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Reconstitution Protocol

HAMA 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 for reconstituting lyophilized hyaluronic acid methacrylate (HAMA Lyophilizate) for the use in 3D cell culture. Add a photoinitiator (PI), such as LAP or Irgacure 2959, to enable photocuring of the reconstituted HAMA bioink. 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.

Material needed

- HAMA Lyophilizate (100 mg)*
- 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 100 mg of HAMA Lyophilizate to obtain bioinks of different concentrations. All HAMA concentrations are calculated weight of HAMA per total weight of HAMA and Reconstitution Agent P (w/w).

Step	Title	Material	Description
1	Prepare HAMA	- HAMA Lyophilizate	- Take HAMA Lyophilizate 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 HAMA 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.
3	Prepare a HAMA solution	- HAMA Lyophilizate - Reconstitution agent with added PI - Stir bar	- Add your desired amount of reconstitution agent with PI to the HAMA Lyophilizate vial, see Table 2 for suggested HAMA concentrations. - Add a sterile stir bar to the container. - Stir the mixture at room temperature for ~60 min, or until dissolved. - Double check that pH is in 6.5-7.4 range. If needed, balance with small volumes of NaOH or HCl. Note: Adding additional liquids to adjust the pH dilutes your bioink and PI concentration.
4	Prepare a bioink	- Syringe - Female/female Luer lock connectors - Amber cartridge, 3cc	- Use the HAMA solution as it is or mix with other components of choice. See Figure 1 for viscosity of HAMA solutions of different concentrations. - Transfer the HAMA solution into a syringe. - Transfer HAMA solution from the syringe to an amber cartridge using a Luer lock connector. - Store at 4-8°C, protected from light.

Table 1. Suggestions of PI concentrations for HAMA bioink.

PI concentration in HAMA 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 HAMA concentrations for mixing one vial of 100 mg HAMA.

Desired concentration of HAMA (w/w)	Volume of reconstitution agent needed
3%	3.23 mL
5%	1.9 mL

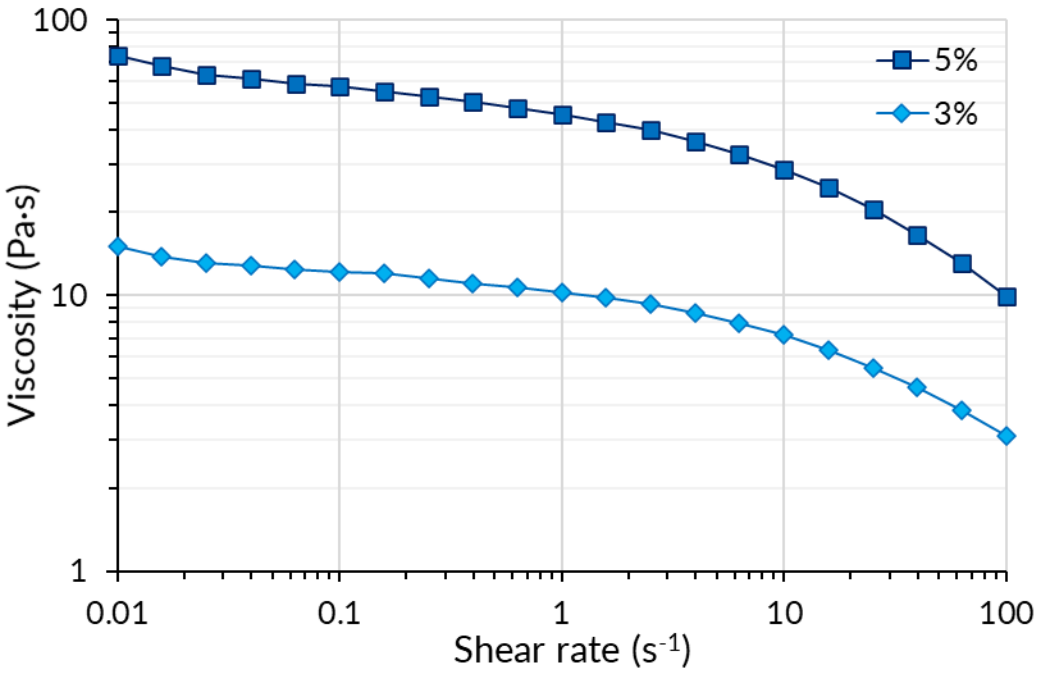

Figure 1. Viscosity of reconstituted HAMA Lyophilizate at various concentrations over a shear rate range of 0.01 to 100 s^{-1}, 25°C.