Ref No: PRP-IK-T20000
Date: 09-JAN-2023
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PREPARATION PROTOCOL

Nanofibrillated Cellulose

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 CELLINKs Nanofibrillated Cellulose (NFC) as a thickener of hydrogels to increase their printability. NFC is a hydrogel of cellulose fibers dispersed in water, with a dry content of 3%.

Materials needed

- Nanofibrillated Cellulose (NFC)*
- Hydrogel to be thickened
- Tubes
- Female/female Luer lock adaptor*
- Syringes with Luer lock connections
- Positive displacement pipette + pipette tips (optional)
- Cells + cell culture medium
- Cartridges, 3cc*
- 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

Nanofibrillated cellulose can be mixed with protein solutions and biopolymers at neutral pH. Salt concentrations above 0.4% may induce flocculation of the nanofibrils of pure NFC in water, causing a slightly less homogeneous hydrogel. No significant effect is normally seen on the flow viscosity on mixed formulations with NFC.

1. Calculations

DESCRIPTION

- Record the desired volume of hydrogel and NFC mixture (V_F).
- Record the desired final % concentration of NFC in the mixture (c_F).
- NFC has a concentration of 3%, thus c_{NFC}=3.
- See Figure 1 for difference in viscosity of NFC of different concentrations.
- Calculate the volume of NFC (V_{NFC}) to be used: $V_{NFC} = \frac{V_F \cdot c_F}{c_{NFC}}$
- Calculate the volume of your hydrogel (V_H) to be mixed with the NFC: $V_H = V_F - V_{NFC}$

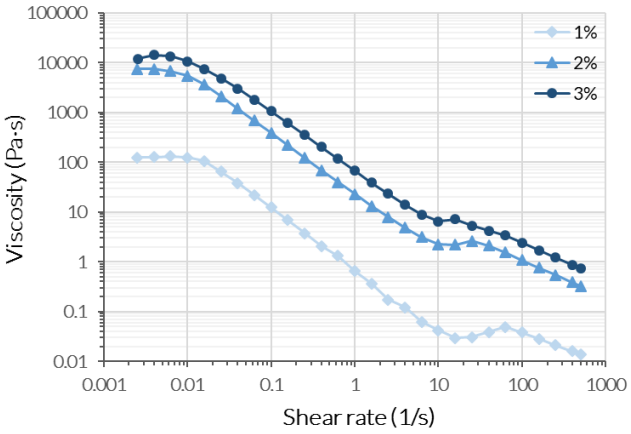

Figure 1. Viscosity of CELLINK Nanofibrillated Cellulose diluted with Reconstitution Agent M (sugar solution, available at CELLINK web store) to different concentrations over a shear rate range of 0.002 to 500 s-1, 25°C.

2. Mixing NFC and hydrogel

MATERIAL

Nanofibrillated Cellulose

Hydrogel

Tube

Female/female Luer lock adaptor

Syringes with Luer lock connections

Positive displacement pipette + pipette tips (optional)

DESCRIPTION

- Transfer the calculated volume of NFC and hydrogel into a sterile tube, or the container of your choice.
- Mix with a spatula to combine.
- Vortex the gel mixture at high speed until it appears homogeneous and of uniform viscosity.
- If vortexing is not enough, instead use two syringes to mix the gels. 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 which can be removed by centrifuging the syringe for 1-2 min at 200 – 500 *g*.

- *If not printing with cells, transfer the mixture to a cartridge and move directly to Step 5.*

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. Bioprinting

MATERIAL

Bioprinting nozzles or needles

3D bioprinter

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 and connect to the air adapter. Place the well plate or Petri dish on the print bed.
- 3D bioprint the hydrogel mixture according to desired application.