Casting Protocol

ColMA

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 ColMA Solution or ColMA 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 thermal or light induced gelation.

Material needed

- ColMA* or ColMA Kit*, reconstituted (Refer to *Reconstitution Protocol ColMA Solution or 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 ColMA Solution or Kit reconstitution protocol prior to following this protocol. See *Reconstitution Protocol ColMA Kit or Reconstitution Protocol ColMA Solution*. This protocol works best with the BIO X and the Temperature Controlled Printhead. If using

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the INKREDIBLE+ system, the dispensing procedure should be performed fast, to prevent the ColMA solution from warming and gelling in the cartridge prior dispensing.

Protocol for casting of cell embedded ColMA solution

Step	Title	Material	Description
1	Prepare Bioink	- Reconstituted ColMA solution - 3 ml syringe	- Cool down ColMA solution on ice for 10 minutes to make sure it remains in the liquid state. - Transfer the ColMA solution into a 3 ml syringe: remove the syringe plunger, cap the syringe and pour in the solution into the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air.
2	Mix ColMA with cells	- Cell suspension in syringe - ColMA solution - Female/female luer lock adaptor	If not casting with cells move directly to step 3. At this point, mix ten parts solution 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 ColMA solution syringe to the syringe with cell suspension, with a female/female luer lock adaptor. - Carefully mix the solution with the cell suspension by gently pushing the solutions 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 solution and the cell suspension, carefully pre-fill the luer lock adaptor with ColMA solution before attaching the syringe with the cell suspension. If preparing for quantities > 2ml of ColMA, it is recommended to use the CELLMIXER.
3	Load the cartridge	- UV shielding orange cartridge, 3cc	- Transfer the cell containing ColMA solution to the orange cartridge and cap it. - If using the BIO X, pre-cool the printhead to 15°C; if using the INKREDIBLE-series, cool down the cartridge on ice if needed
4	Cool the cartridge	- UV shielding cartridges, 3cc loaded with ColMA solution (and cells)	- Place the cartridge in the printhead and cap with a bioprinting nozzle of choice.

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		- Sterile Conical Bioprinting nozzles	
5	Casting	- Bioprinter (BIO X or INKREDIBLE series)	- Dispense the required volume of solution in the mould or in a well plate. Note: If waiting too long between extrusions the solution can warm in the nozzle causing it to clog. If this occurs, replace with new nozzle.
6	Crosslinking	- 365/405 UV module	ColMA can be photocrosslinked using the 365 or 405 nm UV module or thermal crosslinked. If using both, begin with thermal crosslinking. - Thermal crosslinking: warm the construct to 37°C until gelation occurs, approximately 10-15 min. The BIO X heated printbed or incubation can be alternatively used. - UV-curing (optional). Suggested distance of UV module from the sample set at 3 cm; crosslinking time set at 30s. 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 ColMA solution

Step	Title	Material	Description
1	Prepare Bioink	- Reconstituted ColMA solution - 3 ml syringe	- Cool down ColMA solution on ice for 10 minutes until the ink is liquid. - Transfer the solution into a 3 ml syringe: remove the syringe plunger, cap the syringe and pour in the solution into the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air.

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2	Load the cartridge	- UV shielding orange cartridge, 3cc	- Transfer the ColMA solution to the orange cartridge and cap it. - If using the BIO X, pre-cool the printhead to 15°C; if using the INKREDIBLE system, cool down the cartridge on ice if needed.
3	Cool the cartridge	- UV shielding cartridges, 3cc loaded with ColMA (and cells) - Sterile Conical Bioprinting nozzles	- Place the cartridge in the printhead and cap with a printing nozzle of choice.
4	Casting	- Bioprinter (BIO X or INKREDIBLE series)	- Dispense the required volume of solution in the mould or in a well plate. Note: If waiting too long between extrusions the solution can warm in the nozzle causing it to clog. If this occurs, replace with new nozzle.
5	Crosslinking	- 365/405 UV module	ColMA can be photocrosslinked using the 365 or 405 nm UV module or thermal crosslinked. If using both, begin with thermal crosslinking. - Thermal crosslinking: warm the construct to 37°C until gelation occurs, approximately 10-15 min. The BIO X heated printbed or incubation can be alternatively used. - UV-curing (optional). Suggested distance of UV module from the sample set at 3 cm; crosslinking time set at 30s. 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 10µl).
7	Incubation	- Cell culture medium	- Incubate for 1 to 2 hours. - Add the desired medium to submerge the constructs and place in incubator.

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