Printing 3D Skin Constructs with the Corning® Matribot® Bioprinter

Application Note

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Introduction

Skin is the largest organ of the human body and as such performs several critical functions such as thermoregulation, excretion, and absorption to name a few[1]. The skin is also essential for providing a barrier between the body and the external environment. There are two major layers that make up the skin: the epidermis and the dermis[2]. The outer epidermal layer is mostly comprised of keratinocytes while the dermis is largely comprised of fibroblasts which provide structural support for the dermal layer[2]. For skin models to be utilized in applications, such as drug screening, chemical and cosmetic testing or even basic research, recapitulating this layered tissue structure is essential. Bioprinters offer a solution for creating 3D skin constructs as they can print multiple layers of cells embedded in hydrogels to create complex structures. These structures can be comprised of different cells, where the thickness of each layer can be controlled, to produce more *in vivo*-like skin models.

The Corning Matribot bioprinter is the first benchtop bioprinter designed to handle temperature-sensitive bioinks such as Corning Matrigel® matrix and high concentration Collagen. This is accomplished through the utilization of a cooling syringe printhead and heated printbed which gives the Matribot bioprinter the ability to have tight temperature control making it an ideal tool for building up layers with temperature-sensitive hydrogels. Here, we demonstrate utilization of the Corning Matribot bioprinter to create a multilayered skin construct consisting of layers of primary human keratinocytes on top of layers of fibroblasts.

Materials and Methods

Cell Preparation

Human Dermal Fibroblasts from neonatal tissue (HDFn, Thermo Fisher C0045C) were thawed into a 175 cm^2 Corning® CellBIND® surface treated U-shaped flask (Corning 3292) and cultured in 50 mL of Dulbecco's Modified Eagle's Medium (DMEM, Corning 10-013-CM) containing 10% fetal bovine serum (FBS, Corning 35-010-CV). Meanwhile, Human Epidermal Keratinocytes from adult tissue (HEKa, Thermo Fisher C0055C) were thawed into 175 cm^2 flask containing 50 mL of EpiLife™ medium, with 60 µM calcium (Thermo Fisher MEPI500CA) and supplemented with Human Keratinocyte Growth Supplement Kit (HKGS Kit, Thermo Fisher S001K) as per vendor's recommendation. Upon reaching confluence, Accutase® cell detachment solution (Corning 25-058-CI) was used to harvest the cells from the flasks. Harvested cells were enumerated prior to centrifugation at 300 x g for 5 minutes. The resulting cell pellet was resuspended in the appropriate media at a volume to attain the cell density as shown in Table 1. Cells were then placed on ice prior to mixing with Collagen.

Bioink Preparation

One and a half milliliters of 10.2 mg/mL Corning Collagen I, high concentration, rat tail (Corning 354249) was dispensed into Axygen® MaxyClear 5.0 mL snaplock microcentrifuge tubes (Corning MCT-500-C-S) that were pre-chilled at 2°C to 8°C overnight. To the 1.5 mL of Collagen, cold (2°C to 8°C) Hank's Balanced Salt Solution (10X) (HBSS, Corning 20-021-CV) and 1M sodium hydroxide (NaOH, Honeywell 35256-1L) were added to tubes based on the volumes listed in Table 1. The NaOH was used to neutralize the pH of the Collagen while the Phenol Red in the HBSS was used as a visual indicator to confirm the pH was between 7 to 8 prior to use as to maintain cell viability and Collagen polymerization. Once the Collagen was neutralized, cells and media were added to bring the final volume of each ink to 3 mL (Table 1). Immediately prior to printing, 2.5 mL of each ink was drawn into a pre-chilled 3 mL syringe (BD 309657) already affixed with a 22G bioprinting nozzle (Corning 6167).

Printing

Thirty minutes prior to printing, the printbed on the Matribot bioprinter was heated to 37°C and the printhead, with a standard nozzle thermal insulator, was cooled to 2°C. The Corning DNA Studio software was used to create a 10 x 10 mm construct consisting of 2 layers of fibroblasts followed by 2 layers of keratinocytes. Instrument settings are shown in Table 2. The fibroblast ink was loaded into the cooled printhead, and automatic calibration for a 12-well cell culture plate (Corning 3513) was performed prior to printing. Once calibrated, 2 layers of fibroblasts were printed, and the plate was incubated for 10 minutes at 37°C to allow the Collagen to polymerize completely prior to printing the keratinocytes. After inserting the keratinocyte ink in the printhead, automatic calibration was performed again using an empty 12-well cell culture plate. A Z-offset of 0.8 mm was added to the print height to account for the fibroblast layer already present in the plate. The keratinocyte print was performed using the same settings as those used for the fibroblasts. After printing the keratinocytes, the plate was incubated for an additional 10 minutes at 37°C to allow complete polymerization of the newly printed layers. Finally, 4 mL of DMEM containing 10% FBS was added to each well containing a construct and cultured for 11 days with media exchanges occurring 5 days after printing and then every 2 to 3 days thereafter.

Table 1.

	Fibroblasts	Keratinocytes
Volume of HBSS (10X)	0.048 mL	0.048 mL
Volume of 10.2 mg/mL Collagen	1.5 mL	1.5 mL
Volume of 1M NaOH	0.042 mL	0.042 mL
Volume of media and cells	1.41 mL	1.41 mL
Cell concentration (cells/mL)	7×10^5	2×10^6
Total volume of bioink	3	3

Table 2.

Temperature printbed	37°C
Nozzle	0.41 mm (22G)
Speed	8 mm/s
Temperature printhead	2°C
Preflow volume	3.5 µL
Extrusion rate	9 µL/s
Retract volume	3.2 µL
Z-offset	0.1 mm for fibroblasts 0.8 mm for keratinocytes
Extra preflow volume	3.2 µL
Infill extrusion multiplier	60%
Retract rate	5 µL/s
Extra retract	0 µL
Postflow stop time	0.3 s
Z-lift	2 mm
Z-lift between wells	30 mm

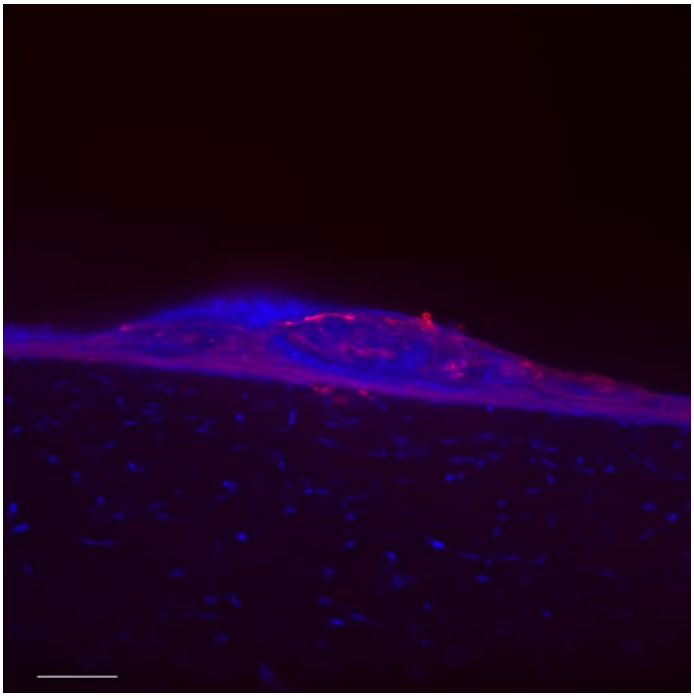

Figure 1. Cross-section view of multilayered 3D printed skin stained with Hoechst (blue) and Cytokeratin (red). Scale bar is 100 µm.

Staining

Skin constructs were prepared for staining by first fixing in 1 mL of 4% paraformaldehyde (Boston Bioproducts BM-155) for 1 hour at room temperature. One to two millimeter cross-sectional slices were hand cut from the construct using a razor blade and transferred to a 24-well cell culture plate (Corning 3524). The slices were washed with 2 mL phosphate buffered saline (PBS, Corning 21-031-CM) followed by permeabilizing of the cells with incubation in 1 mL of 0.5% Triton™ X (Integra Chemical T756.30.30) diluted in PBS for 1 hour at room temperature. Slices were washed with 1 mL of PBS and then incubated in 1 mL PBS solution containing 10 µL of 1 mg/mL Hoechst 34580 (Thermo Fisher H21486) and pan-cytokeratin antibody (Novus NBP2-33200AF647) for 2 hours at room temperature. After staining, constructs were washed twice with 2 mL PBS and imaged with a 10X objective using the Thermo Fisher CellInsight™ High Content Analysis Platform.

Results and Discussion

To create the most *in vivo*-like skin model it is essential to be able 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. Using the Corning® Matribot® bioprinter we were able to recapitulate a less dense layer of fibroblasts underneath a denser layer of keratinocytes, representing the *in vivo* situation. The photomicrograph (Figure 1) shows the difference in the densities of the cell layers based on nuclear staining with Hoechst, as well as the cytokeratin positive staining, indicative of keratinocytes, that can be seen on the upper layer.

Conclusions

The Corning Matribot bioprinter is capable of multilayered printing with the added ability of temperature control. The cooling printhead, maintains the printability of temperature-sensitive bioinks by preventing premature polymerization. Additionally, temperature of the printbed can also be controlled in order to quickly polymerize printed bioink into its desired shape. The unique temperature control capabilities of the Corning Matribot bioprinter, in addition to the extrusion volume range from 1 to 2,500 µL, make this bioprinter an ideal tool for generating 3D models such as human skin constructs.

References

1. Brohem CA, et al. Artificial skin in perspective: concepts and applications. Pigment Cell & Melanoma Research 24.1 (2011):35-50.
2. Yan WC, et al. 3D bioprinting of skin tissue: from pre-processing to final product evaluation. Adv. Drug Deliv. Rev. 132 (2018):270-295.

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