

# Corning® Matribot® Bioprinter

CORNING

## Demo Protocol Experiment 4:

Mimicking Corning Matrigel® matrix printing in a dish from an SD card using Corning Pluronics sacrificial ink

### Introduction

The aim of this protocol is to provide instructions for bioprinting single layer grids with Corning Pluronics sacrificial ink using the Corning Matribot bioprinter. Corning Pluronics sacrificial ink can mimic the handling and bioprinting process of 9 mg/mL Corning Matrigel matrix solution. This document covers bioprinting parameters and procedures for bioprinting into a Petri dish using a g-code loaded in an SD card. All parameters are selected using the LCD interface on the Matribot bioprinter. This protocol was optimized for Pluronics sacrificial ink diluted to 25% with water.

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

### Materials

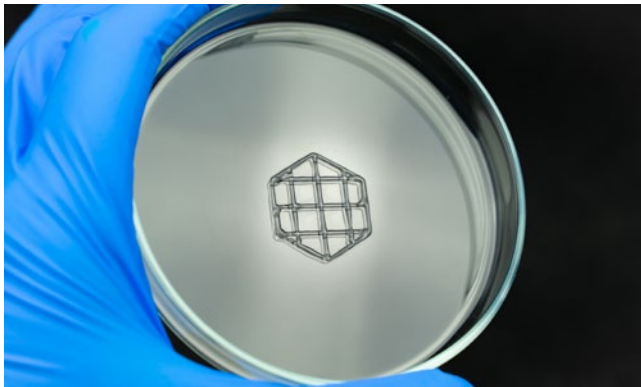
- ▶ Corning Pluronics sacrificial ink, 2.7 mL/syringe (Corning 6157)
- ▶ Corning Matribot bioprinter (Corning 6150)
- ▶ Corning standard conical bioprinting nozzles, 22G-410 µm (blue; Corning 6167)
- ▶ Corning 100 x 15 mm Petri dish with cover (Corning 70165-101) or Corning 100 x 10 mm Petri dish with cover (Corning 70165-100)
- ▶ SD card containing g-code file
- ▶ Purified water, 5 mL pre-chilled at 4°C
- ▶ 3 mL syringe with BD Luer-Lok™ tip (BD 309657)
- ▶ Syringe Luer-Lok cap (BD 408531)
- ▶ 15 mL conical tube
- ▶ Serological pipet or pipet tips pre-chilled at 4°C

### Protocol

This protocol has been optimized for use with the Corning Matribot bioprinter, which has a cooled printhead. However, clogging of Corning Pluronics sacrificial ink at the nozzle tip may still occur. Set the printhead temperature prior to loading the Corning Pluronics sacrificial ink syringe as recommended in Step 1. Keep the Corning Pluronics sacrificial ink refrigerated or on ice until loaded into the pre-cooled Corning Matribot bioprinter printhead. When preparing 25% Corning Pluronics sacrificial ink solution, pre-chill all plastics coming into contact with the Corning Pluronics sacrificial ink such as pipet tips.

Step	Title	Material	Description
1	Set Printhead temperature	<ul style="list-style-type: none"><li>• Corning Matribot bioprinter</li><li>• 100 x 10 mm or 100 x 15 mm Petri dish</li></ul>	<ul style="list-style-type: none"><li>• Connect the printer to the computer using the provided USB cable.</li><li>• Turn on the Matribot bioprinter using the power switch.</li><li>• On the LCD interface, scroll to the Utilities menu and select Enable SD Print.</li><li>• On the LCD interface, return to the main screen by scrolling and choosing Back. Select the Prepare Bioprint menu. Enable temperature control by selecting Enable Temp, and set Printhead Temp to 4°C.</li><li>• Attach the standard thermal insulator.</li><li>• Place a Petri dish (without a cover) on the printhead.</li><li>• Place the SD card containing the DEMO EXP 4.gcode file into the SD card slot.</li></ul>
2	Prepare 25% Pluronics solution	<ul style="list-style-type: none"><li>• Corning Pluronics sacrificial ink</li><li>• Conical tube</li><li>• Purified water</li><li>• 3 mL syringe</li><li>• Syringe Luer-Lok cap</li><li>• Ice bath</li></ul>	<ul style="list-style-type: none"><li>• Prepare 25% Pluronics solution by diluting Corning Pluronics sacrificial ink, which contains 40% Pluronics, in purified water.</li><li>- Prior to starting, place on ice a Corning Pluronics sacrificial ink syringe, a conical tube, a tube containing purified water, and a capped 3 mL syringe with the plunger removed.</li><li>- Use the plunger on the Corning Pluronics sacrificial ink syringe to push the ink into the empty 15 mL conical tube until the Pluronics ink reaches the desired fill line (e.g., 2 mL).</li><li>- Using a pre-chilled serological pipet or pipet tip, transfer the appropriate amount of cold purified water (e.g., 1.2 mL) into the same tube to dilute the Pluronics to a 25% solution. Slowly mix to form a homogenous solution taking care not to let the solution warm. As the solution warms, the viscosity increases, complicating mixing.</li><li>• Transfer 2.7 mL of 25% Pluronics solution to the prepared 3 mL syringe and place the plunger into the syringe. While holding the plunger in place, flip the syringe with the cap end up, and replace the syringe cap with a 22G nozzle. Push on the plunger to remove air until the 25% Pluronics solution reaches approximately halfway through the nozzle.</li></ul>

Step	Title	Material	Description
3	Load the syringe	<ul style="list-style-type: none"> <li>Corning® Matribot® bioprinter</li> <li>3 mL syringe of 25% Corning Pluronics solution</li> <li>Standard conical bioprinting nozzle, 22G-410 µm</li> </ul>	<ul style="list-style-type: none"> <li>Remove air bubbles from the syringe by tapping the syringe.</li> <li>After the printhead has reached 4°C, place the loaded syringe into the pre-cooled printhead.</li> <li>Rotate the syringe counterclockwise until the syringe tabs are locked in place.</li> <li>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.</li> <li>Rotate the syringe plunger holder arm over the syringe plunger.</li> </ul>
4	Machine Calibration (manual)	<ul style="list-style-type: none"> <li>Corning Matribot bioprinter</li> <li>Petri dish</li> </ul>	<ul style="list-style-type: none"> <li>Extrude 30 µL using the Extrude Volume function on device's LCD interface in the Prepare Bioprint menu. Hold a lab tissue under the printing nozzle to catch the extruded material.</li> <li>In the Prepare Bioprint menu select the Home Axes function.</li> <li>In the Prepare Bioprint menu, use the Move Z function to move the stage up by 1 mm and 0.1 mm increments until the end of the nozzle is as close to the surface of the dish as possible without touching it. (<b>NOTE:</b> the Z values will decrease as the stage moves up).</li> <li>In the Prepare Bioprint menu, select the Calibrate Z function.</li> <li>Perform machine calibration each time a new syringe is placed in the printhead or a new surface type is used.</li> </ul>
5	Nozzle priming	<ul style="list-style-type: none"> <li>Corning Matribot bioprinter</li> </ul>	<ul style="list-style-type: none"> <li>Immediately before each print, prime the nozzle by extruding 2 to 3 drops. Priming can be performed by extruding 1 µL increments by using the Extrude Volume function on the LCD interface. If any material has gelled at the tip of the nozzle, ensure it is fully extruded prior starting a print.</li> </ul> <p><b>NOTE:</b> If the system has been idle for an extended period the Pluronics sacrificial ink 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 Pluronics sacrificial ink, or until the gelated part is extruded. If it is not possible to extrude the gelated Pluronics sacrificial ink, replace the clogged nozzle with a new nozzle. Always ensure the nozzle is fully primed prior to printing.</p>
6	Printing	<ul style="list-style-type: none"> <li>Corning Matribot bioprinter</li> </ul>	<ul style="list-style-type: none"> <li>In the Bioprint menu select DEMO EXP 4.file to start the bioprinting process.</li> <li>See Figure 1 for a reference grid.</li> </ul>
7	Finishing	<ul style="list-style-type: none"> <li>Corning Matribot bioprinter</li> </ul>	<ul style="list-style-type: none"> <li>After the print has finished, scroll to the Utilities menu on the LCD interface and select Disable SD Print. Also scroll to the Prepare Bioprint menu and select Disable Temp to disable the printhead temperature control.</li> </ul>



**Figure 1.** Grid acquired after bioprinting with 25% Corning Pluronics solution with a g-code file using the Corning Matribot bioprinter.

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