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Preparation Protocol

CELLINK Glucomannan

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 using CELLINK Glucomannan as a thickener of hydrogels to increase their printability. CELLINK Glucomannan is a sterile powder that may be dissolved in water, a buffer solution or a hydrogel. This document includes Protocol A, describing dissolution of the thickener in a liquid or very low viscosity solution, and Protocol B, describing mixing of the dissolved thickener (glucomannan gel) with a hydrogel for thickening effect.

Materials needed

- CELLINK Glucomannan*
- Female/female Luer lock adaptor*
- Cartridges, 3 cc*
- BIO X* or INKREDIBLE series* 3D Bioprinter
- Bioprinting nozzles* or needles*
- Well plate or Petri dish*
- Syringes with Luer lock connections
- Tubes (1-50 mL)
- Spatulas/spoons
- Laboratory balance
- Reconstitution liquid (e.g. water, PBS, HBSS, cell culture medium etc.)
- Hydrogel to be thickened
- Water bath or laboratory oven (for heating)
- Positive displacement pipette + pipette tips (optional)
- Cells + cell culture medium
- CELLMIXER* (optional)

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

Protocol A – Preparing a glucomannan gel

Step	Title	Material	Description
1	Desired gel properties		- Record the desired final concentration of glucomannan (c_G). - Record the desired volume of gel to prepare (V_L). - See Figure 1 for difference in viscosity of glucomannan gels of different concentrations.
2	Calculation		- Calculate the amount of glucomannan to be used. $m_G = 10 \cdot V_L \cdot c_G$ See Table 1 with calculations for suggested c_G. Note: this equation gives a final concentration in weight/volume. However, depending on the concentration of thickener the mixture may swell resulting in a slightly increased final volume.
3	Heat up the reconstitution liquid	- Reconstitution liquid - Tube	- Transfer V_L of the reconstitution liquid to a sterile tube or container of your choice. - To speed up the dissolution of glucomannan, heat up your reconstitution liquid to ~50°C using a water bath, laboratory oven or similar. Note: if your liquid cannot withstand heating, this part can be skipped.
4	Weigh up glucomannan	- Spatula/spoon - Tube - CELLINK Glucomannan	- Into a tube, weigh up m_G of glucomannan powder using a spatula/spoon.
5	Dissolve glucomannan	- Reconstitution liquid - CELLINK Glucomannan	- Into the tube with reconstitution liquid, add the glucomannan powder. To reduce the formation of clumps, add the glucomannan in increments and mix with a spatula. - Vortex the mixture at high speed until dissolved. If clumps form, crush them with a spatula. - Let the glucomannan gel rest for a minimum of two hours for it to reach its final viscosity.
6	Storage	- Glucomannan gel	- Store at 4-25°C.

Table 1. Suggested concentrations and the corresponding amount of glucomannan powder used for the preparation of 5 mL glucomannan gel.

Concentration of glucomannan, c_G (%)	Volume of prepared gel, V_L (mL)	Mass of glucomannan, m_G (mg)
0.5	5	25
1	5	50
2	5	100
3	5	150

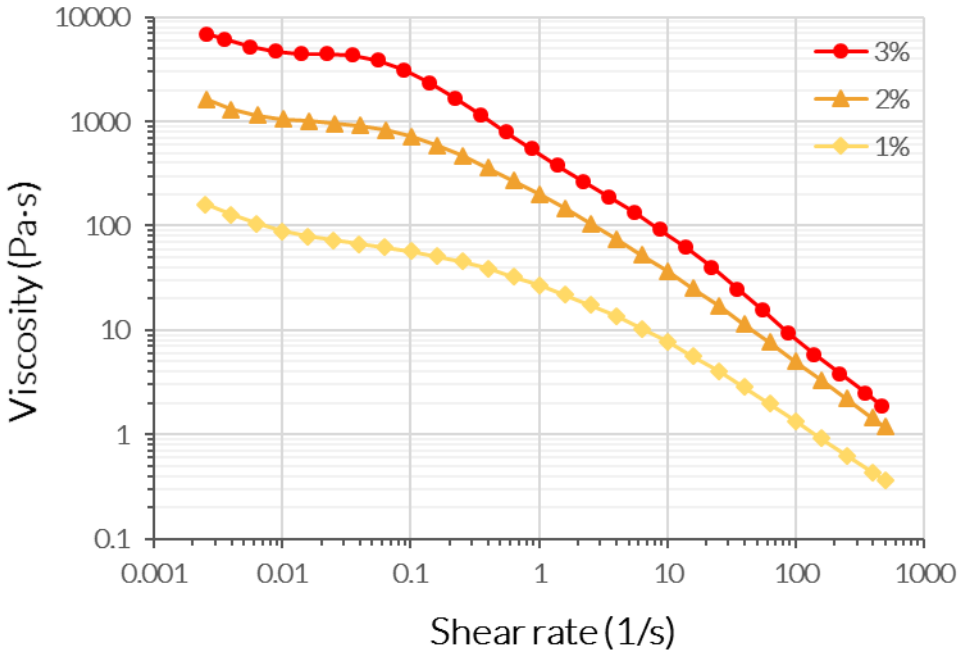

Figure 1. Viscosity of CELLINK Glucomannan dissolved in PBS at different concentrations over a shear rate range of 0.002 to 500 s^{-1}, 25°C.

Protocol B – Thickening a hydrogel using glucomannan

This approach may be preferred when the hydrogel is based on a high molecular weight biopolymer, is temperature sensitive, has a high solid content or similar.

Step	Title	Material	Description
1	Desired final properties		- Record the desired volume of hydrogel and glucomannan gel mixture (V_2). - Record the desired final concentration of glucomannan in the mixture (c_2).
2	Calculation		- Record the concentration of glucomannan gel (c_1). Use protocol A to prepare a glucomannan gel of desired concentration. - Calculate the volume of glucomannan gel (V_1) to be used. $V_1 = \frac{V_2 \cdot c_2}{c_1}$ - Calculate the volume of hydrogel (V_H) to be mixed with the glucomannan gel. $V_H = V_2 - V_1$
3	Mix glucomannan gel and hydrogel	- Glucomannan gel - Hydrogel - Spatula - Tube - Female/female Luer lock adaptor - Syringes with Luer lock connections - Positive displacement pipette + pipette tips (optional)	- Transfer the calculated volume of glucomannan gel and hydrogel into a sterile tube or the container of your choice. - Mix with a sterile spatula. - Vortex the gel mixture at high speed until it appears homogenous. - If vortexing is not enough for mixing, use two syringes instead. Transfer the mixture to a syringe, connect the syringe with another syringe of the same size using a female/female Luer lock adaptor. Mix by pushing the gel back and forth between the two syringes. This method may introduce air bubbles that can be removed by centrifuging the syringe for 1-2 min at 1 500-2 500 rpm. Note: transferring viscous gels may be difficult using a normal pipette. If available, use a positive displacement pipette instead.
4	Mix with cells	- Cell suspension - CELLMIXER (optional) - Female/female Luer lock adaptor	If not printing with cells move directly to step 5. Mix ten parts of hydrogel with one part of cell suspension without introducing air bubbles to the mixture.

		- Syringes with Luer lock connections	- Transfer the cell suspension to the 1 mL cell syringe (PART 1) using a female/female Luer lock adaptor. - Transfer the hydrogel to the 12 mL syringe (PART 2) using a female/female Luer lock adaptor. - Clip both syringes to the Dispensing unit (PART 3). - Connect the two syringes to the Mixing unit (PART 4), then connect the Empty cartridge (PART 5) to the Mixing unit's other side. - Apply gentle pressure onto the Dispensing unit to mix the content of both syringes and transfer it into the empty cartridge. Note: to avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the Luer lock adaptor with hydrogel before attaching the two syringes. If preparing for quantities <2 mL of your hydrogel, it is recommended to connect two Luer lock syringes and slowly mix back and forth between the syringes until homogeneous consistency is reached.
5	3D print	- Female/female Luer lock adaptor - 3 cc cartridge - Bioprinting nozzles or needles - 3D Bioprinter	- Connect the syringe with the hydrogel with a cartridge using a female/female Luer lock adaptor and transfer the hydrogel into the cartridge. If printing with cells, start from here: - Cap the cartridge with a bioprinting nozzle or a needle. - Place the cartridge in the printhead of the 3D bioprinter. - 3D print the hydrogel mixture.