Development of High-Throughput *In Vitro* Human Alveolar Tissue Models Utilizing Novel Electrospun Scaffolds

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[1]PJ Hayden, [1]R Kassab, [1]KC Golden, [2]A Heng, [1]J Chakravarty, [1]NF Long, [1]LM Fitzgerald, [3]L Willetts, [4]GR Gaudette, [1]MD Phaneuf

[1]BioSurfaces, Inc., Ashland, MA, [2]Worcester Polytechnic Institute, Worcester, MA, [3]Corning Inc., Life Sciences, Tewksbury, MA, [4]Boston College, Boston MA

Introduction

Alveolar tissue damage is a hallmark of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus disease 2019 (COVID-19). *In vitro* models of human alveolar air-blood barrier (ABB) tissues are needed for COVID-19 research and therapeutics development. However, high throughput (HTP) formats of these tissue models are lacking. The goal of the current work is to develop HTP human ABB tissue models by adapting novel Bio-Spun™ electrospun scaffolds onto HTS Transwell®-24 and 96 well permeable support plates.

Material/Methods

Scaffold plate fabrication and preparation: Bio-Spun™ biodegradable poly(lactic-co-glycolic acid) (PLGA) scaffolds (BioSurfaces, Inc.) were prepared using solution electrospinning and post-treatment processes which produced scaffolds with thicknesses as low as 6 µm (Figure 1). Scaffolds were bonded to HTS Transwell®-24 and 96 well permeable support plates (Corning Life Sciences) (Figure 1). The scaffolds were pre-wetted for 2 hours at 37°C with DPBS containing 10% FBS and then coated with bovine plasma fibronectin (5 µg/cm^2) overnight at 37°C before use.

Cells and culture medium: Human Pulmonary Microvascular Endothelial Cells (HPMEC) were obtained from Lonza (Walkersville, MD) at passage (P)3 and expanded to P5 before use. Human Alveolar Epithelial Cells (HAEpiC) were obtained from Cell Biologics (Chicago, IL) at P1 and expanded to P3-P11 in epithelial growth medium (ScienCell or Cell Biologics) supplemented with 10 µM Y27632 and 10 µM A83-01 (Tocis) before cryopreservation.

Antibodies/stains: Phalloidin-iFluor 488, Rabbit Anti-ACE2 (ab153480), Mouse Anti-Podoplanin (ab10288), Mouse Anti-CD31 (ab24590), Rabbit Anti-Prosurfactant Protein C (ab90716), Goat Anti-Mouse Alexa Fluor 488 (ab150113) and Goat Anti-Rabbit Alexa Fluor 555 (ab150078) were from Abcam (Cambridge, UK). Rabbit Anti-ABCA3 (PAS-52478) and Anti-ZO-1 (61-7300) were from Invitrogen.

Fluorescence Imaging: Fluorescence images were obtained using an Olympus IX51 fluorescence microscope. Confocal fluorescence imaging was performed on a Leica TCS SP5 Spectral Confocal with a z-galvo stage for z-stacks and 3D-projections using 20X objective. Images were processed with LAS X Core (Leica) and ImageJ (NIH) software.

Transepithelial Electrical Resistance (TEER) Measurement: TEER was measured using a REMS automated TEER instrument (World Precision Instruments, Sarasota, FL).

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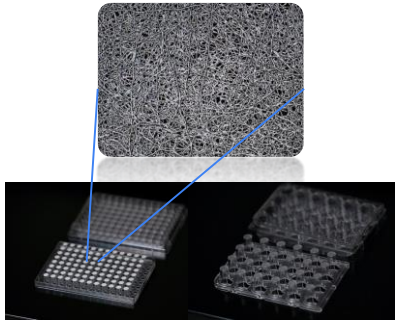


Figure 1. HTS Transwell®-24 and 96 well permeable support plates constructed with BioSpun™ biodegradable poly(lactic-co-glycolic acid) (PLGA) scaffolds. Electrospun PLGA scaffolds utilized in the current experiments were either 6 or 12 µm thickness. Solution electrospinning of PLGA produced randomly-oriented biodegradable fibers that resemble natural *in vivo* extracellular matrix. (50x magnification).

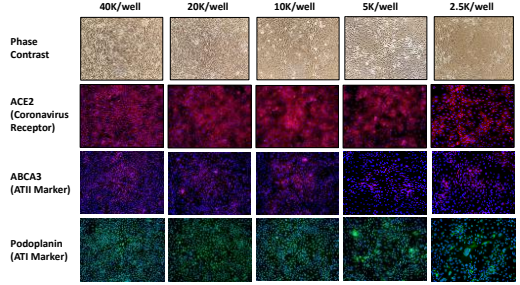


Figure 2. Characterization of Human Alveolar Epithelial Cells (HAEpiC). HAEpiC (P6, final passage without Y-27632 or A83-01) were seeded into standard 24-well tissue culture plates at various seeding densities. The indicated densities represent the number of cells/well of a corresponding 96-well Bio-Spun™ plate. HAEpiC were cultured for 5 days until all of the seeding densities had formed confluent monolayers, prior to fixing in 10% formalin and imaging (10X objective). Phase contrast images of cell morphology show very tightly packed small cells at the highest densities, with progressively increasing size of the cells as the seeding density was decreased. ACE2 (Coronavirus receptor) and ABCA3 (ATII marker) staining also show progressive decrease in staining, while large cells that display bright podoplanin (ATI marker) staining increase as the seeding density was decreased. **These results demonstrate that the HAEpiC cells maintain predominately ATII phenotype and high ACE2 expression when seeded at high densities, and show progressive trans-differentiation to an ATI phenotype and loss of ACE2 expression as the seeding density is deceased.**

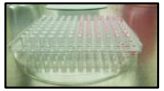


Figure 3. Seeding of Human Pulmonary Microvascular Endothelial Cells (HPMEC). HPMEC (P6) were seeded onto the underside of 96-well Bio-Spun PLGA scaffold plate by applying 25,000 cells/well in a volume of 20 µl. Cells were allowed to adhere for 1 hour at room temperature in a culture hood with the sash closed and the airflow turned off. The plate was then turned upright and placed into a reservoir plate containing 250 µl/well of Human Endothelial Cell Growth Medium (EGM, Cell Biologics). An addition 100 µl of EGM was then added to the apical compartment of the 96-well Bio-Spun™ PLGA scaffold plate.

Figure 4 Seeding of HAEpiC. On day 1 following seeding of HPMEC, HAEpiC were seeded into the apical well compartments of the 96-well Bio-Spun™ PLGA scaffold plate. Plates were cultured under submerged conditions for 5 days before airlifting. A 3D-printed reservoir plate height extender was utilized to allow application of 520 µl/well of culture medium (EGM supplemented with 1 µM dexamesthasone) into the wells of the reservoir plate. Culture medium was aspirated out of the apical well compartments of the 96-well Bio-Spun™ PLGA scaffold plate and the apical scaffold surfaces were left with only a slight film of liquid under airlift conditions.

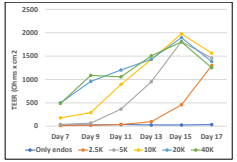
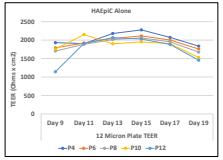


Figure 5. Effect of seeding density on barrier formation of ABB models. Co-culture models containing P6 HPMEC (25,000 cells/well basolateral seeding) and indicated densities of P6 HAEpiC cells (final passage without Y-27632 or A83-01, apical seeding) were cultured for the indicated times on the 96-well Bio-Spun™ PLGA scaffold plate. Barrier development was measured by TEER. **Lower seeding densities of HAEpiC took longer to develop barrier but all densities eventually achieved similar barrier development.**



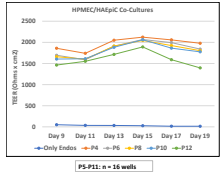


Figure 6. Effect of HAEpiC passage number on barrier formation of ABB models. Cryopreserved HAEpiC from P3, P5, P7, P9 and P11 were thawed and expanded 1 final time without Y-27632 or A83-01 prior to seeding onto 96-well Bio-Spun™ PLGA scaffold plates at 40,000 cells/well. **Results show that all HAEpiC cells passages either alone or in co-culture with HPMEC (25,000 cells/well) on Bio-Spun™ PLGA scaffolds maintain the ability to develop ABB models with very tight and robust alveolar epithelial barriers.**

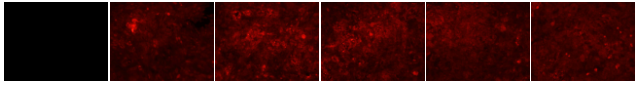


Figure 7. Effect of HAEpiC passage number on expression of ACE2 coronavirus receptor. Cryopreserved HAEpiC from P3, P5, P7, P9 and P11 were thawed an expanded 1 final time without Y-27632 or A83-01 prior to seeding onto 96-well Bio-Spun™ PLGA scaffold plates. HAEpiC (40K cells/well, apical) were cultured on the scaffold plates without HPMEC for 19 days prior to fixation in 10% formalin and immunostaining for ACE2 expression. **Results show that HAEpiC ABB models maintain robust expression of ACE2 coronavirus receptor to at least p12 when cultured on Bio-Spun™ PLGA scaffolds.**

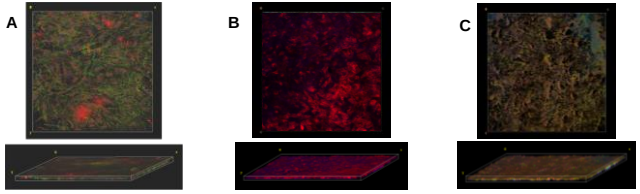


Figure 8. Confocal imaging of: A) HPMEC alone (basolateral seeding); B) HAEpiC alone (apical compartment seeding); and C) HPMEC (basolateral)/HAEpiC (apical) co-cultures. P6 HPMEC (25,000 cells/well and/or HAEpiC (final passage without Y-27632 or A83-01, 40,000 cells/well) were cultured 19 days on 96-well Bio-Spun™ PLGA scaffold plates prior to fixation in 10% formalin and immunostaining. A) CD31 (green); ACE2 (red); Hoechst (blue). B) ZO-1 (red); Hoechst (blue. C) Pro-surfactant protein C (red); CD31 (green); Hoechst (blue). **Results show that HPMEC or HAEpiC each form continuous confluent monolayers when cultured separately on the 12 µm biodegradable Bio-Spun™ PLGA scaffold. However, co-cultures of HPMEC and HAEpiC show complex 3D interactions within the biodegradable scaffold while still maintaining robust barrier function.**

Conclusion

These results indicate that HTP *in vitro* human ABB models produced from primary HPMEC and/or HAEpiC cultured on novel Bio-Spun™ electrospun biodegradable PLGA scaffolds are unique and useful models for SARS-CoV-2/COVID-19 research. These models will likely find utility for wider applications in respiratory infection, toxicology and drug delivery as well.

For further information or to receive a copy of this poster, contact: patrick.hayden@biosurfaces.us