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FRESH BIOPRINTING PROTOCOL

Alginate

This is a suggested procedure, please adjust it 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 of complex 3D structures with low viscosity alginate using FRESH printing method. It covers the steps of pre-print procedures, printing, and post-print crosslinking. Changing the parameters in the protocol might change printing conditions such as pressure and speed. This protocol was optimized for the Temperature-controlled printhead using the BIO X.

Materials needed

- Alginate Lyophilizate (100 mg vial)*
- Reconstitution Agent M* or other buffer
- Magnetic stir bar
- Crosslinking Agent (50 mM $CaCl_2$)*
- LifeSupport™*
- 3 mL syringes with Luer lock connections
- Well plate or Petri dish*
- BIO X*, BIO X6* 3D bioprinter
- Cells in suspension and cell culture media
- Female/female Luer lock adaptors*
- Cartridge, 3cc*
- 22G Conical bioprinting needle (1-inch length)*

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

Protocol

This protocol is adjusted for printing scaffolds at a final alginate concentration close to 2% (w/v) prepared from Alginate Lyophilizate (CELLINK). For other alginate materials and concentrations, recalculations need to be made but the same protocol can be followed. Once compacted, LifeSupport™ should be kept in a fridge at 4°C and used within 12 hours. The ambient temperature should not exceed 23°C during handling or printing.

1. Preparing Alginate 2% (w/v)

MATERIAL

Alginate Lyophilizate

Reconstitution Agent M

Magnetic stir bar

3 mL syringes with Luer lock connections

DESCRIPTION

- Take Alginate Lyophilizate from storage and let it reach room temperature.
- Add 5 mL of a reconstitution agent to the Alginate Lyophilizate vial.

Note: Reconstitution Agent M is a specially designed buffer that maintains a physiologic pH in the final alginate bioink. It also has a low concentration of ions to prevent premature crosslinking.

- Add a sterile stir bar to the container.
- Stir the mixture at room temperature for ~60 minutes, or until dissolved.
- Transfer the dissolved alginate to a syringe with Luer lock connection.

2. Preparing LifeSupport™ bath with 0.1% (w/v) $CaCl_2$

MATERIAL

Crosslinking Agent

Reconstitution Agent M

LifeSupport™

Well plate or Petri dish

DESCRIPTION

- Prepare 40 mL of 0.1% (w/v) $CaCl_2$ solution by mixing 7.2 mL of Crosslinking Agent with 32.8 mL of Reconstitution Agent M. Put the solution in a fridge (4°C) for cooling.
- Add 40 mL of cold $CaCl_2$ solution to LifeSupport™ tube (2 g of sterile powder).

Note: 2 g of sterile LifeSupport™ powder corresponding to 15 mL of LifeSupport™ bath. For detailed directions, visit https://www.cellink.com/wp-content/uploads/2022/03/FluidformDirectionsforUseVersion6-rev-Sep-2021.pdf.

- Vortex for 1 minute.
- Put the tube into the fridge (4°C) for 15 minutes.
- Centrifuge for 5 minutes at 400 *g*.
- Gently pour off or aspirate the liquid supernatant.
- Grab the tube by the cap, hold it horizontally, and gently tap it against a palm 15 times.
- Shake the tube containing dislodged LifeSupport™ vigorously for 10 seconds. Shake along the length of the tube.
- Centrifuge for further 5 minutes at 400 *g*.

- The LifeSupport™ should now be compacted at the bottom of the centrifuge tube. Gently pour off or aspirate any remaining liquid supernatant to leave only the compacted LifeSupport™ in the bottom of the tube.
- Transfer the resulting LifeSupport™ bath with a sterile spatula into well plates or Petri dish and store it in a fridge until use.

3. Preparing for printing

MATERIAL

Alginate 2% (w/v)

BIO X or BIO X6 bioprinter

Cell suspension in syringe

Luer lock adaptors

Cartridge, 3cc

22G Conical bioprinting needle (1-inch length)

DESCRIPTION

- Set the BIO X print bed at 10°C to guarantee LifeSupport™ bath stability.
- To prepare 1 mL of an alginate bioink for printing, mix 900 µL of Alginate 2% (w/v) solution with 100 µL of cells suspension between syringes using a Luer lock adaptor until complete homogenization.

Note: To avoid an air gap and bubbles during mixing, carefully pre-fill the Luer lock adaptor with alginate before attaching the syringe with the cell suspension.

- Load a cartridge with the alginate and cells mixture using a Luer lock adaptor. Place the cartridge in the printhead and cap with the printing needle.

4. Printing

MATERIAL

BIO X or BIO X6 bioprinter

Well plate or Petri dish previous filled with LifeSupport™ bath

Cartridge with the alginate bioink

DESCRIPTION

- Place the well plate or Petri dish previously filled with LifeSupport™ bath and place it on the print bed. Print constructs using suggested parameters:
    - pressure at 18-20 kPa.
    - speed at 3.5 mm/s.

Note: If printability is not as desired, adjust the pressure and/or speed to up/down to extrude more/less material at different speeds.

5. Incubation and crosslinking

MATERIAL

Cell culture medium

DESCRIPTION

- After printing, incubate the constructs for 30 minutes at 37 °C (5% CO_2 and 95% relative humidity) for bioink crosslinking and LifeSupport™ melting.

Note: Large volumes may require longer times for the supporting bath to fully melt, though too long crosslinking time can cause shrinkage of the constructs.

- Remove melted LifeSupport™ by replacing it with a warm cell medium to avoid handling the printed construct. For example, if you printed into a 6-well plate, this can be done by carefully aspirating 2 mL of melted LifeSupport™ out and adding 2 mL of warm cell media. Repeat this process until most of the support bath has been replaced by the medium.