# Corning® Matribot® Bioprinter: Bioprinting with Corning Matrigel® Matrix

## Protocol

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This is a suggested procedure, please use this as a starting point and adjust according to your experimental needs. To maintain the sterility of the product, work under aseptic conditions.

### Introduction

The aim of this protocol is to provide recommendations for dispensing droplets and bioprinting single-layered grids and multi-layered constructs with Corning Matrigel matrix at different concentrations using the Corning Matribot bioprinter. Droplets of Corning Matrigel matrix can be used to create spheroids or organoids with a variety of cell types. Corning Matrigel matrix can also be used to bioprint grids, creating reproducible structures that allow for effective nutrient and gas exchange that can be used for various applications. This document covers bioprinting parameters and procedures for printing with and without cells on Petri dishes, multiwell plates, or microplates (96- or 384-well). This protocol was optimized for Corning Matrigel matrix at a range of concentrations from 5 to 10 mg/mL.

For more details on operating the Corning Matribot bioprinter, please refer to the Corning Matribot Bioprinter Instruction Manual (CLS-AN-641DOC).

### Materials

- Corning Matribot bioprinter (Corning 6150)
- Corning Matrigel matrix
- Serum-free culture media
- 3 mL syringe with BD Luer-Lok™ tip (BD 309657)
- Syringe Luer-Lok cap (BD 408531)
- Ice bath or Corning CoolBox™ container
- Petri dish, multiwell plate, or microplate
- High precision conical bioprinting nozzles, 25G (Corning 6169) or standard conical bioprinting nozzles, 25G (Corning 6166)*

*It is recommended to use high precision conical nozzles for applications where having low variation in droplet size of samples is crucial.

**NOTE:** Keep Corning Matrigel matrix on ice and protected from heat until loaded into the pre-cooled Matribot printhead.

### Protocol

This protocol has been optimized for use with the Corning Matribot bioprinter, which has a cooled printhead. Set the printhead temperature prior to preparing the Matrigel matrix solution as recommended in Step 1. Keep the Matrigel matrix solution cooled on ice until loaded into the pre-cooled Matribot printhead. Pre-chill all plastics in contact with the Matrigel matrix solution such as pipet tips. Clogging of Matrigel matrix at the nozzle tip may still occur if the Matrigel matrix solution is not kept cold.

| Step | Title | Material | Description |
|---|---|---|---|
| 1 | Set Printhead temperature | Corning Matribot bioprinter | • Set the Matribot bioprinter printhead temperature to 2°C. This can be done by navigating through the Matribot bioprinter LCD display or in Corning DNA Studio software.<br>- If using the LCD display, use the dial to navigate to and select Prepare Bioprint. Scroll down to Printhead Temp and select to enter the desired temperature. Scroll down to select Enable Temperature to activate temperature control.<br>- If using Corning DNA Studio software, select Utilities from the Tools drop down menu. In the Utilities window, select the Temperature tab. Enter the desired printhead temperature and select the slidebar to activate temperature control. |
| 2 | Prepare Matrigel matrix | • Corning Matrigel matrix<br>• Serum-free culture media<br>• Ice bath or Corning CoolBox container | **Refer to the Corning Matrigel Basement Membrane Matrix Guidelines for Use for a detailed description on how to handle the Matrigel matrix solution.**<br>• Keep Matrigel matrix solution and all other materials cool on ice. Dilute to desired concentration in serum-free media. If you will be mixing with cells, take this volume into account when determining the concentration to prepare. |

| Step | Title | Material | Description |
|---|---|---|---|
| 3 | Mix Matrigel matrix with cells | • Cell suspension<br>• Corning® Matrigel® matrix solution | **If not printing with cells, move to Step 4.**<br>• Mix the Matrigel matrix solution with cell suspension, taking care not to introduce air bubbles to the mixture. |
| 4 | Cool and load the syringe | • 3 mL syringe<br>• Corning Matrigel matrix solution<br>• Standard conical bioprinting nozzle, 25G | • Transfer the Matrigel matrix solution to a 3 mL syringe, and cap with a bioprinting nozzle.<br>• Attach the thermal insulator to the cooling block on the Matribot bioprinter by inserting it from below and rotating counterclockwise. Use the short insulator for printing in 384-well microplates.<br>• Place the loaded syringe into the pre-cooled printhead. Rotate the syringe counterclockwise until the syringe tabs are locked in place.<br>• Adjust the position of the syringe plunger holder arm by navigating on the LCD interface to Prepare Bioprint. Select Raise Plunger to raise the plunger arm to its maximum height, and use Extrude Volume to lower the plunger arm until it aligns with the height of the syringe plunger.<br>• Rotate the syringe plunger holder arm over the syringe plunger.<br>• Use the Extrude Volume function on the LCD interface in the Prepare Bioprint menu to prime until the Collagen solution fills the nozzle and a few droplets are dispensed. Hold a lab tissue under the printing nozzle to catch the extruded material. |
| 5 | Printing Parameter selection | Corning DNA Studio software | • Use Corning DNA Studio software to select parameters based on your application and select Print on the toolbar when complete:<br>- See Table 1 for droplet dispensing for dome formation as single droplets or droplet arrays.<br>- See Table 2 for droplet dispensing in a 384-well microplate to fill the well bottoms.<br>- See Table 3 for printing single-layer grids.<br>- See Table 4 for printing multi-layered constructs up to four layers.<br>**NOTE:** The values in Tables 1 through 4 are only a reference point for starting parameters. The actual values needed for your given application will depend on the preparation procedures (i.e., concentration and temperature of Matrigel matrix solution) as well as the print surface.<br>**NOTE:** Parameter selection can also be performed prior to Matrigel matrix preparation. |
| 6 | Machine calibration (manual or automatic) | • Corning Matribot® bioprinter<br>• Petri dish, multiwell plate, or microplate | • Place a Petri dish, multiwell plate, or microplate on the printbed.<br>• Perform machine calibration each time a new syringe is placed in the printhead or a new plate type is used.<br>• If the printbed is not leveled, perform Automatic bed-leveling.<br>**NOTE:** Manual calibration is recommended for 96-well microplates and is necessary for 384-well microplates. Ensure the nozzle tip is placed in the center of the well, since manual calibration results in x, y, and z calibration. |
| 7 | Nozzle priming | Corning Matribot bioprinter | • Immediately before each print, prime the nozzle by extruding 2 to 3 drops. If any material has gelled at the tip of the nozzle, ensure it is fully extruded prior to starting a print.<br>**NOTE:** If the system has been idle for an extended period, the Matrigel matrix solution in the nozzle can dry or gel causing it to clog. If this occurs, purge the nozzle by extruding 30 to 60 µL of the Matrigel matrix solution, or until the gelated part is extruded. If the clog cannot be removed, replace with a new nozzle. Always ensure the nozzle is fully primed with liquid Matrigel matrix solution prior to printing. Cells may sediment in the Matrigel matrix solution if idle for extended periods. Remove the syringe from the printhead and flip back and forth a few times to redistribute the cells. Place the syringe back in the printhead and repeat Steps 6 and 7. |
| 8 | Printing | Corning Matribot bioprinter | • Press Start to start the printing process.<br>- See Figure 1 for reference droplets in a 96-well microplate, Figure 2 for grid structures, and Figure 3 for multi-layered constructs.<br>- If the printed structures are not as desired, adjust the extrusion rate up or down by 0.1 µL to extrude more or less material.<br>**NOTE:** If printing does not begin right away, it is most likely because the printhead or printbed has not yet reached the temperature set-point. |
| 9 | Polymerization | Incubator | Matrigel matrix polymerizes at temperatures above 10°C.<br>• For polymerization: leave the plate or dish on the heated printbed or place in a 37°C humidified incubator for approximately 3 to 25 min. based on construct size. Check periodically if sufficiently polymerized. |
| 10 | Incubation | Cell culture medium | • After polymerization, add the desired medium to the constructs and place in the incubator.<br>• Incubate the constructs in cell culture medium in standard culture conditions (37°C, 5% $CO_2$, and 95% relative humidity) or according to your application. |

**Table 1.** Recommended printing parameters for dispensing Corning® Matrigel® matrix droplets for dome formation at 5, 7, and 9 mg/mL protein concentration without cells, and 10 mg/mL protein concentration with cells using the Droplet Print function on the Corning Matribot® bioprinter. The density of cell suspension may alter the flow rate. In the software Surface tab, select your desired plate type and number of droplets per well.

| Parameters | 5 mg/mL | 7 mg/mL | 9 mg/mL | 10 mg/mL |
|---|---|---|---|---|
| Cell concentration | No cells | No cells | No cells | 5 million/mL |
| Temperature printbed* | Disabled | Disabled | Disabled | Disabled |
| Temperature printhead | 2°C | 2°C | 2°C | 2°C |
| Extrusion rate | 20 µL/s | 60 µL/s | 60 µL/s | 15 µL/s |
| Extrusion volume | 8 µL | 8 µL | 8 µL | 20 µL |
| Retract volume | 5 µL | 5 µL | 5 µL | 10 µL |
| Droplet volume | 3 µL | 3 µL | 3 µL | 10 µL |
| Z-offset | 0 mm | 0 mm | 0 mm | 0.2 mm |
| Extra preflow volume | 0.5 µL | 0.5 µL | 0 µL | 0 µL |
| Retract rate | 60 µL/s | 60 µL/s | 60 µL/s | 15 µL/s |
| Postflow stop time | 0 s | 0 s | 0 s | 0 s |

*The printbed temperature can be set to 37°C according to application, for example if printing with cells or if experiencing poor shape fidelity of the droplet. The heated printbed can also be used for polymerization of Matrigel matrix. However, keep in mind that using a heated printbed could result in faster evaporation of smaller droplets.

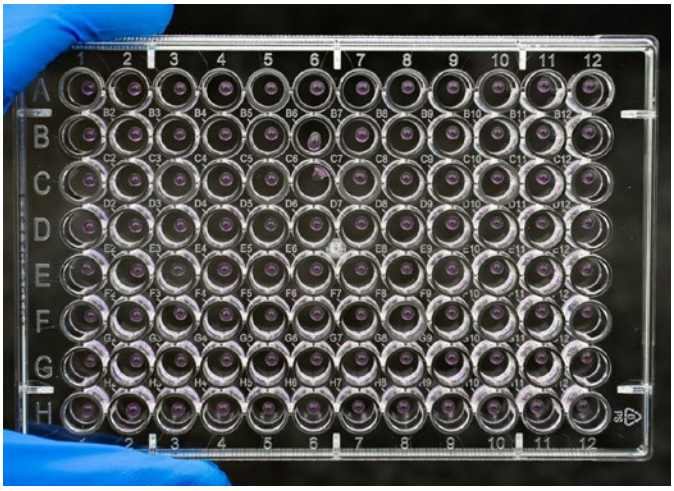

**Figure 1.** Droplets of 9 mg/mL Corning Matrigel matrix without cells dispensed using the parameters in Table 1.

**NOTE:** The droplets may spread or move to the edges of the wells which can be because of the coating of the well plate, poor plate calibration, or if there are residues of bioink on the sides of the nozzle. This can be minimized by changing to a different kind of surface, with proper calibration of the nozzle to the surface, or by wiping the sides of the nozzle with 70% ethanol or isopropanol wipes.

**Table 2.** Recommended settings used for dispensing 4 µL or 10 µL droplets of Corning Matrigel matrix without cells in a 384-well microplate using the Droplet Print function on the Matribot bioprinter.

| Parameters | 5 mg/mL | 10 mg/mL |
|---|---|---|
| Cell concentration | No cells | No cells |
| Temperature printbed* | Disabled | Disabled |
| Temperature printhead | 2°C | 2°C |
| Extrusion rate | 50 µL/s | 50 µL/s |
| Extrusion volume | 13 µL | 10 µL |
| Retract volume | 3 µL | 6 µL |
| Droplet volume | 10 µL | 4 µL |
| Z-offset | 0.0 mm | 0.0 mm |
| Extra preflow volume | 0 µL | 0 µL |
| Retract rate | 50 µL/s | 50 µL/s |
| Postflow stop time | 0 s | 0 s |

*Heating the printbed may cause smaller droplets to evaporate at a faster rate.

**NOTE:** When dispensing into 384-well microplates, do not leave the printer idle for longer than 5 minutes since the shorter thermal insulator exposes the nozzle tip to ambient temperature which increases the risk of clogging. Make sure to prime the nozzle before the start of a new print.

**NOTE:** If printing droplets larger than 7 µL, the full 384-well microplate can't be filled from a single syringe. If printing droplets smaller than 10 µL, the bottom of the well might not be completely covered by the gel.

**Table 3.** Recommended settings used for printing single-layered grids (20 x 20 mm) without cells at three different Corning® Matrigel® matrix concentrations. If diluting the Matrigel matrix with cells, the density of the cell suspension may alter the flow rate and the parameters may need to be adjusted.

| Parameters | 5 mg/mL | 7 mg/mL | 9 mg/mL |
|---|---|---|---|
| Temperature printbed | 25°C | 25°C | 25°C |
| Nozzle | 0.25 mm | 0.25 mm | 0.25 mm |
| Speed | 10 mm/s | 10 mm/s | 10 mm/s |
| Temperature printhead | 2°C | 2°C | 2°C |
| Preflow volume | 3.5 µL | 3.5 µL | 3.5 µL |
| Extrusion rate | 0.9 µL/s | 1 µL/s | 1 µL/s |
| Retract volume | 3.0 µL | 3.2 µL | 3.2 µL |
| Z-offset | 0 mm | 0 mm | 0 mm |
| Extra preflow volume | 2.5 µL | 2.5 µL | 2.5 µL |
| Infill extrusion multiplier | 100% | 100% | 100% |
| Retract rate | 5 µL/s | 10 µL/s | 10 µL/s |
| Extra retract | 0 µL | 0 µL | 0 µL |
| Postflow stop time | 0.3 s | 0.3 s | 0.3 s |
| Z-lift | 2.0 mm | 2.0 mm | 2.0 mm |

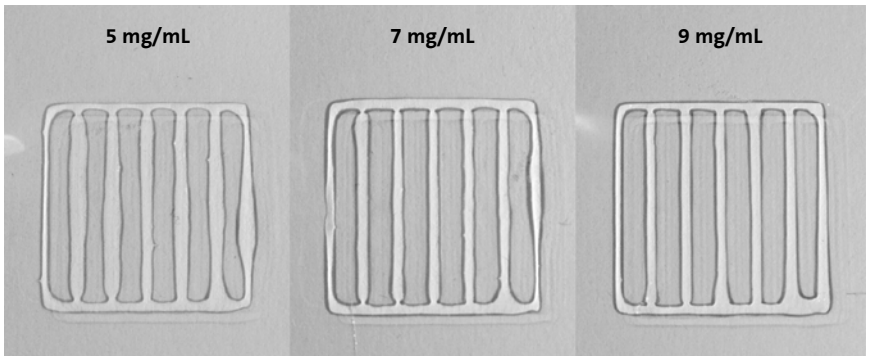


**Figure 2.** Grid structures acquired after printing with the parameters from Table 3 using Corning Matrigel matrix at different concentrations.

**Table 4.** Recommended settings used for printing a three-layered hexagon and four-layered concentric circle with 9 mg/mL Corning Matrigel matrix without cells. If diluting the Matrigel matrix with cells, the density of the cell suspension may alter the flow rate and the parameters may need to be adjusted.

| Parameters | Hexagon | Circle |
|---|---|---|
| Temperature printbed | 36°C | 36°C |
| Nozzle | 0.25 mm | 0.25 mm |
| Speed | 5 mm/s | 5 mm/s |
| Temperature printhead | 2°C | 2°C |
| Preflow volume | 3.7 µL | 3.7 µL |
| Extrusion rate | 0.5 µL | 0.4 µL |
| Retract volume | 3.2 µL | 3.2 µL |
| Z-offset | 0.3 mm | 0.4 mm |
| Extra preflow volume | 1.6 µL | 1.6 µL |
| Infill extrusion multiplier | 60% | 100% |
| Retract rate | 3.0 µL/s | 3.0 µL/s |
| Extra retract | 0 µL | 0 µL |
| Postflow stop time | 0.3 s | 0.3 s |
| Z-lift | 2.0 mm | 2.0 mm |

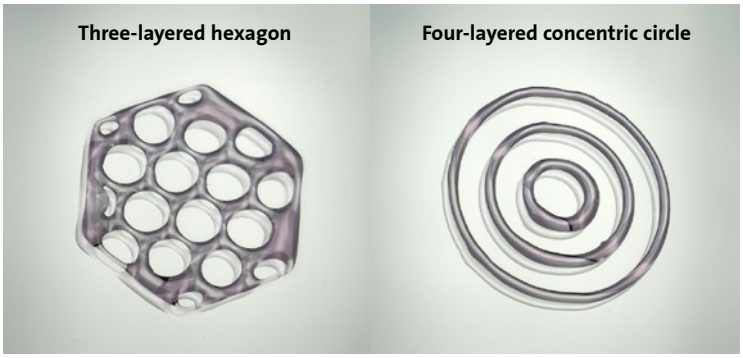


**Figure 3.** Multi-layered structures acquired after printing with the parameters from Table 4 with 9 mg/mL Corning Matrigel matrix.

The printing parameters included in Tables 1-4 of this protocol are a good starting point, but might require some adjustments for your particular application. For more details on how printing parameters affect printing or dispensing, please see the Corning Matribot Bioprinter Parameters (CLS-AN-648).

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