Bioprinting Protocol

GelMA HA

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 GelMA HA using the BIO X. This document covers pre-print mixing with cells, 3D bioprinting and post-print processes of crosslinking through photocuring. This protocol was optimized for GelMA HA 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.

Materials needed

- GelMA HA*
- UV shielding cartridges, 3 cc*
- Sterile conical bioprinting nozzles, 22-27G
- BIO X* 3D Bioprinter
- Temperature-controlled Printhead*
- 365/405 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

This protocol works best using the BIO X with the cooled print bed at 10°C and the Temperature-controlled printhead at 24°C with the thermal nozzle cover. GelMA HA can also be extruded using the BIO X pneumatic printheads or the INKREDIBLE series bioprinters, but with decreased shape fidelity if ambient temperature exceeds 24°C. If using the INKREDIBLE series, the printing substrates such as Petri dishes or well plates should be placed on ice or another cooled surface before and after printing to thermally gel bioprinted constructs prior to photocrosslinking.

Step	Title	Material	Description
1	Prepare bioink	- GelMA HA	- Heat up GelMA HA in a cartridge to 37°C until it is liquid (5 min is usually enough). This can be tested by flipping the cartridge and observing if air bubbles move freely. Note: Prolonged and repeated heating could negatively affect the photoinitiator stability and the homogeneity of the bioink. - Set the Temperature-controlled printhead to 24°C. Pre-cool the print bed to 10°C
2	Mix GelMA HA with cells	- Cell suspension - CELLMIXER - Female/female Luer lock adaptor - 3 mL syringes with Luer lock connections - Prewarmed GelMA HA	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 GelMA HA 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 GelMA HA 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, 3cc loaded with GelMA HA (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 GelMA HA 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 for 5-10 min until the bioink reaches 24°C.
4	Printing	- BIO X 3D bioprinter	- 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: If the bioink seems too liquid and the pressure needs to be decreased with >5 kPa, the printhead temperature might need to be shifted down to 23.5°C. If the bioink comes out chunky, heat it again at 37°C for 3 min, repeat step 3 and increase the printhead temperature to 24.5°C. Note: GelMA HA becomes more solid over extended printing time (>20 min). If printing is paused and extrusion does not occur, heat the bioink again at 37°C for 3 min and repeat step 3 to 'reset' the bioink.
5	Crosslinking	- 405/365 LED modules for photocuring	GelMA HA with LAP can be crosslinked with photoinitiation using either the 405 or 365 nm photocuring module. - Photocrosslinking time required is usually in 10-30 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 GelMA HA construct is thermally gelled after printing by cooling the print bed on the BIO X for 30 s

			or placing the well plate or Petri dish on ice for 10 s. - If photocrosslinking during bioprinting, set the crosslinking parameters appropriately in the printhead setup page for the BIO X. - 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 GelMA HA 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.