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Author: AB, VK, MG. Version: 1

Dilution Protocol

GelMA 20%

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 dilute GelMA 20% (w/w) 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 formulation. 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 20%*
- Reconstitution Agent P* or an alternative buffer of choice
- Photoinitiator*
- Female/female Luer lock adaptor*
- 0.22 µm sterile syringe filter
- BIO X* or INKREDIBLE series* 3D Bioprinter
- Syringes with Luer lock connections
- Sterile containers

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

Protocol

Step	Title	Material	Description
1	Defining desired concentrations		- Record the desired final concentration of GelMA (c_F). - Record the desired final volume of GelMA bioink to prepare (V_F). - Record the desired final concentration of PI (c_{PI_F}). Common concentrations are between 0.01% and 0.5%. See Figure 1 for difference in temperature behavior of GelMA solutions at different concentrations.
2	Calculations		- Calculate the volume of GelMA 20% to be used. $V_{GelMA20\%} = \frac{V_F \cdot c_F}{20\%}$ See Table 1 for suggested c_F. - Calculate the volume of Reconstitution Agent P, V_R, to be used. $V_R = V_F - V_{GelMA20\%}$ - Calculate the needed concentration of PI in the reconstitution buffer, C_{PI_R}, to achieve your desired final concentration of PI. $c_{PI\_R} = \frac{V_F \cdot c_{PI\_F}}{V_R}$ - Calculate the amount of PI, m_{PI}, needed to prepare V_R and 1 mL extra to account for absorption in the syringe filter. $m_{PI} = c_{PI\_R} \cdot (V_R + 1\,mL)$
3	Prepare PI and reconstitution agent	- Photoinitiator - Reconstitution Agent P - Syringe - 0.22 µm sterile syringe filter - Container	- Dissolve m_{PI} in V_R + 1 mL of Reconstitution Agent P. - Sterile filter using a syringe and 0.22 µm sterile syringe filter into a container. Note: always remember to protect all PI containing solutions from light.
4	Prepare GelMA bioink	- PI and reconstitution agent - GelMA 20% - Syringes	- Transfer V_R of the prepared reconstitution agent with PI into a sterile syringe that can accommodate minimum V_F. Heat it to ~35°C. - Heat the GelMA 20% at ~35°C until it is liquid.

		- Luer lock adaptor	- Transfer $V_{GelMA20\%}$ of GelMA 20% into another syringe. - Connect the two syringes using a Luer lock adaptor, make sure there are no air bubbles present. Mix the two solutions by passing them back and forth between the syringes until homogenized. Note: if air bubbles are introduced into the mixture, centrifuge the heated solution at 1500-2000 rpm for 1-2 min to remove them.
5	Storage	- GelMA bioink	- Store at 4-8°C protected from light.
6	Bioprinting	- BIO X or INKREDIBLE + - GelMA bioink	- For an example on bioprinting GelMA 10% with cells, see the *Bioprinting Protocol GelMA Bioink*. - For bioprinting GelMA 5% with cells, the *Bioprinting Protocol GelMA Fibrin* can be used as a reference with slight modifications in pressure and no thrombin crosslinking.

Table 1. Suggested concentrations and the corresponding volume of GelMA 20% and reconstitution agent used for the preparation of 5 mL of GelMA bioink.

Final concentration of GelMA, c_F (%)	Volume of GelMA 20%, $V_{GelMA20\%}$ (mL)	Volume of reconstitution agent with PI, V_R (mL)
5	1.25	3.75
10	2.5	2.5

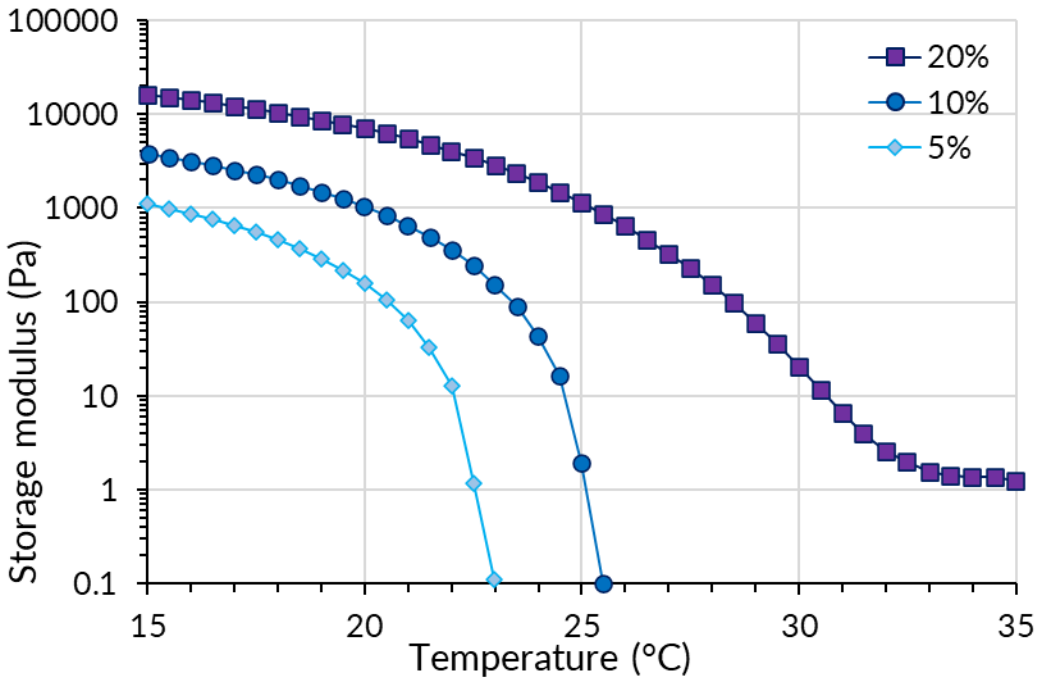

Figure 1. Decrease of storage modulus for GelMA hydrogels at various concentrations over an increasing temperature.