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Preparation Protocol

# CELLINK 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 CELLINK 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

- CELLINK Nanofibrillated Cellulose (NFC)*
- Female/female Luer lock adaptor*
- Cartridges, 3cc*
- 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 pipet + pipet tips (optional)
- Cells + cell culture medium
- CELLMIXER* (optional)

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

## 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. Diluting the pure NFC with sugar solutions such as Reconstitution Agent M does not alter the nanofiber interactions or viscosity.

| Step | Title | Material | Description |
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| 1 | Desired gel properties | | - Record the desired volume of hydrogel and NFC mixture ($V_F$).<br>- Record the desired final % concentration of NFC in the mixture ($c_F$).<br>- NFC has a concentration of 3%, thus $c_{NFC}$=3.<br>- See Figure 1 for difference in viscosity of NFC of different concentrations. |
| 2 | Calculation | | - Calculate the volume of NFC ($V_{NFC}$) to be used.<br>$V_{NFC} = \frac{(V_F \cdot c_F)}{c_{NFC}}$<br>- Calculate the volume of your hydrogel ($V_H$) to be mixed with the NFC.<br>$V_H = V_F - V_{NFC}$ |
| 3 | Mix NFC and hydrogel | - CELLINK Nanofibrillated Cellulose<br>- Hydrogel<br>- Spatula<br>- Tube<br>- Female/female Luer lock adaptor<br>- Syringes with Luer lock connections | - Transfer the calculated volume of NFC and hydrogel into a sterile tube, or the container of your choice.<br>- Mix with a spatula to combine.<br>- Vortex the gel mixture at high speed until it appears homogeneous and of uniform viscosity.<br>- 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 1 500-2 500 rpm.<br><br>Note: Transferring viscous gels may be difficult using a normal pipet, if available use a positive displacement pipet instead. |

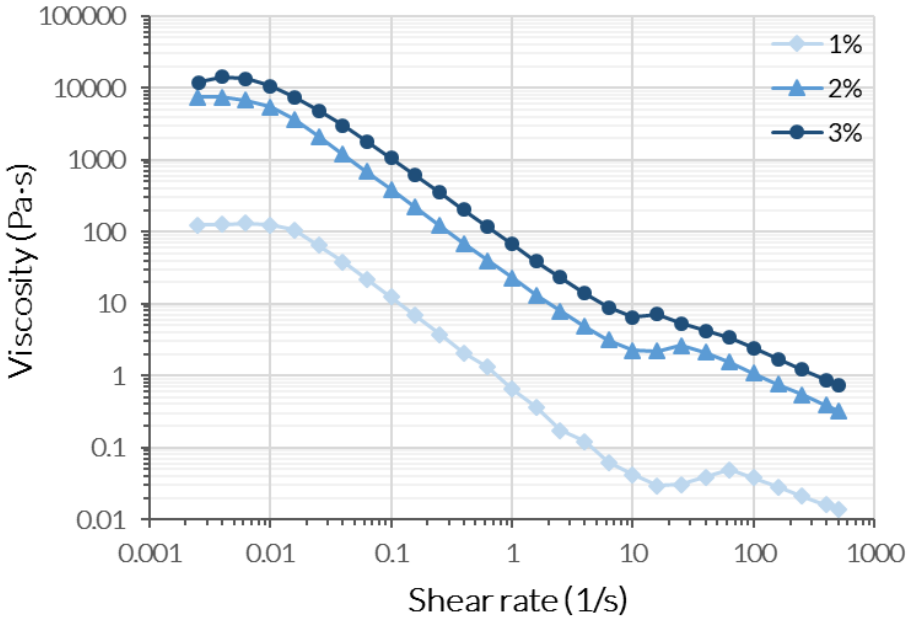


**Figure 1.** Viscosity of CELLINK Nanofibrillated Cellulose diluted with Reconstitution Agent M (sugar solution, available at CELLINK web store) to different concentrations over a shear rate range of 0.002 to 500 $s^{-1}$, 25°C.

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| 4 | Mix with cells | - Cell suspension<br>- CELLMIXER<br>- Female/female Luer lock adaptor<br>- Syringes with Luer lock connections<br>- Hydrogel | If not printing with cells move directly to step 5.<br><br>Mix ten parts of hydrogel with one part of cell suspension without introducing air bubbles to the mixture.<br>- Transfer the cell suspension to the 1 mL cell syringe (PART 1) using a female/female Luer lock adaptor.<br>- Transfer the hydrogel to the 12 mL syringe (PART 2) using a female/female Luer lock adaptor.<br>- Clip both syringes to the Dispensing unit (PART 3).<br>- Connect the two syringes to the Mixing unit (PART 4), then connect the Empty cartridge (PART 5) to the Mixing unit's other side.<br>- Apply gentle pressure onto the Dispensing unit to mix the content of both syringes and transfer it into the empty cartridge.<br><br>Note: To avoid an air gap when mixing the hydrogel and the cell suspension, carefully pre-fill the Luer lock adaptor with hydrogel before attaching the two syringes. |

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| | | | 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 | - Syringe<br>- Female/female Luer lock adaptor<br>- 3cc cartridge<br>- Bioprinting nozzles or needles<br>- 3D Bioprinter | - Transfer the hydrogel mixture into a syringe.<br>- Connect the syringe with a cartridge using a female/female Luer lock adaptor. Transfer the hydrogel into the cartridge.<br><br>If printing with cells, start from here:<br>- Cap the cartridge with a bioprinting nozzle or a needle.<br>- Place the cartridge in the printhead of the 3D bioprinter.<br>- 3D print the hydrogel mixture. |