Preparation Protocol

CELLINK Xanthan Gum

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 use CELLINK Xanthan Gum as a thickener of hydrogels to increase their printability. CELLINK Xanthan Gum 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 (xanthan gum gel) with a hydrogel for thickening effect.

Materials needed

- CELLINK Xanthan Gum*
- 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
- Hydrogel to be thickened
- 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 xanthan gum gel

Step	Title	Material	Description
1	Desired gel properties		- Record the desired final concentration of xanthan gum (c_X). - Record the desired volume of gel to prepare (V_L). - See Figure 1 for difference in viscosity of xanthan gum gels of different concentrations.
2	Calculation		- Calculate the amount of xanthan gum to be used. $m_X = 10 \cdot V_L \cdot c_X$ See Table 1 with calculations for suggested c_X. 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 xanthan gum, 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 xanthan gum	- Spatula/spoon - Tube - CELLINK Xanthan Gum	- Into a tube, weigh up m_X of xanthan gum powder using a sterile spatula/spoon.
5	Dissolve xanthan gum	- Reconstitution liquid - CELLINK Xanthan Gum	- Into the tube with reconstitution liquid, add the xanthan gum powder. To reduce the formation of clumps, add the xanthan gum 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 xanthan gum gel rest for a minimum of six hours for it to reach its final viscosity.
6	Storage	- Xanthan gum gel	- Store at 4-25°C.

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

Concentration of xanthan gum, c_X (%)	Volume of prepared gel, V_L (mL)	Mass of xanthan gum, m_X (mg)
0.5	5	25
1	5	50
2	5	100
3	5	150

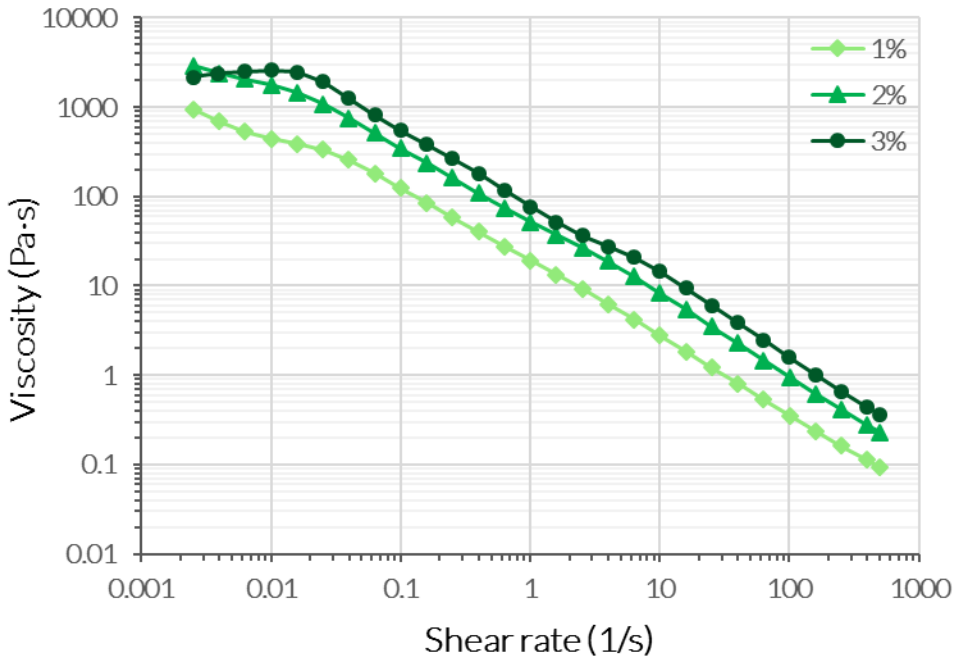

Figure 1. Viscosity of CELLINK Xanthan Gum dissolved in PBS at different concentrations over a shear rate range of 0.002 to 500 s^{-1}, 25°C.

Protocol B – Thickening a hydrogel using xanthan gum

Step	Title	Material	Description
1	Desired final properties		- Record the desired volume of hydrogel and xanthan gum gel mixture (V_2). - Record the desired final concentration of xanthan gum in the mixture (c_2).
2	Calculation		- Record the concentration of xanthan gum gel (c_1). Use Protocol A to prepare a xanthan gum gel of desired concentration. - Calculate the volume of xanthan gum 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 xanthan gum gel. $V_H = V_2 - V_1$
3	Mix xanthan gum gel and hydrogel	- Xanthan gum 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 xanthan gum 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 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. - Transfer the cell suspension to the 1 mL cell syringe (PART 1) using a female/female Luer lock adaptor.

		- Syringes with Luer lock connections	- 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 a cartridge using a female/female Luer lock adaptor. 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.