Ref No: BOBPR-IKS400000503
Date: 21-Mar-2024
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BIO ONE BIOPRINTING PROTOCOL

Pluronics 40%

This is a suggested procedure, please adjust it according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for dispensing droplets and bioprinting multi-layered grids with Pluronics 40% using the BIO ONE. Droplets and grids printing reveals the versatility of the sacrificial ink Pluronics 40% for simple and complex structures used in several applications for sacrificial purposes. This document covers bioprinting parameters and procedures for printing without cells on Petri dishes or in well plates.

Materials

- Pluronics 40%*
- 3 mL BD Plastipak™ Syringes with Luer-Lok™ Tip (Ref#309658)
- Conical bioprinting nozzles, 22-27G recommended*
- BIO ONE 3D bioprinter*
- Well plate or Petri dish*
- Cold PBS

*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.

1. Preparation for printing

MATERIAL

Pluronics 40%

3 mL BD syringes with Luer lock connections

Female/female Luer lock adaptor

Conical bioprinting nozzles, 22-27G recommended.

DESCRIPTION

- Transfer Pluronics 40% to the 3 mL syringe using the Luer lock adapter and cap the syringe with a printing nozzle of choice, 22-27G recommended.
- Let Pluronics 40% reach room temperature. If instead printing with cool Pluronics, make sure that the thermal insulator is attached to the cooling block by inserting it from below and rotating counterclockwise.
- 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.

2. 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.

3. 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 Pluronics 40% 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	30 µL/s
Extrusion volume	35 µL
Retract volume	30 µL
Z-offset	0.5 mm
Extra preflow volume	0 µL
Retract rate	120 µL/s
Postflow stop time	0.3 s
Z-lift between wells	30.0 mm

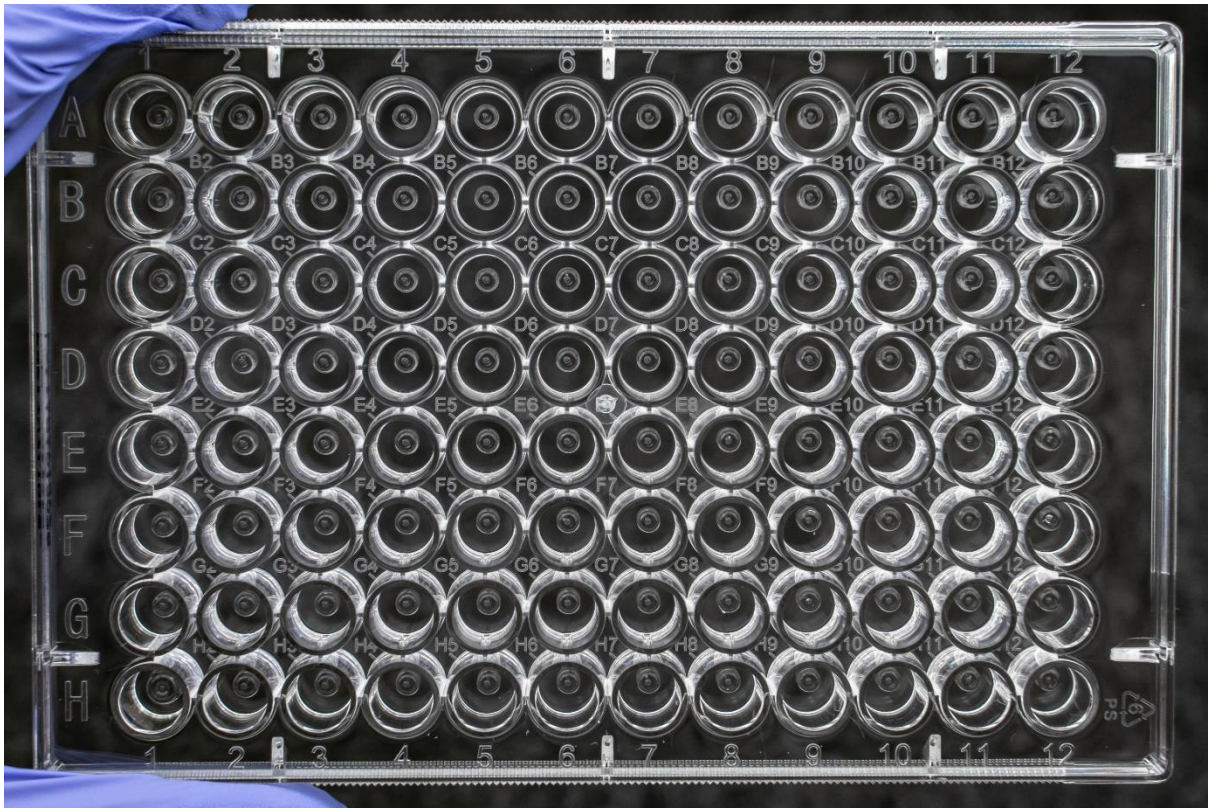

Figure 1. 5 µL droplets of Pluronics 40% dispensed in a 96-well plate, using parameters in Table 1.

Table 2. Recommended settings in DNA Studio Core used for printing three-layered grids (20 x 20 mm) of Pluronics 40%.

Parameters	
Surface	Petri dish
Printbed temperature	-
Nozzle	0.2 mm (27G)
Speed	5 mm/s
Printhead temperature	-
Preflow volume	37.5 µL
Extrusion rate	1.2 µL/s
Retract volume	40 µL
Z-offset	0.2 mm
Extra preflow volume	4.5 µL
Infill extrusion multiplier	100%
Retract rate	60 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 Pluronics 40%.

4. Removal of Pluronics 40%

MATERIAL

Cold PBS or liquid of choice

DESCRIPTION

- Pluronics 40% cannot be crosslinked. Pluronics 40% becomes liquid at low temperatures and may thus be removed by washing with cold PBS, or your liquid of choice. Prior to washing, make sure to crosslink any other bioinks printed to retain their structure.
- Cover the printed construct in cold PBS and remove by pipetting. Repeat until all Pluronics 40% is removed. If the Pluronics 40% is embedded inside a construct, wash with cold PBS and use negative pressure to remove the ink. Acellular and large constructs benefit from being placed on ice for faster liquification of the Pluronics 40%.