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BIOPRINTING PROTOCOL

BIO CONDUCTINK

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 of Bio Conductink using the BIO X or BIO X6. This document covers pre-print mixing with cells, 3D bioprinting and post-print processes such as photocuring. This protocol was optimized for Bio Conductink containing 0.25% LAP as a photoinitiator. Changing the bioink to cell suspension ratio and thus the concentration of photoinitiator, will change the photocrosslinking time. Refer to the *Photocrosslinking Optimization Protocol* to adjust and determine these numbers. This protocol was optimized using the Temperature-controlled printhead with the BIO X.

Materials needed

- Bio Conductink*
- Cells* + cell culture medium*
- Female/female Luer lock adaptor*
- 3 mL syringes with Luer lock connections
- CELLMIXER* (optional)
- UV shielding cartridges, 3 cc*
- Conical bioprinting nozzles, 22-27G recommended*
- Temperature-controlled printhead*
- BIO X* or BIO X6* 3D bioprinter
- Well plate or Petri dish*

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

KEEP THE BIOINK PROTECTED FROM LIGHT IF TRANSFERRED FROM THE ORANGE UV PROTECTED CARTRIDGES TO AVOID CROSSLINKING BEFORE PRINTING. WORK WITH 3D PRINTERS IN DARK MODE. THE PHOTOINITIATOR IS SENSITIVE TO REPEATED OR PROLONGED EXPOSURE TO HEAT.

Protocol

Bio Conductink has been optimized for the BIO X system equipped with the Temperature-controlled printhead, a thermal nozzle cover and a cooled print bed. While the bioink can be used with the INKREDIBLE+ system due to its ability to heat the bioink, secondary steps are necessary to cool the printed structure to pre-gel it prior to photocrosslinking. In addition, clogging may still occur due to lack of temperature control at the nozzle. Therefore, it is not recommended to use the bioink with the INKREDIBLE system since the bioink will not perform as expected and resulting filament characteristics may be inconsistent. First time users of GelMA based bioinks are recommended to optimize the printing conditions without cells before proceeding to bioprint with cells. Perform the desired dilution using medium or PBS.

1. Preparing the bioink

MATERIAL

Bio Conductink

Temperature-controlled printhead

DESCRIPTION

- Heat up Bio Conductink in the cartridge at 37°C until it is liquid. The heating of the Bio Conductink can be performed in a printhead or incubator and usually requires 30-60 min if the bioink is taken directly from the fridge.
- Set the Temperature-controlled printhead to 25°C.
- *If not printing with cells, move directly to Step 3.*

2. Mixing the bioink with cells

MATERIAL

3 mL syringes with Luer lock connections

Female/female Luer lock adaptor

Pre-warmed Bio Conductink

Cell suspension

UV shielding cartridge, 3cc

CELLMIXER (optional)

DESCRIPTION

- At this point, mix ten parts of bioink with one part of cell suspension, taking care not to introduce air bubbles to the mixture. For detailed instructions see the *Mixing cells with bioink Protocol.*
- If preparing for quantities < 2 mL of Bio Conductink, it is recommended to connect two 3 mL Luer lock syringes, one with the bioink and the other with the cell suspension and gently mix back and forth between the syringes until homogeneous. Transfer the mixture to an empty 3cc cartridge by connecting the syringe to the cartridge using the Luer lock adaptor. Cap the cartridge with a tip cap.
- If using larger quantities, the CELLMIXER can be used:
    - Transfer the cell suspension to the 1 mL cell syringe (PART 1) using a female/female Luer lock adaptor.
    - Transfer Bio Conductink to the 12 mL syringe (PART 2) using a female/female Luer lock adaptor.
    - Clip both syringes to the Dispensing unit (PART 3).

- Connect the two syringes to the Mixing unit (PART 4), then connect the Empty cartridge (PART 5) to the Mixing unit's other side.
- Apply gentle pressure onto the Dispensing unit to mix the content of both syringes into the empty cartridge. Cap the cartridge with a tip cap.

Note: To avoid introducing air when connecting the syringes, carefully pre-fill the Luer lock adaptor with Bio Conductink before attaching it to the syringe with the cell suspension.

Note: Keep the cartridge horizontal when tip and end cap are removed. This is to prevent air from entering the cartridge or bioink from dripping out when liquid.

3. Preparation for printing

MATERIAL

Bio Conductink mixed with cells (if applicable) in UV shielding cartridge

Temperature-controlled printhead (optional)

Conical bioprinting nozzles, 22-27G

DESCRIPTION

- If the cartridge is just taken from the heat or if the cartridge still feels warm after mixing in the cells, place it in the pre-heated Temperature-controlled printhead at 25°C for 10 minutes. If not using the Temperature-controlled printhead, place the cartridge on counter for 5-10 minutes to reach approximately 25°C.
- If the cartridge has cooled down below 24°C, re-heat the cartridge at 37°C for 5 min to reset, then restart equilibration in printhead.
- Cap the cartridge with a bioprinting nozzle and place the Bio Conductink in the printhead. Connect the cartridge to the air pressure adapter. If using the BIO X or BIO X6, pre-cool the print bed to 15°C.

Note: When printing with Bio Conductink, the recommended printhead temperature for the highest printing fidelity is 25°C, though the bioink can be dispensed up to 32°C. Below 24°C the bioink can become too viscous resulting in chunky filaments and too high extrusion pressures needed.

Note: Be careful not to touch the printhead with the nozzle tip and if using very liquid materials, make sure that the bioink does not drip through the nozzle especially when attaching the air adapter. Alternatively, the cartridge can be placed in the printhead with the tip cap on and when in place switched to a nozzle.

Note: Test the flow of the bioink after the calibration is performed and start with a low pressure and increase stepwise.

4. Printing

MATERIAL

BIO X or BIO X6

Well plate or Petri dish

DESCRIPTION

- Calibrate the nozzle to the well plate or Petri dish surface. Test the flow of the bioink first after calibration and start with a low pressure and increase stepwise. Bioprint structures into the well plate or Petri dish. If printability is not as desired, adjust the pressure up/down by 1 kPa to extrude more/less material. The printing pressure is inversely proportional to a nozzle diameter and printing speed.

Example: If printing continuous filaments with a Temperature-controlled printhead set to 25°C, a 25G nozzle, a printing speed of 5 mm/s and with 300 ms pre-flow delay, the suggested pressure range is between 23-33 kPa without cell suspension dilution and 18-28 kPa if diluted with cell suspension.

- If proper viscosity and printability is not achieved by extending temperature equilibration time or tuning pressure:
    - Too low viscosity (wide filaments despite using low pressure): decrease the printhead temperature 0.5-1°C to increase the viscosity and equilibrate an additional couple of minutes.
    - Too high viscosity (chunky filaments and high pressure required): increase the printhead temperature 0.5-1°C to decrease the viscosity and equilibrate an additional couple of minutes. If unsuccessful, the bioink might have over-gelled. In this case, re-heat the cartridge at 37°C for 5 min to reset and choose a 1°C higher printhead temperature than before. Let the bioink reach the new temperature before starting to print again.
- During print sessions longer than 20 min at 25°C the bioink can become too viscous due to continued gelling resulting in chunky filaments and too high extrusion pressures needed. To avoid this, a 0.5°C increase in printhead temperature after 20 min printing can extend the time the bioink remains at good printability.
- If the regular pneumatic printheads are used for a long period, they might heat up above the desired printing temperature. Then the bioink also heats up which decreases its viscosity, observed as extrusion of very thick filaments even at low pressures. Remove the cartridge from the printhead and allow to cool down to 25°C. In addition, remove the printheads from the BIO X/BIO X6 to let them cool down before continuing to print.

Note: If waiting too long between extrusions the bioink can dry in the nozzle causing it to clog. If this occurs, replace with new nozzle.

5. Crosslinking

MATERIAL

405/365 nm LED modules for photocuring

DESCRIPTION

- Bio Conductink with LAP can be crosslinked with photoinitiation using either the 405 or 365 nm photocuring module. The photocrosslinking time required is usually in the range of 15-30 s for a construct thickness below 3 mm. More time might be required depending on the construct thickness and desired final stiffness. Ensure that the bioprinted Bio Conductink construct is thermally gelled after printing by letting the construct sit on the cooled print bed for 30 s before starting photocrosslinking.
- If photocrosslinking during bioprinting, set the crosslinking parameters appropriately in the printhead setup page for the BIO X or BIO X6. To achieve the better structural integrity when printing thicker constructs, it is recommended to apply 3 or 5 seconds photocrosslinking with 365 or 405 nm light respectively, every second or fourth layer.
- Let the structure sit for 3-5 min to allow crosslinking after the light source is turned off.

Note: It is recommended to use the 405 nm LED module instead of 365 nm if possible. Overexposure might damage the cells.

Note: To verify that the photocrosslinking is sufficient, add 37°C medium to one printed well and observe that it doesn't dissolve.

6. Incubation

MATERIAL

Cell culture medium

DESCRIPTION

- After photocrosslinking, add the desired medium to the construct and place in the 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. Replace medium regularly.