GelMA 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) Lyophilized GelMA Precursor
2) Solution of Phosphate Buffered Saline (PBS) or other relevant buffer
3) Irgacure 2959 lyophilizate (or other photoinitiator)

Preparation of Solutions and Calculations

1) Resuspend GelMA Precursor lyophilizate in the solution of Phosphate Buffered Saline (PBS) or another relevant buffer in desired concentration to obtain GellMA Precursor Solution. Calculate the volume of GelMA and the mass of Irgacure 2959 necessary to achieve the desired final concentrations. GelMA 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%(w/v).

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

3) Prepare GelMa solutions

B. Dissolve lyophilized GelMa in PBS that consist of 80% of the desired final volume.
   a. In this case, 1g of GelMa was dissolved (for 10% concentration of the final solution) in 8 ml of dPBS (80% of 10ml)
   b. Cover the GelMa solution with foil, and place it in a 40°C water bath and mix it for 30 minutes (or until GelMa fully dissolved).
   c. All the procedures have to be done under the sterile conditions
   d. 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 mCollMA is photosensitive. Make sure to protect from light.**

C. Once both precursor solutions are prepared, transfer the Irgacure 2959 solution to the GelMA solution.
D. Mix well for 15 minutes under heat (40 °C) to ensure full mixing will be protected from light.

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

E. Transfer the ready-to-print GelMA Bioink into the printing cartridges

Bioprinting Process

1) Let the Foldink GelMA bioink cool down 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 will become homogenous and the colour of the final ink becomes similar into the whole volume of bioink
7) As the solution is 35°C, remove all air bubbles
8) Cap the bioink with syringe caps and let the bioink cooldown
9) If the bioprinter has temperature controlled printhead, set 18°C place the bioink into the printhead, cool for 5-10 minutes, or place the bioink in the fridge for 5-10minutes
10) Cap with sterile printing nozzle or conical tip of the desired diameter
11) Turn on UV-crosslinking in the printer or from the other light source - 365nm lamp.
12) Crosslinking time will vary depending on light source intensity from 2-5 minutes, fig.1

Distance (cm) / Time (sec)	30	60	120	180	240	300	Intensity (mW/cm2)
2.5							40
3							27.75
5							10
7							5.1
10							5

Figure 1. Dependence of cross-linking on UV intensity. Testing was performed with the UV-LED spot system HTLD-4. Cells marked with **yellow** show good crosslinking properties.

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

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

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