mColMA Preparation and Bioprinting Protocol

⚠ **This protocol has been optimized for general bioprinters, depends on extruder type, parameters may vary**

⚠ **Room temperature assumes 22 °C, times and printing parameters may vary at a different room temperature**

Materials

1) ColMA Precursor Powder or Lyophilizate
2) Solution of Phosphate Buffered Saline (PBS) or other relevant buffer
3) Irgacure 2959 Powder (or other photoinitiator)

Preparation of Solutions and Calculations

1) Resuspend ColMA Precursor in the solution of Phosphate Buffered Saline (PBS) or other relevant buffer in desired concentration to obtain ColMA Precursor Solution. Calculate the volume of ColMA and the mass of Irgacure 2959 necessary to achieve the desired final concentrations. ColMA precursor solution should be 80% of the final bioink and the Irgacure 2959 Precursor should be 20% of the final bioink. The final concentration of Irgacure should be 0.5%.
We recommend 10-20% final concentration of ColMA in the hydrogel for better rheology.

⚠ **The following ratio values were calculated for a 10 mL final volume of bioink. (For easy calculation)**

2) Prepare precursor solutions

A. Dissolve the Irgacure 2959 powder in PBS that consists of 20% of the desired final volume.
    a. In this case, 0.05 g of Irgacure 2959 is dissolved in 2 mL of PBS (20% of 10 mL).
    b. Cover the Irgacure precursor solution with foil to protect from light, and place it in a 70°C water bath for ~30 minutes.
    c. Vortex after 20 minutes and ensure that Igracure is dissolved through the absence of undissolved solids.
    d. Sterilize Irgacure solution via filtering with a 0.22µm filter.

3) Prepare ColMa solutions

B. In this case, 1g of ColMa was dissolved (for 10% concentration of the final solution) in 8 ml of dPBS (80% of 10ml)
C. Cover the ColMa solution with foil, and place it in a 40°C water bath and mix it for 30 minutes (or until ColMa fully dissolved).
D. All the procedures have to be done under the sterile conditions
E. We also recommend adding antibiotic/antimycotic mixture

⚠ **Irgacure solution has a shelf-life of 2 weeks, prepare only the necessary volume of Irgacure for the current experiment**.

⚠ **Irgacure, as well as ColIMA is photosensitive. Make sure to protect from light.**

F. Once both precursor solutions are prepared, transfer the Irgacure 2959 solution to the ColMA solution.
G. Mix well for 15 minutes with heating (40°C) and ensure that the contents are protected from light throughout the whole mixing process.

⚠ **The mixed solution can be stored at this point at 4°C *protected from light* for future use.**

H. Transfer the ready-to-print ColMA Bioink into the printing cartridges

Bioprinting Process

1) Let the Foldink ColMA bioink reach to 35°C before mixing with cells.
2) Calculate the desired number of living cells.
3) Create 500-1000µL cell suspension with culture medium
4) Place cell suspension into the Luer lock syringe
5) Connect the cartridge of bioink with the syringe with cell suspension
6) Gently mix until the mixture becomes homogenous and the color of the final ink spreads evenly throughout the whole volume of bioink

1) As the solution is 35°C, remove all air bubbles
2) Cap the bioink with syringe caps and let the bioink cooldown
3) If the bioprinter has temperature controlled printhead, set 15°C place the bioink into the printhead, cool for 5-10 minutes, or place the bioink in the fridge for 5-10 minutes
4) Cap with sterile printing nozzle or conical tip of the desired diameter
5) Turn on the UV-crosslinking feature of the bioprinter. Otherwise, if the bioprinter does not have this feature, use another UV light source. The desirable wavelength is 365nm.
6) Crosslinking time will vary depending on light source intensity, typically from 2 to 5 minutes

⚠ **Note, longer exposure of the scaffold to UV light may affect cell viability**

7) If printing is performed in cool conditions, crosslinking may be applied after the printing.
8) Print test lines to adjust the necessary pressure or extrusion speed (if mechanical extruder)
9) If filament characteristics are sufficient, replace nozzle, and print as planned
10) If filament characteristics are non-ideal due to too low of a viscosity (high temperature) wait another minute for additional cooling and retest.
11) If filament characteristics are non-ideal due to too high of a viscosity (low temperature), increase pressure or reheat and repeat steps
12) Adjust cooling time as necessary.
13) Print

⚠ **If performed correctly, an approximate 15-20 minute bioprinting window exists where viscosity will be ideal.**