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DROPLET-IN-DROPLET BIOPRINTING PROTOCOL

BIO ONE

This is a suggested procedure, please adjust according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

The aim of this protocol is to provide instructions for bioprinting Droplet-in-Droplet (DiD) geometry using the BIO ONE. In this process, an inner droplet is encapsulated by a second, outer droplet. The DiD geometry can be utilized to mimic multilayered tissues in a miniaturized form, making it adaptable for multi-well plates. This setup can be used for invasion and migration assays.

Materials needed

- Cells + cell culture medium
- Incubator
- Positive displacement pipette and tips
- 3 mL BD Plastipak™ Syringes with Luer-Lok™ Tip (Ref#309658)
- Female/female Luer lock adaptor*
- Bioink 1 for the inner droplet
- Bioink 2 for the outer droplet
- Conical bioprinting nozzles, 18-22G recommended*
- BIO ONE 3D bioprinter**
- Well plate (6-48 wells)

*The product can be purchased in the CELLINK store at www.cellink.com/shop/.
**BIO ONE can be quoted at Get a Quote - CELLINK or by contacting sales.

Protocol

For the outer droplet, this protocol applies to bioinks that can undergo thermal, photo, or chemical crosslinking. The inner droplet should be limited to bioinks suitable for thermal or photo crosslinking only. For cell migration studies, it is well documented that cells migrate from the inner layer to the outer layer in collagen-based hydrogels. In any case, it is always recommended to test various bioink and cell concentrations and combinations for the inner and outer droplets to achieve the desired biological activity.

1. Software setup

MATERIAL

BIO ONE 3D bioprinter

DESCRIPTION

Create the droplet tab 1 for the inner droplet and navigate through:

- **Surface:** select the well plate and the wells to be printed in.
- **Printer:** Select if photocuring or regulation of printbed temperature are required according to the bioprinting protocols for the bioink 1.
- **Printhead:** select the bioink profile according to the bioink 1. Adjust the extrusion volume to 5 µL, as the minimum recommended volume.

Create the droplet tab 2 for the outer droplet and navigate through:

- **Surface:** same as used for the tab 1.
- **Printer:** Select if photocuring or regulation of printbed temperature are required according to the bioprinting protocols for the bioink 2.
- **Printhead:** select the bioink profile according to the bioink 2. Change the z-offset to 2 mm and increase the extrusion volume to 30 µL as the minimum recommended volume.
- Proceed to the print page.

Note: Avoid using the wells where the surface probe touches during autocalibration and ABL. The probe might poke the inner droplet during autocalibration before printing the outer droplet in the droplet-in-droplet. If using autocalibration avoid using single droplet or droplet array with central droplets.

Note: Increasing the volume of the outer droplet enhances the likelihood of encapsulating the first droplet. The recommended starting proportion in volume for inner and outer droplets is 1:6.

Note: the z-offset for inner and outer droplets must be adjusted according to their volumes. Higher volumes require higher z-height.

2. Bioink preparation

MATERIAL

Cells + cell culture medium

Positive displacement pipette and tips

Female/female Luer lock adaptor

3 mL BD Plastipak™ Syringes

Bioink 1 for the inner droplet

Bioink 2 for the outer droplet

DESCRIPTION

Prepare the bioink and transfer it into 3 mL Syringes according to the BIO ONE bioprinting protocols specific for the chosen bioinks and available at Bioprinting Protocols – MyCELLINK - Knowledge Center.

3. Setting up bioink 1 for inner droplets

MATERIAL

Syringe containing the bioink 1 for the inner droplet (Syringe 1)

Conical bioprinting nozzles (20 or 22 G)

BIO ONE

DESCRIPTION

- Attach 20 or 22 G nozzle to the Syringe 1 to be used for the inner droplet.
- Attach the thermal insulator to the cooling block, by inserting it from below and rotating counterclockwise.
- Insert the syringe into the printhead and lock it by rotating it counterclockwise.

Note: If working with temperature sensitive materials, pre-set the target temperature before loading the Syringe 1 into the printhead.

Note: Always use the thermal insulator, even when working with non-temperature-sensitive materials. This ensures precise nozzle positioning, leading to more accurate droplet placement.

Note: 20 G nozzles are recommended for materials with low viscosity.

4. Calibration and nozzle priming

MATERIAL

Syringe 1

BIO ONE

Well plate (6-48 wells)

DESCRIPTION

- Place a well plate on the printbed, ensuring that the A1 well is positioned in the top left corner when viewed from the front of the BIO ONE.
- Perform either manual or automatic calibration according to the BIO ONE manual.
- Prime the nozzle by extruding a couple of drops. The nozzle is primed when it is filled with bioink completely.
- Wipe the nozzle with a tissue paper

Note: Before starting the print, test the flow of the bioink using the Test extrude button (Utilities tab) with the recommended starting parameters retrieved from the bioink profile.

5. Printing and crosslinking inner droplets

MATERIAL

Syringe 1

BIO ONE 3D bioprinter

Well plate (6-48 wells)

DESCRIPTION

- Optional: print few droplets in another plate or plate lid using the corresponding bioprinting protocols to ensure the droplets are homogeneous in size and there is no bioink oozing out the nozzle. Parameters might change after mixing the biomaterials with cells. Adjust the parameters if they are not optimal, following the Parameter Guidelines & Print Troubleshooting - MyCELLINK - Knowledge Center.
- Optional: To enhance the volume precision, after testing the bioprinting parameters in another plate, avoid priming and wiping before printing.
- Open the Tab 1 setup at step 1.
- Print the droplets into the well plates.
- Crosslink the droplets according to the bioink bioprinting protocol.
- Remove the Syringe 1 from the printhead and store it according to the temperature requirements for further use (if necessary).

6. Bioprinting bioink 2 for outer droplets

MATERIAL

Syringe containing the bioink 2 for the outer droplets (Syringe 2)

BIO ONE

Well plate (6-48 wells)

DESCRIPTION

- Repeat step 3 of this protocol for Syringe 2, attaching 18 or 20 G nozzle to it.

Note: The nozzles for printing the outer droplets should have a smaller gauge (larger orifice diameter) compared to those used for printing the inner droplets.

Note: 18 G nozzles are recommended for materials with low viscosity.

- Go to the printhead tab for Syringe 2, set previously at step 1 of this protocol.
- Repeat step 4 using the same well plate and Syringe 2.
- Repeat step 5 using Syringe 2.

7. Incubation

MATERIAL

Incubator

Cell culture medium

Well plate (6-48 wells) with the bioprinted DiDs

DESCRIPTION

- After crosslinking of outer droplets, add the desired medium to the printed DiDs and place the plate into 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.