Ref No: BPR-IK-200011
Date: 14-JUN-2021
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Bioprinting Protocol

CELLINK A-RGD

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® A-RGD 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 crosslinking. This protocol was optimized for CELLINK A-RGD, undiluted as well as using a 10+1 cell suspension dilution. Changing the parameters in the protocol might change the crosslinking time required. This protocol was optimized using the pneumatic printhead using the BIO X.

Material needed

- CELLINK A-RGD*
- Green 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)

- 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 A-RGD	If not printing with cells move directly to step 3. - Warm up CELLINK A-RGD in a cartridge to room temperature.
2	Mix CELLINK A-RGD with cells	- 3 mL syringes with Luer lock connections - Prewarmed CELLINK A-RGD - 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 A 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 units 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 A-RGD before attaching the syringe with the cell suspension. If preparing for quantities < 2 mL of CELLINK A-RGD, 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	- Green cartridges, 3cc loaded with CELLINK A-RGD (and cells) - Sterile Conical Bioprinting nozzles	- Place the room tempered CELLINK A-RGD in the printhead and cap with a printing nozzle of choice.

4	Printing	- Bioprinter (BIO X, BIO X6 or INKREDIBLE series recommended) - Well plate or Petri dish	- 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. Note: For printing complicated structures, it is recommended that CELLINK A-RGD is used in conjugation with CELLINK SUPPORT or CELLINK START to aid in its stability or with the use of the Syringe Pump Printhead to better control extrusion rates.

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	3 / 4	3 / 4	4 / 4	4 / 5
25	4 / 4	4 / 5	5 / 6	6 / 7
27	5 / 6	6 / 8	7 / 9	10 / 11

***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 24°C and with a bioink dilution with a low concentration of cells.*

Step	Title	Material	Description
5	Crosslinking	- Crosslinking solution - Cell culture medium	CELLINK A-RGD can be crosslinked with ions using the $CaCl_2$ crosslinking solution. - Submerge the cell-laden constructs in the crosslinking solution for 30 s to 5 min depending on construct size. Remove crosslinking solution and rinse constructs with basal culture media once.
6	Incubation	- Cell culture medium	- After crosslinking and washing, add the desired medium to the constructs and place in incubator. - Incubate the constructs in cell culture medium in standard culture conditions (37°C, 5% CO_2 and 95% relative humidity) or according to your application.