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Author: PG, JH. Version: 1

BIO ONE BIOPRINTING PROTOCOL

CELLINK RGD

This is a suggested procedure, please adjust it according to your experimental needs. Work under aseptic conditions.

Protocol aim

The aim of this protocol is to provide instructions for dispensing droplets and bioprinting multi-layered grids with CELLINK RGD using the BIO ONE bioprinter. Droplets and grids printing reveals the CELLINK RGD printability of simple and complex structures for 3D cell culture. This document covers procedures for printing with cells on Petri dishes or in well plates including bioprinting parameters as well as post-print ionic crosslinking.

Materials

- CELLINK RGD*
- Crosslinking Agent, included with the bioink
- Cells + cell culture medium
- 3 mL BD Plastipak™ Syringes with Luer-Lok™ Tip (Ref#309658)
- Female/female Luer lock adaptor*
- Conical bioprinting nozzles, 22-25G recommended*
- BIO ONE 3D bioprinter*
- Well plate or Petri dish*

*The product can be purchased in the CELLINK shop at www.cellink.com/shop.

Protocol

The protocol has been optimized for use with the BIO ONE bioprinter. This protocol can be performed with printhead and printbed at room temperature, where room temperature is between 20-25°C

1. Bioink preparation

MATERIAL

CELLINK RGD

3 mL BD syringes with Luer lock connections

Female/female Luer lock adaptor

DESCRIPTION

- Mix the CELLINK RGD a few times to make sure it is homogenous. Gently mix the bioink back and forth between two 3 mL syringes connected with a Luer lock adaptor to homogenize the bioink, taking care not to introduce air.
- Let the bioink reach room temperature, only warm the needed volume. Calculate the volume of bioink and cell suspension needed for a 10:1 bioink to cell suspension dilution.

2. Mixing the bioink with cells

MATERIAL

CELLINK RGD

Cell suspension

3 mL BD syringes with Luer lock connections

Female/female Luer lock adaptor

DESCRIPTION

- At this point, mix the CELLINK RGD with cell suspension, taking care not to introduce air bubbles to the mixture.
- In brief, prepare a cell suspension with the desired number of cells. It is recommended to connect two 3 mL syringes with the Luer lock and divide the bioink between the two syringes. Disconnect the two syringes and pipette the cell suspension into one of the syringes very gently while pulling on the plunger to create room for the cell suspension in the syringe. Remove any air introduced into the syringe and connect the two syringes again. Gently mix back and forth between the syringes until the mixture is homogeneous. If detecting any air bubbles during mixing, disconnect the syringes and evacuate the air. Mix until homogeneous. The number of mixing cycles will depend on the cell type and bioink volume, optimize depending on application.

Note:

- The maximum filling level of the 3 mL syringes is 2.7 mL.

3. Preparation for bioprinting

MATERIAL

CELLINK RGD mixed with cells in 3 mL BD syringe.

Conical bioprinting nozzles, 22-25G recommended.

DESCRIPTION

- Cap the syringe with a bioprinting nozzle of choice, 22-25G recommended.
- Place the syringe into the printhead. Rotate the syringe plunger holder arm over the plunger and twist the syringe by the tabs counterclockwise to ensure it is locked in place.

Note:

- The recommended nozzle size is 22- 25G. Decrease the nozzle diameter to achieve smaller filament diameter, however this also increase the risk of the bioink clogging.

4. Calibration and nozzle priming

MATERIAL

BIO ONE

Well plate or Petri dish

DESCRIPTION

- Place a Petri dish or well plate on the printbed and perform either manual- or automatic calibration. Perform calibration each time a new syringe is placed in the printhead. If needed, perform Autobed levelling.
- Right before each print, prime the nozzle by extruding a couple of drops of material. Make sure the nozzle tip is clean before starting the print.

Notes:

- Before starting the print, test the flow of the bioink using the Test extrude button with the recommended starting parameters in Table 1 or 2, or even lower extrusion rate to avoid losing material during the nozzle priming procedure.
- If the system has been idle for an extended period, the bioink in the nozzle can dry causing it to clog. If this occurs purge the nozzle by extruding 10 to 50 µL of the bioink or until the dried part is extruded. If the clog cannot be removed, replace with a new nozzle. Always ensure the nozzle is fully primed with bioink prior to printing.

5. Printing

MATERIAL

BIO ONE

Well plate or Petri dish

DESCRIPTION

- Dispense droplets or print three-layered grids with parameters according to Table 1 or Table 2 respectively, in a well plate or Petri dish.
- See Figure 1 for reference droplets and Figure 2 for three-layered grid structures.
- If the printed structures are not as desired, adjust the extrusion rate up/down by 0.1 µL to extrude more/less material or refer to the BIO ONE Protocol Parameter Guidelines.

Note:

- The values are only a reference of starting parameters. The actual values needed to print will vary depending on the preparation procedures well as the print surface.

Table 1. Recommended settings in DNA Studio Core used for dispensing 5 µL droplets of CELLINK RGD through a 22G nozzle in a 96-well plate using the Droplet Print function on the BIO ONE bioprinter.

Parameters	
Well plate	96-well plate
Printbed temperature	-
Printhead temperature	-
Extrusion rate	60 µL/s
Extrusion volume	11 µL
Retract volume	6 µL
Z-offset	0.3 mm
Extra preflow volume	0 µL
Retract rate	10 µL/s
Postflow stop time	0.3 s
Z-lift between wells	30.0 mm

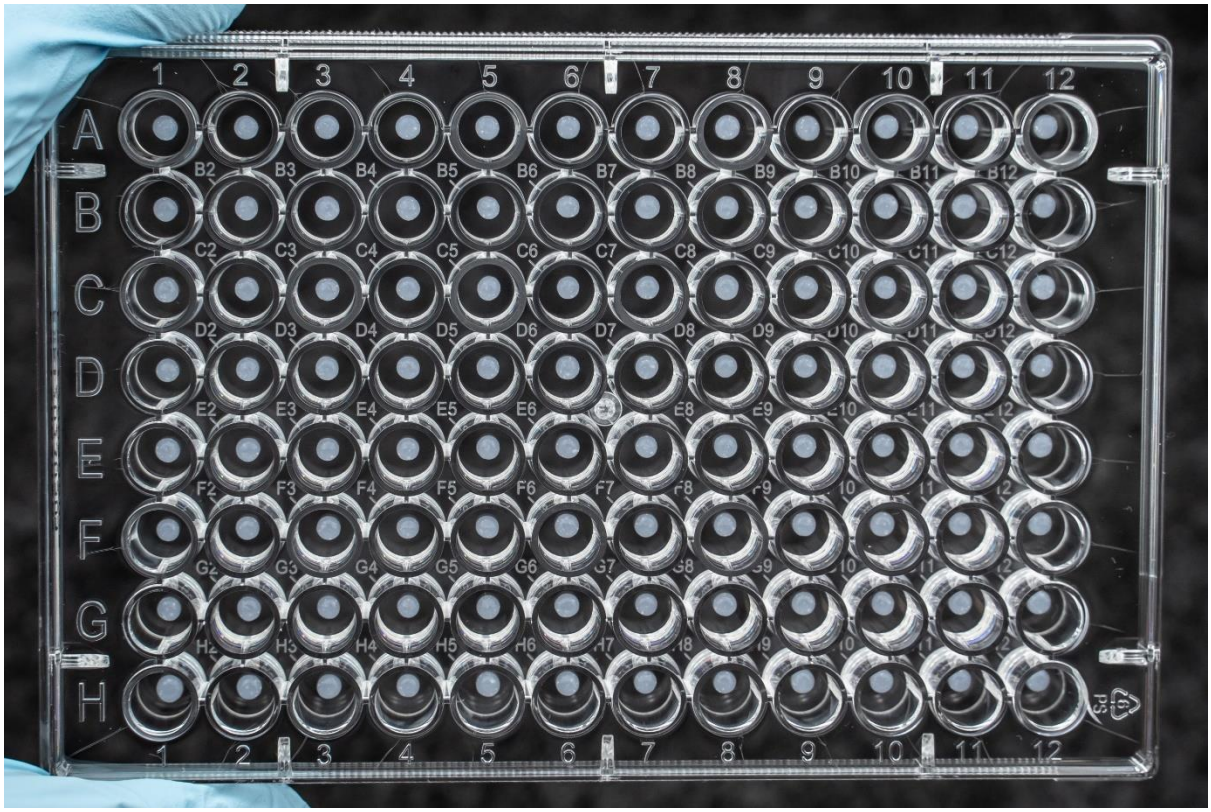

Figure 1. 5 µL droplets of CELLINK RGD mixed with PBS (10:1), simulating bioink dilution with cell suspension, dispensed in a 96-well plate, using parameters in Table 1. The picture was taken prior to ionic crosslinking.

Table 2. Recommended settings in DNA Studio Core used for printing three-layered grids (20 x 20 mm) of CELLINK RGD mixed with PBS (10:1), simulating bioink dilution with cell suspension.

Parameters	
Surface	Petri dish
Printbed temperature	-
Nozzle	0.25 mm (25G)
Speed	7 mm/s
Printhead temperature	-
Preflow volume	10 µL
Extrusion rate	2 µL/s
Retract volume	10 µL
Z-offset	0.2 mm
Extra preflow volume	4 µL
Infill extrusion multiplier	100%

Retract rate	20 µL/s
Extra retract	0 µL
Postflow stop time	0.3 s
Z-lift	3.0 mm

Figure 2. Three-layered grid structure, 20 x 20 mm, acquired after printing with the parameters in Table 2 with CELLINK RGD mixed with PBS (10:1). The picture was taken prior to ionic crosslinking.

6. Crosslinking and incubation

MATERIAL

Crosslinking Agent

Cell culture medium

Incubator

DESCRIPTION

- CELLINK RGD is crosslinked using the $CaCl_2$-containing Crosslinking Agent. Submerge the constructs in the Crosslinking Agent for 30 seconds to 5 minutes depending on construct size, infill density and desired construct stiffness.
- Remove the Crosslinking Agent and rinse the constructs with basal culture media once.
- After crosslinking and washing, add the desired medium to the constructs and place them in an incubator. Incubate in standard culture conditions (37°C, 5% CO_2 and 95% relative humidity) or according to your application.