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Casting Protocol

Coll I

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 Coll I 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. Coll I is crosslinked through thermal induced gelation. This protocol was optimized for Coll I undiluted as well as a 10+1 cell suspension dilution. Changing the concentration of solution to cell suspension ratio will change the gelation time.

Material needed

- Coll I*, reconstituted (Refer to *Reconstitution Protocol Coll I*)
- Cartridges, 3cc*
- BIO X* or INKREDIBLE-series* 3D Bioprinter
- Sterile Conical Bioprinting nozzles*

- Cells + 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/*.

Protocol

Make sure to follow the Coll I reconstitution protocol prior to following this protocol. See *Coll I Reconstitution Protocol*. This protocol works best with the BIO X and the Temperature Controlled Printhead. If using the INKREDIBLE+ system, the dispensing procedure should be performed fast, to prevent the solution from warming and gelling in the cartridge prior dispensing.

Protocol for casting of cell embedded Coll I solution

Step	Title	Material	Description

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1	Prepare Bioink	- Reconstituted Coll I solution - 3 ml syringe	- Cool down Coll I on ice for 10 minutes to make sure it remains in the liquid state. - Transfer the Coll I solution into a 3 ml syringe: remove the syringe plunger, cap the syringe and pour in the solution in the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air.
2	Mix Coll I with cells	- Cell suspension in syringe - Cooled Coll I solution - Female/Female luer lock adaptor	If not casting with cells move directly to step 3. Mix ten parts Coll I 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 Coll I solution syringe to the syringe with cell suspension, with a female/female luer lock adaptor. - Carefully mix the solutions 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 Coll I solution before attaching the syringe with the cell suspension. - If preparing for quantities > 2ml of Coll I, it is recommended to use the CELLMIXER.
3	Load the cartridge	- Cartridge, 3cc	- Transfer the cell containing solution to the 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	- Cartridges, 3cc loaded with Coll I (and cells) - Sterile Conical Bioprinting nozzles, 25G	- Place the cartridge in the printhead and cap with a bioprinting nozzle of choice.
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.

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6	Thermal crosslinking		Coll I can be thermally crosslinked. - Warm the construct to 37°C until gelation occurs, approximatly 10-15 min. The BIO X heated printbed or incubation can be alternatively used.
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 Coll I solution

Step	Title	Material	Description
1	Prepare Bioink	- Reconstituted Coll I solution - 3 ml syringe	- Cool down Coll I solution on ice for 10 minutes to make sure it remains in the liquid state. - Transfer the solution into a 3 ml syringe: remove the syringe plunger, cap the syringe and pour in the Coll I solution into the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air.
2	Load the cartridge	- Cartridge, 3cc	- Transfer the Coll I solution to the 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
3	Cool the cartridge	- Cartridges, 3cc loaded with Coll I (and cells) - Sterile Conical Bioprinting nozzles, 25G	- Place the cartridge in the printhead and cap with a bioprinting nozzle of choice.
4	Casting	- Bioink - Bioprinter (BIO X or INKREDIBLE series recommended)	- 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	Thermal crosslinking		Coll I can be thermally crosslinked. - Warm the construct to 37°C until gelation occurs, approximatly 10-15 min. The BIO X heated printbed or incubation can be alternatively used.

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6	Cell seeding	- Cell suspension	- Dispense the cell suspension in the middle of the casted 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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