Suggested Protocol

CELLINK VasKit

This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for the CELLINK VasKit using the INKREDIBLE, INKREDIBLE+, or BIO X, with and without cells. This document covers the preparation of channeled tissues within the CELLINK VasKit using CELLINK GelMA C and CELLINK PLURONICS. Additionally, the protocol describes the incorporation of cells within the CELLINK GelMA C.

Materials needed

CELLINK VasKit* containing:

- CELLINK VasKit Perfusion Device
- CELLINK PLURONICS (2 x 3 mL)
- CELLINK GelMA C with LAP photoinitiator (2 x 3 mL)
- Bioprinting nozzles
- Luer adapters (2psc)
  Empty cartridge, 3cc

- BIO X* or INKREDIBLE-series* 3D Bioprinter
- 3 mL syringe
- Photocuring UV module, 365 nm or 405 nm
- Cells + culture medium
- 3 mL syringes with luer lock connections
- Female/Female luer lock adaptor*
- CELLMIXER*
- Peristaltic pump

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

KEEP THE INK PROTECTED FROM LIGHT IF TRANSFERRED FROM THE ORANGE UV PROTECTED CARTRIDGES TO AVOID CROSSLINKING BEFORE PRINTING. WORK WITH 3D PRINTERS IN DARK MODE. THE PHOTOINITIATOR IS SENSITIVE TO REPEATED OR PROLONGED EXPOSURE TO HEAT.

Arvid Wallgrens Backe 20
413 46 Gothenburg
SWEDEN

100 Franklin St,
Boston, MA 02110
USA

Med-Pharm Collaboration
Building, 46-29 Yoshida-Shimo
Kyoto, JAPAN

Set up for casting of cell embedded bioink

This protocol works best with the INKREDIBLE+ system.

Step	Title	Material	Description
1	Prepare VasKit	- CELLINK VasKit	- Transfer the sealed sterile CELLINK VasKit perfusion device inside the LAF bench and open the package.
2	Warming	- CELLINK GelMA C	- Heat the CELLINK GelMA C to 37°C in an incubator or bean heater.
3	Casting CELLINK GelMA	- CELLINK GelMA C	- Transfer sterile CELLINK GelMA C and spread it over the device bottom glass to completely cover the surface.
4	Load the bioprinter	- CELLINK GelMA C - CELLINK PLURONICS - Luer adapters - Empty cartridge, 3cc - Bioprinting nozzles - Bioprinter (BIO X or INKREDIBLE+ series recommended)	- Transfer chilled and liquid CELLINK PLURONICS from the syringe to the empty cartridge by connecting the two with a luer lock adapter. Allow to heat to room temperature to solidify. Place the filled cartridge in the printhead and cap with the printing nozzle. - Print your channel structure using the CELLINK PLURONICS onto the casted GelMA C inside the device. - You may need to manually fill any pluronics gaps between the gel connector and the printed channel. - If using our Ink series of bioprinter, see suggested G-Codes, found in Bioverse (http://bioverse.co/). Relevant stl model files are included is using other bioprinter models.
5	Channel embedding with CELLINK GelMA C	- CELLINK GelMA C	- Add 1-2 mL warm liquid CELLINK GelMA C on top of the printed channel structure to embed the channel and make sure that it is covered completely. If embedding with cells, see section 9.
6	Cooling	- 365/405 UV module - $CaCl_2$ solution	- Allow the CELLINK VasKit device to cool down in room temperature to induce gelation of the GelMA C. Crosslink with UV 365/405 nm, and/or $CaCl_2$ solution.
7	Evacuation	- 5 mL syringe - 1 mL syringe	- Chill the device to liquefy the pluronics. - Connect a 1 mL syringe to the luer connector on the VasKit device. Gently and slowly evacuate the pluronics by withdrawing the plunger. For best results, have a 5 mL open syringe (without plunger) to the other channel end with chilled $CaCl_2$ solution inside. This will

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			flush away the pluronics while crosslinking the channel lumen.
8	Crosslinking	- 365/405 UV module	GelMA C can be photocrosslinked using the 365/405 nm UV emitter. - Suggested distance of UV module from the sample set at 3 cm and crosslinking time of 60 seconds. The crosslinking time is to be adjusted based on the construct depth. Ensure that the bioprinted constructs are thermally gelled before UV crosslinking. - Let the CELLINK VasKit device reach room temperature.
	Cell seeding	- CELLINK GelMA C - Cell suspension in syringe - 3 mL syringes with luer lock connections - Female/Female luer lock adaptor - CELLMIXER	- For mixing GelMA C with cells to a cell concentration of up to 10 x 10^6 cells/mL bioink, use a cell suspension that is composed of a 1:2 ratio between HUVECs and Human Dermal Fibroblasts. - Transfer the GelMA C to a 3 mL syringe using a Female/Female luer lock adaptor. - Attach the bioink syringe to the syringe with cell suspension. - Carefully mix the GelMA C bioink with the cell suspension by gently pushing the bioink back and forth. - Transfer the cell containing bioink back to the orange cartridge and cap it. Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the luer lock adaptor with GelMA C bioink before attaching the syringe with the cell suspension. - If preparing for quantities > 2ml of GelMA, it is recommended to use the CELLMIXER. - Cover the pluronics channel with the GelMA C/cell blend.
10	Crosslinking	- 365/405 UV module	- Induce photoassisted crosslinking for the CELLINK GelMA C containing the cells as in Step 8. Note: Over exposure of UV to the constructs might damage the cells.
11	Addition of media	- Cell culture media or PBS	- If required, add a thin layer of cell culture media or PBS on top of the cell containing CELLINK GelMA C, to moisture in the chamber.

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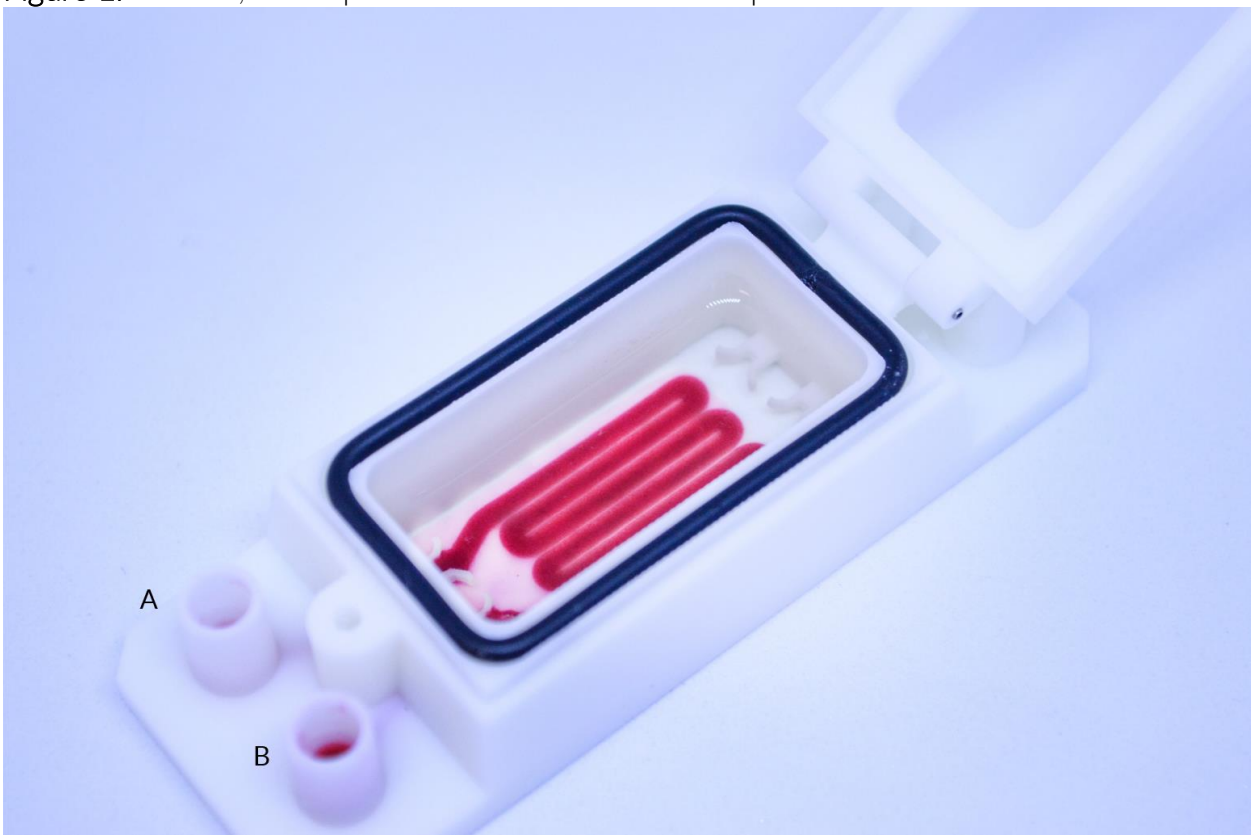
www.cellink.com

12	Perfusion	- Peristaltic pump	- Connect the CELLINK VasKit device to a peristaltic pump with a recommended flow of 10-100 µL/min, see Figure 1.
13	Incubation		- Close the lid of the CELLINK VasKit device and tighten up the screws. - Place your CELLINK VasKit including the perfusion system into the incubator at 37°C.

Figure 1. Show A, luer slip connector out and B luer slip connector in.



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