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Reconstitution Protocol

GelMA Lyophilizate

This is a suggested procedure, please adjust according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

The aim of this protocol is to provide instructions on how to reconstitute GelMA Lyophilizate (gelatin methacrylate) to your desired concentration using Reconstitution Agent P. The obtained GelMA hydrogel can be used as a bioink on its own or as a component in other bioink formulations. Addition of a photoinitiator (PI) and use of 365 or 405 nm LED modules ensure stable and controlled photocrosslinking of GelMA constructs for 3D cell culturing.

Materials needed

- GelMA Lyophilizate (500 mg), sterile*
- Reconstitution Agent P* or an alternative buffer of choice
- Photoinitiator*
- Magnetic stir bar
- Syringes
- 0.22 µm sterile syringe filter
- 15 mL Falcon tube or equivalent
- Female/female Luer lock adaptors*
- Amber cartridge, 3cc*

*The product can be purchased in the CELLINK store at *www.cellink.com/store/*.

Protocol

This protocol describes reconstitution of 500 mg of GelMA Lyophilizate to obtain bioinks of different concentrations. All GelMA concentrations are calculated as weight of GelMA per total weight of GelMA and Reconstitution Agent P (w/w).

Step	Title	Material	Description
1	Prepare GelMA	- GelMA	- Take GelMA from storage and let it reach room temperature.
2	Prepare a reconstitution agent with added PI	- Reconstitution Agent P - Photoinitiator (PI) - Syringe - 0.22 µm sterile syringe filter - 15 mL Falcon tube	- Prepare 12 mL of a reconstitution agent. Note: Reconstitution Agent P is a specially designed buffer that maintains a physiologic pH in the final GelMA bioink. - Mix in the desired amount of PI in the reconstitution agent, see Table 1 for suggested PI concentrations. Remember to protect all PI containing mixtures from light. - Sterile filter the PI solution into a sterile 15 mL Falcon tube using a syringe and 0.22 µm sterile syringe filter. - Heat the sterile PI solution to ~50°C.
3	Prepare a GelMA solution	- GelMA Lyophilizate - Reconstitution agent with added PI - Stir bar	- Add your desired amount of reconstitution agent with PI to the GelMA vial, see Table 2 for suggested GelMA concentrations. - Add a sterile stir bar to the container. - Stir the mixture at ~50°C for ~60 min, or until dissolved. - Double check that pH is in the 7.0-7.4 range since pH is important for the proper viscosity of the bioink. If needed, balance with small volumes of NaOH or HCl. Note: Adding additional liquids to adjust the pH dilutes your bioink and PI concentration. In addition, be careful with addition of NaOH since high pH degrades GelMA.
4	Prepare a bioink	- Syringe - Female/female Luer lock connectors - Amber cartridge, 3cc	- Use the GelMA solution as it is or mix with other components of choice. See Figure 1 for viscosity and printing temperature of GelMA solutions of different concentrations. - Heat the GelMA solution at 35°C until it is liquid. Transfer necessary amount of GelMA solution into a syringe.

			- Transfer GelMA solution from the syringe to an amber cartridge using a Luer lock connector. - Store at 4-8°C. - See the *Bioprinting Protocol GelMA Bioink* for an example of printing GelMA with cells.

Table 1. Suggestions of PI concentrations for GelMA bioink.

PI concentration in GelMA bioink	PI mass for 12 mL of reconstitution agent
0.05% (0.5 mg/mL)	6 mg
0.10% (1 mg/mL)	12 mg
0.25% (2.5 mg/mL)	30 mg

Table 2. Suggestions of final GelMA concentrations for reconstitution of 500 mg GelMA Lyophilizate.

Concentration of GelMA (w/w)	Volume of reconstitution agent needed
5%	9.5 mL
10%	4.5 mL
20%	2 mL

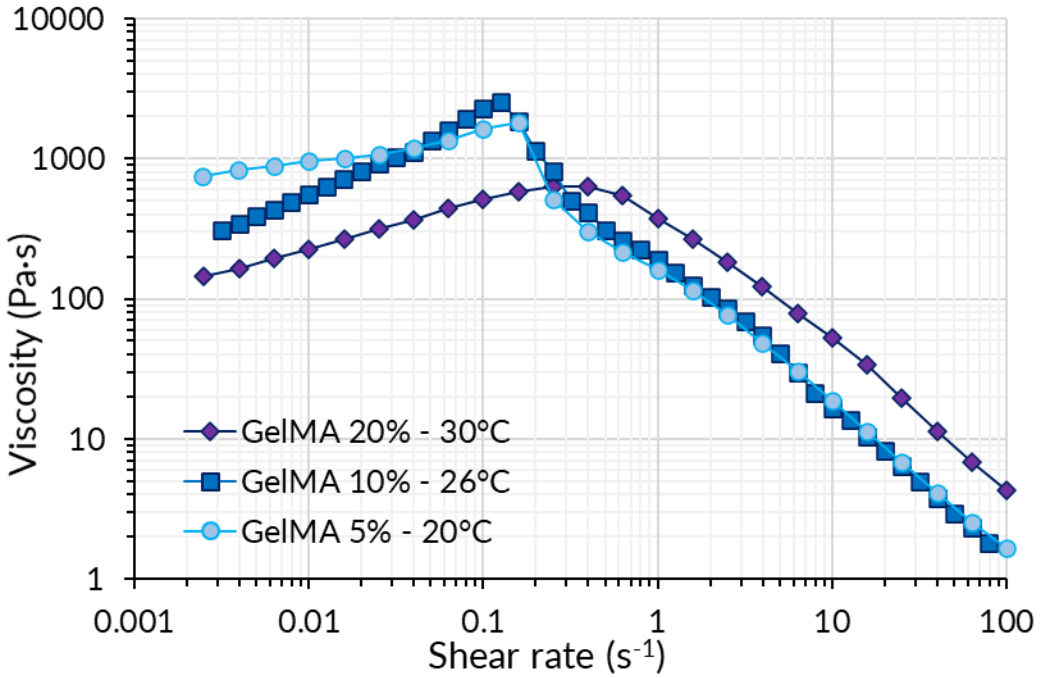

Figure 1. Optimized viscosity profiles of reconstituted GelMA Lyophilizate at various concentrations over a shear rate range of 0.01 to 100 s^{-1}, measured at the suggested printing temperature.