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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* (PI)
- 0.22 µm sterile syringe filter
- Syringes with Luer lock connections
- Female/female Luer lock adaptor*
- Cartridges, 3cc*
- Conical bioprinting nozzles*
- BIO X*, BIO X6* or INKREDIBLE series* 3D bioprinter

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

KEEP THE BIOINK PROTECTED FROM LIGHT IF TRANSFERRED FROM THE ORANGE UV PROTECTED CARTRIDGES TO AVOID CROSSLINKING BEFORE PRINTING. WORK WITH 3D PRINTERS IN DARK MODE. THE PHOTOINITIATOR IS SENSITIVE TO REPEATED OR PROLONGED EXPOSURE TO HEAT.

Protocol

1. Calculations

DESCRIPTION

- 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.
- 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 Agent P, 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.

  $$m_{PI} = c_{PI\_R} \cdot (V_R + 1\ mL)$$

Table 1. Suggested concentrations and the corresponding volume of GelMA 20% and Reconstitution Agent P 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

2. Preparing PI and Reconstitution Agent P

MATERIAL

Reconstitution Agent P

PI

0.22 µm sterile syringe filter

Syringe with Luer lock connections

DESCRIPTION

- Dissolve mPI 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.

3. Preparing GelMA bioink

MATERIAL

PI and Reconstitution Agent P

GelMA 20%

Syringes with Luer lock connections

Female/female Luer lock adaptor

DESCRIPTION

- Transfer V_R of the prepared Reconstitution Agent P 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.
- 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 1000 x *g* for 1 min to remove them.

Note: If not using the GelMA bioink right away, store at 4-8°C protected from light.

4. Bioprinting

MATERIAL

GelMA bioink

Cartridges, 3cc

Conical bioprinting nozzles

BIO X, BIO X6 or INKREDIBLE+

DESCRIPTION

- Transfer the GelMA bioink to a cartridge and cap with a bioprinting nozzle. Place in the printhead and print according to application.
    - 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.

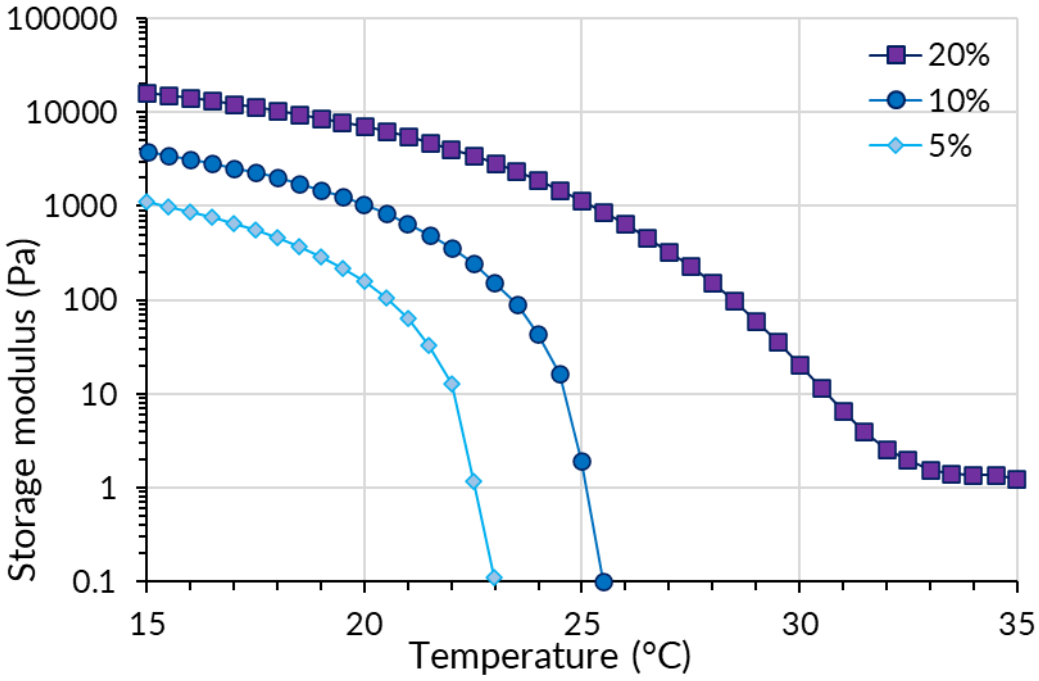

Figure 1. *Decrease of storage modulus for GelMA hydrogels at various concentrations over an increasing temperature.*