

BIO ONE BIOPRINTING PROTOCOL

CELLINK A-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 A-RGD using the BIO ONE bioprinter. Droplets and grids printing reveals the Alginate 5% 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 A-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, 22-27G 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 A-RGD

3 mL BD syringes with Luer lock connections

Female/female Luer lock adaptor

DESCRIPTION

- Warm up CELLINK A-RGD to room temperature.
- 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 A-RGD

Cell suspension

3 mL BD syringes with Luer lock connections

Female/female Luer lock adaptor

DESCRIPTION

- At this point, mix the CELLINK A-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 A-RGD mixed with cells in 3 mL BD syringe.

Conical bioprinting nozzles, 22-27G recommended.

DESCRIPTION

- Cap the syringe with a bioprinting nozzle of choice, 22-27G 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.

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.

Notes:

- 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.
- Due to the low viscosity of this bioink, avoid printing more than three layers structures. For printing complicated structures, it is recommended that CELLINK A-RGD is used in conjugation with a sacrificial ink to aid in its stability.

Table 1. Recommended settings in DNA Studio Core used for dispensing 5 μL droplets of CELLINK A-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 $\mu\text{L/s}$
Extrusion volume	10 μL
Retract volume	5 μL
Z-offset	0.5 mm
Extra preflow volume	0 μL
Retract rate	10 $\mu\text{L/s}$
Postflow stop time	0.5 s
Z-lift between wells	30.0 mm

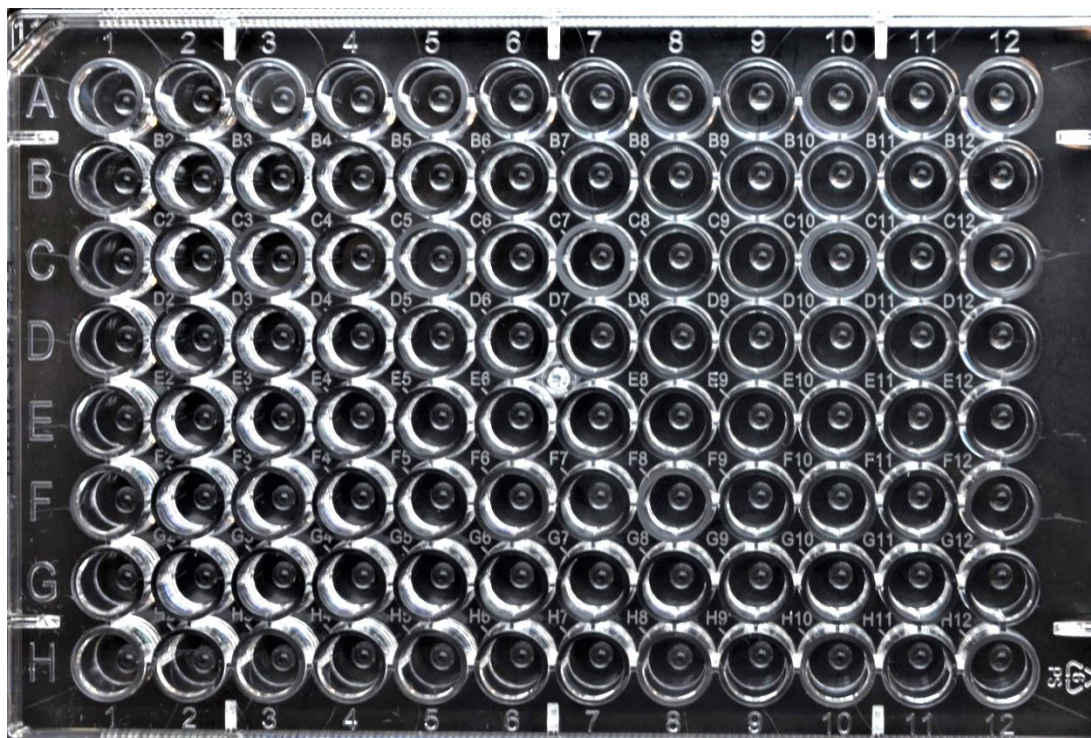


Figure 1. 5 μL droplets of CELLINK A-RGD mixed with PBS (10:1), simulating bioink dilution with cell suspension, dispensed in a 96-well plate, using parameters in Table 1. The picture was taken prior to ionic crosslinking.

Table 2. Recommended settings in DNA Studio Core used for printing three-layered grids (20 x 20 mm) of CELLINK A-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	20 μL
Extrusion rate	0.7 $\mu\text{L/s}$
Retract volume	20 μL
Z-offset	0.2 mm
Extra preflow volume	3 μL
Infill extrusion multiplier	100%
Retract rate	30 $\mu\text{L/s}$
Extra retract	0 μL
Postflow stop time	0.3 s
Z-lift	3.0 mm

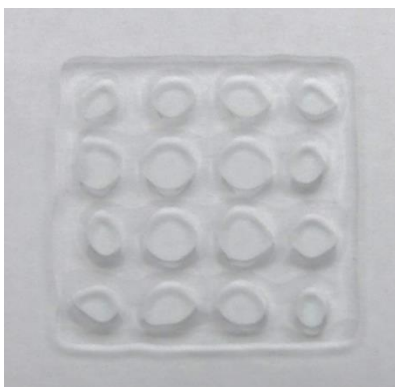


Figure 2. Three-layered grid structure, 20 x 20 mm, acquired after printing with the parameters in Table 2 with CELLINK A-RGD mixed with PBS (10:1). The picture was taken prior to ionic crosslinking.

6. Crosslinking and incubation

MATERIAL

Crosslinking Agent
Cell culture medium
Incubator

DESCRIPTION

- CELLINK A-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.

Note:

- Longer crosslinking times will lead to stiffer constructs, optimize according to application.