An HTS-Compatible Plate For Highly Miniaturized Cultures Of Primary Human Bronchial Epithelial Cells At Air-Liquid Interface

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Overview

Primary human bronchial epithelial cells (HBE) cultured at air liquid interface (ALI) exhibit striking similarity to the in vivo situation, including both tissue architecture and ion channel functionality. Cultures of this type serve as a gold standard for predicting therapeutic activity in airway diseases such as cystic fibrosis. An obstacle to use of HBE-ALI models for high throughput screening (HTS) has been the severely limited supply of tissue samples from patients with unusual CFTR mutations. We have developed a plate for highly miniaturized, arrayed HBE-ALI cultures suitable for use in automated drug screening and mechanistic studies (µALI Plate). Cells cultured on the µALI Plate exhibit hallmarks of HBE biology including beating cilia and mucus production, but require less than one tenth the number of cells compared to conventional Transwell filters. Cells grown in this manner can be visualized directly from the apical surface by immunohistochemistry without the requirement to image through a filter support. Primary cells grown on the µALI Plate will be useful for testing therapeutic interventions directed towards patients with uncommon CFTR variants. Studies of ion channel function, cilia beat frequency and airway surface liquid depth are currently underway. Among other things, the µALI technology has the potential to facilitate personalized approaches to CF therapy, including drug development.

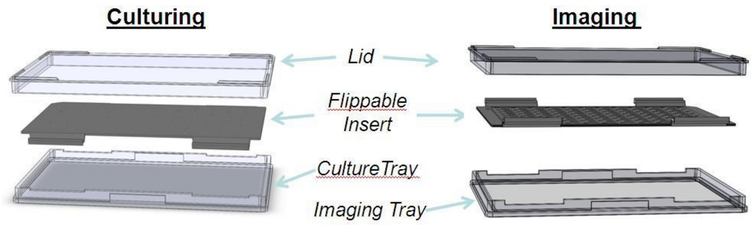

Figure 1: Schematic of the µALI Plate. The µALI Plate consists of a single 96-well flippable insert which allows liquid handling access and contains both the basal and apical sides of the device. The insert can be placed in a culturing tray for cell culture or removed and placed in an imaging tray for high content imaging.

Workflow for Cell Culture and Analysis

	µALI Plate	Transwell- 12mm
Primary Cell Type	NHBE	NHBE
Cell #/well	10,000	200,000
Basal Media Volume	32 µL	1500 µL
Apical Volume	12 µL	500 µL
Media Change Frequency	Daily	Daily
PBS Wash	1/week	1/week
Airlift	Day 3	Day 3
Evaporation Control	2° Plastic Plate	None
Assay Duration	4 weeks	4 weeks
Replicate Value	256 wells	2-wells
Liquid Handling	Automated	Manual
Assay Readout	Cilia β-tubulin ICC	Cilia β-tubulin ICC

Table 1: Experimental Setup. Shown is a comparison of the experimental conditions used to evaluate the BBL ALI plate with a 12-mm Transwell® plate. Differences in experimental conditions are highlighted in orange. Note that the assay conditions were designed conservatively to increase chances of successful differentiation. Cell number, media volume, media change frequency, and assay duration could potentially be decreased to facilitate higher throughput.

µALI Plate Design and Operation

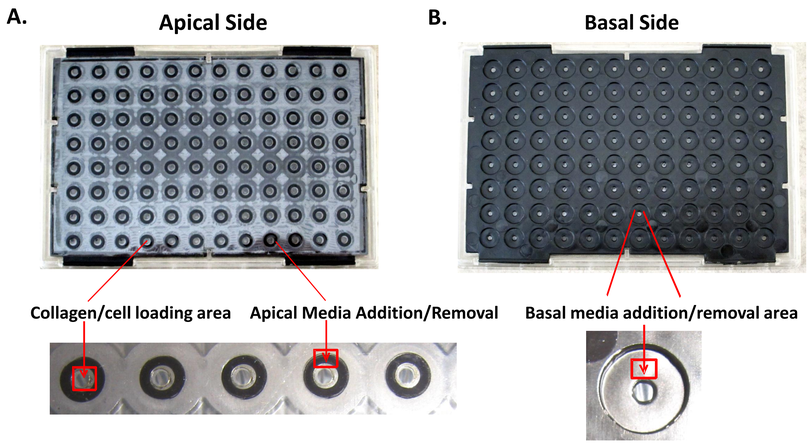

Figure 2: Device operation. A) The apical side consists of a flat surface embedded with a porous membrane located above ports in a standard 96-well array. Collagen and cells are loaded directly onto the apical surface of the membrane. An apical media addition and removal area is located directly adjacent to the membrane. **B)** The insert is flipped to access the basal side of the device. The basal side consists of shallow wells for media, which is retained by surface tension in the inverted position.

Differentiation of Primary Bronchial Epithelial Cells

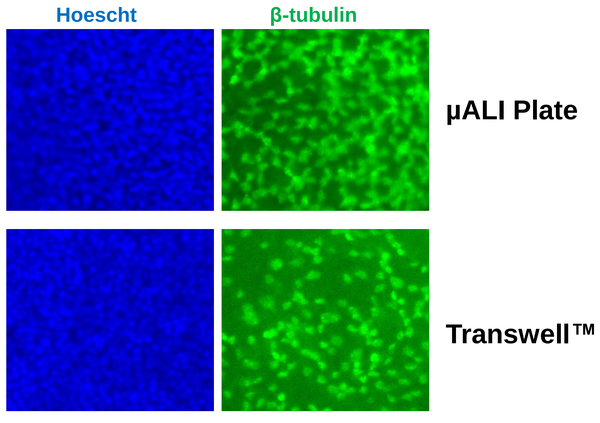

Figure 3: Representative images of NHBE differentiation into cilia. Normal primary human bronchial epithelial cells (CF Center Tissue Procurement and Cell Culture Core at University of North Carolina) were cultured for 4 weeks side by side in the 12-well Transwell® plate and the µALI plate. Shown is nuclear staining with Hoescht and cilia staining with β-tubulin antibody. Beating cilia were also observed and captured with live phase contrast microscopy (data not shown).

Automated High Content Analysis of ALI Cultures

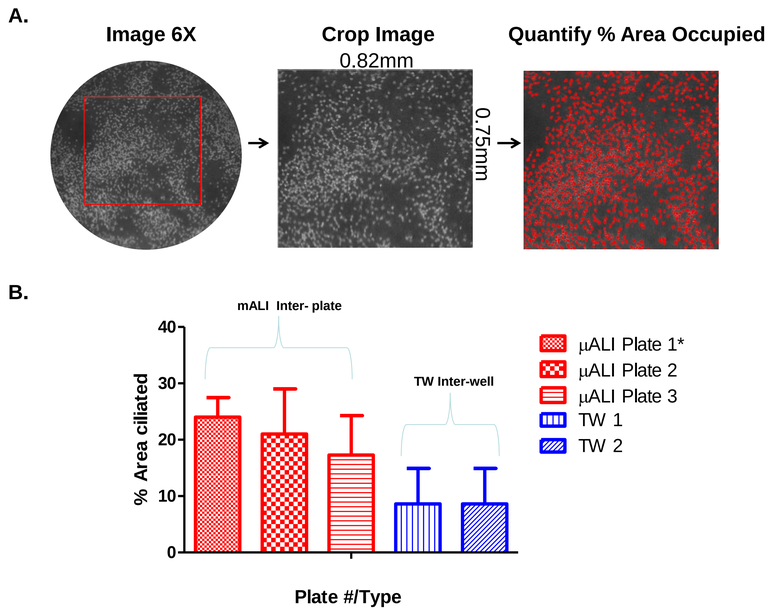

Figure 4. Image analysis. A. Method. Data was analyzed buy acquiring images of cilia stained with β-tubulin antibody using a Nikon Eclipse TE2000U microscope with a 4X objective. For µALI devices, one Image was cropped per well and the % area occupied was quantified using MetaMorph (Molecular Devices) count nuclei algorithm. For Transwell® devices, six images were cropped from the center and edge of each of two replicate wells (not shown). **B. Results.** Shown are the mean and std. dev. of % area of ciliated cells for each plate. Due to the high cell requirement of the Transwell device, only two wells were analyzed. 6 images (3 center and 3 edge) for each Transwell® well were acquired and used to calculate mean and standard deviation of each well.

Conclusions

- A proptotype 96-well µALI plate was developed y that requires no more than 10,000 cells / well and supports differentiation of primary airway epithelial cells
- Differentiation as quantified by cilia formation was found to be at least equal to or above that of the Transwell® cell culture inserts.
- The µALI device supports high content image analysis and automated liquid handling operations.

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