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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 dissolution of the thickener in a liquid or very low viscosity solution as well as mixing of the dissolved thickener (glucomannan gel) with a hydrogel for thickening effect.
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Materials needed

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

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

Protocol for preparing a glucomannan gel

1. Calculations

DESCRIPTION

- 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.
- Calculate the amount of glucomannan to be used: $m_G = 10 \cdot V_L \cdot c_G$. See Table 1 with calculations for suggested cG.

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.

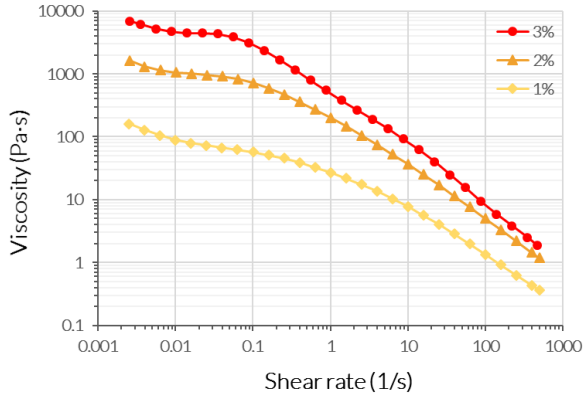

Figure 1. *Viscosity of CELLINK Glucomannan dissolved in PBS at different concentrations over a shear rate range of 0.002 to 500 s^{-1}, 25°C.*

Table 2. Suggested concentrations and the corresponding amount of glucomannan powder used for the preparation of 5 mL glucomannan gel.

Concentration of , 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

2. Heating up the reconstitution liquid

MATERIAL

Reconstitution liquid

Tube

DESCRIPTION

- 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. If your liquid cannot withstand heating, this part can be skipped.

3. Weighing up glucomannan

MATERIAL

Tube

CELLINK Glucomannan

DESCRIPTION

- Into a tube, weigh up m_G of glucomannan powder.

4. Dissolving glucomannan

MATERIAL

Reconstitution liquid

CELLINK Glucomannan

DESCRIPTION

- 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.
- Store at 4-25°C.

Protocol for 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.

1. Calculating desired final properties

DESCRIPTION

- Record the desired volume of hydrogel and glucomannan gel mixture (V_2).
- Record the desired final concentration of glucomannan in the mixture (c_2).
- Record the concentration of glucomannan gel (c_1). Follow the protocol above 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: $V_H = V_2 - V_1$

2. Mixing glucomannan gel and hydrogel

MATERIAL

Glucomannan gel

Hydrogel

Tube

Female/female Luer lock adaptor

Syringes with Luer lock connections

Positive displacement pipette + pipette tips (optional)

DESCRIPTION

- 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 300 *g*.

Note: Transferring viscous gels may be difficult using a normal pipette. If available, use a positive displacement pipette instead.

3. Mixing with cells

MATERIAL

3 mL syringes with Luer lock connections

Female/female Luer lock adaptor

Hydrogel

Cell suspension

Cartridge, 3cc

CELLMIXER (optional)

DESCRIPTION

- At this point, mix ten parts of hydrogel with one part of cell suspension, taking care not to introduce air bubbles to the mixture. For detailed instructions see the *Mixing cells with bioink Protocol.*
- If preparing for quantities < 2 mL of hydrogel, it is recommended to connect two 3 mL Luer lock syringes, one with the bioink and the other with the cell suspension and gently mix back and forth between the syringes until homogeneous. Transfer the mixture to an empty 3cc cartridge by connecting the syringe to the cartridge using the Luer lock adaptor. Cap the cartridge with a tip cap.
- If using larger quantities, the CELLMIXER can be used:
    - 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 into the empty cartridge. Cap the cartridge with a tip cap.

Note: To avoid introducing air when connecting the syringes, carefully pre-fill the Luer lock adaptor with hydrogel before attaching it to the syringe with the cell suspension.

4. Printing

MATERIAL

BIO X, BIO X6 or INKREDIBLE series bioprinter

Bioprinting nozzles or needles

Well plate or Petri dish

DESCRIPTION

- Cap the cartridge with a bioprinting nozzle or a needle.
- Place the cartridge in the printhead of the 3D bioprinter. Place the well plate or Petri dish on the print bed.
- 3D bioprint the hydrogel mixture according to the application.