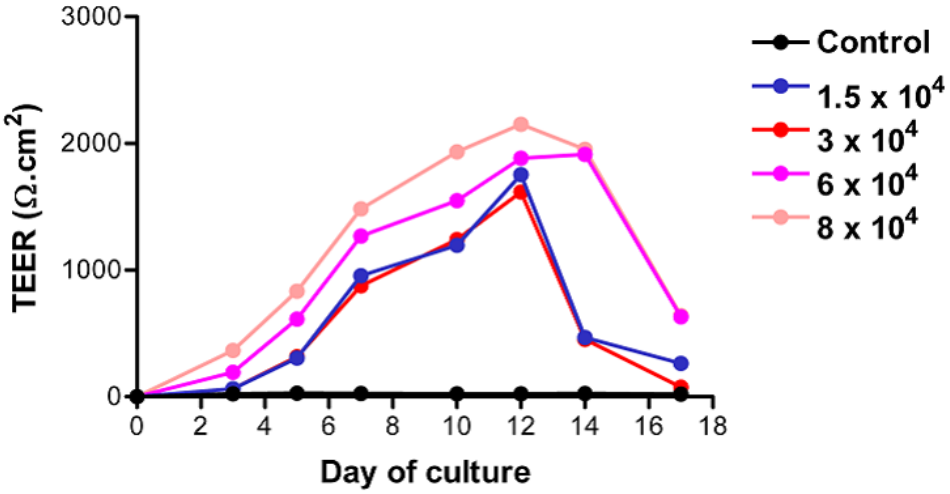

Figure 2. CFBE41o- cell cultures were grown on semi-permeable supports until a polarized epithelium was obtained. TEER values were taken every 48h over 18 days of cultures at varying cell densities. Optimal TEER (>1000Ω.cm2) was obtained in 10-14 days utilizing 8 x 10^4 cells (C. Greene, Dublin).