

Table 1: Previous studies that compared different commercial differentiation media

Publications	Expansion by CR	Differentiation Media	Bronchial/ Nasal/Tracheal Cultures	Thickness of cultures	Resistance	Cilia density	Cilia beat frequency	Ion channel function	Cell morphology (Cell size)	Mucosecretory markers (MUC5AC, goblet cells)	Virus infectivity	Inflammatory chemokines
Current study	Yes (F media)	AB-ALI vs PN-ALI	Bronchial (adult)	Thicker in PN-ALI	Lower in PN-ALI	Higher density and FOXJ1 expression in PN-ALI	Higher in AB-ALI	Similar CFTR currents, higher CaCC currents	Smaller, more tightly packed cells in PN-ALI	Higher MUC5AC, but not significant in PN-ALI	More RSV infected cells in PN-ALI but not significant	Similar in both media
Livnat et al., 2023 (14)	No	MEM USG-ALI ALI from UNC PN-ALI	CFBE41o Cells (bronchial) stably expressing cell line	-	-	-	-	Higher wt-CFTR currents in MEM media.	-	-	-	-
Luengen et al., 2020 (7)	No	mAir, PN-ALI, Epi, EMM	Nasal (donors between 10-40 years old)	-	Measured but not mentioned which media had better resistance	Increased ciliation in mAir media. Lower in PN-ALI and EMM	-	-	-	Higher MUC5AC in mAir cultures. PN-ALI, Epi and EMM cultures were negative for MUC5AC	-	-
Leung et al., 2020 (12)	No (BEBM - Lonza)	Comparison of AB-ALI vs PN-ALI	Bronchial (adult)	Thicker in PN-ALI	Lower in PN-ALI	Higher in PN-ALI	Similar in both media	-	-	Higher MUC5AC in PN-ALI	-	-
Broadbent et al., 2020 (11)	No	Promocell vs PN-ALI media	Nasal (paediatric)	-	Similar in both media	Increased ciliated cells in PN-ALI but not significant	-	-	Smaller, more tightly packed cells in PN-ALI	Similar MUC5AC in both media	More RSV infected cells in PN-ALI	-
Saint-Criq et al., 2020(13)	Yes (F media)	Comparison of AB-ALI vs PN-ALI	Bronchial (adult)	Thicker in PN-ALI	Lower in PN-ALI	Higher in PN-ALI	-	Higher CFTR and baseline currents in PN-ALI	-	No difference in MUC5AC across both media	-	-
Ruiz García et al., 2019 (10)	No (BEBM - Lonza)	Comparison of AB-ALI vs PN-ALI	Nasal (adult)	-	-	Higher in PN-ALI	-	-	-	No detection of MUC5AC or SCGB1A1 in AB-ALI	-	-
Lee et al., 2019 (22)	Yes (F media)	Comparison of BEGM, PromoCell, LHC-8 and PN-ALI	Nasal (adult)	-	Lower in PN-ALI	Higher in BEGM	Lower in PN-ALI	-	-	Higher MUC5AC expression in PN-ALI	-	-

MEM: minimum essential media, **USG:** Ultrosor-G (TM), **PN-ALI:** PneumaCult ALI, **50:50 BEBM and DMEM:** AB-ALI, **BEGM:** bronchial epithelial growth medium, **BEBM:** bronchial epithelial cell growth basal medium, **F media:**, **CaCC:** Calcium activated currents, **AECGM:** Promocell, **CR:** conditionally reprogrammed culture # PN-Explus media to apical and basal side for initial 4 days on transwells, **mAir:** modified AECGM, **Epi:** MucilAir culture medium, **EMM:** EGM2-MucilAir-mixture, **wt-CFTR:** wild type CFTR, **MUC5AC:** Mucin 5AC.