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Dilution Protocol

HAMA 5%

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 for how to dilute HAMA 5% to your desired concentration and adding a photoinitiator (PI). The obtained HAMA hydrogel can be used as a bioink on its own or as a component in other bioink formulation. Addition of a photoinitiator such as LAP or Irgacure 2959 enables photocuring of HAMA to ensure stable constructs for cell culture. HAMA solution with a concentration less than 3% w/w will not photocrosslink efficiently and is thus not suggested for use in cell culture on its own. If lower concentrations are used, make sure the bioink contains other crosslinkable components.

Materials needed

- HAMA 5%*
- Photoinitiator (PI)*
- Reconstitution Agent P* or an alternative buffer of choice
- Female/female Luer lock adaptor*
- BIO X* or INKREDIBLE series* 3D Bioprinter
- Syringes with Luer lock connections
- 0.22 µm sterile syringe filter
- Containers

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

Protocol

Step	Title	Material	Description
1	Note desired concentrations		- Record the desired final concentration of HAMA (c_F). - Record the desired final volume of HAMA 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 viscosity of HAMA solutions of different concentrations and Figure 2 for their temperature dependence.
2	Calculations		- Calculate the volume of HAMA 5% to be used. $V_{HAMA5\%} = \frac{V_F \cdot c_F}{5\%}$ See Table 1 for suggested c_F. - Calculate the volume of Reconstitution Agent P, V_R, to be used. $V_R = V_F - V_{HAMA5\%}$ - 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. Note: if c_F of HAMA is lower than 3%, incorporate other crosslinking polymers into V_R as well after sterile filtering to ensure the stability of the bioink post-printing.

4	Prepare HAMA bioink	- PI and reconstitution agent - HAMA 5% - Syringes - Luer lock adaptor	- Transfer V_R of the prepared reconstitution agent with PI into a sterile syringe that can accommodate minimum V_F. - Transfer $V_{HAMA5\%}$ of HAMA 5% 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 at 1500-2000 rpm for 1-2 min to remove them.
5	Storage	- HAMA bioink	- Store at 4-8°C and protect from light.
6	Droplet printing	- BIO X or INKREDIBLE + - HAMA bioink	See the *Bioprinting Protocol HAMA 5%* for a step by step instruction of droplet printing using a HAMA bioink.

Table 1. Suggested dilution options for HAMA 5% allowing the preparation of 5 mL of HAMA bioinks with different final concentration of HAMA. Though 1% HAMA solution is not crosslinkable on its own, it can be used as an additive to a customized bioink.

Final concentration of HAMA, c_F (%)	Volume of HAMA 5%, $V_{HAMA5\%}$ (mL)	Volume of reconstitution agent with PI, V_R (mL)
1	1	4
3	3	2

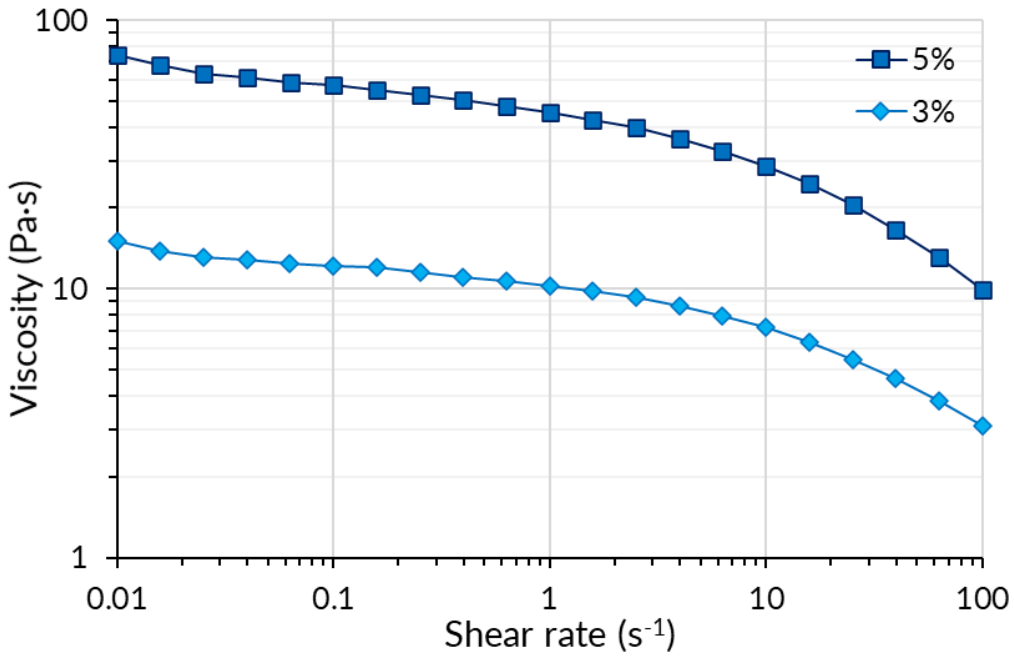

Figure 1. Viscosity of reconstituted HAMA at various concentrations over a shear rate range of 0.01 to 100 s^{-1}, 25°C.

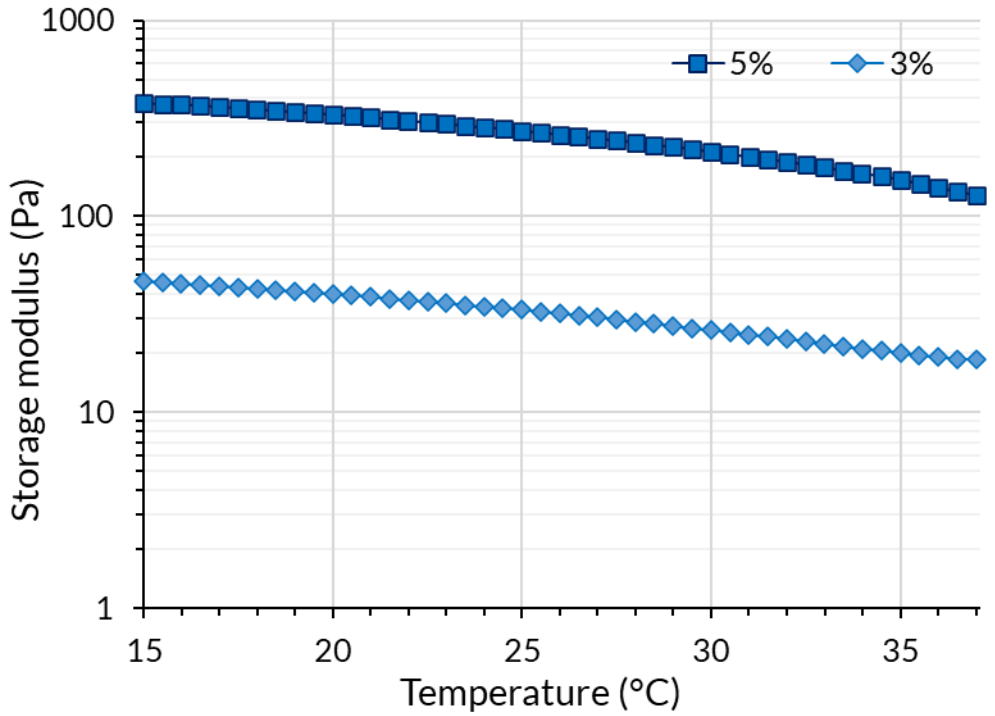

Figure 2. Storage modulus of reconstituted HAMA at various concentrations over a temperature range of 15 to 37°C.