

Optimization of RPMI 2650 Cells as a Model for Nasal Mucosa

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INTRODUCTION

In the past few years, a human nasal epithelial cell line derived from septum carcinoma (RPMI 2650) has been proposed as a potential *in vitro* model for screening nasally delivered drugs [1-3]. However, these studies have left some unanswered questions in terms of the validation of the *in vitro* model.

In particular, no clear agreement was found with respect to several parameters, such as the seeding density, the time for switching cell culture from Liquid Covered Culture (LCC) to air liquid interface (ALI) conditions, or the day at which cell cultures have to be used for transport experiments, when these cells are cultured *in vitro*.

Hence, the aim of this study was to expand on the previous *in vitro* cell models to better define the fundamental parameters to be used as a tool for studying drug deposition and transport through the nasal mucosa.

METHODS

RPMI 2650, human nasal septum carcinoma derived cells (ATCC, USA) were grown in 75 cm² flasks in complete Minimum Essential Medium (Life Technologies, Australia) containing 10% (v/v) fetal bovine serum, 1% (v/v) non-essential amino acid solution and 2 mM L-glutamine, which was incubated in a humidified atmosphere of 95% air/5% CO₂ at 37°C.

Cells were seeded on Snapwell™ polyester membrane (1.13 cm², 0.4 μm pore size, Corning Costar, USA; coated with 200 μL of 1 μg/mL collagen solution in PBS 24 hours before seeding) at three different seeding densities: 2.5x10⁵, 5.0x10⁵, and 1.0x10⁶ cells/well. After 24 hours, the medium in the apical chamber was removed to switch cells from the LCC to ALI

configuration, in order to develop a differentiated cell layer for further deposition and transport studies. The cells were cultured for 21 days and were characterized for mucus production and tight junction functionality through trans-epithelial electrical resistance (TEER) and permeability of fluorescein sodium (flu-Na).

TEER measurements were recorded every two to three days with an EVOM2 Epithelial Voltometer (WPI, USA) to evaluate the monolayer integrity and suitability for transport studies of the RPMI 2650 cell layers.

Concurrently, permeability studies using flu-Na, a marker of paracellular transport, were performed to evaluate barrier formation and tight junction functionality. Briefly, 250 μL of 2.5 mg/mL flu-Na solution were added on the apical chamber and 200 μL samples were taken from the basal chamber at pre-determined time points. Fresh buffer was added to maintain sink conditions. In addition, the Snapwells were stained according to a previously described method [4] at specific time points to assess the ability of RPMI 2650 for mucus production. Cells were fixed using 4% w/v paraformaldehyde, stained using Alcian blue (1% w/v in acetic acid, 3% v/v pH 2.5) and air-dried. Alcian blue stains mucus glycoproteins allowing the visualization of mucus production when cells are cultured in the ALI configuration. The intensity of the blue color is related with the amount of mucus. Images were taken using an Olympus BX60 microscope equipped with an Olympus DP71 camera.

RESULTS AND DISCUSSION

Culturing conditions to switch from LCC to ALI were evaluated at four different times (1, 2, 4, and 8 days after seeding, respectively). Results showed that RPMI 2650 could be changed from the LCC to the ALI configuration just after 24 hours, since no significant benefits on cell growth was observed from prolonged culture in liquid covered conditions (data not shown).

In order to evaluate the progressive formation of a viable monolayer over time, TEER measurements were performed. Figure 1 shows the profile of TEER values, for each seeding density at different days. TEER values increased steadily from day 1 and reached a plateau at day 15 around 90-100 $\Omega \cdot \text{cm}^2$. These values are similar to TEER previously reported for the same cell line and considered suitable for drug permeation experiments [1, 2]. TEER values slightly decreased at day 21, suggesting a loss of cell viability.

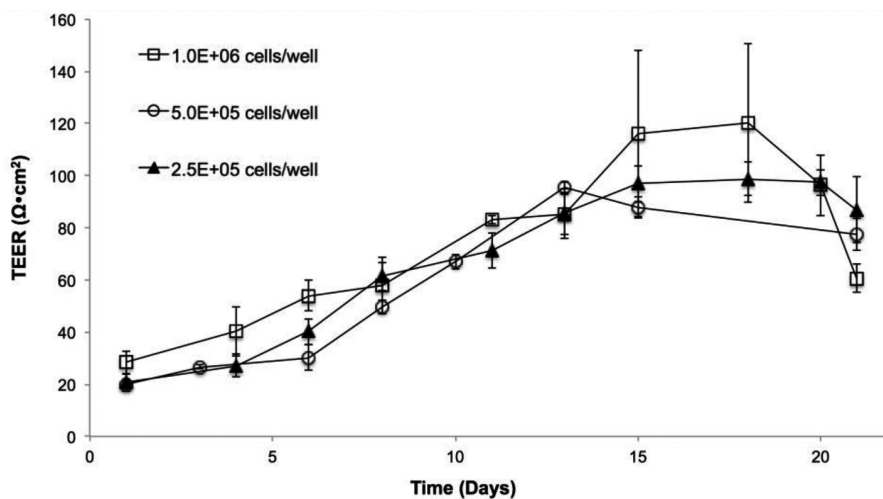


Figure 1. TEER values ($\Omega \cdot \text{cm}^2$) of RPMI2650 grown on Snapwell at different seeding densities ($n = 3, \pm \text{StDev}$).

Permeability studies of the paracellular marker flu-Na were conducted at 1, 2, and 3 weeks after seeding. Figure 2 shows the apparent permeability (P_{app}) calculated for all the three seeding densities. A significant decrease of the flu-Na permeability values was observed after the first week. The P_{app} reached a minimum value and was maintained after two and three weeks of culture, with values nearly halved in comparison to week one. The P_{app} at week two and three were not significantly different ($p > 0.05$), suggesting that the monolayer formed tight junctions and differentiated after two weeks of culture in the ALI configuration. Furthermore, permeability values found in our model were in close agreement with value reported in literature for human nasal mucosa ($3.12 \pm 1.99 \times 10^{-6}$ cm/s) [4], at two and three weeks in culture.

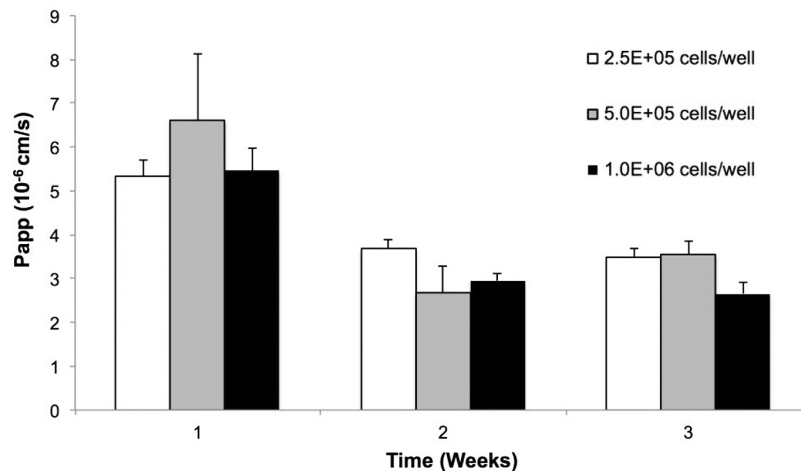


Figure 2. Values of Flu-Na apparent permeability (10^{-6} cm/s) on RPMI 2650 cells grown under air-liquid interface as function of weeks in culture ($n = 3$, \pm StDev).

Since this *in vitro* cell model should be representative of the physiological nasal mucosa, RPMI 2650 grown under ALI were analyzed to assess their ability to secrete mucus, an important aspect not previously investigated. Figure 3 shows the development of a mucus layer on the surface of RPMI 2650 cells during the first two weeks for the intermediate seeding density.

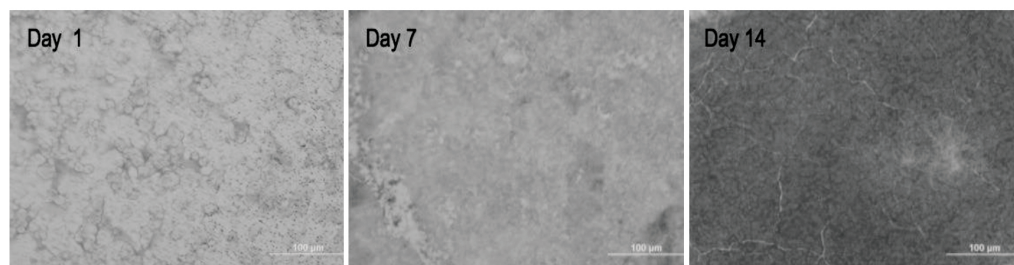


Figure 3. Microscopic images of mucus stained with Alcian blue (dark gray) on the surface of RPMI 2650 nasal cells seeded on Snapwell at 5×10^5 cells/well and cultured in ALI configuration at day 1, 7, and 14.

Only slight differences in mucus production were observed for the three different seeding densities. Qualitative observation of the images suggested that all cells seeding presented a homogeneous mucus layer from the second week of culture onwards.

CONCLUSIONS

This study has shown that RPMI 2650 human nasal carcinoma cells grown on Snapwells represent a promising *in vitro* model for nasal drug delivery studies. These cells grown in air-liquid interface configuration form monolayers with viable tight junctions and secrete a homogeneous layer of mucus after two weeks in culture. For the range evaluated, seeding densities appear to have a limited impact on these properties. Further studies, i.e., drug permeation and expression of transporter proteins, are required to complete the validation of this *in vitro* model before it can be used as a screening tool for nasally delivered formulations.

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