Bioprinting of Corning® Matrigel® Matrix with the Corning Matribot® Bioprinter: Evaluating the Precision of the Temperature-controlled Syringe Pump Printhead

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Application Note

Introduction

Corning Matrigel matrix is commonly used in 3D cell culture, as it provides structure and signaling cues necessary for 3D and organoid models. In these models, Matrigel matrix is typically either used to coat the bottom of a microplate surface or it is dispensed as droplets to form domes. This latter method, which is commonly used when working with precious samples such as organoids, can be time-consuming and variable between users due to the Matrigel matrix's viscosity and temperature sensitivity.

The Corning Matribot bioprinter is a 3D bioprinter that contains a temperature-controlled printhead, enabling an ideal system for Matrigel matrix dispensing. This syringe-based system can be used to accurately and precisely dispense small volumes of hydrogels with or without cells in a semi-automated throughput. Here we demonstrate the precision and accuracy of the Matribot bioprinter for dispensing Matrigel matrix as 10 µL and 50 µL droplets.

Materials and Methods

Corning Matrigel matrix solution with a protein concentration of 9 mg/mL was dispensed as single droplets into pre-weighed weigh boats using the Corning Matribot bioprinter dispense parameters listed in Table 1. The volume of each dispensed droplet, as represented by mass, was measured by determining the mass difference of the weigh boat before and after Matrigel matrix addition.

Results and Discussion

When programming a target volume of 10 µL droplets of 9 mg/mL Corning Matrigel matrix in the Corning Matribot bioprinter, we were able to dispense 9.63 ± 0.80 mg of Matrigel matrix droplets with a coefficient of variation (CV) of 8.3% and systematic error under 4% (Table 2 and Figure 1).

When programming a target volume of 50 µL droplets of 9 mg/mL Corning Matrigel matrix in the Corning Matribot Bioprinter, we were able to dispense 49.16 ± 1.39 mg of Matrigel matrix droplets with a CV of 2.8% and systematic error under 2% (Table 2 and Figure 2).

Table 1. Conditions for dispensing 10 µL and 50 µL droplets of 9 mg/mL Corning Matrigel matrix solution.

Target volume	10 µL	50 µL
Nozzle type	27G needle, 0.5"	27G needle, 0.5"
Volume loaded in 3 mL syringe	2.5 mL	2.6 mL
Set temperature in printhead	0°C	0°C
Set temperature of printbed	37°C	37°C
Room temperature	23°C	23°C
Volume purged before experiment	200 µL	200 µL
Distance between nozzle and substrate	1 mm	1 mm
Sample size	48	47

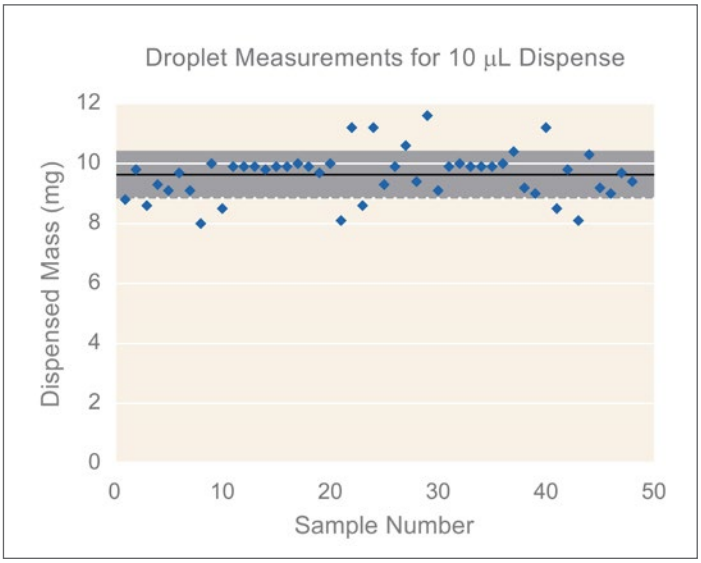

Figure 1. The dispensed droplet volume for 10 µL dispensing of 9 mg/mL Corning Matrigel matrix as measured by mass. The average mass for 48 dispenses is represented by a black line and the standard deviation is shown by gray shading.

Table 2. Mean dispensed mass from dispensing 10 µL and 50 µL droplets of 9 mg/mL Corning Matrigel matrix solution.

Target volume	10.00 µL	50.00 µL
Mean dispensed mass	9.63 mg	49.16 mg
Standard Deviation	0.80 mg	1.39 mg
Trueness/systematic error	3.7 %	1.7 %
% CV	8.3	2.8

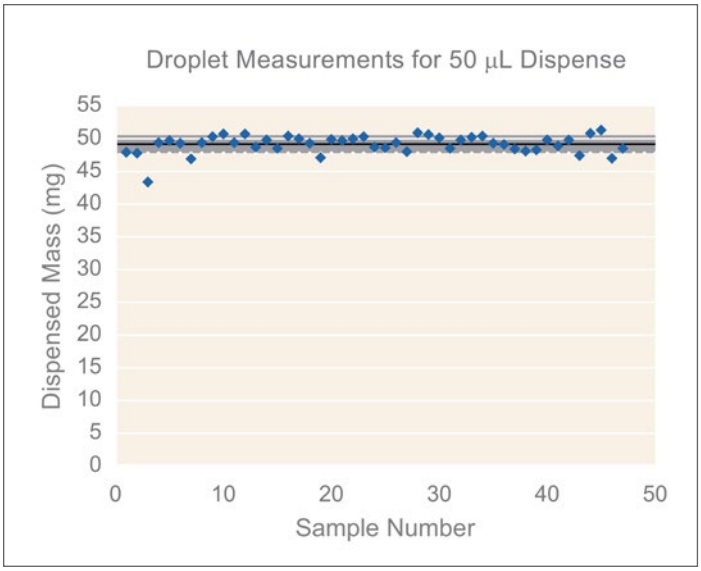

Figure 2. The dispensed droplet volume for 50 µL dispensing of 9 mg/mL Corning Matrigel matrix as measured by mass. The average mass for 47 dispenses is represented by a black line and the standard deviation is shown by gray shading.

To determine systematic error (% SE)

$$\overline{V} = \frac{1}{N}\sum_{i=1}^{N} V_i \qquad \%SE = \frac{\overline{V} - V_T}{V_T} \text{ x } 100\%$$

$\overline{V}$ is the average of all measured volumes;

N is the number of replicate deliveries;

V_i is a single measured volume;

V_T is the target volume, the volume intended to be delivered.

To determine random error (% CV)

$$\%CV = \frac{100\%}{\overline{V}} \sqrt{\frac{\sum_{i=1}^{N}(V_i - \overline{V})^2}{N-1}}$$

Conclusions

The Corning® Matrigel® matrix solution dispensed by the temperature-controlled syringe pump printhead as measured by mass matched the target volumes with a high accuracy and reproducibility.

We also found the dispensing precision to be dependent on the target volume, where it has a higher precision for a volume of 50 µL (CV of 2.8%) compared to 10 µL (CV of 8.3%). In both cases, the systematic error was well below 5%, indicating the robustness of this bioprinting technology.

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Corning® Matribot® Bioprinter Parameters

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Introduction

The Corning Matribot Bioprinter is a 3D bioprinter that can dispense and print bioinks as well as extracellular matrices such as Corning Matrigel® matrix and Collagen. Corning DNA Studio software enables the user to adjust several parameters in order to optimize dispensed volumes and printed structures for their particular application. These parameters, which are located in the Printhead tab in Corning DNA Studio, are explained in detail below for both Droplet Dispensing and Bioprinting projects. This document also includes illustrations to demonstrate how adjusting these parameters can resolve common printing issues.

Parameters in Droplet Dispensing Mode in Corning DNA Studio

Basic Parameters

Basic Parameters	Description	Printing Impact
Temperature	The temperature of the printhead.	Changing the temperature of some materials can alter their viscosity. Check which temperature the material prints best at. Certain materials, such as Corning Matrigel matrix and Collagen, require a cooled printhead to keep from premature polymerization.
Extrusion rate	The rate at which the bioink flows from the nozzle with respect to µL per seconds.	Increasing the extrusion rate will increase the flow of the bioink from the nozzle. Decrease if the droplet size is inconsistent. It is recommended to set the extrusion rate between 0.5 to 60 µL/s.
Extrusion volume	The volume of the bioink that flows from the nozzle.	The extrusion volume can range from 1 to 2500 µL. If a droplet array is selected in the Surface tab, the maximum volume will be dependent upon the plate type and number of droplets per well.
Retract volume	The volume of bioink which is drawn into the nozzle after dispensing a droplet before the printhead moves to the next location.	Increase the retract volume if material continues to extrude after completing a droplet or if droplet size is inconsistent. It is recommended to set the retract volume between 5 to 20 µL for droplet volumes above 10 µL and to set it between 2 to 5 µL for droplet volumes below 10 µL. Set the retract volume at least 1 µL lower than the extrusion volume.
Droplet volume	The net volume of bioink dispensed for each droplet. This is equal to the extrusion volume minus the retract volume.	The droplet volume is automatically calculated by the software. It can be changed by adjusting the extrusion volume or retract volume.
Z-offset	The distance the nozzle is offset above the calibrated reference point.	Increase the z-offset if the nozzle is calibrated too close to the surface. It is recommended to set 0.2 to 0.4 mm as default.

Advanced Parameters

Advanced Parameters	Description	Printing Impact
Extra preflow volume	The amount of bioink that is extruded at the start of the very first droplet.	Increase the extra preflow volume if the first droplet is too small. This can occur if the nozzle is not fully primed at the start of the print. Decrease this setting if the first droplet is too large. It is recommended to set between 0 to 4 µL.
Retract rate	The speed at which the bioink is retracted back into the nozzle with respect to µL per seconds.	Increase the retract rate if experiencing a delay in printhead movement due to waiting for the retract move to finish. It is recommended to use a retract rate of 3 to 25 µL/s.
Postflow stop time	The time the print movement is delayed after each droplet.	Increase the postflow stop time if viscous materials are oozing between droplets. It is recommended to use 0.3 s as default.
Z-lift between wells	The lowering of the printbed when moving between wells.	Increase the z-lift for deep well plates. Decrease to speed up the process in 384-well micro plates.

Parameters in Bioprinting and Generate Modes in Corning® DNA Studio

Basic Parameters

Basic Parameters	Description	Printing Impact
Nozzle	The size of the nozzle inner diameter.	Select the nozzle size that corresponds to the nozzle loaded on the syringe. Changing the nozzle size will result in thinner or thicker printed lines. It is recommended to use a nozzle size between 20 to 27G.
Speed	The speed of the printhead during printing moves.	Increasing the printing speed will result in a shorter printing time, while decreasing the speed can give higher resolution of the printed structures. It is not recommended to use a speed higher than 40 mm/s.
Temperature	The temperature of the printhead.	Changing the temperature of some materials can alter their viscosity. Check which temperature the material prints best at. Certain materials, such as Corning Matrigel® matrix and Collagen, require a cooled printhead to keep from premature polymerization.
Preflow volume	The amount of bioink that is extruded before starting a new filament.	Increase the preflow volume if the bioink is not extruding at the start point of each new filament. Decrease the preflow if there is an accumulation of bioink at the start point. It is recommended to set between 2 to 50 µL.
Extrusion rate	The rate at which the bioink flows from the nozzle with respect to µL/s.	Increasing the extrusion rate will increase the flow of the bioink from the nozzle. Decrease if the filament is too thick or overflowing. It is recommended to set between 0.5 to 60 µL/s.
Retract volume	The volume of bioink which is drawn into the nozzle during a non-print move.	Increase the retract volume if material continues to extrude after completing an extrusion move. It is recommended to set between 2 to 30 µL.
Z-offset	The distance the nozzle is offset in the z direction from the calibrated reference point.	Increase the z-offset if the nozzle is calibrated too close to the surface. It is recommended to set 0.2 to 0.4 mm as default.

Advanced Parameters

Advanced Parameters	Description	Printing Impact
Extra preflow volume	The amount of bioink that is extruded at the start of the very first filament.	Increase the extra preflow volume if the bioink is not extruding at the start point. This can occur if the nozzle is not fully primed at the start of the print. Decrease this setting if there is an accumulation of bioink at the starting point. It is recommended to set between 0.5 to 4 µL.
Infill extrusion multiplier	A scaling factor of the extrusion rate with respect to the infill verses the perimeter paths.	Increase the infill extrusion multiplier if the perimeter is as desired but the infill is too thin.
Retract rate	The speed at which the bioink is retracted back into the nozzle with respect to µL per seconds.	Increase the retract rate if experiencing a delay in printhead movement due to waiting for the retract move to finish. It is recommended to use a retract rate of 3 to 25 µL/s.
Extra retract	The volume of bioink drawn into the nozzle after the print is complete.	If printing with temperature sensitive materials, increase the extra retract to protect the bioink from heat until the start of a new print. It is recommended to use 30 µL to prevent gelation at the nozzle tip between prints. If not using a temperature-sensitive material, this value can be set to 0 µL.
Postflow stop time	The time the print movement is delayed after each filament.	Increase the postflow stop time if the end of the filament is not complete due to the next move starting before the bioink is properly extruded. It is recommended to use 0.3 s as default.
Z-lift	The lowering of the printbed when performing non-print moves.	Increase the z-lift if the nozzle is dragging in a previously printed filament during a non-print move. It is recommended to use 1 mm as default.

Common Bioprinting Issues that can be Resolved by Parameter Adjustment

The Basic Bioprinting parameters include nozzle size, print speed, printhead temperature, preflow volume, extrusion rate, retract volume, and z-offset. By modifying these values, improvements to the shape of your print can be made. The parameters in the Advanced settings bar can be adjusted to improve the structures even further. These advanced parameters include extra preflow volume, infill extrusion multiplier, retract rate, extra retract, postflow stop time, and z-lift parameters.

Below you can find common printing issues and how adjusting these parameters can influence your print.

NOTE: Some parameters are closely connected and can be adjusted in relation to one another. For example, if the filament thickness is as desired, but the speed is reduced by half, combine this change with reducing the extrusion rate by half to maintain the same filament thickness when printing at the lower speed.

Issue	Resolution
1. The resolution of the print is too low.	By decreasing the print speed, a higher resolution in corners can be achieved. Adjust the print speed in direct relation to the extrusion rate. Liquids with low viscosity can also overflow (see No. 12 for more details).

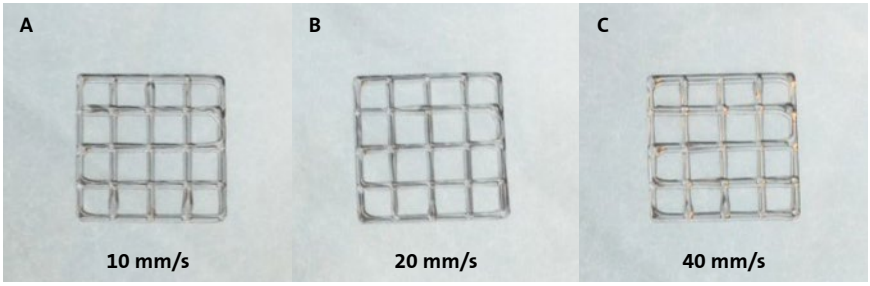

Figure 1. Adjusting the print speed, and in relation changing extrusion rate. The extrusion rate was doubled each time the print speed was doubled.

2. The filaments of a temperature sensitive bioink like Collagen and Corning Matrigel matrix, are uneven.	Certain materials require a cooled printhead to keep from premature gelation, such as Corning® Matrigel® matrix and Collagen. **NOTE:** Make sure that the printhead is already cooled when inserting a temperature sensitive material to prevent premature gelation in the nozzle. At 2°C the Matrigel matrix is completely liquid which can result in the filaments overflowing. Increasing the printhead temperature to 12°C results in a more viscous material making the filament thicker. At 20°C the Matrigel matrix polymerizes and will print unevenly. Changing the temperature of other materials can also alter their viscosity, so it is best to verify at which temperature the material prints best.

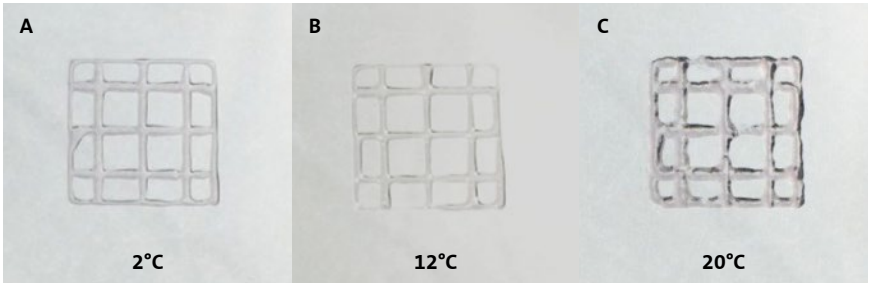

Figure 2. Adjusting the printhead temperature.

3. The filaments are broken.	Broken filaments can be due to a low extrusion rate. Try increasing the extrusion rate to compensate for this effect. If the broken lines are at the start of each new filament in the infill, increase the preflow volume. If the filament is broken at the very start of a new print, change the extra preflow volume (see No. 6). Bubbles in the material can also cause breaks in the print. To get rid of bubbles in the material when preparing the bioink, centrifuge the syringe at 1600 x g for 2 to 3 min.

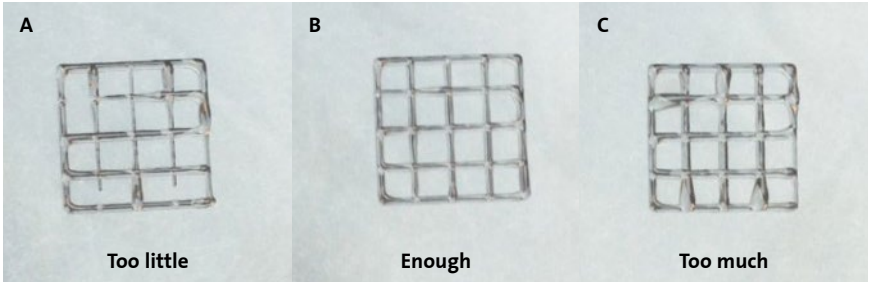

Figure 3. Adjusting preflow volume.

Issue	Resolution
4. The filaments are too thick.	Try to decrease the extrusion rate and/or increase the printing speed. A smaller nozzle can also help to get thinner filaments. If the filaments in the perimeter or in the infill are too thick in relation to one another, change the infill extrusion multiplier (see No. 7).

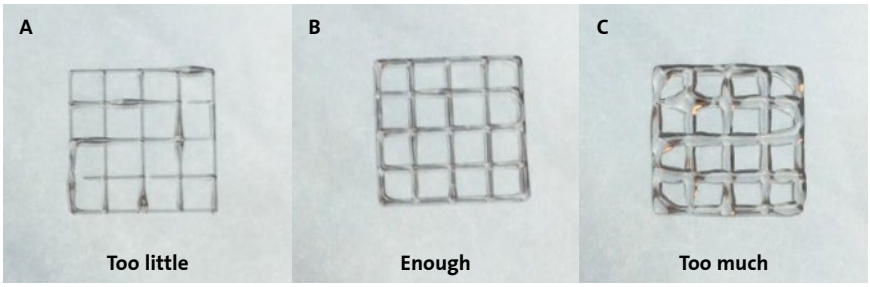

Figure 4. Adjusting extrusion rate.

5. The material continues to extrude out of the nozzle during non-print moves.	Highly viscous materials tend to continue extruding from the nozzle even after the extrusion move is finished. Increase the retract volume and/or rate to minimize this effect. Increasing the postflow stop time can also aid in preventing this problem by allowing the extrusion to fully extrude.

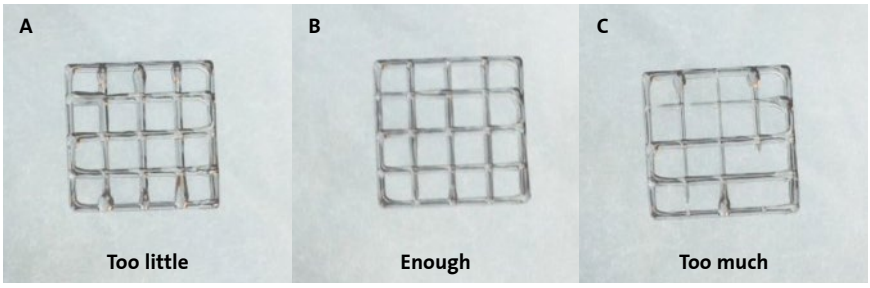

Figure 5. Adjusting retract volume.

6. The material does not extrude at the starting point of the print.	Ensure that the nozzle is fully primed before starting a print. Increase the extra preflow volume if the bioink is not extruding at the starting point of the print. Decrease the extra preflow volume if there is an accumulation of bioink at the starting point.

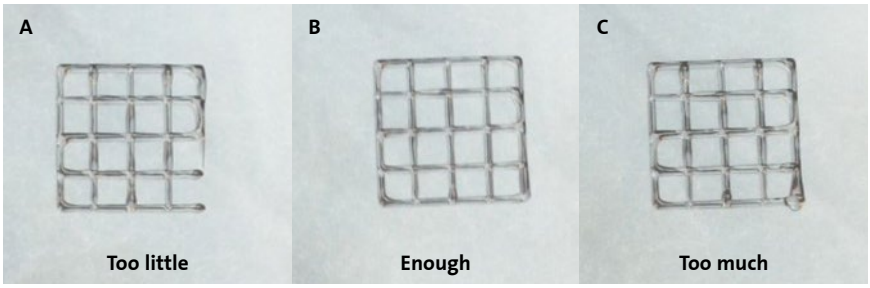

Figure 6. Adjusting extra preflow volume.

7. The infill is too thin while the perimeter is as desired.	Increase the infill extrusion multiplier if the perimeter is as desired, but the infill is too thin. Decrease the infill extrusion multiplier if the perimeter is as desired, but the infill is too thick.

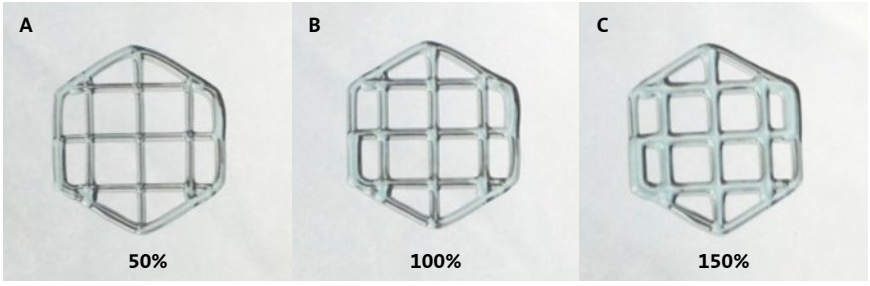

Figure 7. Adjusting infill extrusion multiplier.

Issue	Resolution
8. There is a pause in the print after each filament.	This can be due to a low retract rate value. The printer will stop after each filament in the infill to allow for the plunger to retract. Adjusting the retract rate has little impact on the printed structure, however, too slow of a retract rate can extended the print time, which can be stressful for cells.

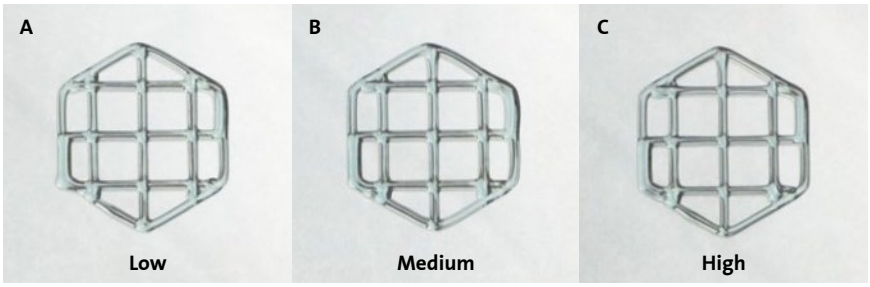

Figure 8. Adjusting retract rate.

9. There is gelated/dried bioink in the nozzle between each print.	The bioink in the nozzle tip can gelate or dry if left idle for a time. To try to avoid this issue, use a higher extra retract volume to retract the bioink up into the nozzle after a finished print. Keep in mind to fully prime the nozzle before starting the next print.
10. There is a gap at the end of the filament.	If the end of the filament does not completely extrude before the start of the next move, increase the postflow stop time. Adjusting the postflow stop time has little impact on the printed structure, however, a long postflow stop time can extend the print time, which can be stressful for the cells.

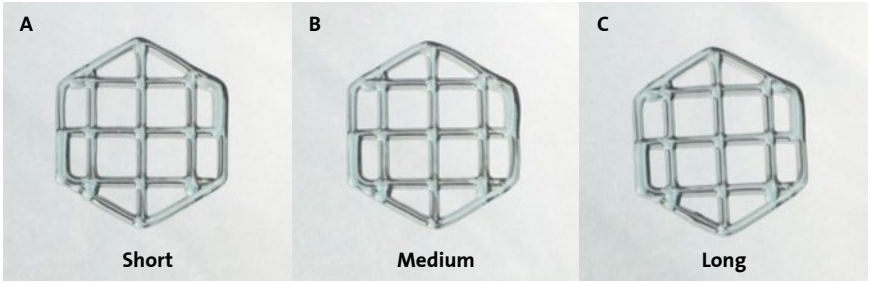

Figure 9. Adjusting the postflow stop time.

11. The nozzle is dragging in the printed structure.	If the nozzle is dragging in a previously printed filament during a non-print move, increase the z-lift. Generally, set z-lift to at least 1 mm. This can also happen if the layer height is set lower than the extruded filament height, if the actual nozzle size is larger than the set value, or if the extrusion rate is too high.
12. The filaments are overflowing.	This can be a material property. Certain materials with low viscosity have problems holding their shape after extrusion. If printing with Corning Matrigel matrix or Collagen, which can thermally gel, try heating the printbed to 37°C for the material to polymerize. **NOTE:** Adjusting the printbed temperature to above 37°C has little impact on the printed structure but can stress cells. Avoid printing tall constructs if the material has poor shape fidelity. Using lower printing speeds can facilitate in obtaining higher print resolution.

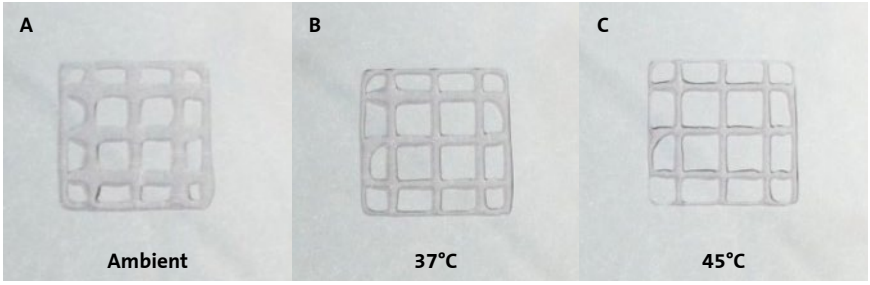

Figure 10. Adjusting the printbed temperature.

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Bioprinting with the Corning® Matribot® Bioprinter for High Throughput 3D Cell Culture using Corning Matrigel® Matrix

Application Note

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Introduction

In vitro cell cultures and animal experiment models are crucial instruments in basic research and preclinical studies[1]. 3D cell culture involves expanding cells in a volumetric space as aggregates, spheroids, or organoids. This technique creates a more accurate *in vitro* environment and provides an alternative to that used *in vivo* for fundamental cell biology and physiology research[2]. The complexities of assay development and challenges associated with validating and transferring assays into high throughput modes are well known bottlenecks in the drug discovery process, especially for those using 3D cell culture models[3]. Hence, 3D bioprinting is emerging as a promising technology for high throughput handling of extracellular matrices that support cell growth and differentiation into 3D structures[4].

The Corning Matribot bioprinter can dispense and print bioinks and extracellular matrices such as Corning Matrigel matrix and Collagen. It enables fast, accurate, and high throughput bioink handling for 3D cell culture with promising applications in medical research, drug discovery, toxicity testing, and other pre-clinical studies.

Materials and Methods

Printing with Corning Matrigel matrix

Corning Matrigel matrix (Corning 354230) was drawn up into a syringe and the syringe was inserted into the printhead of a Corning Matribot bioprinter (Corning 6150). The bioprinter dispensed droplets of Matrigel matrix at 9 mg/mL using Corning DNA Studio software with the parameters shown in Table 1. A single droplet printing pattern was selected for each well of a 24-well plate or a 96-well microplate (Figures 1A and 1C) while a "droplet array" was selected to dispense four droplets in each well of a 24-well plate (Figure 1B). The plates were then placed at 37°C for 15 minutes to facilitate polymerization of Matrigel matrix.

NOTE: More details on operating the Corning Matribot bioprinter can be found in the Corning Matribot Bioprinter Instruction Manual (CLS-AN-641DOC) and Corning Matribot Bioprinter Matrigel Matrix Protocol (CLS-AN-645).

Printing with cell suspensions

A549 and MDCK cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Corning 10-013-CV) with 10% fetal bovine serum (FBS, Corning 35-081-CV) until reaching 90% confluence. The medium was removed by aspiration, cells were washed with phosphate buffered saline (PBS, Corning 21-040-CV) and dissociated into single cells using 0.25% trypsin-EDTA (Corning 25-053-CI). An equal volume of complete medium (DMEM + 10% FBS) was added to the cells and mixed. The suspension was centrifuged at 1,000 rpm for 3 minutes to pellet the cells. The supernatant was discarded, and the pellet was resuspended with undiluted Matrigel matrix (Corning 356231) and the final cell density was adjusted to 50 cells/µL. The mixture of Matrigel matrix and cells was transferred into the syringe, inserted into the printhead of the bioprinter, and dispensed using the parameters stated in Table 2. The plates were incubated at 37°C for 15 minutes followed by the addition of 1 mL complete medium to each well. The plates were incubated at 37°C and 5% CO_2, and the medium was exchanged every 2 to 3 days.

Mouse intestinal organoids (STEMCELL Technologies 70931) were thawed, cultured, and passaged in complete IntestiCult™ organoid growth medium (STEMCELL Technologies 06005) according to the manufacturer's instructions until a cell density of approximately 150 organoids per well was achieved. A pre-wet 1000 µL pipet was used to break up the organoids by pipetting up and down twenty times, followed by centrifugation at 290 x g for 5 minutes at 4°C. The supernatant was carefully discarded, and the pellet was resuspended in undiluted Matrigel matrix by pipetting up and down ten times. The Matrigel matrix mouse intestinal organoid suspension was transferred into the syringe, inserted into the printhead of the bioprinter, and dispensed using the parameters stated in Table 2. The plates were incubated at 37°C for 15 minutes followed by the addition of 1 mL complete IntestiCult organoid growth medium to each well, and the medium was exchanged three times per week.

Results and Discussion

Corning Matribot bioprinter enables fast, accurate, and high throughput Corning Matrigel matrix dispensing

Corning Matrigel matrix was dispensed into each well of a 24-well plate by the Corning Matribot bioprinter (Figure 2A). This automated method is faster and more uniform compared to manual dispensing which has higher technical requirements. Printing patterns using several droplets per well improves the cellular utilization rate of the culture medium (Figure 2B), while printing individual droplets in 96-well microplates is designed for high throughput assays (Figure 2C).

Table 1. Printing parameters for dispensing Corning® Matrigel® matrix droplets using different patterns.

Printhead Parameters			
Printhead settings	Corning Matrigel matrix (9 mg/mL)		
Vessel selection	24-well plate	24-well plate	96-well microplate
Droplet pattern per well	Single droplet	Four droplets	Single droplet
Droplet volume	10 µL	5 µL	3 µL
Temperature of printbed	37°C		
Temperature of printhead	4°C		
Extrusion rate	20 µL/s		
Extrusion volume	12 µL	7 µL	5 µL
Retract volume	2 µL	2 µL	2 µL
Z-offset	0.3 mm		
Advanced			
Extra preflow volume	0 µL		
Retract rate	5 µL/s		
Postflow stop time	0 s		
Z-lift between wells	30 mm		

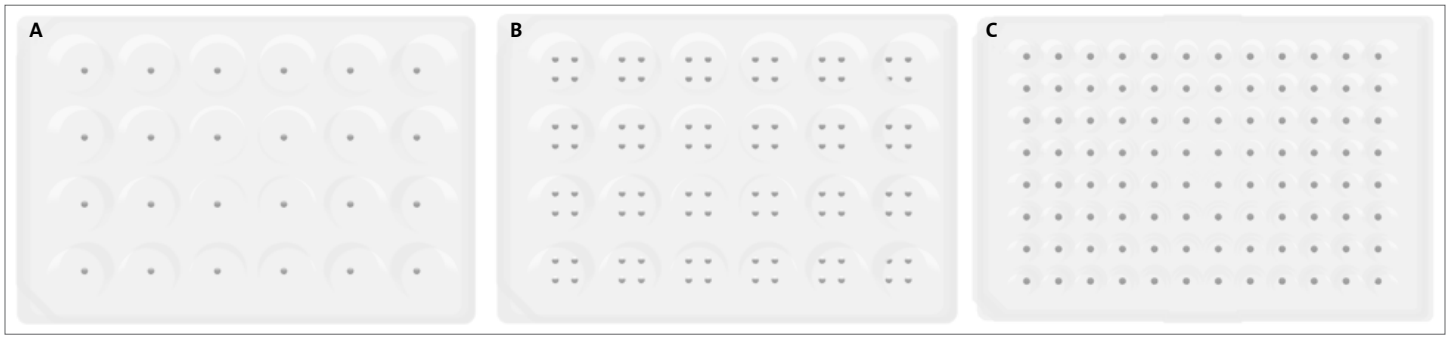

Figure 1. Different printing patterns. (A) Single droplet per well in a 24-well plate. (B) Multiple droplets per well in a 24-well plate using a square pattern of four droplets. (C) Single droplet per well in a 96-well microplate.

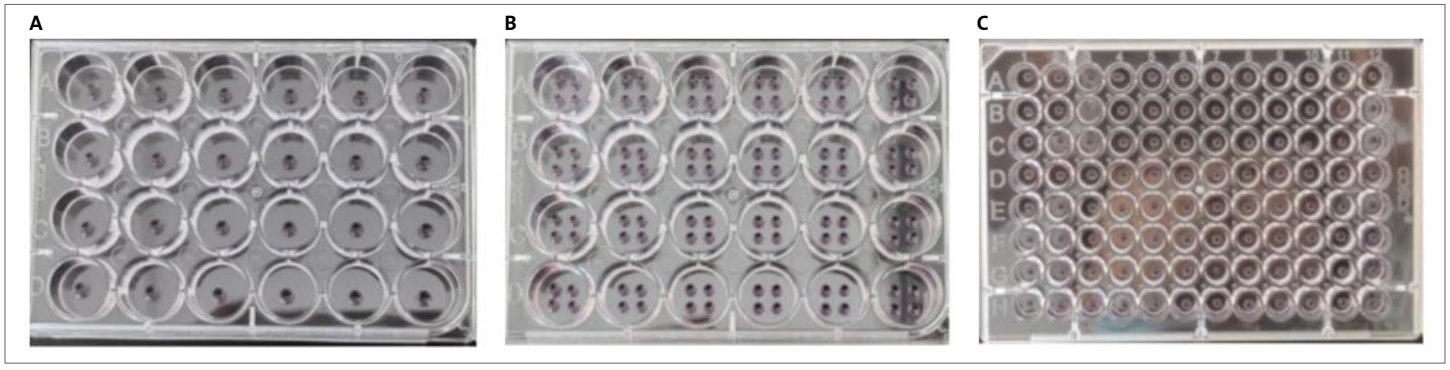

Figure 2. Uniform domes of 9 mg/mL Corning Matrigel matrix formed in each well.

Bioprinting with the Corning® Matribot® bioprinter supports spheroid and organoid formation

A549 or MDCK cells suspended in Matrigel matrix and dispensed using the Matribot bioprinter formed spheroids after 10 days of culture (Figures 3 and 4). Complex, multi-lobed mouse intestinal organoid structures were observed after 5 to 7 days of culture following passage into fragments and automatic dispensing using the Matribot bioprinter (Figure 5).

Table 2. Printing parameters for dispensing cell suspensions and mouse intestinal organoid fragments.

Printhead Parameters	
Printhead settings	Cell suspension/Corning Matrigel matrix (9 mg/mL)
Vessel selection	24-well plate
Droplet pattern per well	Three droplets
Droplet volume	10 µL
Temperature printbed	37°C
Temperature printhead	4°C
Extrusion rate	20 µL/s
Extrusion volume	12 µL
Retract volume	2 µL
Z-offset	0.3 mm
Advanced	
Extra preflow volume	0 µL
Retract rate	5 µL/s
Postflow stop time	0 s
Z-lift between wells	30 mm

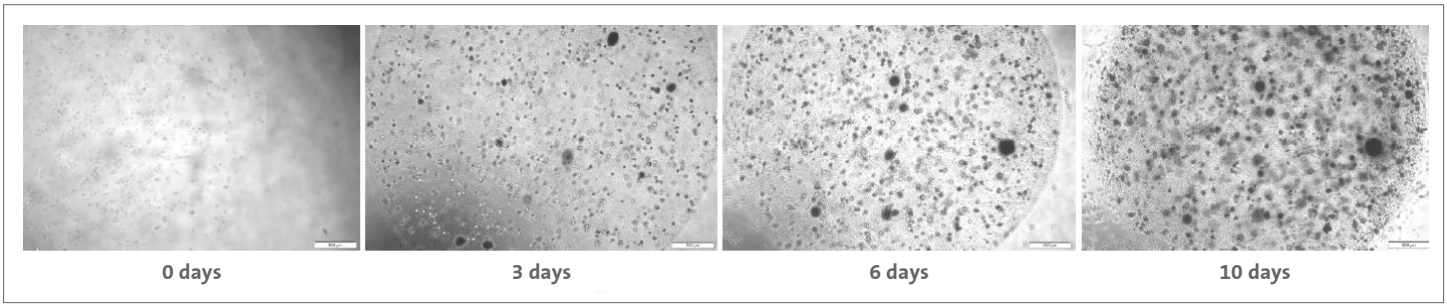

Figure 3. Corning Matrigel matrix dispensed with the Corning Matribot bioprinter supports spheroid formation of A549 cells. Representative photomicrographs of A549 spheroids after 0, 3, 6, and 10 days in culture. Images were captured at 40X magnification with an Olympus IX53 microscope. Scale bars are 500 µm.

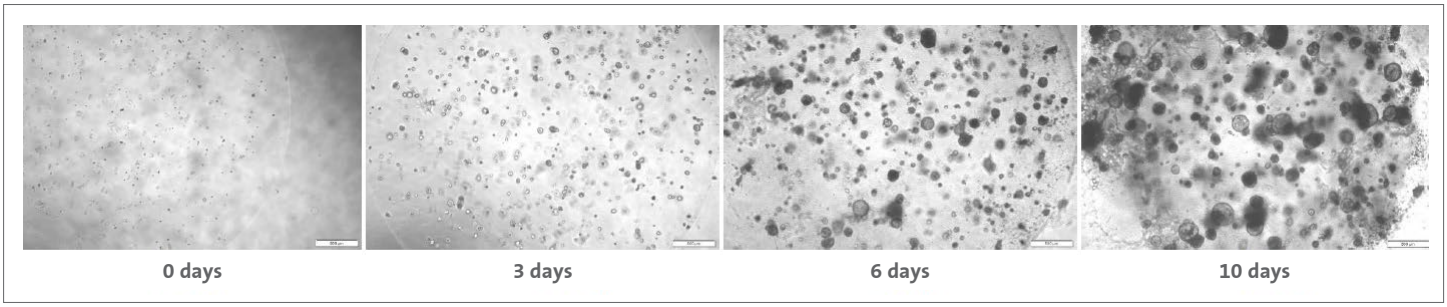

Figure 4. Corning Matrigel matrix dispensed with the Corning Matribot bioprinter supports spheroid formation of MDCK cells. Representative photomicrographs of MDCK spheroids after 0, 3, 6, and 10 days in culture. Images were captured at 40X magnification with an Olympus IX53 microscope. Scale bars are 500 µm.

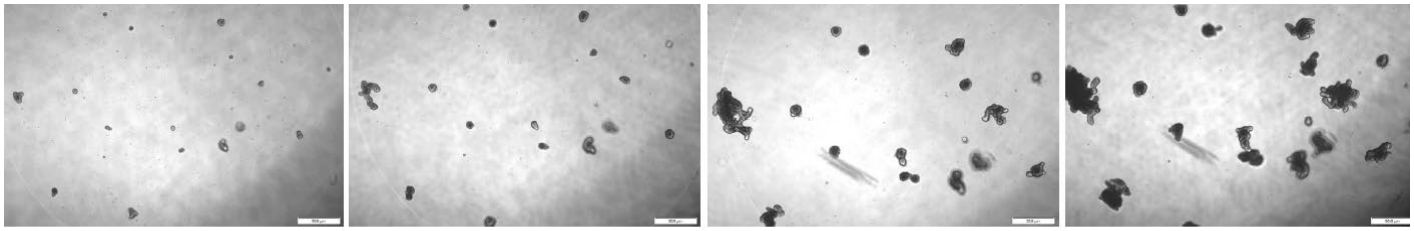

Figure 5. Corning Matrigel matrix dispensed with the Corning Matribot bioprinter supports the formation of mouse intestinal organoids following passaging. Representative photomicrographs of mouse intestinal organoids after 0, 2, 5, and 7 days in culture. Images were captured at 40X magnification with an Olympus IX53 microscope. Scale bars are 500 µm.

Conclusions

- The Corning® Matribot® bioprinter provides a quick, high throughput platform for dispensing designated volumes of bioinks and extracellular matrices such as Corning Matrigel matrix using single droplet or multiple droplet patterns in different vessels.
- This method supports the formation of spheroid-forming cells (A549 and MDCK) from single cells and organoid-forming cells (mouse intestinal organoids).

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Corning® Matribot® Bioprinter: An Easy-to-Use Tool for 3D Cell Culture

CORNING

Application Note

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Introduction

Bioprinting technology has grown along with stem cell research and has become a vital tool with diverse applications in the biological and medical fields.[1,2] *In vitro* 3D cell culture techniques create an accurate *in vitro* environment and provide an alternative to *in vivo* models used for fundamental cell biology and physiology research.[3] However, these applications are currently hampered by organoid variability, low throughput, and limited scale. Although bioprinting technology is expected to be applied in regenerative medicine and drug discovery, there are enduring concerns regarding the impact of cell dispensers on cell integrity.

The Corning Matribot bioprinter exhibited cell-friendly dispensing performance similar to that of the manual dispensing operation. The culture dispensed by the Corning Matribot bioprinter were suitable for further molecular and protein analysis as well as downstream applications including high throughput drug screening.

Materials and Methods

Bioprinting with cell suspensions and intestinal organoid fragments

The Corning Matrigel® matrix was pre-thawed and aliquoted according to the manual. All the tips and syringe used for handling spheroids or organoids were pre-chilled. The mediums were equilibrated to room temperature (15°C to 25°C) before use. A549 and MDCK cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Corning 10-013-CV) supplemented with 10% Fetal Bovine Serum (FBS; Corning 35-081-CV) until they reached 90% confluence. The medium was removed by aspiration, and the cells were washed with Phosphate-Buffered Saline (PBS; Corning 21-040-CV) and dissociated into single cells using 0.25% Trypsin-EDTA (Corning 25-053-CI). An equal volume of complete medium (DMEM + 10% FBS) was then added to the cells and mixed. The suspension was centrifuged at 300 x g for 3 min., the supernatant was discarded, and the pellet was resuspended in undiluted Corning Matrigel® matrix (Corning 356231). The final cell density was adjusted to 50 cells/µL. The mixture of the Matrigel matrix and cells were transferred to a syringe (BD 309657), inserted into the printhead of the Corning Matribot bioprinter (Corning 6150), and dispensed using the parameters listed in Table 1. Manual dispensing was performed using a single channel pipettor. The plates were incubated at 37°C for 15 min., followed by the addition of complete medium (1 mL) to each well. The plates were incubated at 37°C and 5% CO_2, and the medium was changed every 2 to 3 days.

Mouse intestinal organoids (MIOs; STEMCELL Technologies 70931) were thawed, cultured, and passaged in complete IntestiCult™ organoid growth medium (STEMCELL Technologies 06005), according to the manufacturer's instructions, until a density of approximately 150 organoids/well was achieved. A pre-wetted 1,000 µL pipet was used to break up the organoids by pipetting up and down 20 times, followed by centrifugation at 290 x g for 5 min. at 4°C. The supernatant was carefully discarded, and the pellet was resuspended in undiluted Matrigel matrix by pipetting up and down 10 times. The mixture of Matrigel matrix and MIO suspension was transferred to a syringe, inserted into the printhead of the Corning Matribot bioprinter, and dispensed using the parameters listed in Table 1. Manual dispensing was performed using a single-channel pipettor. The plates were incubated at 37°C for 15 min., followed by the addition of complete IntestiCult organoid growth medium (1 mL) to each well. The medium was changed three times per week.

Table 1. Printing parameters for dispensing cell suspensions and mouse intestinal organoid fragments.

Printhead Parameters	
Printhead settings	Cell suspension/Corning Matrigel matrix (9 mg/mL)
Vessel selection	24-well plate
Droplet pattern per well	Single droplet
Droplet volume	50 µL
Temperature of printbed	37°C
Temperature of printhead	4°C
Extrusion rate	20 µL/s
Extrusion volume	55 µL
Retraction volume	5 µL
Z-offset	0.3 mm
Advanced	
Extra preflow volume	0 µL
Retract rate	5 µL/s
Postflow stop time	0 s
Z-lift between wells	30 mm

Immunohistochemical analysis of 3D cell cultures

As described in the Guidelines for Use (CLS-AN-528)[4], A549 and MDCK spheroids or MIOs were collected from Matrigel matrix using Cell Recovery solution (Corning 354253), washed several times with cold PBS, and fixed with 4% paraformaldehyde at 4°C overnight. The 3D cultures were washed with PBST (PBS + 0.05% Tween® 20) and permeabilized with 0.2% Triton™ X-100 for 30 min. The 3D cultures were washed with PBST several times prior to staining. For immunostaining, the 3D cultures were incubated overnight at 4°C with primary antibodies (Table 2) at 1:100

dilution. The next day, the 3D cultures were washed with PBST, incubated with fluorescent secondary antibodies, and the nuclei were stained with 2 µg/mL 4',6-diamidino-2-phenylindole (DAPI). Images were captured using a CQ1 Image cytometer.

Table 2. Antibodies for immunofluorescence.

Antibody	Vendor	Cat. No.
Alex Fluor™ 647 Phalloidin	Thermo Scientific	A22287
E-Cadherin Monoclonal Antibody, Alexa Fluor 488	Thermo Scientific	53-3249-82
ZO-1 Monoclonal Antibody, Alexa Fluor 647	Thermo Scientific	MA3-39100-A647
Villin Polyclonal Antibody	Thermo Scientific	PA5-29078
MUC2 Polyclonal Antibody	Thermo Scientific	PA5-103083
Lysozyme Monoclonal Antibody	Thermo Scientific	MA5-32154

Gene expression analysis

After dissociation from the Matrigel matrix, the 3D cultures were washed several times with cold PBS (Corning 21-040-CV). RNA was extracted using a Magnetic Tissue/Cell/Blood Total RNA kit (TIANGEN DP761). One-Step TB Green® PrimeScript™ RT-PCR kit (Takara Bio RR066A) with primers synthesized by GENEWIZ (Table 3) was used with a LightCycler® system (Roche) for real-time quantitative polymerase chain reaction (qPCR) analysis.

Table 3. Primers for gene expression analysis.

Gene	Primer Sequence (5' to 3')
CD14	GCCGCTGTGTAGGAAAGAAG
	AGGTTCGGAGAAGTTGCAGA
MD2	AATCTTCCAAAGCGCAAAGA
	GGGCTCCCAGAAATAGCTTC
TLR4	TGAGCAGTCGTGCTGGTATC
	CAGGGCTTT TCTGAGTCGTC
Rab 5	TTTTAGTGCAGTGGGAAACA
	GAACGCCATCAATTTACCAA
Rab 7	TGAACCCATCAAACTGCACA
	CTGTGCTCTGCTCTCACTCG
Survivin	CGGGGTGAGAAGGTTACAAG
	AAGCCCAGATGCTTCACTGT
LGR5	CCTACTCGAAGACTTACCCAGT
	GCATTGGGGTGAATGATAGCA
Vimentin	CGTCCACACGCACCTACAG
	GGGGGATGAGGAATAGAGGCT
TFF3	TTGCTGGGTCCTCTGGGATAG
	TACACTGCTCCGATGTGACAG
Villin	ACTGAGAACCTAAAGATGCTGC
	TCCCTGGGTAGCTTTTGGACT
Chromogranin-A	ATCCTCTCTATCCTGCGACAC
	GGGCTCTGGTTCTCAAACACT
Chromogranin-B	AGCTCCAGTGGATAACAGGGA
	GATAGGGCATTTGAGAGGACTTC
Lysozyme	CTTCAGCATAGTCGCAGAATCC
	AGCCAGTTTCCAAGGGCAAAT

Results and Discussion

We demonstrated that 3D cultures dispensed by using the Corning® Matribot® bioprinter were comparable with those obtained via manual operation. Both methods had brought similar morphology, component cell types, and gene expression profile.

As illustrated in Figure 1A, the morphology of A549 spheroids generated from bioprinter-dispensed and manually dispensed samples seemed to have normal shapes. After culturing for 7 days, the bioprinter-dispensed and manually dispensed MDCK spheroids showed obvious and typical cystic structures (Figure 1B). Complex, multilobed MIO structures were also observed after 5 days of culture (Figure 1C). Moreover, the densities of the formed 3D cultures were comparable between the bioprinter-dispensed and manually dispensed samples (Figures 1A-C).

Immunofluorescence staining was performed to investigate the presence of specific cell type markers in the 3D cultures. Significant junctional F-actin was observed at the cell-cell contact points within the A549 spheroids, indicating that stable and strong intercellular adhesive interactions were formed.[5] Moreover, 3D cultures of A549 cells showed bright and continuous E-cadherin labeling on the cell surface and at cell-cell contact sites.[5] Hence, immunofluorescence staining confirmed that F-actin and E-cadherin were highly expressed in A549 spheroids generated by both bioprinting and manual dispensing (Figure 2A). Töyli M, et al.[6] reported that when MDCK cells are cultured in a 3D environment, they form cell cysts with the apical domain facing a lumen, and with E-cadherin delineating the lateral membranes and ZO-1 at the tight junctions within the lumen. In agreement, the present experiment showed that MDCK spheroids generated by both methods expressed E-cadherin and ZO-1 (Figure 2B). Moreover, immunohistochemical analysis of the MIOs cultured using bioprinted and manual methods showed that the 3D cultures contained differentiated intestinal cells, including enterocytes, goblet cells, and Paneth cells, as demonstrated by the expression of Villin, Mucin-2, and Lysozyme, respectively (Figure 2C), which was consistent with a previous report.[7]

In accordance with Liu J, et al.,[5] the lipopolysaccharide receptors CD14, MD2, and TLR4, were upregulated in 3D cultures as compared with 2D cultures. Herein, the levels of these receptors in both bioprinted and manually generated spheroids were comparable and higher than those in monolayer cell cultures (Figures 3A-C). The formation of MDCK spheroids was previously reported to be accompanied by a reduced expression of the apoptosis inhibitor Survivin and of the vesicle transport effector Rab7, but with increased Rab5 expression.[6] Consistent with these findings, herein Rab5 was also found to be upregulated, whereas Rab7 and Survivin were downregulated in MDCK cultures compared with 2D cultures (Figure 3D-F). In addition to remarkable proliferative activity, the cellular composition of the intestinal epithelium is extremely diverse, reflecting a high degree of heterogeneity within its major lineages.[8] In line with the intestinal epithelium cellular nature, qPCR analysis confirmed that the experimental MIOs expressed the coding genes of Lgr5, Villin, Vimentin, TFF3, Lysozyme, and chromogranin-A and -B, which are specific to intestinal stem cells, enterocytes, mesenchymal cells, goblet cells, Paneth cells, and enteroendocrine cells, respectively (Figure 3G). Noteworthy, a high level of consistency of gene expression profiles was observed between 3D cultures generated by the Corning Matribot bioprinter and manual dispensing.

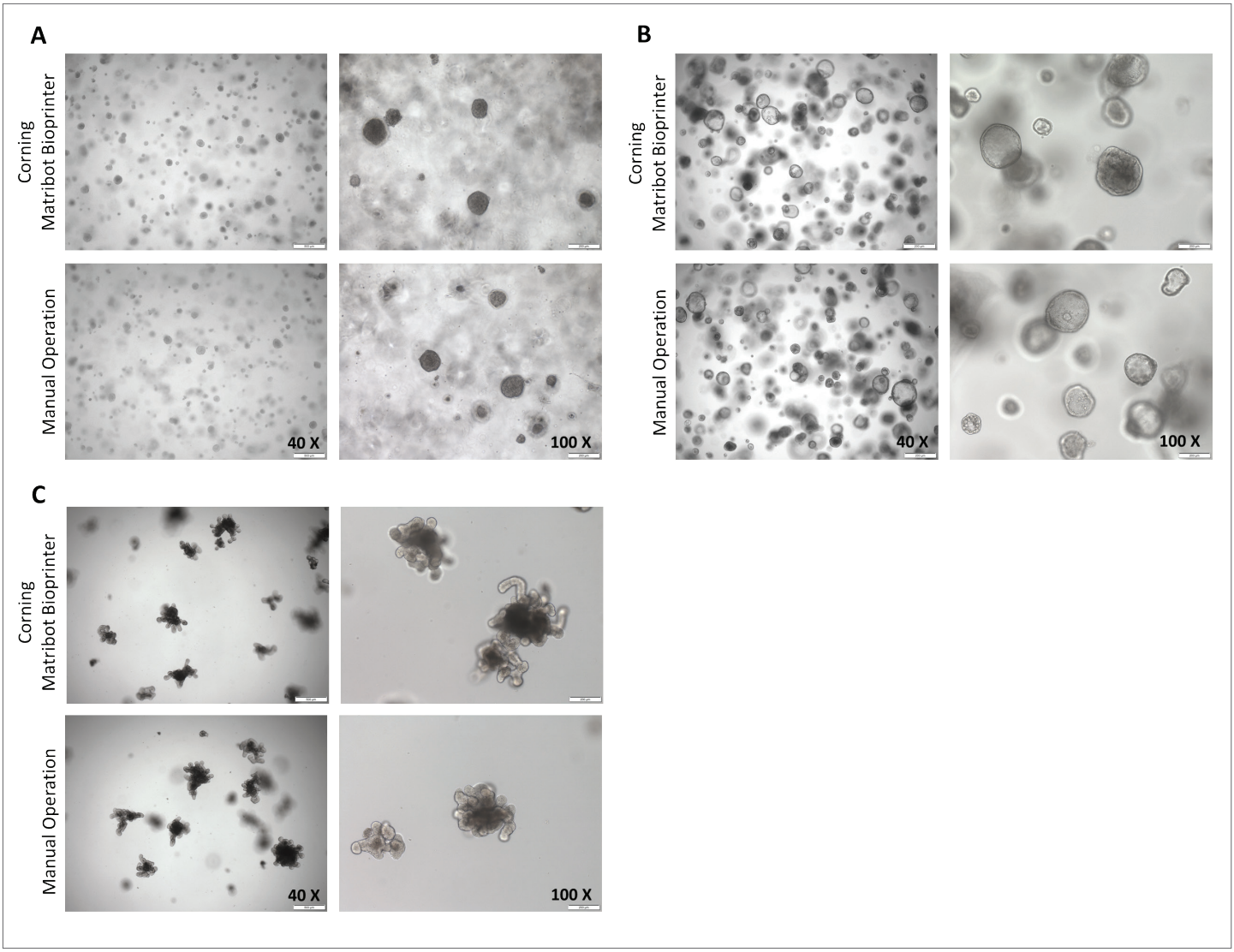

Figure 1. Representative photomicrographs of A549 and MDCK spheroids, and mouse intestinal organoids (MIOs). Brightfield of manual generated and bioprinted 3D cultures. (A) A549 spheroids after 10 days in culture. (B) MDCK spheroids after 7 days in culture. (C) MIOs after 5 days in culture. Images were captured at 40X and 100X magnifications with an Olympus IX53 microscope.

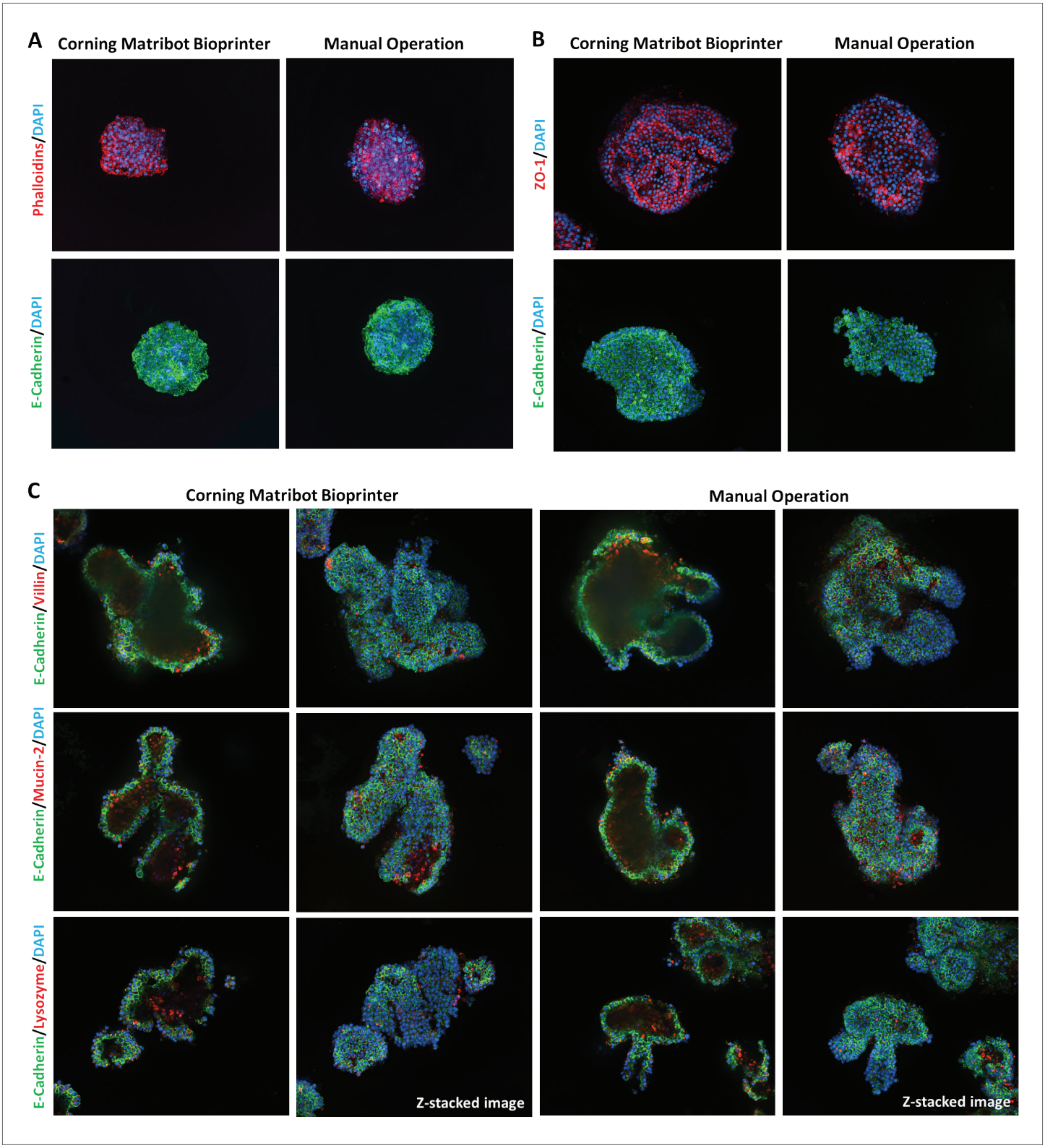

Figure 2. Immunohistochemical staining of specific cell types in the 3D cell cultures. Representative photomicrographs of fluorescently stained (A) A549 spheroids, (B) MDCK spheroids, and (C) mouse intestinal organoids. Images were captured at 200X magnification with an YOCOGAWA CQ1 Image Cytometer.

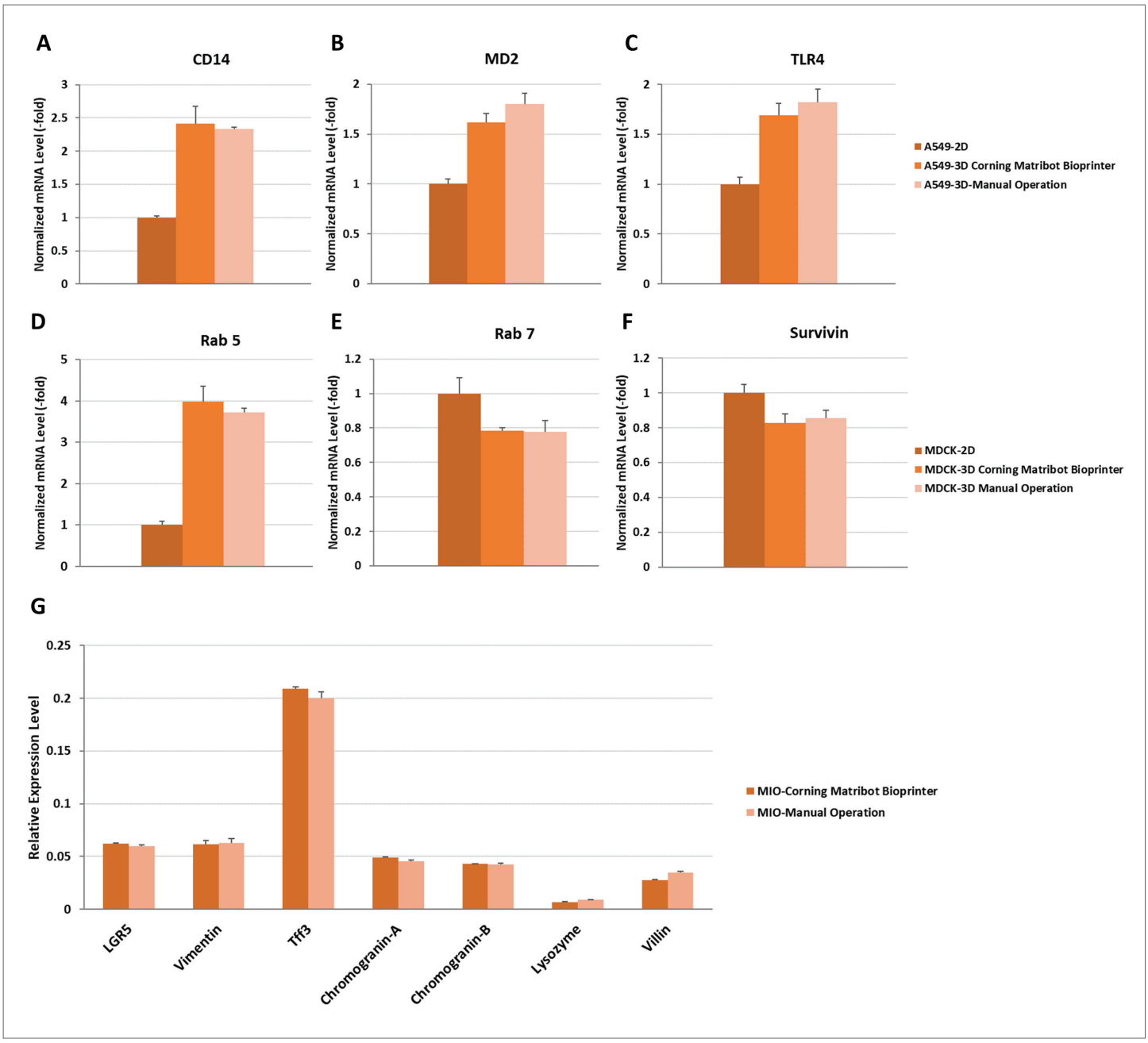

Figure 3. Gene expression profile in A549 and MDCK spheroids, and mouse intestinal organoids (MIOs). (A) CD14, (B) MD2, and (C) TLR expression in A549 monolayer and spheroid 3D cultures. (D) Rab5, (E) Rab7, and (F) Survivin relative mRNA expression in MDCK monolayer and spheroid 3D cultures. (G) Cell - specific maker expression in MIOs. Data represent mean ± SD of experimental triplicates.

Conclusions

- 3D cell cultures generated using the Corning® Matribot® bioprinter were similar to those obtained by manual dispensing in terms of morphology, component cell types, and gene expression profile.
- The culture dispensed by the Corning Matribot Bioprinter were suitable to be analyzed by various techniques, including observation under a microscope, immunohistochemical analysis, qPCR, etc.

NOTE: Should you intend to use the HUB Organoid Technology methods for commercial purposes, please contact HUB at info@hub4organoids.nl for a commercial use license.

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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

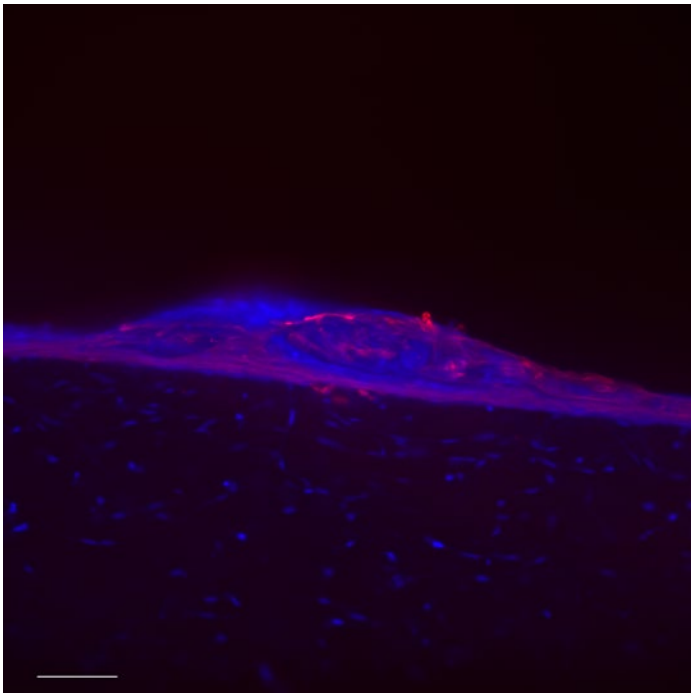

Figure 1. Cross-section view of multilayered 3D printed skin stained with Hoechst (blue) and Cytokeratin (red). Scale bar is 100 µm.

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.

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

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Application Note

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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).

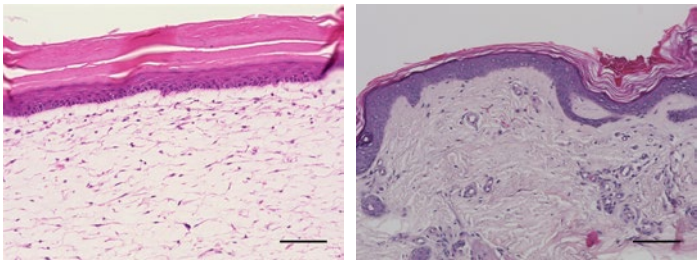

Figure 1. Cross-section view of hematoxylin-eosin stained Corning Matribot bioprinter printed skin model (left) and human skin sample (right). Scale bar is 100 µm.

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.

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Considerations when Optimizing Corning® Matribot® Bioprinter Dispensed Dome Assays

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Since its first publication, dome or droplet culture has become a widely used method for propagating and assaying epithelial derived organoids[1]. The technique involves mixing stem cells or pieces of tissue containing stem cells with an extracellular matrix (ECM) such as Corning Matrigel® matrix and dispensing this mix as droplets onto a cell culture surface. The cell filled domes are then polymerized and overlaid with media optimized for the organoid of interest. Use of the Corning Matribot bioprinter to dispense droplets can increase the accuracy and precision of dome size and placement. Unfortunately, this seemingly simple process, if not properly implemented, can result in poor growth and unwanted differentiation. The following information will highlight some important factors to consider to better optimize the printing of organoids in an ECM matrix. Additional information on optimizing Matribot bioprinter settings can be found in the document Corning Matribot Bioprinter Parameters (CLS-AN-648).

Dome Size

Optimizing the size of the organoid dome is a key factor for successful organoid health as nutrient diffusion into ECMs can be a limiting factor[3]. Mouse organoids have been traditionally cultured in a single 50 µL dome in each well of a 24-well plate[2] whereas human organoids, which can be more sensitive to growth factor diffusion from surrounding culture media, often do better in smaller domes that enable better growth factor penetration[4]. Differences in organoid morphology between the center and edge of a dome is likely an indication that a reduction in dome size is warranted. In addition to health of the organoids, the purpose of the culture should be considered when choosing dome size. Smaller domes are easier to visualize due to the more uniform focal plane of the organoids. Inversely, larger domes are more challenging to image as multiple focal planes are required due to dome thickness but produce more organoids per dome which can be beneficial for scaling up cultures.

Creating Sturdy Domes

For organoid cultures to be successful, it is essential that Matrigel matrix domes maintain their integrity for the duration of culture. One of the most important aspects to consider is Matrigel matrix concentration. Matrigel matrix with higher protein concentrations can result in stiffer and more stable domes. If domes are breaking down after several days of culture, consider increasing the Matrigel matrix concentration, considering the final concentration of Matrigel matrix after cell and media additions. It should be noted that repeated freeze/thaw cycles of the Matrigel matrix can degrade the stability and ability to maintain printed structures. It is recommended that Matrigel matrix be aliquoted and frozen into single-use volumes when initially thawed. Also, ensure domes are fully polymerized before addition of pre-warmed cell culture medium, keeping in mind that larger domes take longer to polymerize than smaller ones. Finally, using cold medium during exchanges may break down the Matrigel matrix over time and should be avoided.

Plate Type and Preparation

For most applications, a tall dome of organoids is desired to prevent cells from contacting the plastic plate surface where a thin spread-out dome would bring cells closer to the plastic growth surface and increase the likelihood of attachment and differentiation. Ideally, a tissue culture-treated surface is recommended to provide enough treatment for the dome to remain attached during media exchanges but does not cause the dome to spread out. We have also had success with non-treated plates depending on the length of culture and frequency of media exchanges. Incubating plates in a cell culture incubator, for at least 24 hours prior to printing, has been found to result in taller domes that polymerize more quickly. Using the heated printbed of the Corning Matribot bioprinter can help domes polymerize faster as well. If the experiment requires a short duration for organoid culture (i.e., for an imaging assay) the use of the heated printbed may not be desired as it might be better to allow the organoids to settle into a more uniform focal plane before polymerization is complete.

Corning Matribot Printing Optimization

The temperature controlled printhead of the Matribot bioprinter is one of the main features that make it well suited for printing Matrigel matrix domes. To keep Matrigel matrix less viscous and therefore printable, the temperature must be maintained under 10°C. It is essential to ensure that all consumables in contact with Matrigel matrix are chilled prior to use including pipet tips, syringes, and printing nozzles.

In addition to keeping ink printable, there are several factors that can impact the success of droplet dispenses such as the height at which the domes are dispensed in relation to the bottom of the plate. If the droplet is dispensed too high from the plate surface, the Matrigel matrix droplet could remain attached to the nozzle and be carried to the next print location resulting in some wells with less domes than other wells or with domes of varying sizes within a given well. The automatic calibration function that is integrated into the Corning DNA Studio software will help to determine the appropriate Z height for printing.

In certain instances (e.g., the print nozzle becomes warped or when using a non-standard receiver plate) it might be desired to perform a manual calibration instead of automatic. This can easily be done with a clear plate following the Corning® Matribot® Bioprinter Instruction Manual (CLS-AN-641DOC). Manual calibration can also be implemented if the plate being printed into has opaque side walls by using a clear equivalent plate. If a clear equivalent plate is not available, manual calibration of Z height can be accomplished by using the printbed as a reference point and then increasing the Z height by the thickness of the plate bottom. The plate bottom height is available from the plate manufacturer and is typically referred to as the well bottom elevation.

Other factors that are important for consistency of droplet dispensing are ensuring a clean nozzle and a fully primed printhead. If liquid is coating the outside of the print nozzle opening, the surface tension can pull the dispensing ink towards that liquid resulting in an inadequate dispense for one dome and a larger dome in the next print location. Using a sterile alcohol wipe to clean the nozzle and allowing it to dry, just prior to printing, can reduce the likelihood of this occurring. A printhead that is not fully primed will result in unprinted locations until the ink reaches the tip of the nozzle. This can be prevented by ensuring that the ink is fully primed all the way to the tip of the nozzle prior to printing. If the first dome is still not being printed, increase the extra pre-flow volume.

Format/Throughput

The Corning Matribot bioprinter has been designed to use with BD Luer-Lok™ 3 mL Syringes (BD 309657). Syringes should be filled with no more than 2.7 mL of ink. This means the number of domes and plates that can be filled with a single fill will vary based on volume per well and plate format used. Corning DNA Studio software comes with pre-loaded droplet dispense settings allowing for single or multiple domes in each well depending on the configuration of the microplate being used. Alternatively, if the desired dome format is not available via the Droplet Dispense option, a custom STL file can be generated using a third party design software which can be imported into DNA Studio.

Filling each plate with the number of domes listed in Table 1 takes about 3 minutes (extrusion speed of 60 µL/sec. and a 20 mm Z-lift between wells). Testing the settling rate of cells or organoids in the bioink is an important factor to consider as settling rates for each cell type or organoid can impact the reproducibility across multiple plates. This must be tested empirically, as the settling time can be a dependent on the time to print, ink concentration, temperature, type of bioink, as well as the size of cells or organoids.

Table 1. Domes per well via Droplet Dispense drop down menu.

Plate Format	Maximum Number of Domes/Well
6-well plate	9
12-well plate	5
24-well plate	4
48-well plate	1
96-well microplate	1

Discussion

Optimization of any cell-based assay is essential to achieving consistent and meaningful results. The added challenge of using sensitive systems such as organoids makes dome culture optimization even more compelling. Dome size, ink formulation, plate type, and print settings can all have significant impact on the final product and should be optimized to attain the most benefit of using a bioprinter.

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