Ref No: BPR-IKJ1250000
Date: 28-AUG-2020
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Bioprinting Protocol

HAMA 5%

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 droplets of a HAMA bioink using the INKREDIBLE, INKREDIBLE+, or BIO X. The protocol covers the steps of pre-print mixing with cells, 3D bioprinting and post-print processes such as photocuring. It was optimized for HAMA bioinks diluted with a cell suspension at 10/1 volume ratio and droplet-printed in the BIO X equipped with the pneumatic printhead. To enable photocuring, addition of a photoinitiator is crucial. Changing the parameters in the protocol might change the photocuring time required. For printing complex multi-layered structures, it is recommended to mix HAMA 5% with a thickener such as CELLINK's Nanofibrillated Cellulose, Xanthan Gum or Glucomannan.

Materials needed

- HAMA 5%*
- Photoinitiator*
- BIO X* or INKREDIBLE series* 3D Bioprinter
- Amber cartridges, 3cc*
- Sterile bioprinting nozzles 22-27G* or needles*
- Well plate or Petri dish*
- Female/female Luer lock adaptors*
- Syringes with Luer lock connections
- Positive displacement pipette + pipette tips (optional)
- CELLMIXER*
- Cells + cell culture medium

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

Protocol

This protocol can be performed with printheads and print bed set to room temperature, *i.e.* 20-25°C.

Step	Title	Material	Description
1	Preparation of the bioink	- HAMA bioink - Photoinitiator	- Heat up your HAMA bioink to room temperature. - For dilution to your desired concentration and addition of photoinitiator, see the *Dilution Protocol HAMA 5%*. Remember to protect all photoinitiator containing bioinks from light.
2	Mixing the bioink with cells	- Cell suspension - CELLMIXER - Female/female Luer lock adaptor - Syringes with Luer lock connections - Prewarmed HAMA bioink. - Positive displacement pipette + pipette tips (optional)	If not printing with cells move directly to step 3. - Mix ten parts of the bioink with one part of cell suspension without introducing air bubbles to the mixture. Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the Luer lock adaptor with HAMA bioink before connecting the syringe with the cell suspension. Note: Transferring viscous HAMA bioinks may be facilitated by using a positive displacement pipette. Note: If preparing bioink quantities >2 mL, use the CELLMIXER. If preparing bioink quantities <2 mL, it is recommended to connect two 3 mL Luer lock syringes and mix the bioink and the cell suspension back and forth between the syringes until homogeneous.
3	Preparation for printing	- Amber cartridge, 3cc - HAMA bioink (mixed with cells if applicable) - Sterile bioprinting nozzles 22-27G	- Load a cartridge with the bioink. - Place the bioink in the printhead and cap with a printing nozzle or needle of choice. - Choose the desired printing temperature for the bioink (refer to Figure 1).
4	Printing	- Bioprinter (BIO X or INKREDIBLE series recommended) - Well plate or Petri dish	- Bioprint droplets using suggested parameters (Table 1). If printing is not as desired, adjust the pressure up/down by 1 kPa to extrude more/less material. If the pressure needed is too high, increase the extrusion time.

			Note: The Syringe Extrusion Printhead allows for better control of extrusion rates.
5	Photocuring	- Photocuring module (installed in bioprinter)	HAMA bioinks can be photocured, if a photoinitiator has been added, using either the 405 or 365 nm photocuring module. For both 1% and 3% HAMA with 0.25% LAP, a minimum 30 s of photocuring at 405 nm, 5 cm from the light source, is recommended. Higher concentration of HAMA and longer exposure time will result in stiffer constructs. Note: It is not recommended to expose cells to low wavelength light for longer than 150 s, as this may negatively affect the cell viability.
6	Incubation	- Cell culture medium	- After photocuring, add the desired medium to the constructs and place them in an 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.

Table 1. Recommended extrusion pressure* (±2 kPa) and extrusion time used for bioprinting droplets at 20-25°C, using diluted HAMA bioink and a 22 G nozzle. 'Diluted' assumes a mixture of one part of PBS to ten parts of bioink, which is the simulation of bioink and cell suspension mixing conditions. Undiluted bioink needs higher pressure, or longer extrusion time, to achieve the same droplet size as diluted bioink.

HAMA concentration	Extrusion time (s)	Printing pressure (kPa)	
		10 µL droplet	20 µL droplet
1%	0.1	12	20
3%	0.5	30	42

**Note this is only a recommended reference of starting pressures. The actual pressure needed will vary depending on the preparation procedures (amount of bioink and actual temperature of the bioink) as well as the fitting of the piston in the cartridge and the leveling of the print surface. This table was generated with printhead temperature set to 24°C and with a bioink/PBS volumetric ratio of 10/1.*

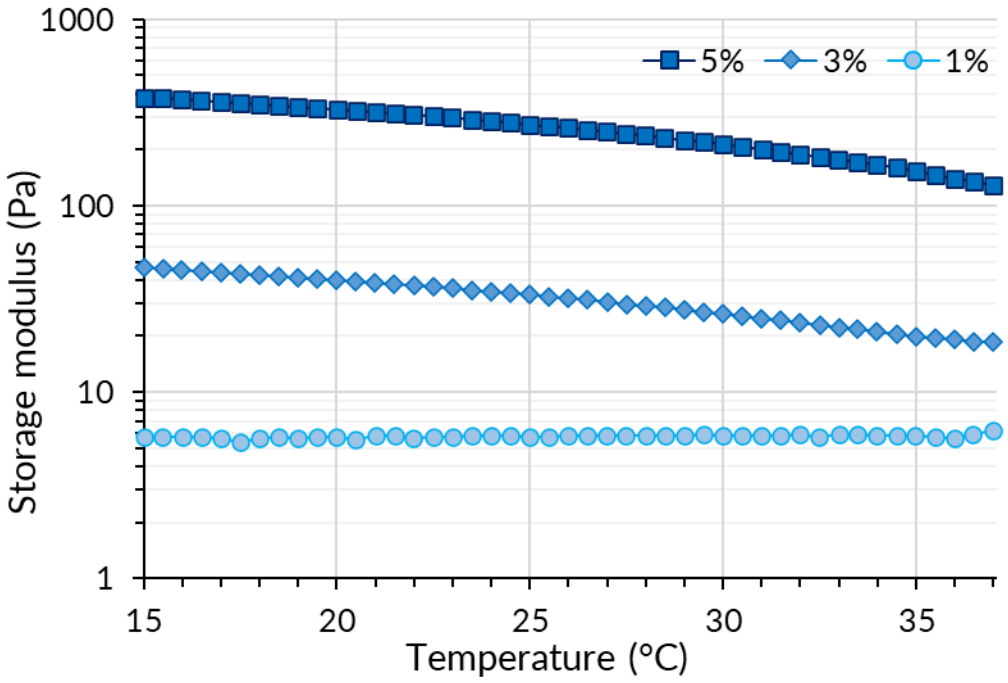

Figure 1. Storage modulus of reconstituted HAMA at various concentrations over a temperature range of 15 to 37°C.