Corning® Matribot® Bioprinter: Bioprinting with Collagen

Protocol

CORNING

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 instructions for dispensing droplets and bioprinting single layered grids and multi-layered constructs with Collagen at different concentrations using the Corning Matribot bioprinter. Droplets of Collagen can be used in an effective contraction assay. Collagen can also be used to bioprint grids, creating reproducible porous structures that allow for nutrient and gas exchange that then can be used for various applications. This document covers bioprinting parameters and procedures for printing with and without cells on Petri dishes or well plates up to 384-well microplates. This protocol was optimized for Collagen at a range of concentrations from 2 mg/mL to 8 mg/mL. Changing the neutralization procedure or the Collagen concentration may change the bioprinting parameters.

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)
- Collagen (e.g., Corning Collagen I, high concentration, rat tail (Corning 354249)
- Sterile neutralization material (e.g., 1 N NaOH)
- 3 mL syringe with BD Luer-Lok™ tip (BD 309657)
- Ice bath or Corning CoolBox™ module
- 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 bioprinting nozzles for applications where having low variation in size of samples is crucial.

NOTE: Keep Collagen on ice and protected from heat until loaded into the pre-cooled Matribot bioprinter printhead.

Protocol

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

Step	Title	Material	Description
1	Set Printhead temperature	• Corning Matribot bioprinter • Corning DNA Studio software	• Set the Matribot bioprinter printhead temperature to 2°C. This can be done by navigating through the Matribot LCD display or in Corning DNA Studio software. - 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. If using Printbed temperature control, enter the desired temperature in Bed Temp. - 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 and printbed temperatures and select the slidebars to activate temperature control.

Step	Title	Material	Description
2	Prepare Collagen	• Collagen solution • Ice bath or Corning® CoolBox™ module	**Refer to the preparation protocol of the Collagen manufacturer for a detailed description on how to neutralize the Collagen solution.** • Keep the Collagen and all other materials cool on ice. • Make sure the pH is between 6.9 to 7.4. Adjust pH as necessary. • After neutralization to desired concentration, keep on ice, and begin the next step immediately. If mixing with cells, take this volume into account.
3	Mix neutralized Collagen with cells	• Cell suspension • Neutralized Collagen solution	**If not printing with cells, move to Step 4.** • Mix the Collagen solution with cell suspension, taking care not to introduce air bubbles to the mixture.
4	Cool and load the syringe	• Corning Matribot® bioprinter • 3 mL syringe • Collagen solution • Conical bioprinting nozzle, 25G	• Transfer the Collagen solution to a 3 mL syringe, and cap with a bioprinting nozzle. • 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. • Place the loaded syringe into the pre-cooled printhead. Rotate the syringe counterclockwise until the syringe tabs are locked in place. • 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. • Rotate the syringe plunger holder arm over the syringe plunger. • 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: - See Table 1 for droplet dispensing as single droplets or droplet arrays in multiwell plates or 96-well microplates, or for filling 384-well microplates. - See Table 2 for printing single-layer grids in a Petri dish. - See Table 3 for printing multi-layered constructs up to three layers. **NOTE:** The values in Tables 1 through 3 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 the Collagen solution) as well as the print surface. **NOTE:** Parameter selection can also be performed prior to Collagen preparation.
6	Machine calibration (manual or automatic)	• Corning Matribot bioprinter • Petri dish, multiwell plate, or microplate	• Place a Petri dish or well plate on the printbed. • Perform manual or automatic calibration following the software prompts. Perform machine calibration each time a new syringe is placed in the printhead or a new plate type is used. • If the printbed is not leveled, perform Automatic bed-leveling. **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. **NOTE:** If the system has been idle for an extended period, the Collagen 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 Collagen 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 Collagen prior to printing. Cells may sediment in the Collagen solution if idle for extended periods. Remove the syringe from the printhead and flip back and forth a few times. Place back in the printhead and repeat Steps 6 and 7.
8	Printing	• Corning Matribot bioprinter • Petri dish, multiwell plate, or microplate	• Press Start to start the printing process. - See Figure 1 for reference droplets, Figure 2 for grid structures, and Figure 3 for multi-layered constructs. - 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. **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	• Neutralized Collagen polymerizes more quickly at warmer temperatures. - 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 incubator. • 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 Collagen droplets at 2 mg/mL with cells and at 8 mg/mL without cells in a variety of well plates using the Droplet Print function on the Corning® Matribot® bioprinter. The density of cell suspension may alter the flow rate. Plate type and number of droplets per well can be selected in the Surfaces tab.

Parameters	2 mg/mL	8 mg/mL	8 mg/mL
Well plate	48-well plate	96-well microplate	384-well microplate
Cell concentration	0-0.4 million	No cells	No cells
Temperature printbed*	25°C	37°C	Disabled**
Temperature printhead	4°C	2°C	2°C
Extrusion rate	30 µL/s	30 µL/s	50 µL/s
Extrusion volume	30 µL	5 µL	13 µL
Retract volume	7 µL	2 µL	3 µL
Droplet volume	23 µL	3 µL	10 µL
Z-offset	0.2 mm	0.2 mm	0.0 mm
Extra preflow volume	0 µL	0 µL	0 µL
Retract rate	30 µL/s	30 µL/s	50 µL/s
Postflow stop time	0 s	0.5 s	0.0 s
Z-lift	20 mm	20 mm	20 mm

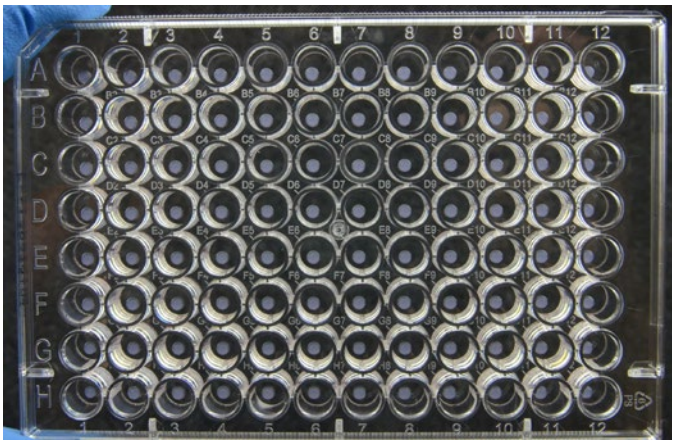

Figure 1. Droplets of 8 mg/mL Collagen without cells dispensed into a 96-well microplate using the parameters in Table 1.

*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 faster polymerization of Collagen. However, keep in mind that using a heated printbed could result in faster evaporation of smaller droplets.

**Do not heat the printbed since smaller droplets can evaporate.

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 dispensing 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 2. Recommended settings used for printing single layered grids (20 x 20 mm) without cells at four different Collagen concentrations. If diluting the Collagen with cells, the density of the cell suspension may alter the flow rate and the parameters may need to be adjusted.

Parameters	2 mg/mL	4 mg/mL	6 mg/mL	8 mg/mL
Temperature printbed	25°C	25°C	25°C	25°C
Nozzle	0.25 mm	0.25 mm	0.25 mm	0.25 mm
Speed	10 mm/s	10 mm/s	10 mm/s	10 mm/s
Temperature printhead	2°C	2°C	2°C	2°C
Preflow volume	3.5 µL	3.5 µL	3.5 µL	3.5 µL
Extrusion rate	0.7 µL/s	0.9 µL/s	1.2 µL/s	1.0 µL/s
Retract volume	3.2 µL	3.2 µL	3.2 µL	3.2 µL
Z-offset	0.2 mm	0.2 mm	0.2 mm	0.2 mm
Extra preflow volume	2.5 µL	2.5 µL	2.5 µL	2.5 µL
Infill extrusion multiplier	100%	100%	100%	100%
Retract rate	5.0 µL/s	5.0 µL/s	5.0 µL/s	10.0 µL/s
Extra retract	0 µL	0 µL	0 µL	0 µL
Postflow stop time	0.5 s	0.5 s	0.3 s	0.3 s
Z-lift	2.0 mm	2.0 mm	2.0 mm	2.0 mm

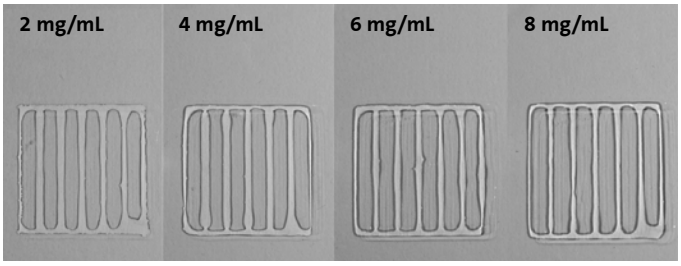

Figure 2. Grid structures acquired after printing Collagen with the parameters from Table 2.

Table 3. Recommended settings used for printing a two-layered concentric circle and three-layered hexagon with 8 mg/mL Collagen without cells. If diluting the Collagen with cells, the density of cell suspension may alter the flow rate and the parameters may need to be adjusted.

Parameters	Circle	Hexagon
Temperature printbed*	37°C	37°C
Nozzle	0.25 mm	0.25 mm
Speed	5 mm/s	8 mm/s
Temperature printhead	2°C	2°C
Preflow volume	4.5 µL	4.5 µL
Extrusion rate	0.4 µL/s	0.8 µL/s
Retract volume	4.2 µL	4.2 µL
Z-offset	0.2 mm	0.3 mm
Extra preflow volume	2.2 µL	2.2 µL
Infill extrusion multiplier	100%	60%
Retract rate	5.0 µL/s	5.0 µL/s
Extra retract	10 µL	10 µL
Postflow stop time	0.3 s	0.3 s
Z-lift	2.0 mm	2.0 mm

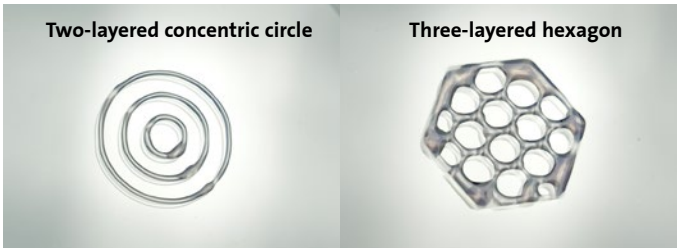

Figure 3. Multi-layered grid structures acquired after printing with the parameters from Table 3 with 8 mg/mL Collagen.

*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 construct). However, keep in mind that using a heated printbed could result in faster evaporation of smaller constructs.

The printing parameters included in Tables 1-3 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).

CORNING

Corning Incorporated
Life Sciences

www.corning.com/lifesciences

NORTH AMERICA
t 800.492.1110
t 978.442.2200

ASIA/PACIFIC
Australia/New Zealand
t 61 427286832
Chinese Mainland
t 86 21 3338 4338
India
t 91 124 4604000
Japan
t 81 3-3586 1996
Korea
t 82 2-796-9500
Singapore
t 65 6572-9740
Taiwan
t 886 2-2716-0338

EUROPE
CSEurope@corning.com
France
t 0800 916 882
Germany
t 0800 101 1153
The Netherlands
t 020 655 79 28
United Kingdom
t 0800 376 8660

All Other European Countries
t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400