Improved Expansion, Differentiation, and Cystic Fibrosis Transmembrane Regulator Function of Human Nasal Epithelial Cells Supporting Drug Screening

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INTRODUCTION

Human nasal epithelial cells (HNECs) are useful for studying cystic fibrosis transmembrane conductance regulator (CFTR) function in the context of airway pathophysiology and drug testing. HNECs with rare CFTR variants can be obtained from people with cystic fibrosis (CF) by nasal brushing. However, culturing and differentiating HNECs are limited by currently available expansion media. To address these limitations, we assessed PneumaCult™-NGEx Medium, an optimized expansion medium for robust passaging and biobanking of HNECs.

METHODS

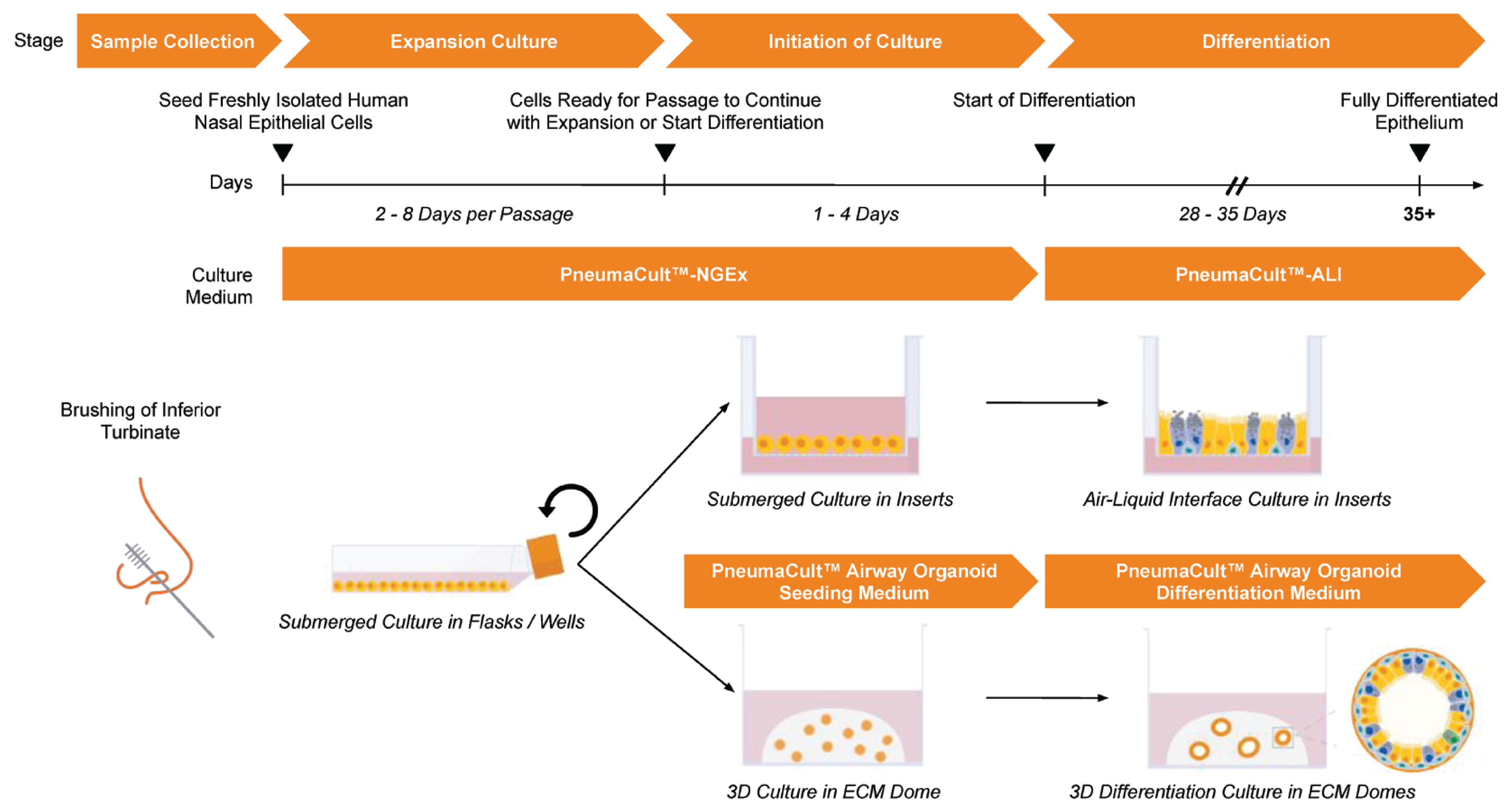


FIGURE 1. Workflow for PneumaCult™-NGEx Medium

Freshly isolated human nasal epithelial cells (HNECs) from CF and non-CF donors are expanded in PneumaCult™-NGEx Medium until 60 - 80% confluent, then passaged. Cells were seeded into cell culture inserts and differentiated at the air-liquid interface (ALI) using PneumaCult™-ALI. PneumaCult™-NGEx is also compatible with 3D organoid applications using PneumaCult™ Airway Organoid Kit.

Immunofluorescence Staining: Briefly, expanded basal cells or ALI-differentiated cultures were processed for whole-mount immunocytochemistry (ICC) according to STEMCELL Technologies Inc. protocol 'ICC Staining Protocol for Monolayer or Air-Liquid Interface Cultures'.

Electrophysiology Measurements: CFTR activity of CF and non-CF ALI-differentiated cultures was measured by electrophysiology in Ussing chambers. CFTR induction and inhibition values were determined by comparing peak CFTR short-circuit current (Isc) induction and inhibition post-drug addition with resting Isc baseline before drug addition.

Forskolin Induced Swelling Assay: CFTR activity of CF and non-CF airway organoid cultures was measured by forskolin induced swelling (FIS). Organoid swelling with CFTR activation was determined by quantifying the organoid surface area 6 hours after drug addition compared to time zero.

CFTRm Treatment: HNEC cultures for electrophysiology or FIS were treated for 24 hours with DMSO or CFTR modulators: 3 μm VX-445 (elexacaftor), VX-661(tezacaftor), VX-121 (vanzacaftor).

RESULTS

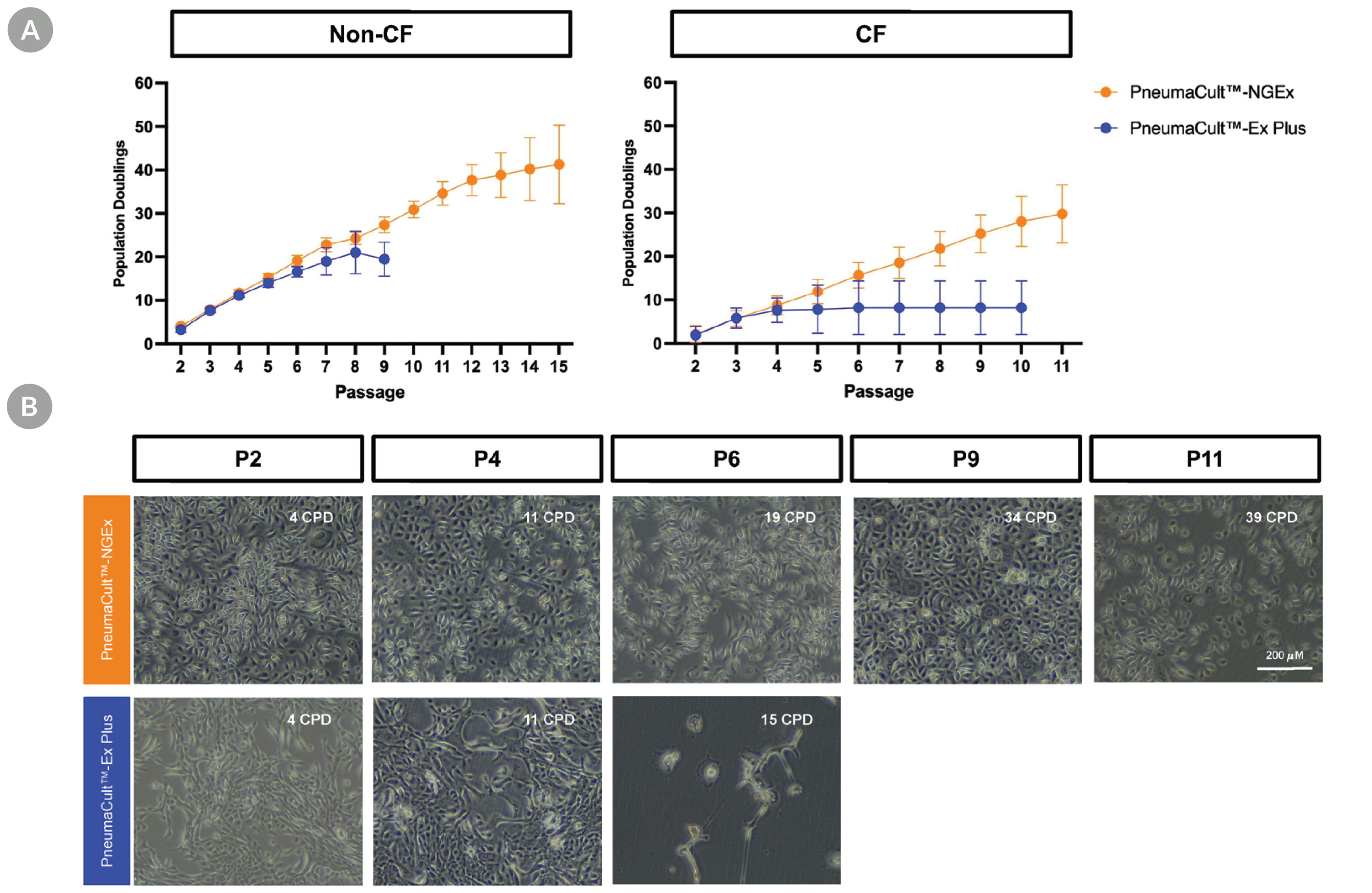
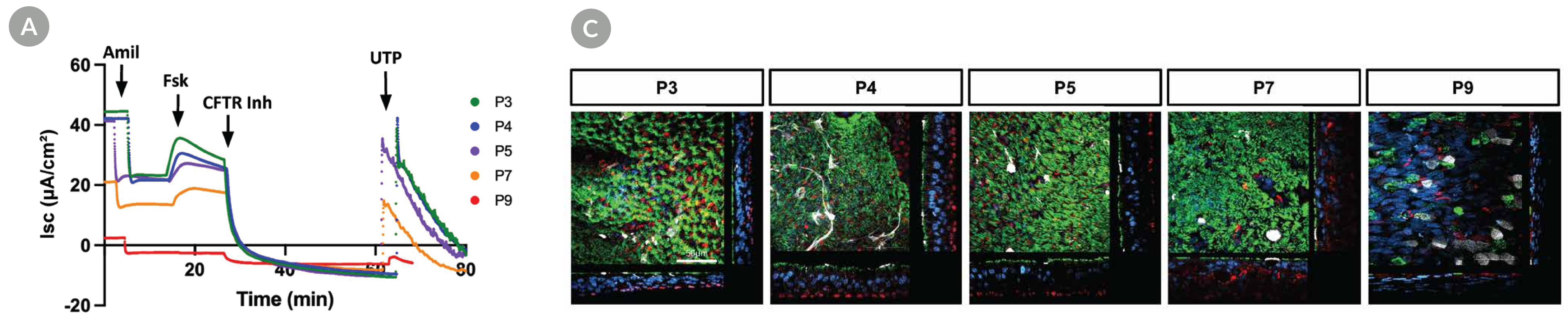


FIGURE 1. PneumaCult™-NGEx Supports Enhanced Expansion of Human Nasal Epithelial Basal Cells Through Extended Passaging

(A) Cumulative population doublings (CPD) were measured after expanding HNECs in the specified PneumaCult™-NGEx or PneumaCult™-Ex Plus media from initial brush collection until senescence. HNECs expanded in PneumaCult™-NGEx for an additional 22 population doublings compared to PneumaCult™-Ex plus in non-CF and CF donors. Each data point represents the mean ± SD (n = 3 donors). **(B)** Representative brightfield images of a CF donor's HNECs cultured in PneumaCult™-NGEx and -Ex plus with CPD in white. Notably, cobblestone morphology was observed in PneumaCult™-NGEx and was preserved until passage 11. Scale bar = 200 μm. This indicates that HNECs expanded in PneumaCult™-NGEx retain their basal cell identity even with long-term passaging. Taken together, the data suggest that PneumaCult™-NGEx demonstrates superior population doublings over long-term passaging and higher cumulative cells maintaining optimal morphology.



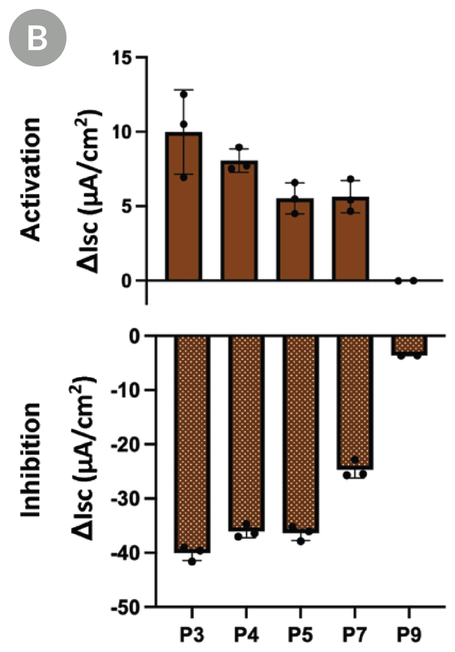


FIGURE 3. Expansion in PneumaCult™-NGEx Supports Robust Electrophysiological Function and Critical Markers for Respiratory Disease Studies After Differentiation in PneumaCult™-ALI

(A) Ion channel activities for ALI cultures at 28 days post-air-lift were characterized using HNECs expanded in PneumaCult™-NGEx followed by differentiation on cell culture inserts using PneumaCult™-ALI. Cells were placed in Ussing chambers and allowed to equilibrate to a baseline short circuit current (Isc). Representative electrophysiology traces. Arrows 1 - 4 indicate time points at which compounds were added to induce ENaC inhibition (amiloride; Amil), followed by CFTR activation (forskolin; Fsk), inhibition (CFTR inhibitor 172; CFTR Inh), and lastly, activation of CaCCs (UTP). The change in Isc after forskolin addition was measured as the CFTR activation. The reduction in Isc after CFTRinh-172 addition was measured as the CFTR inhibition. **(B)** CFTR activation and inhibition were measured at passages (P) 3 - 5, 7, and 9 in PneumaCult™-NGEx (mean ± SD; n = 1, non-CF donor). In PneumaCult™-NGEx, CFTR function gradually decreased with passaging, but remained quantifiable at later passages, notably with no significant differences observed up to P7. **(C)** After expansion in PneumaCult™-NGEx and differentiation in PneumaCult™-ALI, immunofluorescence staining shows the presence of ciliated cell marker Acetylated tubulin (green), goblet cell marker MUC5AC (white), basal cell marker P63 (red) and nuclei marker DAPI (blue). Scale bar = 50 μm. These results demonstrate that PneumaCult™-NGEx preserves normal airway epithelium and CFTR function even in later passage ALI cultures.

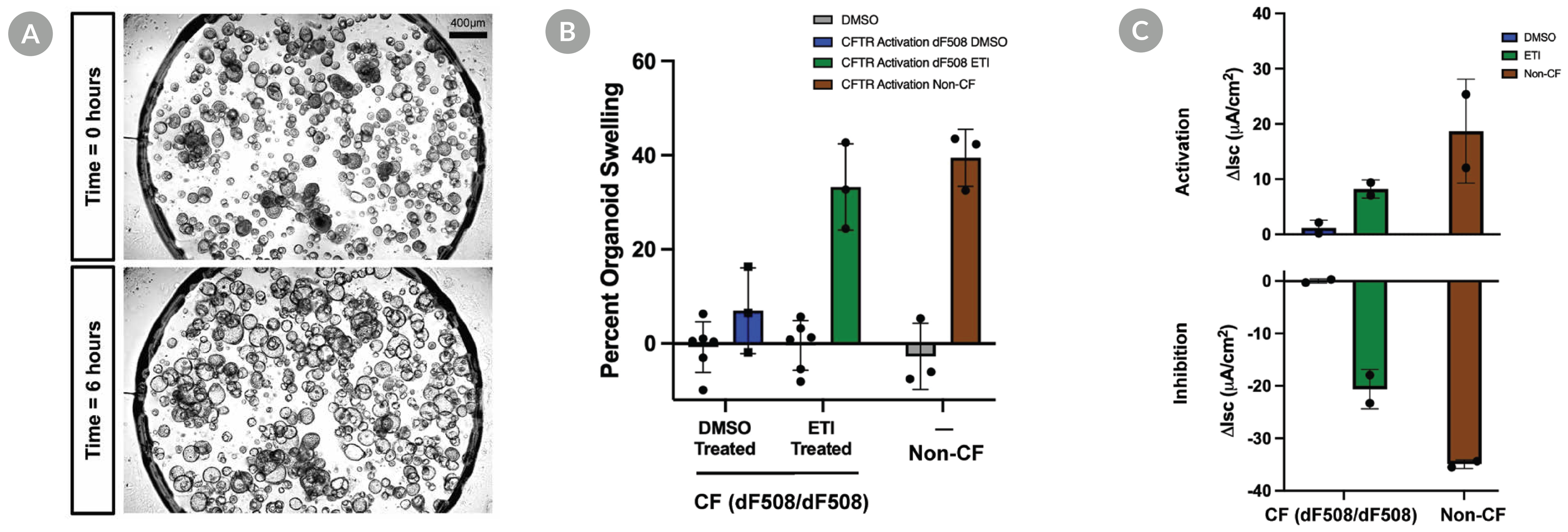


FIGURE 4. 2D and 3D Cultures Derived From CF Nasal Epithelial Cells Display Rescuable CFTR Activity by Electrophysiology and Forskolin-Induced Swelling

(A) Bright-field images show visible swelling of passage 3 nasal airway organoids derived from HNECs expanded in PneumaCult™-NGEx and differentiated in PneumaCult™-Airway Organoid Kit 6 hours after treatment with amiloride, forskolin, and genistein. Scale bar = 400 μm. **(B)** After 6 hours of treatment, CF dF508/dF508 airway organoids corrected with elexacaftor/tezacaftor/ivacaftor (ETI) increased in surface area (mean ± SD, n = 1 donor), and as expected, non-CF airway organoid controls without addition of ETI increased in surface area (n = 1 donor). **(C)** Passage 3 HNEC ALI cultures from the same CF (dF508/dF508) and non-CF donor were used to quantify CFTR activity by short-circuit current (Isc). In the Ussing chamber the following compounds were added to induce ENaC inhibition (Amiloride), followed by CFTR activation (Forskolin), potentiation for modulator treated cultures (Ivacaftor), inhibition (CFTR Inhibitor 172), and lastly, activation of CaCCs (UTP). These results demonstrate that 2D and 3D cultures derived from nasal airway epithelial cells express functional CFTR proteins and are rescuable by CFTR modulators in a CF donor.

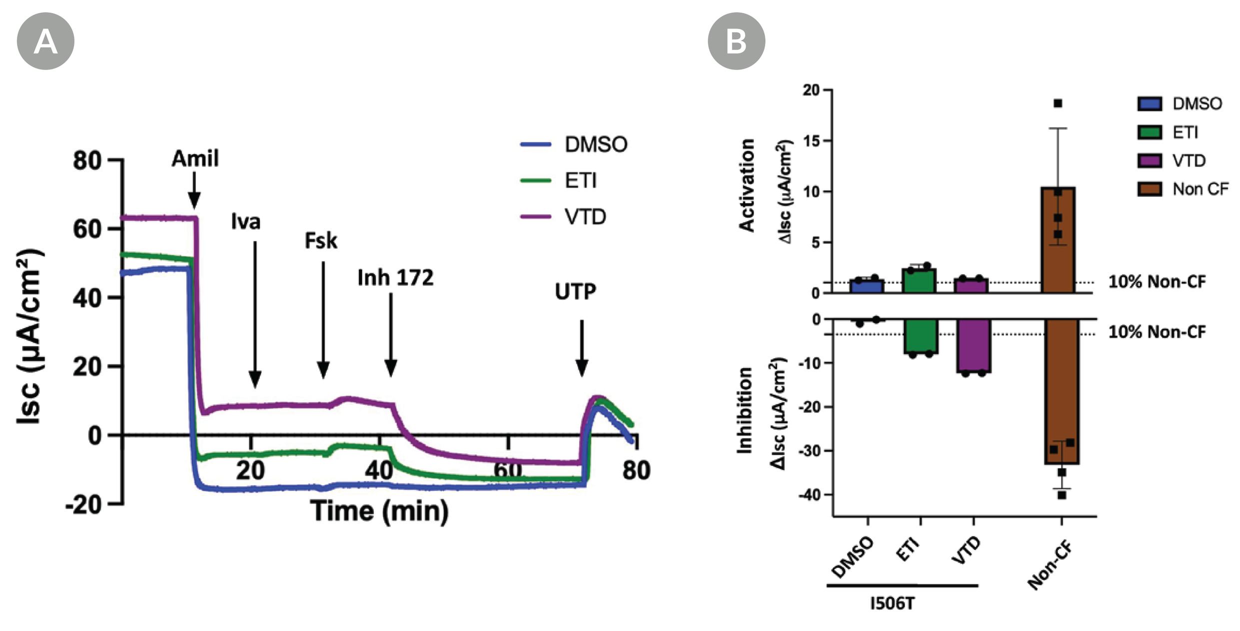


FIGURE 5. Nasal Epithelial Cells Derived from Rare CF donor (I506T/1898+3A>G) Demonstrate CFTR Rescue with CFTR modulators Leading to Clinical Drug Access for Individual

Ion channel activities for ALI cultures at 28 days post-air-lift for rare CF donor (I506T/1898+3A>G) were characterized using HNECs expanded in PneumaCult™-NGEx and differentiated at passage 3. **(A)** Representative CF Using chamber trace for cells treated with DMSO-vehicle control, elexacaftor/tezacaftor/ivacaftor (ETI) or vanzacaftor/tezacaftor/deuterated(D)-ivacaftor (VTD). Arrows 1 - 5 indicate time points at which compounds were added to induce ENaC inhibition (Amiloride), followed by CFTR activation (Forskolin), potentiation (Ivacaftor or Deuterated ivacaftor)inhibition (CFTR Inhibitor 172), and lastly, activation of CaCCs (UTP). **(B)** Quantification of CFTR activation and inhibition for I506T HNECs with CFTR modulator treatments (mean ± SD, n = 2) and comparative non-CF function (n = 4). Using the following data, this individual has been granted access to trial Trikafta™ by the British Columbia Health Ministry (Canada).

SUMMARY

PneumaCult™-NGEx Medium represents a significant advancement in developing robust, scalable, and physiologically relevant nasal epithelial culture models because it:

- Supports extended passaging of HNECs while preserving basal cell morphology.
- Promotes differentiation of HNECs into a pseudostratified epithelium exhibiting appropriate functional marker expression under air–liquid interface conditions.
- Maintains CFTR channel activity in ALI cultures, even at later passages.
- Drives robust organoid formation of HNECs and is compatible with forskolin-induced swelling assay.
- Provides high quality data that can be used to advocate for drug access.

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