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Casting Protocol

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 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

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

- 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

Make sure to follow the HAMA Kit reconstitution protocol prior to following this protocol. See *Reconstitution Protocol HAMA Kit*.

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Protocol for casting of cell embedded bioink

Step	Title	Material	Description
1	Prepare Bioink	- Reconstituted HAMA solution - 3 ml syringe	- Prewarm 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. Keep the bioink protected from light by covering the syringe with aluminium foil or use an amber syringe.
2	Mix HAMA with cells	- Cell suspension in syringe - Prewarmed 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*. - Attach the 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 HAMA bioink before attaching the syringe with the cell suspension. - If preparing for quantities > 2 ml of HAMA, it is recommended to use the CELLMIXER.
3	Load the cartridge	- UV shielding cartridge, 3cc	- Transfer the bioink to the UV shielding cartridge and cap it. - Warm the printhead to 37°C.
4	Load the cartridge	- UV shielding cartridges, 3cc loaded with HAMA (and cells) - Sterile Conical Bioprinting nozzles	- Place the cartridge in the printhead and cap with a bioprinting nozzle of choice.
5	Casting	- Bioprinter (BIO X or	- Dispense the required volume of bioink in the mould or in a well plate.

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		INKREDIBLE series)	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.
6	Crosslinking	- 365/405 nm photo curing module	HAMA can be photocrosslinked using the 365 or 405 nm photocuring module. - Suggested distance of photocuring module from the sample set at 3 cm; crosslinking time minimum 30-60 sec. 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 does not 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	- Reconstituted HAMA solution - 3 ml syringe	- Prewarm 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. Keep the bioink protected from light by covering the syringe with aluminium foil or use an amber syringe.
2	Load the cartridge	- UV shielding cartridge, 3cc	- Transfer the HAMA bioink to the UV shielding cartridge and cap it. - Warm the printhead to 37°C.
3	Load the cartridge	- UV shielding cartridges, 3cc loaded with 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 dry in the nozzle causing it to clog. If this occurs, replace with new nozzle.
5	Crosslinking	- 365/405 nm photocuring module	HAMA can be photocrosslinked using the 365 or 405 nm photocuring module. - Suggested distance of photocuring from the sample set at 3 cm; crosslinking time minimum 30-60 sec. 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 does not 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 10 µl).
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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