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

Material needed

- Bio Conductink*
- UV shielding cartridges, 3 cc*
- Sterile conical bioprinting nozzles, 22-27G
- BIO X* or BIO X6* 3D Bioprinter
- Temperature-controlled Printhead*
- 365/405 nm LED modules for photocuring
- Petri dish* or well plate
- Cells + cell culture medium
- 3 mL syringes with Luer lock connections
- Female/female Luer lock adaptor*
- CELLMIXER*

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

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 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. 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.

Step	Title	Material	Description
1	Prepare bioink	- Bio Conductink	- Heat up Bio Conductink in a cartridge to 35°C until it is liquid. This can be tested by flipping the cartridge and observing if air bubbles move freely. The heating of the bioink can be performed in an incubator. - Set the Temperature-controlled printhead to 26°C. Pre-cool the print bed to 9°C
2	Mix Bio Conductink with cells	- Cell suspension - CELLMIXER - Female/female Luer lock adaptor - 3 mL syringes with Luer lock connections - Prewarmed Bio Conductink	If not printing with cells move directly to step 3. 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 Protocol GelMA Series.* - 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 units from the other side. - Apply gentle pressure onto the Dispensing unit to mix the content of both syringes into the empty cartridge. Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the Luer lock adaptor with Bio Conductink before attaching the syringe with the cell suspension.

			If preparing for quantities <2 mL of the bioink, it is recommended to connect two 3 mL Luer lock syringes and mix back and forth between the syringes until the bioink becomes homogeneous.
3	Cool and load the cartridge	- UV shielding cartridges, 3 cc loaded with Bio Conductink (and cells) - Sterile conical bioprinting nozzles, 22-27G	- Cap the cartridge with a tip cap and place it horizontally on the cooled print bed for 30 s or until air bubbles are no longer moving when flipping the cartridge. Note: This is to prevent cell sedimentation in the liquid Bio Conductink if placed in the printhead when over 26°C. - Cap the cartridge with a 22-27G bioprinting nozzle and place in the Temperature-controlled Printhead and wait for 5-10 min until the bioink reaches 26°C.
4	Printing	- BIO X or BIO X6	- Bioprint structures using the bioink. 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 using a 22G nozzle and 10 mm/s printing speed, start at 10 kPa and adjust as needed. Note: Over time the bioink will become more solid (15-20 min). If printing is paused and this happens, replace the nozzle. If extrusion does not occur, repeat Step 1 and 3 to "reset" the cartridge.
5	Crosslinking	- 405/365 nm LED modules for photocuring	Bio Conductink with LAP can be crosslinked with photoinitiation using either the 405 or 365 nm photocuring module. - Photocrosslinking time required is usually in 5-30 s range 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 cooling the print bed for 30 s. - If photocrosslinking during bioprinting, set the crosslinking parameters appropriately in the printhead setup page for the BIO X or BIO X6.

			- 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 photocuring module instead of 365 if possible when photocuring Bio Conductink with LAP. Exposure at the 365 nm wavelength over 2 min might damage the cells. Note: If crosslinking is unsure, add media to one printed well at 37°C to validate that it doesn't dissolve.
6	Incubation	- Cell culture medium	- After photocrosslinking, add the desired medium to the constructs and place in 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.