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Casting Protocol

GelMA-HAMA Kit

This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for casting the reconstituted GelMA-HAMA Kit using the INKREDIBLE, INKREDIBLE+, or BIO X, with and without cells. This document covers the dispensing of cell encapsulated gels and post seeding of casted gels, obtained through light induced gelation.

Material needed

- GelMA-HAMA Kit*, reconstituted (Refer to *Reconstitution Protocol* GelMA-*HAMA Kit*)
- Photocuring UV module
- BIO X* or INKREDIBLE-series* 3D Bioprinter
- UV shielding cartridges, 3cc*
- Sterile Conical Bioprinting nozzles*

- Cells + cell culture medium
- 3ml 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 INK 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

Make sure to follow the GelMA-HAMA Kit reconstitution protocol prior to following this protocol. See *Reconstitution Protocol GelMA-HAMA Kit*. This protocol works best with the BIO X and the Temperature Controlled Printhead. If using the INKREDIBLE+ system, the

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dispensing procedure should be performed fast, to prevent the bioink from warming and gelling in the cartridge prior dispensing.

Protocol for casting of cell embedded bioink

Step	Title	Material	Description
1	Prepare Bioink	- Reconstituted GelMA-HAMA solution - 3 ml syringe	- Prewarm GelMA-HAMA bioink to 37°C. - Transfer the bioink into a 3 ml syringe: remove the syringe plunger, cap the syringe and pour in the bioink into the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air.
2	Mix GelMA-HAMA with cells	- Cell suspension in syringe - Prewarmed GelMA-HAMA bioink - Female/female luer lock adaptor	If not printing with cells move directly to step 3. At this point, mix ten parts bioink with one part cell suspension, taking care to not introduce air bubbles to the mixture. For detailed instructions see the *Mixing cells Protocol GelMA series*. - Attach the GelMA-HAMA bioink syringe to the syringe with cell suspension, with a female/female luer lock adaptor. - Carefully mix the bioink with the cell suspension by gently pushing the bioink back and forth between the syringes. Note: Suggested cell suspension density is $5x10^6$ cells/ml to $10x10^6$ cells/ml. Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the luer lock adaptor with GelMA-HAMA bioink before attaching the syringe with the cell suspension. If preparing for quantities > 2ml of GelMA-HAMA, it is recommended to use the CELLMIXER.
3	Load the cartridge	- UV shielding orange cartridge, 3cc	- Transfer the cell containing bioink to the orange cartridge and cap it. - Warm the printhead to 37 °C.
4	Load the cartridge	- UV shielding cartridges, 3cc loaded with GelMA-HAMA (and cells) - Sterile Conical Bioprinting nozzles	- Place the cartridge in the printhead and cap with a bioprinting nozzle of choice.

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5	Casting	- Bioprinter (BIO X or INKREDIBLE series)	- Dispense the required volume of bioink in the mould or in a well plate. Note: If waiting too long between extrusions the bioink can cool down in the nozzle causing it to clog. If this occurs, replace with new nozzle.
6	Crosslinking	- 365/405 UV module	GelMA-HAMA can be photocrosslinked using the 365 or 405 nm UV module. - Suggested distance of UV module from the sample set at 3cm; crosslinking time minimum 30-60 seconds. The crosslinking time is to be adjusted based on the construct depth. Ensure that the bioprinted constructs are thermally gelled after printing. Note: Over exposure of UV to the constructs might damage the cells. If crosslinking is unsure add 37°C media to one printed well to validate that it doesn't dissolve.
7	Incubation	- Cell culture medium	- Add the desired medium to submerge 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.

Protocol for post-seeding of casted bioink

Step	Title	Material	Description
1	Prepare Bioink	- GelMA-HAMA bioink - 3 ml syringe	- Prewarm GelMA-HAMA bioink to 37°C. - Transfer the bioink into a 3ml syringe: remove the syringe plunger, cap the syringe and pour in the bioink into the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air.
2	Load the cartridge	- UV shielding cartridge, 3cc	- Transfer the GelMA-HAMA bioink to the orange cartridge and cap it. - Warm the printhead to 37 °C.
3	Load the cartridge	- UV shielding cartridges, 3cc loaded with GelMA-HAMA (and cells) - Sterile Conical Bioprinting nozzles	- Place the cartridge in the printhead and cap with the printing nozzle.

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4	Casting	- Bioprinter (BIO X or INKREDIBLE series recommended)	- Dispense the required volume of ink in the mould or in a well plate. Note: If waiting too long between extrusions the bioink can cool down in the nozzle causing it to clog. If this occurs, replace with new nozzle.
5	Crosslinking	- 365/405 UV module	GelMA-HAMA can be photocrosslinked using the 365 or 405 nm UV module. - Suggested distance of UV module from the sample set at 3cm; crosslinking time minimum 30-60 seconds. The crosslinking time is to be adjusted based on the construct depth. Ensure that the bioprinted constructs are thermally gelled after printing. Note: Over exposure of UV to the constructs might damage the cells. If crosslinking is unsure add 37°C media to one printed well to validate that it doesn't dissolve.
6	Cell seeding	- Cell suspension	- Dispense the cell suspension in the middle of the hydrogel. Suggested cell suspension density: $20x10^3$ cells/cm^2 to $50x10^3$ cells/cm^2 (a highly concentrated cell suspension is suggested to use, for not more than 10µl).
7	Incubation	- Cell culture medium	- Incubate for 1 to 2 hours. - Add the desired medium to submerge 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.

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