Next-Generation Full-Thickness Human Bronchial Epithelial Model Produced using 3D Electrospun Scaffolds and Animal-Component-Free Culture Media

Jayashree Chakravarty, Elexa M. McFadden, Jamie A. Rangel, Rayan K. Hodroj, Lisa M. Fitzgerald, Jacob T. Le Do, Nathaniel F. Long, Matthew D. Phaneuf, Patrick J. Hayden

BioSurfaces, LLC, Ashland, MA 01721, USA

SOT SAN DIEGO, CA 65th Annual Meeting & ToxExpo MARCH 22-25, 2026

5240/M814

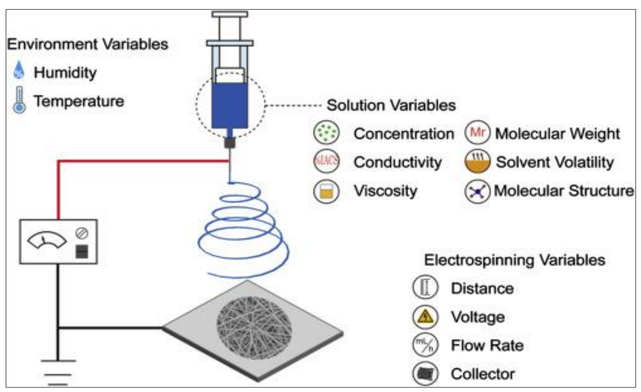

Figure 1. Basic process variables for creating electrospun scaffolds by solution electrospinning. Polymer solutions are dispensed across a high voltage field and collected on a grounded surface. Source: Kurecic, Manja. (2013). Electrospinning: Nanofibre Production Method. Tekstilec. 56. 4-12. 10.14502/Tekstilec2013.56.4-12.

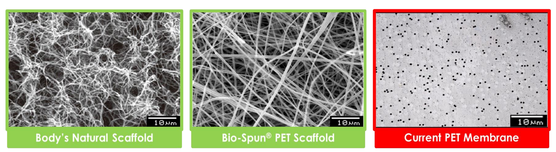

Figure 2. Scanning electron micrograph of *in vivo* extracellular matrix, Bio-Spun® PET scaffold, and a film-based microporous membrane. 3D randomly oriented nanofiber scaffolds are similar to 3D *in vivo* extracellular matrix. The film-based, porous PET membrane is a highly-rigid 2D surface.

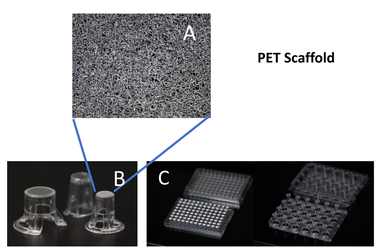

Figure 3. Electrospun scaffold insert products. Bio-Spun® scaffolds (A) are bonded to various sizes of individual inserts (B) and 24-and 96-well HTS plate format components (C). Inserts and plates are shown in the upside-down orientation to highlight the scaffold component. The HTS formats are compatible with robotic plate handlers and individual inserts are compatible with several common organ-on-a-chip fluidic systems.

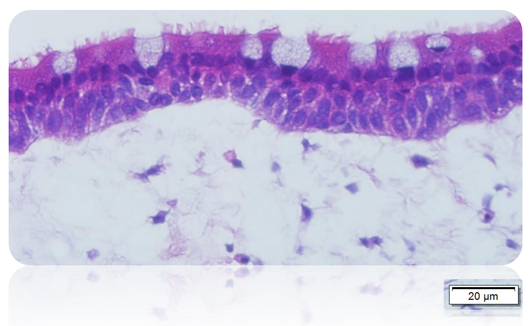

Figure 4. Bio-Spun® FT Bronchial Model

Background

In vitro human bronchial tissue models are important tools for testing environmental pollutants and chemicals, screening of new pharmaceuticals, and human disease modeling research. Stromal-epidermal interactions play a key role in regulation of epithelial proliferation, differentiation, wound healing and barrier function, and are involved in the pathogenesis of aging/photoaging, immune responses and numerous respiratory diseases. Full-thickness skin (FT-Bronchial) models therefore have the potential to provide more comprehensive and *in vivo*-relevant experimental data compared to partial-thickness models. However, these models commonly utilize animal-derived collagen as a main structural element of the stromal matrix. Animal-derived collagen constructs suffer from stability and contraction issues, resulting in a short lifespan and poor reproducibility. Additionally, culture media utilized to produce these models commonly contain undesirable animal-derived components including fetal bovine serum (FBS) and bovine pituitary extract (BPE). To address these shortcomings, we developed full-thickness human bronchial models without animal-derived collagen using electrospun scaffolds as structural components of the stromal constructs, together with FBS/BPE-free culture media formulations.

Methods

Electrospun polyester (Bio-Spun®-PET) scaffolds are composed of randomly-oriented fibers with nm to low µm diameters, similar in structure to native extracellular matrix. The scaffolds were attached to Transwell® inserts in place of typical 2D microporous membrane supports. The electrospun scaffold inserts can be attached to a variety of insert formats including 6-, 12- and 24-well individual Transwell® inserts, as well as 24-well and 96-well Transwell® high throughput screening (HTS) formats. Scaffold thickness can also be customized based on the specific application.

For this study, the stromal components were produced using 24-well inserts with Bio-Spun® PET scaffold (IIC24-200; 150µm thickness; BioSurfaces, LLC) that were seeded with primary human lung fibroblasts (Lifeline Cell Technology) and cultured under submerged conditions in FBS-free medium (Lifeline Cell Technology or Lonza) supplemented with ascorbic acid and TGF-β1. FBS in the fibroblast medium was replaced with human platelet lysate or human serum. Primary human bronchial epithelial cells (Lifeline Cell Technology) were then seeded onto the stromal components. The constructs were cultured at the air-liquid interface (ALI) using an ALI lifter (ALI-LC24; BioSurfaces, LLC) containing FBS/BPE-free ALI differentiation medium (PneumaCult™ ALI, STEMCELL Technologies) to produce the fully-developed 3D organotypic full-thickness bronchial tissue models. Functional tissue barrier was evaluated by measuring transepithelial electrical resistance (TEER). Histochemical (H&E staining) and immunohistochemical staining of formalin-fixed paraffin sections were utilized to evaluate morphological features of the tissue models.

Results

H&E-stained paraffin sections of Bio-Spun® FT-Bronchial models revealed robust stromal components populated with viable fibroblasts. The fibroblasts proliferated within the synthetic scaffolds and synthesized native collagen and extracellular matrix materials that self-assembled *in situ* to produce robust and stable stromal matrices within 4-10 days. Copious amounts of *in situ*-produced stromal extracellular matrix material was evident throughout the scaffold. H&E, alcian blue and immunostained paraffin sections also showed well-developed pseudostratified epithelium by Day 21 of ALI culture, consisting of basal, club, ciliated (a-tubulin) and goblet cells (mucin). A uniform viable epithelium with 3-4 cell layers and TEER of ~200 Ω x cm^2 was maintained out to at least Day 35 after ALI (longest timepoint evaluated to date), providing an extended window of useful downstream experimentation time.

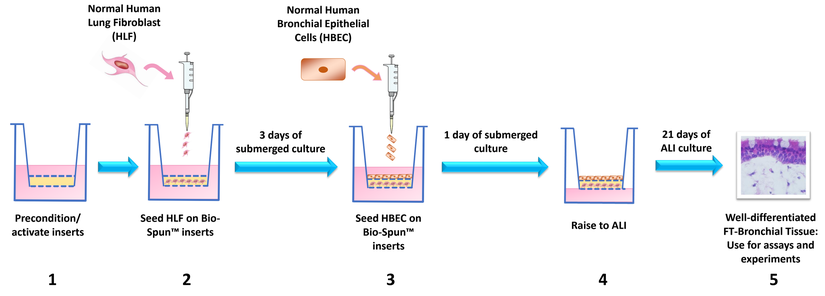

Figure 5. Schematic workflow for Bio-Spun® FT-Bronchial Model Production. 1: Scaffolds are degassed by pre-wetting on Friday. 2: HLF are seeded onto the apical surface of the electrospun scaffold. Sub-epithelial stromal components are fed under submerged conditions for 3 days. 3: HBEC are seeded onto the stromal component and cultured under submerged condition for 1 day. 4: The developing FT-Bronchial tissues are raised to the air-liquid interface (ALI) using an ALI lifter plate and cultured for 21 days. 5: The FT-Bronchial tissues are well-differentiated by day 21 after ALI.

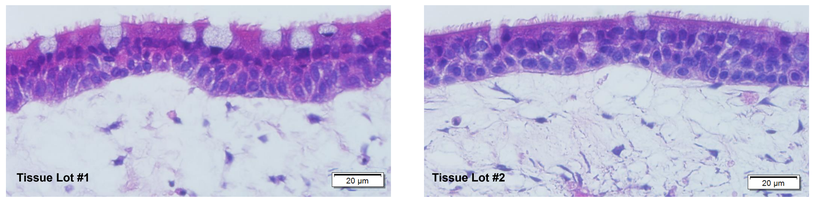

Figure 6. H&E-stained cross section of full-thickness human bronchial model produced on non-degradable Bio-Spun® PET scaffold. The well-differentiated pseudostratified epithelium contains basal, goblet and ciliated cells. Fully human extracellular matrix is secreted by pulmonary fibroblasts.

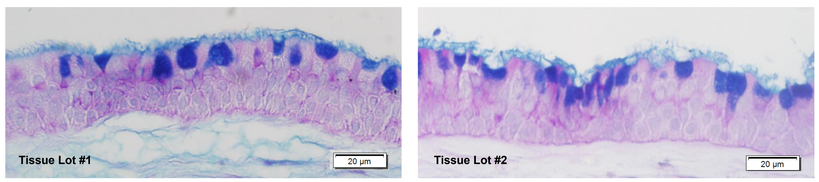

Figure 7. Alcian Blue/PAS-stained cross section of full-thickness human bronchial model produced on non-degradable Bio-Spun® PET scaffold. Purple stain indicates mucins within epithelial goblet cells.

This work was funded, in part, by a SBIR Phase II grant from NIEHS (Grant number R44 ES034681)

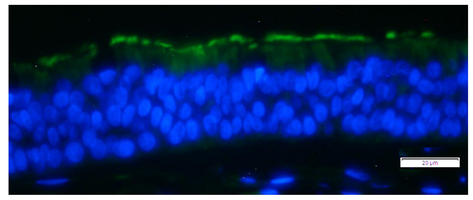

Figure 9. Immunofluorescent staining of full-thickness human bronchial model produced on non-degradable Bio-Spun® PET scaffold. α-tubulin (green) indicates cilia on the apical epithelial surface. Cell nuclei are stained with Hoechst dye (blue).

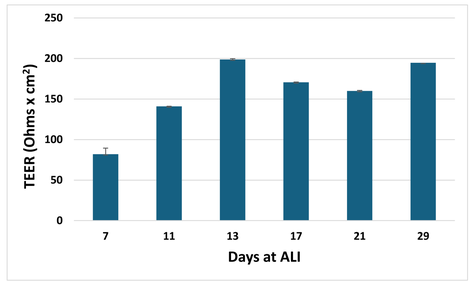

Figure 10. Transepithelial electrical resistance (TEER) of full-thickness human bronchial model produced on non-degradable Bio-Spun® PET scaffold. TEER greater than 150 Ω X cm^2 was obtained by Day 13 and was maintained through Day 29.

Conclusions

- Full-thickness bronchial epithelial models have the potential to provide more comprehensive and *in vivo*-relevant experimental data compared to partial-thickness models.
- Next-generation, *in vitro* full-thickness human bronchial models (Bio-Spun® FT-Bronchial Models) were produced using animal collagen-free 3D electrospun scaffolds and FBS/BPE-free culture media formulations.
- The fully human bronchial models provide long-term stability and do not suffer from contraction and stromal degradation issues.
- The models display a well-developed pseudostratified epithelium, with *in vivo*-like cellular composition, as well as a barrier similar to *in vivo* bronchial tissue.
- These next-generation full-thickness human bronchial models offer promise for completely animal-product-free testing of environmental pollutants and chemicals, tobacco products, screening of new pharmaceuticals and more human-relevant disease modeling.