Generating Full Thickness Skin Models with Transwell® Permeable Supports and the Corning® Matribot® Bioprinter

CORNING

Application Note

Hilary Sherman and Ann Ferrie
Corning Incorporated, Life Sciences
Kennebunk, ME USA

Introduction

The skin provides an essential barrier between the body and the external environment. Being able to model this tissue is important for drug screening, chemical and cosmetic testing, and studying skin diseases[1]. Typically, these models are built upon scaffolds such as Transwell® permeable supports from Corning which enable air exposure to the cells allowing for further differentiation of the skin model[2]. In its most basic form, the skin is primarily made up of two layers: the epidermis and the dermis[3]. The outer epidermal layer, which provides protection from the external environment, is mostly comprised of keratinocytes while the dermis is comprised of fibroblasts and collagen which provides structural support for the dermal layer[3]. To recreate this model, fibroblasts are often mixed with collagen and dispensed into Transwell inserts and later overlayed with epidermal cells. Due to the viscosity and temperature sensitivity of collagen, this can be a challenge to do manually. Here, we utilize the temperature controlled printhead of the Corning Matribot bioprinter to print the dermal layer of a full thickness skin model directly into Transwell inserts.

Materials and Methods

Dermal Bioink Preparation

Human Dermal Fibroblasts from neonatal tissue (HDFn) (Thermo Fisher C0045C) were thawed into a Corning CellBIND® surface T-175 cell culture flask (Corning 3292) and cultured in 50 mL of FGM™-2 Fibroblast Growth Medium-2 BulletKit™ (FGM; Lonza CC-3132). Cells were seeded at a density of 3,500 cells/cm^2 and upon reaching confluence, Accutase® cell detachment solution (Corning 25-058-CI) was used for cell harvests. On the day of printing, cells were harvested as previously described[4] and centrifuged at 300 x g for 5 minutes to pellet the cells. The bioink was prepared with the cell pellet resuspended at a final concentration of 2.5 x 10^6 cells/mL with 4 mg/mL Collagen I high concentration, rat tail (Corning 354249), 15 mM sodium hydroxide (NaOH; Honeywell 35256-1L), 2% 10X Hank's Balanced Salt Solution (HBSS; Corning 20-021-CV), and 100 µM Genipin (Thermo Fisher 466642500) in FGM. The bioink was kept on ice in pre-chilled Axygen® MaxyClear Snaplock 5.0 mL microcentrifuge tubes (Corning MCT-500-C-S) until just prior to use.

Dermal Printing

Twenty minutes prior to printing, the printhead with a standard nozzle thermal insulator, was cooled to 2°C in the biological safety cabinet. A droplet dispense program was designed in Corning DNA Studio to dispense a single 100 µL droplet of dermal bioink per 6.5 mm Transwell insert (Corning Cat. No. 3470) at an extrusion rate of 20 µL/sec. Once the printhead reached 2°C, 2.5 mL of dermal bioink was drawn into a pre-chilled 3 mL syringe (BD 309657) already affixed with a 22G bioprinting nozzle (Corning 6167). Manual calibration was used to orient the syringe nozzle 1 to 2 mm above the Transwell membrane. After dispensing, the Transwell plates were transferred to a 37°C incubator for 45 minutes to fully polymerize. Once polymerization was complete, FGM media containing 50 µg/mL L-ascorbic acid (Fisher Scientific A61-100) was added to each insert: 100 µL apical (Transwell insert) and 700 µL basolateral (receiver well).

Epidermal Seeding

Neonatal-derived Human Epidermal Keratinocytes (HEKn; Thermo Fisher C0015C) were thawed into a Corning CellBIND surface T-175 cell culture flask containing 50 mL of KGM™ Gold Keratinocyte Growth Medium BulletKit (KGM; Lonza 00192060) as per the vendor's recommendation. Upon reaching confluence, Accutase cell detachment solution was used to harvest cells from T-flasks and scaled up to an initial seeding density of 3,500 cells/cm^2. Forty-eight hours post-dermal seeding, HEKn; were harvested and centrifuged at 300 x g for 5 minutes and were resuspended at 5 x 10^5 cells/mL in KGM plus 50 µg/mL L-ascorbic acid. Media was aspirated from Transwell inserts (apical chamber) and replaced with 100 µL of cell suspension per insert and 700 µL of KGM plus 50 µg/mL L-ascorbic acid per basolateral. A complete media exchange was performed 48 hours later.

Airlift

Five days after epidermal seeding, media was removed from each insert (apical chamber), and the media in the basolateral chamber was replaced with 1.5 mL of 50:50 mix of DMEM (Corning 10-090-CV) and DMEM/Ham's F12 (Corning 10-090-CV) containing: 1% penicillin streptomycin (Corning 30-001-CI), 0.5 µM Hydrocortisone (Tocris 4093), 0.5 µm Isoproterenol (Tocris 1747), 0.5 µg/mL insulin (MP Biomedicals 193900), 2.5 µM palmitic acid (Sigma 800508100), 2.5 µM oleic acid (Sigma 4954), 1.5 µM linoleic acid (Sigma 436305), 0.7 µM arachidonic acid (Sigma 181198), 2.2 µM DL-α-tocopherol (Sigma 613420), 2.4 µM BSA (Sigma 126625), and 50 µg/mL L-ascorbic acid. Apical chambers were left exposed to air without media while the media in the basolateral chambers were changed 3 times per week for an additional 21 days.

Histology

Upon completion of airlift culture, media was aspirated, and inserts were fixed for 30 minutes with 4% paraformaldehyde (Boston Bioproducts BM-155) at room temperature. Inserts were then washed twice with phosphate buffered saline (PBS; Corning 21-040-CM) and stored at 2°C to 8°C in 70% ethanol until ready for processing. Inserts were paraffin-embedded, sectioned, and stained by the Histology and Imaging Core at the University of New England, following the Preparation of Transwell Inserts for Histology Guidelines for Use (Corning CLS-AN-335DOC).

Results and Discussion

To create the most *in vivo*-like skin model it is essential to control the composition, thickness, and position of the epidermis and the dermis layers. Creating these models with a bioprinter allows for the level of control necessary to form these distinct cell layers consistently. Additionally, working with temperature-sensitive hydrogels, such as collagen, can be a challenge due to its propensity to prematurely gel at room temperature. Using the Corning® Matribot® bioprinter with Transwell® inserts, full thickness skin models that resemble human tissue were generated (Figure 1).

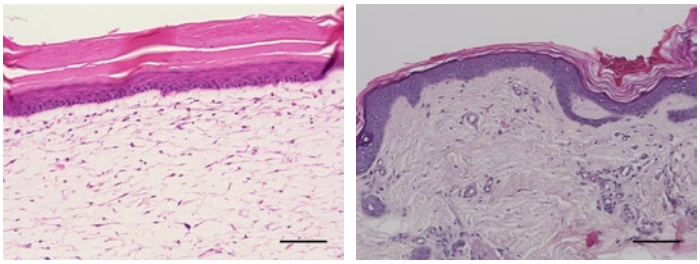

Figure 1. Cross-section view of hematoxylin-eosin stained Corning Matribot bioprinter printed skin model (left) and human skin sample (right). Scale bar is 100 µm.

Conclusions

Corning's Matribot bioprinter is capable of multi-layered printing with the added ability of temperature control. The cooling printhead maintains the printability of temperature-sensitive bioinks (i.e., collagen and Corning Matrigel® matrix) by preventing premature polymerization during printing. Additionally, the temperature of the printbed can also be controlled to quickly polymerize printed bioink into desired shapes. Temperature control capabilities of the printhead and printbed, which are unique to the Corning Matribot bioprinter, along with the ample extrusion volume range (1 to 2500 µL) makes this bioprinter an ideal tool for generating 3D models such as human skin.

Histological preparations and imaging were performed by the Histology and Imaging Core at the University of New England, Biddeford, ME USA.

References

1. Moniz T, Costa Lima SA, and Reis S. Human skin models: From healthy to disease mimetic systems; characteristics and applications. Br J Pharmacol 177.19 (2020): 4314-4329.
2. Sugihara H, et al. Reconstruction of the skin in three-dimensional collagen gel matrix culture. In Vitro Cell Dev Biol 27.2 (1991): 142-146.
3. Yan WC, et al. 3D bioprinting of skin tissue: from pre-processing to final product evaluation. Adv Drug Deliv Rev 132 (2018): 270-295.
4. Typical cell passaging using Accutase protocol (Corning CLS-CG-AN-007).

CORNING

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110.
Outside the United States, call +1.978.442.2200.