Next-Generation Open-Source Full-Thickness Human Skin Models Produced using 3D Electrospun Scaffolds and Animal-Component-Free Culture Media

[1]Patrick J. Hayden, [1]Rayan K. Hodroj, [1]Jayashree Chakravarty, [3]Sar R. Lindner, [1]Lisa M. Fitzgerald, [1]Jacob T. Le Do, [2]Vivek Patel, [2]Holger P Behrsing, [1]Nathaniel F. Long, [1]Matthew D. Phaneuf [1]BioSurfaces, LLC, Ashland, MA, USA, [2]Institute for *In Vitro* Sciences, Inc., Gaithersburg, MD, USA, [3]Department of Cell Biology, Harvard Medical School, Boston, MA, USA

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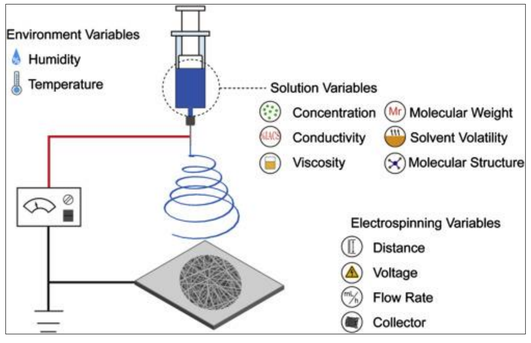


Figure 1. **Basic process variables for creating electrospun scaffolds by solution electrospinning**. Polymer solutions are dispensed across a high voltage field and collected on a grounded surface. Source: Kurecic, Manja. (2013). Electrospinning: Nanofibre Production Method. Tekstilec. 56. 4-12. 10.14502/Tekstilec2013.56.4-12.

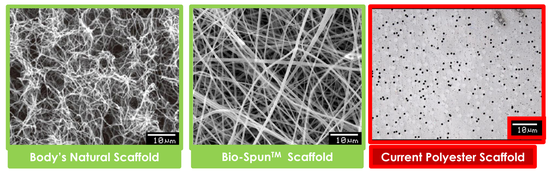


Figure 2. Scanning electron micrograph of *in vivo* extracellular matrix, Bio-Spun™ PET scaffold, and a film-based PET microporous membrane. 3D randomly oriented PET nanofiber scaffolds are similar to 3D *in vivo* extracellular matrix. The film-based PET membrane is a highly rigid 2D surface.

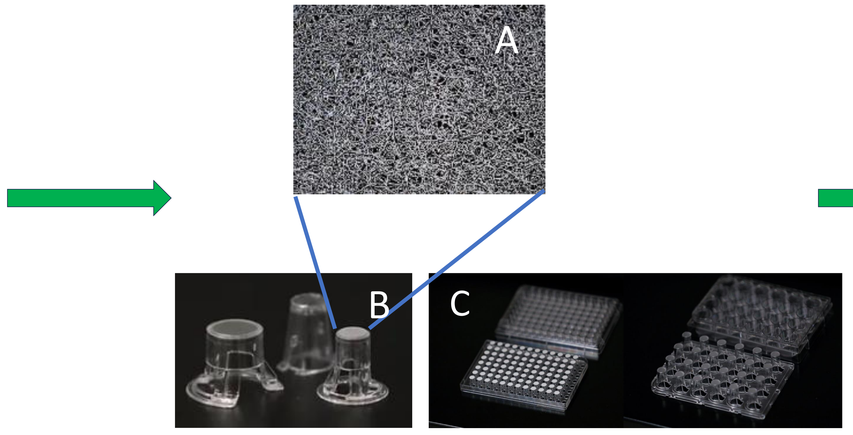


Figure 3. Electrospun scaffold insert products. Bio-Spun™ scaffolds (A) are bonded to various sizes of individual inserts (B) and 24-and 96-well HTS plate format components (C). Inserts and plates are shown in the upside-down orientation to highlight the scaffold component. The HTS formats are compatible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

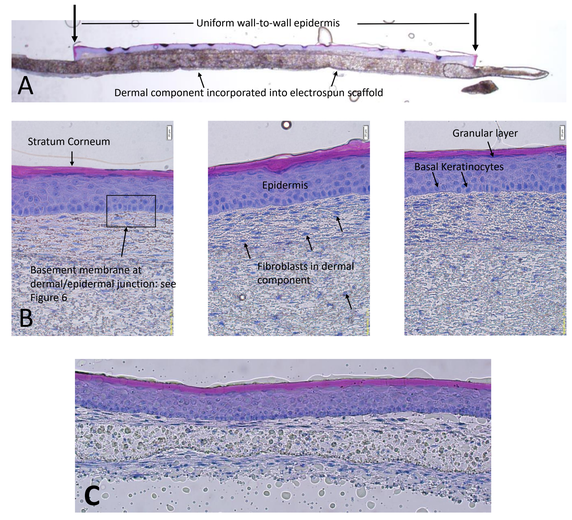


Figure 4. H&E-stained cross section of full-thickness human skin model produced on don-degradable Bio-Spun™ PET and biodegradable PDLGA/PLLA scaffolds. A: Low magnification view showing uniform thickness of the epidermis and wall-to-wall coverage across the entire width of the 6.5 mm insert scaffold with no contraction (7.9X). **B:** High magnification view of PET tissue (252X). **C:** High magnification view of PDLGA/PLLA tissue (252X). The scaffolds are populated with dermal fibroblasts which produce a fully human-derived dermal component without the need for animal collagen. The fully developed stratified epidermis contains basal, spinous, granular keratinocyte layers with a functional stratum corneum.

Abstract

Background and Purpose: *In vitro* full-thickness human skin models are potentially important tools for testing of cosmetics and chemicals, screening of new pharmaceuticals, and human disease modeling research. However, these skin 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 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 skin models without animal-derived collagen using electrospun scaffolds as structural components of the stromal constructs, together with open-source FBS/BPE-free culture media formulations..

Methods: Non-degradable polyester (Bio-Spun™-PET) or biodegradable poly(lactic-co-glycolic acid)/Poly(L-lactide) (Bio-Spun™-PDLGA/PLLA) bilayered electrospun scaffolds are composed of randomly oriented fibers with nanometer to low micron diameters, similar in structure to that of native extracellular matrix. These scaffolds were separately attached to Transwell® inserts in place of the typical microporous 2D 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. The thickness of the scaffolds can also be customized. Scaffolds utilized in the current study were 150 and 100 microns in total thickness, respectively. To produce the dermal component of the models, individual inserts with the respective Bio-Spun™ scaffold (BioSurfaces) were seeded with primary human dermal fibroblasts (Lonza) and cultured under submerged conditions in FBS/BPE-free medium supplemented with ascorbic acid and TGF-β1. FBS in the fibroblast medium was replaced with human platelet lysate (HPL). Primary human epidermal keratinocytes (Lonza or ThermoFisher) were then seeded onto the stromal components. Following 3 days of submerged culture in FBS-free epidermal medium (open-source, contact BioSurfaces for detailed formulation), the constructs were cultured at the air-liquid interface (ALI) in FBS-free ALI epidermal differentiation medium (open-source, contact BioSurfaces for detailed formulation) to produce fully developed 3D organotypic full-thickness epidermal tissue models. Histochemical (H&E staining) and immunohistochemical staining of formalin fixed paraffin sections, and transmission electron microscopy (TEM) were utilized to evaluate morphological and ultrastructural features of the tissue models. Baseline tissue viability was determined by WST-8 assay. Functional tissue barrier was evaluated by measuring resistance to Triton X-100 penetration using a time-to-toxicity (ET50) assay.

Results: H&E-stained paraffin sections 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 6 days. Copious amounts of *in situ* produced stromal extracellular matrix material was evident throughout the scaffold. Immunohistochemical staining revealed uniform collagen I deposition throughout the entire electrospun dermal component. TEM of the dermal-epidermal junction showed large numbers of striated collagen fibers and a well-developed basement membrane with lamina densa, anchoring fibrils and hemidesmosomes. H&E-stained paraffin sections also showed well-developed stratified epidermis by Day 10 following ALI culture, consisting of basal, spinous, granular and stratum corneum components. TEM of the stratum corneum showed abundant production and deposition of lamellar lipid sheets between the corneocytes. A viable epidermal layer with stable thickness of 8-10 cell layers was maintained out to at least Day 35 after ALI (longest timepoint evaluated to date), providing an extended window of useful downstream experimentation time. The production protocol was successfully transferred to an independent contract research laboratory (IIVS), where tissues with comparable histology were readily produced by their team. Functional barrier assessment based on the Triton X-100 ET50 assay conducted by IIVS determined an ET50 of 13.89 hours, vs. ET50 of 13.25 hours as determined by BioSurfaces tissues produced in parallel at BioSurfaces.

Histological, Immunohistochemical and Ultrastructural Characterization

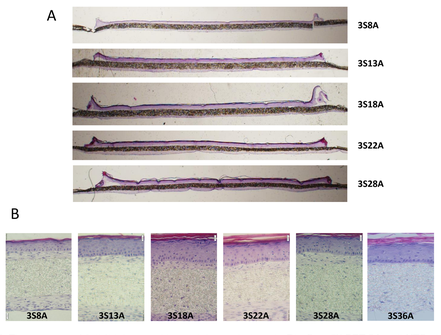


Figure 4. Time course of full-thickness skin model morphology in Bio-Spun™ PET 24-well HTS plate. H&E stained cross sections show tissue development and maintenance of robust viable epidermal structure out to 36 days of ALI culture. **A:** Low magnification (7.9X) shows wall-to-wall insert coverage with uniform thickness and no contraction. **B:** High magnification (252X) shows maintenance of robust epithelium with stable thickness including basal, spinous, granular and stratum corneum components. Note: 3S36A corresponds to 3 days of submerged epithelial culture followed by 36 days at the ALI.

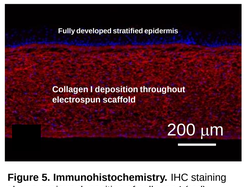


Figure 5. Immunohistochemistry. IHC staining shows copious deposition of collagen I (red) throughout the electrospun dermal component, and cell nuclei of dermal fibroblasts and epidermal keratinocytes (blue).

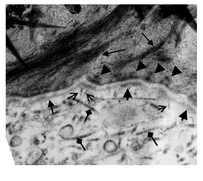


Figure 6. Transmission electron micrograph of basal lamina. Lamina densa (⟶), striated interstitial collagen fibers (⟶) and anchoring fibrils (⟶). Basal keratinocytes have numerous well-developed hemidesmosomes (▼) with associated tonofilament networks (⟶).

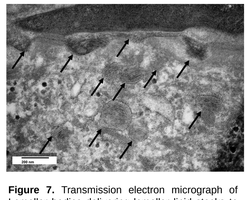


Figure 7. Transmission electron micrograph of Lamellar bodies delivering lamellar lipid stacks to the intercellular space between granular KC and corneocytes

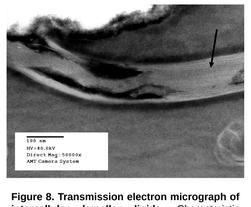
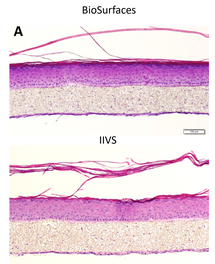


Figure 8. Transmission electron micrograph of intercellular lamellar lipids. Characteristic multilayered pattern of mature lamellar lipids in the stratum corneum intercellular space.

Protocol Transfer



B

Well-to-Well Reproducibility:
Baseline Viability (WST-8 Assay)
Ave OD +/- CV (%), n = 6 tissues

BioSurfaces	IIVS
1.436 +/- 5.87%	1.60 +/- 6.78%

C

Barrier Function: TX-100 ET50 Assay

BioSurfaces	IIVS
13.25 hours	13.89 hours

Figure 8. Protocol transfer. Parallel tissue lots were produced at BioSurfaces (Ashland, MA) and IIVS (Gaithersburg, MD) using the same scaffold inserts, cryopreserved NHDF and NHEK, and culture media. All materials were prepared at BioSurfaces and shipped to IIVS by overnight courier. IIVS staff received previous ½ day of in person training at IIVS and further 1 ½ hour training by zoom conference prior to performing the production run. Histology (A), Well-to-well baseline viability (B) and Barrier function (C) were all highly reproducible between laboratories. The ET50 of ~13 hours further indicates very robust barrier of the models.

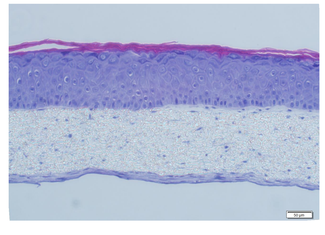


Figure 9. Histology of tissue produced without FBS or BPE medium components during the production process. FBS and BPE-free medium formulations have also been developed. The histology of tissues produced without FBS or BPE during the production process is comparable to tissues that were cultured in the presence of FBS and BPE. Tissue histology is shown at 4 days submerged followed by 14 days of ALI culture.

Conclusions: Next-generation, *in vitro* full-thickness human skin models were produced using animal collagen-free 3D electrospun scaffolds and open-source FBS/BPE-free culture media formulations. The fully human skin models provide long-term stability and do not suffer from contraction and stromal degradation issues. The models display a well-developed stratified epithelium, with *in vivo*-like basement membrane and stratum corneum ultrastructural elements, as well as functional stratum corneum barrier similar to commercially available skin models. Additionally, the production protocol was successfully transferred to an independent laboratory, with tissue productions comparable to that achieved in-house. These next-generation full-thickness human skin models offer promise for completely animal-product-free testing of cosmetics and chemicals, screening of new pharmaceuticals and more human-relevant disease modeling.

Key Benefits/Improvements of Tissue Models Using Electrospun Bio-Spun™ Scaffolds

- ✓ Eliminates Issues with Dermal Contraction and Degradation
- ✓ Robust and Uniform Wall-To-Wall Epidermis
- ✓ Long Lifespan Accommodates Long-term Experiments
- ✓ Easy to Implement Production Protocol
- ✓ Provides Stable Foundation for Development of More Complex Skin Models (Melanocytes, Nerves, Adipocytes, Immune Cells)
- ✓ Can be Produced Completely from Animal-Free Media/Culture Materials
- 🚫 No Animal Collagen or Extracellular Matrix Coating Used
- 🚫 No Fetal Bovine Serum Used in Production
- 🚫 No Bovine Pituitary Extract Used in Production

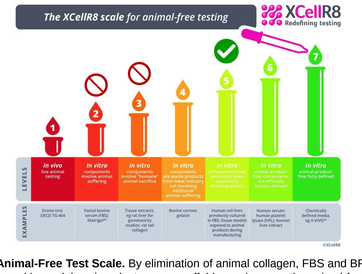


Figure 9 Animal-Free Test Scale. By elimination of animal collagen, FBS and BPE, full-thickness skin models using electrospun scaffolds can improve the animal-free testing scale from 2/3 to 5/6. (*XCellR8 animal free test scale reproduced with permission*)

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