Delayed airway epithelial repair is correlated with airway obstruction in young adults born very preterm

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To the Editor:

We have previously reported a reduced epithelial repair capacity in infant survivors of preterm birth [1]. However, it is not known if this repair defect persists beyond infancy, or whether this has any correlation to respiratory function. Given the persistent and progressive lung disease typical after preterm birth [2], and the finding of delayed repair in infancy, we hypothesised that dysregulated epithelial repair remains a feature of preterm-associated lung disease across the life-course and is an underlying driver of disease pathogenesis. This pilot study specifically tested whether a repair defect is evident in the nasal airway epithelium of young adult survivors of very preterm birth and if repair defects are correlated with lung function in this population.

All participants attended a research visit as part of the Western Australian Lung Health in Prematurity (WALHIP) 19 year follow up [2, 3]. Primary nasal airway epithelial cells were collected from a subset of young adults born at term or very preterm (⩽32 weeks gestation between 1997–2003) *via* brushing of the nasopharyngeal cavity. Bronchopulmonary dysplasia (BPD) was defined as requiring a minimum of 28 days supplemental oxygen as assessed at 36 weeks postmenstrual age [4]. Pulmonary function testing was performed according to American Thoracic Society/European Respiratory Society recommendations [5, 6] and computed tomography imaging scored *via* the criteria described by Aukland *et al.* [7] as detailed in the cohort study [3]. Written informed consent and human ethics approval was obtained for both epithelial and lung function studies (Child and Adolescent Health Service of Western Australia #RGS815, Curtin University #HRE2021-0489).

Primary cells were seeded in fibronectin-coated flasks with irradiated NIH-3T3 feeder cells and conditionally reprogrammed using the rho-associated protein kinase inhibitor Y-27632 (Enzo Life Sciences, Farmingdale, NY, USA) as previously described [8]. Cells were maintained under atmospheric conditions of 37 °C, 5% CO_2 and 95% air. Once confluent, cells were passaged into 96-well image lock plates (Sartorius, Göttingen, Germany) using media free from growth additives. After 24 h, cells were mechanically scratch-wounded (Essen Woundmaker, Essen BioScience, Ann Arbor, USA) and repair tracked using a live-cell imaging system (Incucyte Zoom, Essen BioScience, Ann Arbor, USA). The Incucyte Scratch Wound Analysis software module was used to mask the resulting images and provide the wound confluence for each timepoint.

Statistical analysis was performed using IBM SPSS version 27.01. Differences in demographic data were assessed using chi-square tests for categorical variables and either independent t-tests or Mann–Whitney U-tests for continuous variables, as appropriate to the distribution of data. Correlation between repair and clinical factors was assessed using either Pearson correlation or Spearman's rho correlation as appropriate. Statistical significance for all tests was set at $p<0.05$.

Nasal epithelial brushings were collected from six term and 29 preterm participants at a mean±SD age of 19.2±1.7 years. Monolayer cultures were successfully established from four term (67%) and 15 preterm (52%) samples. Starting cell counts were not different between the successful and unsuccessful cultures (239 500±143 500 *versus* 272 500±159 000 cells). Scratch wounding was successfully performed on all but two established cultures, which failed to survive once growth additives were removed in preparation for

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Nasal epithelial cells from young adults with a history of very preterm birth show delayed closure following scratch-wounding. Repair correlated with lung function, suggesting epithelial barrier integrity may play a role in preterm-associated lung disease. https://bit.ly/4dJnvWO

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TABLE 1 Association between clinical characteristics and epithelial wound closure at 72 h

	Cohort analysis			Repair analysis	
	Term	Preterm			
	Successful	Wounded	Not wounded	Correlation or mean difference[#]	Significance level
Visit demographics					
N (male)	4 (1)	13 (10)	16 (8)	−30.16 (−53.67, −6.65)	0.015*
Age at bushing (years)	20.97 (1.4) (20.02, 21.59)	19.56 (2.74) (17.54, 22.14)	18.07 (1.59) (17.06, 23.79)	−0.058 (−0.536, 0.447)	0.824
Height (cm)	177.5±8.5 (166.7, 187.5)	169.2±7.60 (155.5, 180.3)	169.4±11.77 (148.9, 198.2)	0.235 (−0.284, 0.639)	0.364
Weight (kg)	68.9 (24.3) (47.0, 79.30)	63.0 (15.05) (47.8, 79.0)	64.50 (18.75) (48.6, 180.0)	0.263 (−0.264, 0.669)	0.308
Perinatal data					
Gestational age (weeks)		27.3±2.6 (23.0, 30.0)	27.2±2.4 (24.0, 30.7)	0.235 (−0.372, 0.691)	0.440
n (%) Bronchopulmonary dysplasia		6 (46)	11 (64)	−25.6 (−57.97, 6.82)	0.110
Birthweight (z-score)		−0.09±0.87 (−2.05, 1.00)	−0.10±0.91 (−1.90, 1.32)	0.170 (−0.426, 0.655)	0.579
Mechanical ventilation (days)		4.25 (39.15) (0, 67)	3.04 (15.28) (0, 73)	−0.034 (−0.758, 0.276)	0.255
Supplemental oxygen (days)		27.0 (87.5) (0, 142)	46.0 (88.75) (0, 154)	−0.035 (−0.763, 0.267)	0.243
n (%) received postnatal steroids		5 (38)	3 (19)	−27.57 (−60.30, 5.13)	0.090
Pulmonary function and imaging					
FEV_1 (z-score)	−0.01±0.57 (−0.70, 0.70)	−0.28±0.94 (−1.85, 1.12)	−0.86±1.51 (−3.99, 1.26)	0.630 (0.175, 0.852)	0.009*
FVC (z-score)	−0.01±0.46 (−0.33, 0.64)	0.41±0.95 (−0.87, 2.16)	0.21±1.12 (−1.75, 2.20)	0.111 (−0.410, 0.573)	0.682
FEV_1/FVC (z-score)	0.05±1.4 (−1.94, 1.34)	−1.00±0.99 (−2.58, 0.93)	−1.46±1.25 (−3.92, 0.66)	0.500 (−0.011, 0.792)	0.049*
n (%) with bronchodilator response	0 (0)	2 (15)	4 (25)	−36.53 (−80.81, 7.74)	0.097
F_{ENO} (ppb)	25.4±19.3 (11, 53.5)	23.5±14.9 (5.5, 50.0)	20.8±13.0 (6.5, 53.5)	−0.147 (−0.596, 0.380)	0.586
Aukland CT score	1.5 (3.75) (0, 4)	5 (6.5) (0, 14)	2.5 (5.5) (0, 19)	−0.498 (−0.795, −0.008)	0.042*
n (%) with bronchial wall thickening on CT	0 (0)	1 (8)	4 (25)		
Questionnaire data					
Total no. hospitalisations	0 (0) (0, 0)	0 (1) (0, 10)	1 (2) (0, 10)	−0.677 (−0.911, −0.109)	0.022*

Clinical data is provided for the term cohort and both the successful and unsuccessful preterm cohorts. Cohort data is presented as mean±SD (range) for normally distributed variables or as median (interquartile range) (range) for non-parametric variables. Correlation coefficients were calculated to % repair at 72 h using either Pearson or Spearman's rho correlation as appropriate. Independent t-tests were used to assess if categorical variables altered the average repair. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; F_{ENO}: exhaled nitric oxide fraction; CT: computed tomography; *: $p<0.05$; [#]: Mean difference was provided for non-continuous variables (BPD diagnosis, male sex, postnatal steroids and bronchodilator response).

wounding (despite multiple attempts). Of note, both samples were from preterm born participants with a history of BPD and spirometry parameters below the lower limit of normal. Overall, the mean forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC) ratio was half a z-score higher in the successfully wounded preterm group compared to those that were not successfully wounded (−1.00±0.99 *versus* −1.53±1.15).

Nasal epithelial cells from term participants achieved full repair (99±2% wound confluence) within 72 h of initial wounding. In contrast, epithelial repair at 72 h was significantly reduced in adults born preterm (71±28% repair, p<0.01). Repair varied widely within the preterm cohort (range: 28–100% at 72 h) and was lower in males compared to females (mean difference of 30%; p=0.015). Repair was at least 25% lower in those with BPD, a positive response to bronchodilator and those receiving steroids in the neonatal intensive care unit, however, these predictors failed to reach significance (table 1). No other neonatal factors correlated with repair; however the low culture success meant that the study was only powered to detect a large effect size (0.75, G*Power). Reduced repair of the airway epithelium was associated with lower lung function in young adult survivors of very preterm birth. Both the FEV_1 z-score (r=0.630; p<0.01) and the FEV_1/FVC z-score (r=0.500; p<0.05) positively correlated to the extent of wound closure. A higher number of self-reported respiratory hospitalisations were also associated with a lower repair capacity (r= −0.677; p<0.05), as was the overall CT score (r= −0.498, p=<0.05).

Our study is the first to show that adult survivors of very preterm birth have an airway epithelial wound repair defect, with wound closure rates positively correlated to FEV_1 and the FEV_1/FVC. Aberrant epithelial repair is thought to be a key pathophysiological feature of airway remodelling [9, 10]. The initial injury and dysregulated repair process can trigger exaggerated production of inflammatory cytokines, leading to collateral tissue damage which promotes the release of pro-remodelling factors, such as TGF-β [11, 12]. Dysregulated repair consequently triggers a negative feedback cycle of injury, inflammation and repair, leading to permanent structural changes in the airway and, ultimately, reduced lung function.

Clinical observations support the hypothesis of structural epithelial abnormalities in those with preterm-associated lung disease. Bronchial wall thickening, which typically occurs in conjunction with epithelial remodelling, is more evident in preterm-born children with reduced spirometry trajectories [2]. The overall CT score in adults born preterm, which includes bronchial wall thickening, negatively correlates to measures of airway obstruction [13]. Airway remodelling observed on CT correlates with both FEV_1 and FEV_1/FVC ratio, but not FVC [14], similar to the relationship observed in this study between epithelial repair and lung function.

In this study, numerous cultures obtained from individuals with clinically low pulmonary function failed to establish or expand, biasing the results away from those with more severe respiratory disease. Given the speed of epithelial wound closure is similarly correlated with the degree of airway obstruction in individuals with COPD [15], we speculate that the epithelium may be further disrupted in those preterm-born individuals with more severe lung function deficits. The consequent low sample size limited the ability for comprehensive analysis into the clinical and mechanistic factors driving delayed repair.

In conclusion, a dysregulated nasal epithelial repair process was observed in adults that were born preterm, suggesting preterm birth and its associated confounders may impact the epithelial barrier beyond the acute neonatal period. A more pronounced repair defect was observed in those with reduced lung function, indicating that poor airway repair may underpin preterm-associated lung disease. Whilst additional work is needed to confirm the relationship between epithelial abnormalities and respiratory outcomes in individuals born preterm, these preliminary data provide an intriguing foundation for future research. Reductions in the strength of the epithelial barrier create vulnerability to infection and injury, perpetuating a cycle that can lead to epithelial remodelling and negatively impact overall lung health. We speculate that early intervention to strengthen the epithelial barrier could improve life-long respiratory outcomes for survivors of preterm birth. Future work in a larger cohort should investigate which mechanisms and pathways are driving the reduced epithelial integrity, with the aim to identify potential therapeutic targets.

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