



Tissue Specific Bioink 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 Tissue Specific Bioink 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) Tissue Specific Bioink Solution in 3mL Syringes

Bioprinting Process

- 2) Let the 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/cm ²)
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.**