Comparing the influence of different culture media compositions regarding the responsiveness in human nasal epithelial cells

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1. Abstract

Air-liquid-interface (ALI) cultures are essential for studying respiratory diseases. Previous experiments with human nasal epithelial cells (NECs) showed limited responsiveness to stimulation. This thesis examined how different culture media compositions affect cell response, comparing three conditions: standard PneumaCult medium, a modified PneumaCult medium without hydrocortisone and heparin, and Bronchial Epithelial Cell Growth Medium (BEGM). During the differentiation phase, the cell cultures were characterized in detail using several parameters. In addition to recording the cell morphology and determining the transepithelial electrical resistance (TEER), the cilia function was analyzed based on the ciliary beating frequency (CBF) and active area using reflection microscopy and high-speed video microscopy. After stimulation, an LDH assay was used to quantify any cytotoxic effects of stimulation. Quantitative PCR was used to evaluate the expression of various markers as indicators of cellular responsiveness to the stimuli. BEGM cultures showed poor differentiation, lacking cilia and mucus, and had low RNA quality, hindering analysis. PneumaCult, with and without supplements, produced well-differentiated cultures. Removing hydrocortisone and heparin led to slight improvements in some stimulations, notably with LPS, but results varied across donors. No consistent benefit was observed for other stimuli. The findings highlight donor variability and offer directions for future research.

2. Introduction

The ALI model with NECs is used in various research areas and for diagnosing primary ciliary dyskinesia (PCD). Its key feature is exposing the apical cell surface to air while the basal side contacts liquid medium, mimicking human airway conditions and promoting mucociliary differentiation. This in vitro setup reflects the in vivo respiratory tract and may reduce animal testing. [1] However, the model lacks standardization. Variations in cell types and growth media complicate comparisons across studies [2]

For experiments beyond PCD diagnostics, NECs are collected from healthy donors. Previous studies by the research group of Loretta Müller showed unexpectedly weak responses to stimuli, possibly due to the cells being overly robust in the ALI-model. Heparin and hydrocortisone, additives in the PneumaCult-ALI medium, were suspected to reduce responsiveness [3]

3. Aims and leading questions

Aim 1: Establishment of BEGM cultures.

- Does the quality of the cultures differ from the PneumaCult cultures? If yes, how?

Aim 2: Comparison of PneumaCult (Stemcell), PneumaCult without heparin/hydrocortisone and BEGM (Lonza) regarding the influence of the culture media on the nasal epithelial cells in an ALI culture.

- How does the responsiveness of nasal epithelial cells at ALI change when they are cultured with BEGM instead of PneumaCult?
- How does the responsiveness of nasal epithelial cells at ALI change when they are cultured with PneumaCult without added hydrocortisone and heparin and is there a difference between one week and 24-hour removal?

4. Material and Methods

The NECs for the primary cultures were collected from a total of 14 healthy volunteers (no known airway or lung diseases and no current respiratory infections). The cells were cultivated on the ALI inserts for 28 days until maturity. During these 28 days, various measurements were taken regularly to evaluate the quality of the cultures. On day 29 the cells were stimulated with five stimuli (see Table 1).

Stimuli	Dose of infection	Quantity per Insert (apical)
1. PBS+	negative control	20µl
2. Poly I:C (pIC)	1mg/ml	20µl
3. Lipopolysaccharide (LPS)	1µg/ml	20µl
4. Rhinovirus 16 (RV-16)	MOI 4	118.6µl (after one hour 20µl)
5. BronchoVaxom (BV)	500µg/ml	20µl

Table 1: Overview of the different stimuli concentrations - own presentation

After a 24-hour incubation, the cells were lysed using Trizol. Later on, RNA was isolated and converted to cDNA which was used for qPCR.

5. Results

The BEGM cultures were not used for stimulation due to poor quality.

Three out of 48 comparisons showed significance. One significant difference is the higher TLR 3 expression in the PC+[7d] group compared to the PC-[7d] group under BV stimulation. The other two (shown in Figure 1) are a higher release of MUC5 and IL6 under LPS stimulation in the PC-[7d] group compared to the PC+[7d] group.

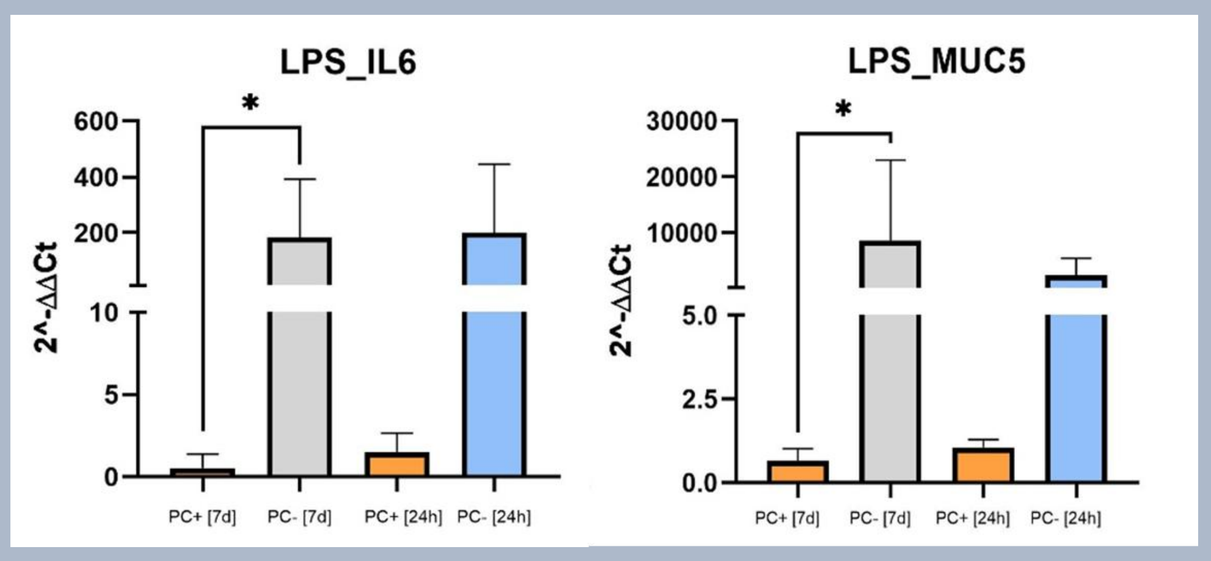

Figure 1: Comparison of IL6 and MUC5 release among the 7d-removal group and 24h-removal group. *p-value <0.05

6. Discussion

To assess the impact of hydrocortisone and heparin removal from PneumaCult medium, cultures were compared after 7-day and 24-hour withdrawal periods. No significant differences in culture quality was observed across both conditions. A slight increase in ciliary activity after 24-hour removal may suggest a short-term cellular response, but further validation is needed. Gene expression analysis showed only three significant results out of 48 comparisons, with several non-significant trends. A high donor variability and some non-responders to the stimuli complicated a clear outcome. The small sample size further limited statistical power.

Overall, while heparin and hydrocortisone removal may enhance responsiveness in specific donors (e.g. LPS stimulation), no consistent effect was observed. Differences between the 7-day and 24-hour removal strategies remain inconclusive.

References

[1] Müller, personal communication, 6th January 2025
[2] Silva et al., 2023, p. 2-3
[3] Stemcell Technologies, 2019

Figures

Figure 1: own presentation, statistical comparison

Tables

Table 1: own presentation, overview stimuli concentrations