Comparing the establishment of Primary Human Nasal Epithelial cells on BETA/CIVIC membranes and PET membranes

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1. Abstract

This study examined whether Primary Human Nasal Epithelial Cells (PHNECs) can be successfully cultured and differentiated on BETA/CIVIC membranes in comparison with the widely established PET membranes. The barrier properties of the epithelial layers were assessed by transepithelial electrical resistance (TEER) measurements, and cell morphology was observed microscopically. PET membranes consistently supported stable epithelial growth, producing high TEER values and showing well-differentiated, ciliated epithelium [1]. In contrast, BETA membranes displayed strong variability between experiments. Many membranes leaked within a few days, which prevented reliable data collection. Even when precoated with extracellular matrix proteins such as Matrigel or PureCol, the TEER values on BETA membranes remained markedly lower than on PET. These findings demonstrate that while PET membranes provide a robust and reproducible system for PHNEC culture, BETA membranes are not yet sufficiently reliable for routine application, although their biomimetic design may hold promise for future use [2].

2. Introduction

Primary Human Nasal Epithelial Cells are considered a valuable model for respiratory research because they better reflect the complexity of in vivo epithelial function than immortalized cell lines [3,4]. To culture these cells under physiologically relevant conditions, the air–liquid interface (ALI) method is commonly used, as it promotes differentiation into a pseudostratified, ciliated epithelium [1]. PET membranes are currently the standard support for ALI cultures because they offer mechanical stability and allow consistent barrier formation. In contrast, the newly developed BETA/CIVIC membranes are designed to be elastic and biomimetic, with the aim of reproducing the mechanical stretch of lung tissue during breathing [2]. This thesis explored whether PHNECs can adhere, grow, and differentiate on BETA/CIVIC membranes and whether there are measurable differences in TEER values and epithelial morphology compared with the PET standard.

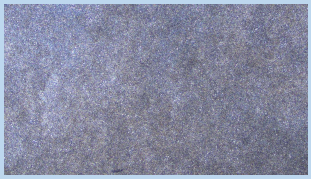


Fig. 1: PHNECs on PET membrane. (Sonderegger, 2025)

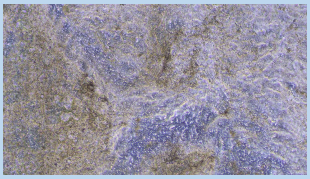


Fig 2. PHNECs on BETA/CIVIC membrane. (Sonderegger, 2025)

3. Aims and leading question

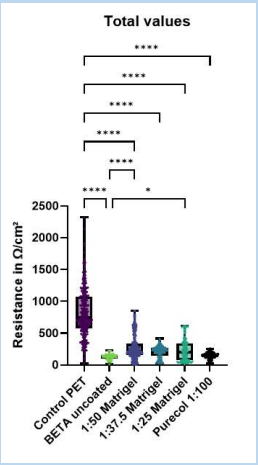
The aim of this project was to compare the ability of PET and BETA/CIVIC membranes to support the culture and differentiation of PHNECs under ALI conditions. The central research question was whether it is possible to culture and differentiate PHNECs on BETA/CIVIC membranes and whether there are differences in TEER values and cell morphology compared with the commonly used PET membranes.

4. Methods and material

PHNECs were obtained by nasal brushings from healthy, non-smoking donors and expanded in PneumaCult-Ex Plus medium. Cells were then transferred either to PET membranes, which served as the control, or to BETA/CIVIC membranes that were used either uncoated or precoated with Matrigel or PureCol. After reaching confluency, cultures were exposed to air–liquid interface conditions using PneumaCult-ALI medium to induce differentiation. TEER values were measured daily over a period of twenty days in order to monitor the integrity of the epithelial barrier. Cell morphology and differentiation were observed macroscopically through mucus production and microscopically by evaluating ciliary beating. In addition, immunofluorescence staining with tubulin, MUC5b, phalloidin and DAPI was applied to visualize cilia, mucus production, actin cytoskeleton and nuclei.

5. Results

PHNECs cultured on PET membranes showed reproducible attachment and growth. TEER values on PET membranes consistently reached high levels, often exceeding 800 Ω/cm² and in some cases surpassing 1500 Ω/cm². These results reflected the development of a tight epithelial barrier. Microscopically, PET cultures exhibited a pseudostratified epithelium with active ciliated cells and mucus production, consistent with successful differentiation. In contrast, BETA membranes produced much lower TEER values, generally in the range of 100 to 300 Ω/cm², and almost half of the membranes failed due to leakage during the culture period. Although Matrigel or PureCol coatings slightly improved adhesion and resulted in somewhat higher TEER values, these modifications did not eliminate the problem of variability and leakage. Morphologically, BETA membranes showed less consistent differentiation, and while some ciliated cells were observed, complete epithelial development was not as robust as on PET.



Graph 1 Total TEER values for every condition. (Sonderegger, 2025)

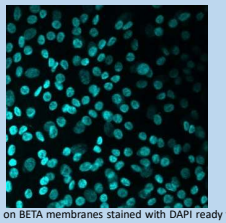


Fig. 3 Nuclei on BETA membranes stained with DAPI ready to use. (Sonderegger, 2025)

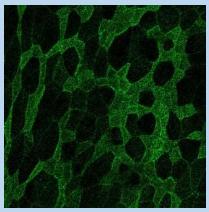


Fig. 4 Cytoskeleton on BETA membrane stained with Phalloidin 488. (Sonderegger, 2025)

6. Discussion

The findings of this project show that PET membranes remain the most reliable support for PHNEC cultures under ALI conditions. They allow reproducible epithelial growth, strong barrier formation as reflected by high TEER values, and the development of physiologically relevant epithelial morphology. In comparison, BETA membranes showed considerable variability and a high rate of failure due to leakage, which significantly limited their usefulness. Even though precoating with extracellular matrix proteins could partly improve their performance, the results remained inconsistent. Nonetheless, the study demonstrated that PHNECs can grow to some extent on BETA membranes, which confirms the basic feasibility of their use. However, before these membranes can be applied in research or diagnostics, their production process must be standardized to reduce variability and leakage. Until then, PET membranes remain the dependable choice for generating stable and differentiated PHNEC cultures.

References

[1] Brocke et al., 2024
[2] Doryab et al., 2021
[3] Hussain et al., 2014, Bukowy-Bieryłło, 2021

Figures

Fig 1. PHNECs on PET membrane. (Sonderegger, 2025)
Fig 2. PHNECs on BETA/CIVIC membrane. K. Sonderegger (2025)
Fig. 3 Nuclei on BETA membranes stained with DAPI ready to use. (Sonderegger, 2025)
Fig. 4 Cytoskeleton on BETA membrane stained with Phalloidin 488. (Sonderegger, 2025)

Graph

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