Ref No: BPR-IK-C20600
Date: 21-JUN-2021
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Bioprinting Protocol

CELLINK FIBRIN

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 bioprinting with the CELLINK® FIBRIN using the INKREDIBLE, INKREDIBLE+, BIO X or BIO X6, and covers steps from pre-print mixing with cells, 3D bioprinting and post-print processes of ionic and enzymatic crosslinking. This protocol was optimized for undiluted CELLINK FIBRIN, as well as using a 10+1 cell suspension dilution. Changing the parameters in the protocol might change the required crosslinking time. This protocol was optimized using the pneumatic printhead using the BIO X.

Material needed

- CELLINK FIBRIN*
- Clear cartridges, 3cc*
- Sterile Conical Bioprinting nozzles*
- BIO X*, BIO X6* or INKREDIBLE-series* 3D Bioprinter
- Well plate or Petri dish
- Crosslinking agent (included with the bioink purchase)
- Vial with 100 U thrombin (included with the bioink purchase)
- Cells + cell culture medium
- 3 mL syringes with Luer lock connections
- Female/female Luer lock adaptor*
  or
- CELLMIXER*

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

Protocol

This protocol can be performed with printheads and print bed at room temperature, where room temperature is between 20-25°C.

Step	Title	Material	Description
1	Prepare bioink	- CELLINK FIBRIN	If not printing with cells move directly to step 3. - Warm up the CELLINK FIBRIN cartridge to room temperature.
2	Mix CELLINK FIBRIN with cells	- 3 mL syringes with Luer lock connections - Prewarmed CELLINK FIBRIN - Female/female Luer lock adaptor - Cell suspension in syringe	At this point, mix ten parts bioink with one part cell suspension, taking care not to introduce air bubbles to the mixture. For detailed instructions see the *Mixing Cells Protocol CELLINK Series.* - Transfer the cell suspension to the 1 mL cell syringe (PART 1) using a female/female Luer lock adaptor. - Transfer the bioink 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. Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the Luer lock adaptor with CELLINK FIBRIN before attaching the syringe with the cell suspension. If preparing for quantities < 2 mL of CELLINK FIBRIN, it is recommended to connect two 3 mL Luer lock syringes and mix back and forth between the syringes until homogeneous.
3	Load the cartridge	- Clear cartridges, 3cc loaded with CELLINK FIBRIN (and cells) - Sterile Conical Bioprinting nozzles.	-Attach a nozzle to the cartridge and mount it into the printhead. The recommended nozzle size is 22G. Decrease the nozzle diameter to achieve smaller filament diameter, however this also increases the risk of the bioink clogging.
4	Printing	- Bioprinter (BIO X, BIO X6 or INKREDIBLE series recommended)	- Bioprint structures with parameters according to Table 1. If printability is not as desired, adjust the pressure up/down by 1 kPa to extrude more/less material.

		- Well plate or Petri dish	Note: If waiting too long between extrusions the bioink can dry in the nozzle causing it to clog. If this occurs, replace with new nozzle.

Table 1. Recommended minimal extrusion pressure** (±2 kPa) used for printing continuous filaments at 21-25°C $^{\text{with cells}}/_{\text{without cells}}$. Again, 'with cells' assumes a mixture of one part cell suspension to ten parts bioink. For highly concentrated cell suspensions, the pressure needs to be increased towards the pressure used for undiluted bioink.

Printing speed (mm/s) → Nozzle size (G) ↓	5	10	15	20
22	6 / 16	7 / 17	8 / 18	9 / 19
25	8 / 16	10 / 17	12 / 21	14 / 21
27	12 / 16	14 / 17	16 / 19	16 / 20

***Note this is only a recommended reference of starting pressures. The actual pressure needed will vary depending on the preparation procedures (amount of bioink and actual temperature of the bioink) as well as the fitting of the piston in the cartridge and the leveling of the print surface. This table was generated with printhead temperature at 23°C and with a bioink dilution with a low concentration of cells.*

Step	Title	Material	Description
5	Crosslinking	- Crosslinking Agent - Thrombin - Cell culture medium	CELLINK FIBRIN is crosslinked with $CaCl_2$ ions and thrombin. Preparation of 10 mL crosslinking solution with a final concentration of 10 U thrombin/mL and 10 mM $CaCl_2$, to use *immediately****: - Add 1 mL of cell culture medium to the thrombin vial of 100 U, swirl to dissolve. - Transfer to a separate tube containing 7 mL cell culture medium. - Transfer 2 mL of the 50 mM $CaCl_2$ Crosslinking Agent to the tube containing 8 mL thrombin in cell culture medium. - Mix gently by pipetting up and down 2-3 times. - Submerge the bioprinted samples in the $CaCl_2$/thrombin containing medium and incubate 2-4 h (depending on construct size and infill density, larger solid constructs need longer time for diffusion) in standard culture conditions (37°C, 5% CO_2 and 95% relative humidity) or according to your application. - Switch to regular culture media and continue cell culture in incubator.

			***The $CaCl_2$/thrombin crosslinking medium does not retain full thrombin activity if stored for longer than a few hours. Instead, prepare small aliquots of pure thrombin solution: - Reconstitute the 100 U thrombin in the vial with 1 mL sterile 0.1% BSA, in cell culture medium of choice, to a 100 U/mL stock solution. - Aliquot in plastic tubes and store in -20°C for up to 4 weeks. - Prepare fresh $CaCl_2$/thrombin crosslinking medium the same day as usage with a 10x dilution of the 100 U/mL thrombin stock and 5x dilution of $CaCl_2$ Crosslinking agent in cell culture media.