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Reconstitution Protocol

ColMA 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 reconstituting and neutralizing the ColMA powder. The reconstituted ColMA can be used for 3D bioprinting as well as casting for 3D cell culture. The kit contains two components, sterile ColMA powder and chosen photoinitiator (PI) e.g. LAP or Irgacure 2959.

Material needed

- ColMA powder (100 mg), sterile*
- LAP or Irgacure 2959 (100 mg)
- Sterile 20 mM acetic acid
- Sterile stir bar
- Ice bath
- Sterile 10X PBS
- Sterile deionized (DI) water
- Sterile 1 M NaOH
- Sterile 15 mL Falcon tube
- Sterile 0.22 µm filter
- UV shielding cartridge, 3cc*

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

Protocol

Be aware to keep the ColMA and PI protected from light.

Step	Title	Material	Description
1	Dissolving the ColMA powder	- Vial of ColMA - Sterile 20 mM acetic acid - Ice bath - Sterile stir bar	- Record the desired final volume of the ink (V_{INK}). - Record the desired final collagen concentration (C_{Fconc}). - Add sterile 20 mM acetic acid solution to the ColMA vial at the desired volume to achieve

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			the target concentration of the stock solution, see Table 1. This is the collagen stock solution (C_{Conc}). Note: C_{FConc} and C_{Conc} cannot be the same, otherwise the solution would not be neutralized - Add sterile stir bar and mix gently over night at 4 °C. Avoid rapid stirring which can generate air bubbles. Alternatively, place the bottle in the fridge and turn the bottle over a couple of times every other hour. - After dissolution, maintain the vial with ColMA stock solution on ice to keep cool.
2	Calculations for neutralization		- Prepare a neutralization solution for the collagen based on the following calculations: - Volume of ColMA Stock Solution: $V_{ColMA} = \frac{C_{Fconc} \times V_{INK}}{C_{Conc}}$ - Volume of 10X PBS: $V_{PBS} = \frac{V_{INK}}{10}$ - Volume of 1 M NaOH: $V_{NaOH} = V_{ColMA} \times 0.011$ - Volume of DI water $V_{DI} = V_{INK} - V_{ColMA} - V_{PBS} - V_{NaOH}$ - See Table 2 for example calculation.
3	Neutralization	- Sterile 10X PBS - Sterile DI water - Sterile 1 M NaOH - Sterile 15 mL Falcon tube - Ice bath - LAP or Irgacure 2959	- Mix following volumes from Step 3; V_{PBS}, V_{NaOH} and V_{DI} in a sterile 15 mL Falcon tube. This is the neutralization solution V_{NS}. $V_{NS} = V_{PBS} + V_{NaOH} + V_{DI}$ - Optional; For photocrosslinking, dissolve the mass of PI in the neutralization solution to achieve a 0.25% final concentration. Mass of PI necessary is $V_{INK} \times 0.0025$. Sterile filter the PI solution using a 0.22 µm filter.

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		- 0.22 µm filter	- Cool the neutralization solution on ice for 10 minutes. - Act fast at this step, once the V_{NS} has been added the collagen solution will begin to self-assemble. - Transfer the V_{ColMA} to the tube containing the neutralization solution, gently mix by pipetting up and down. - Check the pH and if needed adjust with more NaOH to achieve a pH between 6.9-7.4.
4	Casting or bioprinting	- UV shielding cartridge, 3cc	- For casting: Cast structure and warm to 37°C to induce gelation, approximately 10-15 min. See *Casting Protocol ColMA* for more detailed instruction on casting with cells. - For bioprinting; Transfer to a cartridge and print structure and warm to 37°C to induce gelation. - If desired, and photoinitiator has been added, crosslink under UV exposure.

Table 1. Suggestions of desired concentration of ColMA stock solution.

Concentration Stock C_{conc}	Volume Acetic Acid Needed
0.5% (5 mg/mL)	20 mL
1% (10 mg/mL)	10 mL
1.2% (12 mg/mL)	8.33 mL
1.4% (14 mg/mL)	7.14 mL
1.6% (16 mg/mL)	6.25 mL
2% (20 mg/mL)	5 mL
5% (50 mg/mL)	2 mL
10% (100 mg/mL)	1 mL

Table 2. Example of the different components in the ColMA solution.

$$V_{NS} = V_{PBS} + V_{NaOH} + V_{DI}$$

V_{INK}	C_{Fconc}	C_{conc}	V_{ColMA}	V_{NS}	V_{PBS}	V_{NaOH}	V_{DI}
1 mL	5 mg/mL	10 mg/mL	0.5 mL	0.5 mL	100 µL	5.5 µL	394.5 µL
3 mL	5 mg/mL	10 mg/mL	1.5 mL	1.5 mL	300 µL	16.5 µL	1.184 mL
1 mL	15 mg/mL	20 mg/mL	0.75 mL	0.25 mL	100 µL	8.25 µL	141.75 µL
3 mL	15 mg/mL	20 mg/mL	2.25 mL	0.75 mL	300 µL	24.75 µL	425.25 µL

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