Protocol Transfer and Reproducibility of an Electrospun Scaffold-based *In Vitro* Human Full-thickness Skin Model

[1]Patrick J. Hayden, [1]Lisa M. Fitzgerald, [1]Rayan K. Hodroj, [1]Jayashree Chakravarty, [1]Jacob T. Le Do, [1]Jamie A. Rangel, [1]Elexa M. McFadden, [2]Hsin Tzu Keng, [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

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

4175/J658

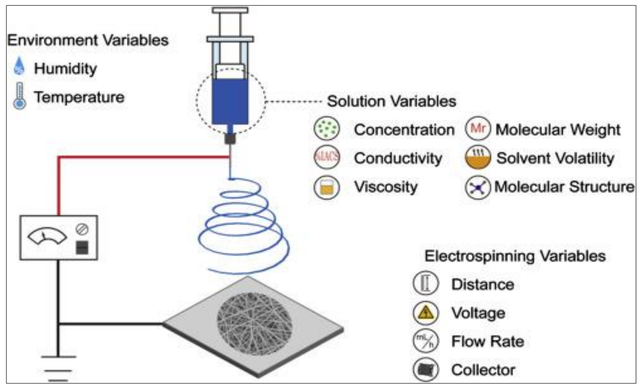

Figure 1. Basic process variables for creating electrospun scaffolds by solution electrospinning. Polymer solutions are dispensed across a high voltage field and collected on a grounded surface. Source: Kurecic, Manja. (2013). Electrospinning: Nanofibre Production Method. Tekstilec. 56. 4-12. 10.14502/Tekstilec2013.56.4-12.

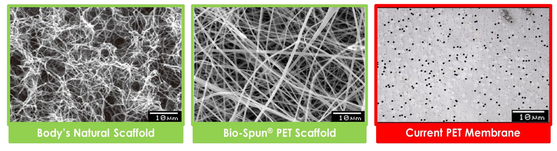

Figure 2. Scanning electron micrograph of *in vivo* extracellular matrix, Bio-Spun® PET scaffold, and a film-based microporous membrane. 3D randomly oriented nanofiber scaffolds are similar to 3D *in vivo* extracellular matrix. The film-based PET membrane is a highly rigid 2D surface.

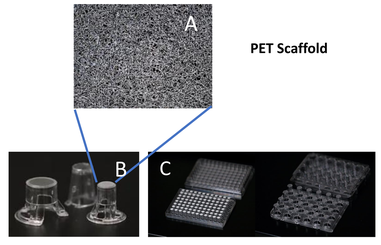

Figure 3. Electrospun scaffold insert products. Bio-Spun® scaffolds (A) are bonded to various sizes of individual inserts (B) and 24-and 96-well HTS plate format components (C). Inserts and plates are shown in the upside-down orientation to highlight the scaffold component. The HTS formats are compatible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

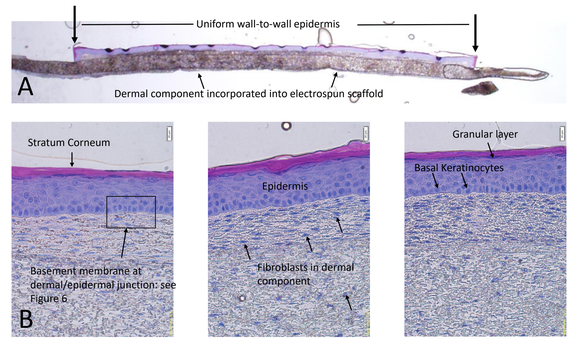

Figure 4. H&E-stained cross section of full-thickness human skin model produced on non-degradable Bio-Spun™ PET scaffolds. A: Low magnification view showing uniform thickness of the epidermis and wall-to-wall coverage across the entire width of the 6.5 mm insert scaffold with no contraction (7.9X). **B:** High magnification view of PET tissue (252X). The fully developed stratified epidermis contains basal, spinous, granular keratinocyte layers with a functional stratum corneum.

Background

In vitro human skin models are important tools for testing cosmetics and chemicals, screening new pharmaceuticals, and human disease research. Dermal-epidermal interactions play a key role in regulation of epidermal proliferation, differentiation, wound healing and barrier function, and are involved in the pathogenesis of aging/photoaging, immune responses and numerous skin diseases. Full-thickness skin (FT-Skin) models therefore have the potential to provide more comprehensive and *in vivo*-relevant experimental data compared to partial-thickness models. However, these models commonly use animal-derived collagen as a main structural element of the stromal matrix, resulting in stability and contraction issues, short lifespan and poor reproducibility. Additionally, culture media used 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 recently developed a full-thickness human skin model (Bio-Spun® FT-Skin model) using electrospun scaffolds as the stromal structural component, together with open-source FBS/BPE-free culture media. In the current work, we evaluated protocol transfer and reproducibility of the Bio-Spun® FT-skin model in a naive laboratory.

Methods

To test the reproducibility and robustness of the Bio-Spun® FT-Skin production protocols and Bio-Spun™ scaffold inserts, Bio-Spun® FT-Skin models were produced by an independent contract research laboratory (the Institute for In Vitro Sciences, IIVS) (see Figure 5 for production protocol). Complete medium formulations and detailed protocols are available from BioSurfaces (info@biosurfaces.us). Tissue morphology and baseline viability were determined by histological analysis and WST-8 metabolism assays, respectively. A time-to-toxicity (ET50) barrier assessment assay that is commonly used as a quality control test for several commonly accepted regulatory assays was also performed. Parallel tissue lots were produced at BioSurfaces (Ashland, MA) and IIVS (Gaithersburg, MD) using identical lots of Bio-Spun® scaffold inserts, cryopreserved NHDF and HEKn, and FBS/BPE-free culture media. All materials were prepared at BioSurfaces and shipped to IIVS by overnight courier. IIVS staff received half day of in-person training at IIVS and further 1.5 hour training by zoom conference prior to performing the production runs.

Results

Well-to-well baseline viability was assessed on n = 6 random tissues from 3 independent production runs from both laboratories using the WST-8 assay. Results show a low coefficient of variation (cv) ranging between 5.87-11.64%, and good concordance between laboratories. ANOVA showed that run number 2 was significantly different from runs numbers 1 and 3 ($p < 0.01$). No statistically significant difference was observed between run numbers 1 and 3 ($p = 0.55$). No overall statistically significant difference was observed between laboratories (2-tailed t-test, $p = 0.67$).

The WST-8 assay is non-destructive, allowing histological assessment of the same tissues following the WST-8 viability assay. The morphology of the Bio-Spun® FT-Skin tissues produced by BioSurfaces and IIVS was virtually indistinguishable. A robust viable epithelium with similar thickness, showing well-developed basal, spinous and granulosum layers was produced by both laboratories. Stratum corneum development between the 2 sets of FT-Skin tissues was also similar.

The Triton X-100 (TX-100) ET50 assay was conducted to assess the functional barrier of the FT-Skin tissues produced by the 2 laboratories in 3 independent production runs. The barrier of the tissues was found to be highly reproducible within and between laboratories. The observed average ET50 of 12.51 ± 0.37 hours (cv = 2.97 %) demonstrated a very robust stratum corneum barrier of the models. No statistically significant differences were found between production runs (1-way ANOVA $p = 0.30$) or laboratories (2-tailed t-test $p = 0.052$).

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

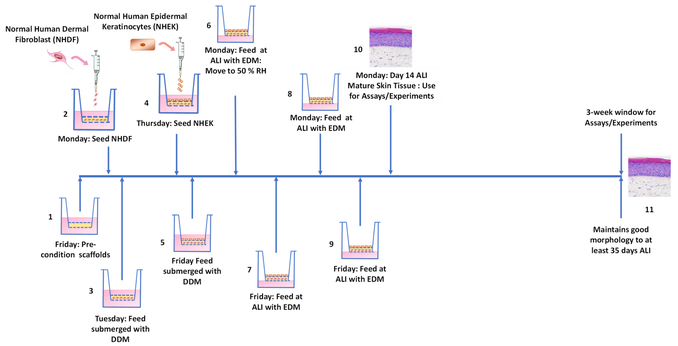

Figure 5. Schematic workflow for Bio-Spun® FT-Skin Model Production. The FT-Skin production protocol minimizes the production time and feedings, and eliminates weekend feedings. NHDF and HEKn/NHEK were seeded directly after thawing without final expansion. Mature FT-Skin tissues are ready on a Monday schedule to maximize experimental assays time during the week. 1: Scaffolds (IIC24-200; 150µm thickness; BioSurfaces, LLC) are activated by pre-wetting on Friday. 2: NHDF are seeded onto the apical surface of the electrospun scaffold on Monday under submerged culture conditions. 3: Dermal components are fed under submerged conditions on Tuesday with DDM. 4: NHEK are seeded onto the dermal component and cultured under submerged conditions in Epidermal Submerged Medium (ESM) to initiate formation of the epidermal component on Thursday. 5: FT-Skin tissues are fed under submerged conditions on Friday with ESM. 6: The developing FT-Skin tissues are raised to the air-liquid interface (ALI) using an ALI lifter (ALI-LC24; BioSurfaces, LLC) and Epidermal Differentiation Medium (EDM) on Monday. 7, 8, 9: The developing FT-Skin tissues are fed at the ALI on the following Friday, Monday and Friday for 24-well individual insert formats. The 24-well HTS insert formats are fed on Wednesdays as well. 10: The fully mature FT-Skin tissues are ready to use the following Monday at Day 14. 11: The FT-Skin tissues may be cultured for at least 3 additional weeks with maintenance of good epidermal thickness and morphology.

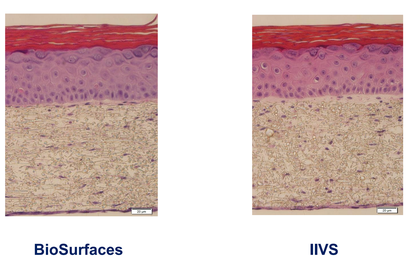

Figure 6. Histological comparison of Bio-Spun® FT-Skin tissues produced at BioSurfaces and IIVS. Parallel tissue lots were produced at BioSurfaces (Ashland, MA) and IIVS (Gaithersburg, MD) using the same lots of Bio-Spun® scaffold inserts, cryopreserved NHDF and HEKn, and DDM, ESM and EDM culture media. Histological assessment of H&E-stained tissue sections shows that the tissues produced by the two laboratories are indistinguishable.

Average OD +/- Standard deviation of n = 6 random FT-Skin tissues

	BioSurfaces	IIVS
Run #1	1.44 +/- 0.08	1.60 +/- 0.11
Run #2	1.24 +/- 0.12	1.10 +/- 0.13
Run #3	1.65 +/- 0.18	1.22 +/- 0.09

Figure 7. Comparison of baseline viability of Bio-Spun® FT-Skin tissues produced at BioSurfaces and IIVS. Parallel tissue lots were produced at BioSurfaces (Ashland, MA) and IIVS (Gaithersburg, MD) using the same lots of Bio-Spun® scaffold inserts, cryopreserved NHDF and HEKn, and DDM, ESM and EDM culture media. Well-to-well baseline viability was assessed on n = 6 random tissues from 3 independent production runs from both laboratories using the WST-8 assay. Run #2 is significantly different then run #1 and run #3: One-way ANOVA $p < 0.01$. Runs # 1 and #3 are not significantly different: one-way ANOVA $p = 0.55$. No significant overall difference between laboratories was observed: two-tailed t-test $p = 0.67$.

1.0% Triton X-100 ET50 (h)

	BioSurfaces	IIVS
Run #1 (2491219 NHEK)	12.68	12.21
Run #2 (2491219 NHEK)	13.14	12.55
Run #3 (2491219 NHEK)	12.36	12.12
Run #1 (2825951 NHEK)	12.8	

Figure 8. Comparison of stratum corneum barrier Bio-Spun® FT-Skin tissues produced at BioSurfaces and IIVS by the TX-100 ET50 assay. Parallel tissue lots were produced at BioSurfaces (Ashland, MA) and IIVS (Gaithersburg, MD) using the same lots of Bio-Spun® scaffold inserts, cryopreserved NHDF and HEKn, and DDM, ESM and EDM culture media. The Triton X-100 (TX-100) ET50 assay was conducted to assess the functional barrier of the FT-Skin tissues produced by the 2 laboratories in 3 independent production runs. The overall average ET50 for 2491219 NHEK-based FT-Skin models was 12.51 ± 0.37 h (cv = 2.97%). There are no significant differences between production runs (one-way ANOVA $p = 0.30$) or laboratories (two-tailed t-test $p = 0.052$)

Conclusions

- Next-generation, Bio-Spun® FT-Skin models were produced using 3D electrospun scaffolds and open-source FBS/BPE-free culture media formulations.
- Advanced features of the models include:
  - ✓ Animal-free production process
  - ✓ Long-term stability; elimination of contraction and stromal degradation issues.
  - ✓ Improved functional lifespan and improved stratum corneum barrier compared to commercially available skin models.
  - ✓ Easily transferable and highly reproducible production protocol – amenable to in-house production for routine and regulatory testing:
    - Reduced reliance on “black box” commercial skin models
    - Eliminates shipping issues
- 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.