GelMA 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**

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

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

Materials

1) GelMA Precursor Solution 10% in 3mL Syringes

Bioprinting Process

2) Let the Foldink GelMA bioink cool down to 35°C before mixing with cells.
3) Calculate the desired number of living cells.
4) Create 500-1000µL cell suspension with culture medium
5) Place cell suspension into the Luer lock syringe
6) Connect the cartridge of bioink with the syringe with cell suspension
7) Gently mix until the mixture will become homogenous and the colour of the final ink becomes similar into the whole volume of bioink
8) As the solution is 35°C, remove all air bubbles
9) Cap the bioink with syringe caps and let the bioink cooldown

⚠ **Store the mixed solution at this point at 4°C *protected from light*.**

10) If the bioprinter has temperature controlled printhead, set the temperature 18°C
11) Place the bioink into the printhead, cool down for 5-10 minutes
12) Cap with sterile printing nozzle or conical tip of the desired diameter
13) 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.
14) 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.

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

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

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