



GelMA HA Printing Protocol

Overview: This protocol presents recommendations for optimizing the crosslinking of GelMA using the INKREDIBLE, INKREDIBLE+, and BIO X 365 and 405 LED. This protocol was optimized for CELLINK GelMA was at 10% w/w concentration with either 0.25% Irgacure or 0.25% LAP, changing these concentrations will change the crosslinking time. Reference the GelMA crosslinking optimization protocol to adjust and determine these numbers.

Note: This protocol works best with the BIO X and the Temperature Control Printhead and the Cooled Printbed.

Note: When using the INKREDIBLE+ system preheat a printhead to 26 °C to achieve the same temperature maintenance as the Temperature Control Printhead. After deposition, transfer the well plate or petri dish printed into onto ice or another cooled surface to quickly thermally gel the construct prior to UV exposure.

GelMA Printing Protocol

This protocol below a recommendation for the crosslinking of GelMA bioink. See below for more detailed numbers and how to optimize the crosslinking for your specific construct or experiment. This protocol below includes post-processing steps that are critical for GelMA crosslinking.

1. Heat up GelMA HA in a cartridge to 35 °C until the GelMA HA is liquid, this can be tested by flipping the cartridge and observing if air bubbles move freely. The heating of the GelMA can be performed in a pneumatic printhead or in a water bath or incubator.
2. Cool GelMA HA cartridge on ice for 30 seconds to 3 minutes. The exact time is going to dependent on the volume of GelMA in the cartridge and your concentration. Every 15-25 seconds remove the GelMA cartridge from the ice and flip. Observe the air bubble movement, once it begins to slow down, the GelMA HA is almost ready to print. The viscosity needs to be like a thick syrup or honey.
3. Place the semi-gelled GelMA in either an INKREDIBLE+ printhead preheated to 24 °C or the Temperature Control Printhead on the BIO X preheated to 24 °C. If using the BIO X, precool the printbed to 10 °C.
4. Print or deposit your GelMA construct. The necessary pressure for extrusion can range from 20 kPa to 60 kPa depending on the nozzle diameter (22 G to 27 G). Recommended translation speed is 600 mm/min (10 mm/sec). When determining the pressure to

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extrude at, start at the lower end of the range. Increase the pressure by increments of 10 kPa during the priming steps. Once the GelMA HA extrudes and forms a filament, fine tune the pressure from there. If the GelMA HA forms a droplet, cool down the GelMA HA more and repeat the pressure generation.

Note: These parameters may need to be refined based on the exact GelMA HA concentration and photoinitiator concentration that is utilized.

Note: Blending with cells will dilute the GelMA HA concentration and lower the desired pressures.

Note: Over time the GelMA HA will become more solid (15-20 minutes). If printing is paused and this happens, replace the nozzle. If extrusion does not occur, repeat steps 1 to 3 to 'reset' the cartridge.

GelMA HA Crosslinking Protocol

See the charts below for recommended crosslinking times for CELLINK GelMA HA. Ensure that the bioprinted GelMA HA construct is thermally gelled through cooling of the printbed if using the BIO X or placement of the printed construct on ice for 10 seconds if using the INKREDIBLE +. If crosslinking during printing, set the crosslinking parameters appropriately in the GCode for the INKREDIBLE+ or the printhead setup page for the BIO X. Refer to the GelMA crosslinking optimization protocol for refinement.

Table 1: Crosslinking success of CELLINK GelMA HA with 0.5% Irgacure 2959.

		CROSSLINKING TIME (SECONDS)								
		15	30	45	60	90	120	180	240	300
DISTANCE FROM LED LIGHT (CM)	3									
	4									
	5									
	6									
	7									

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Table 2: Crosslinking success of CELLINK GelMA HA with 0.05% Irgacure 2959.

		CROSSLINKING TIME (SECONDS)								
		15	30	45	60	90	120	180	240	300
DISTANCE FROM LED LIGHT (CM)	3									
	4									
	5									
	6									
	7									
	7									

Table 3: Crosslinking success of CELLINK GelMA HA with 0.5% Irgacure 2959 in four wells.

		CROSSLINKING TIME (SECONDS)								
		15	30	45	60	90	120	180	240	300
DISTANCE FROM LED LIGHT (CM)	3									
	4									
	5									
	6									
	7									
	7									



Table 4: Crosslinking success of CELLINK GelMA HA with 0.5% LAP.

		CROSSLINKING TIME (SECONDS)								
		15	30	45	60	90	120	180	240	300
DISTANCE FROM LED LIGHT (CM)	3									
	4									
	5									
	6									
	7									

Table 5: Crosslinking success of CELLINK GelMA HA with 0.05% LAP.

		CROSSLINKING TIME (SECONDS)								
		15	30	45	60	90	120	180	240	300
DISTANCE FROM LED LIGHT (CM)	3									
	4									
	5									
	6									
	7									

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