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BIOPRINTING PROTOCOL

Lifeink® 220

This is a suggested procedure, please adjust it 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 of Lifeink® 220 collagen bioink, a pH neutral type I collagen, to create multi-layered 3D constructs without any support. It covers pre-printing procedures, printing, and post-printing thermal self-assembly of the collagen bioink. Changing the parameters in the protocol might change printing conditions such as pressure and speed. This protocol was optimized for the Temperature-controlled printhead using the BIO X.

Materials needed

- Lifeink® 220*
- Temperature-controlled printhead*
- BIO X* or BIO X6* bioprinter
- 3 mL syringes with Luer lock connections
- Cartridges, 3cc*
- Female/female Luer lock adaptor*
- Cell culture medium
- Cells* + cell culture medium*
- 22G Conical bioprinting nozzle*
- Well plate or Petri dish*

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

Protocol

This protocol is adjusted for printing scaffolds at an original Lifeink® 220 concentration of 70 mg/mL with a minor dilution with cell suspension. For other concentrations of collagen, recalculations need to be made but the same protocol can be followed. To avoid premature self-assembly, it's recommended to keep the collagen bioink and all consumables in the fridge prior the printing. It is also recommended to use cold cell culture medium.

1. Preparation for printing

MATERIAL

Temperature-controlled printhead

BIO X or BIO X6 bioprinter

3 mL syringes with Luer lock connections

Luer lock adaptor

Cartridge, 3cc

22G Conical bioprinting nozzle

DESCRIPTION

- Set the Temperature-controlled printhead to 10°C.
- Cool the syringes, cartridge, nozzles and Luer lock adaptors to 4°C.

2. Mixing with cells

MATERIAL

Cool 3 mL syringes with Luer lock connections

Cool female/female Luer lock adaptor

Lifeink® 220

Cell suspension

Cool cartridge, 3cc

Cool 22G conical bioprinting nozzle

DESCRIPTION

- At this point, mix ten parts bioink with one part cell suspension (or dilute according to application), taking care not to introduce air bubbles to the mixture. For detailed instructions see the *Mixing cells with bioink Protocol.*
- Connect two cool 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 a cool empty 3cc cartridge by connecting the syringe to the cartridge using the Luer lock adaptor. Make sure to not heat the bioink while mixing.
- Cap the cartridge with a cool 22G conical bioprinting nozzle and place directly in the cool Temperature-controlled printhead.

Note: To avoid introducing air when connecting the syringes, carefully pre-fill the Luer lock adaptor with Lifeink® 220 before attaching it to the syringe with the cell suspension.

3. Printing

MATERIAL

BIO X or BIO X6 bioprinter

Lifeink® 220 mixed with cells (in the cartridge)

Well plate or Petri dish

DESCRIPTION

- Calibrate to the well plate or Petri dish and after a proper calibration, test the flow of the bioink. Start with a low pressure and increase stepwise.
- Print structures on to the well plate or Petri dish. Example of parameters:
    - For non-diluted collagen: 50 kPa pressure at 5 mm/s printing speed.
    - For a 4+10 cell suspension in collagen bioink: 16 kPa pressure at 3 mm/s printing speed.

Note: If printability is not as desired, adjust the pressure and/or speed to up/down to extrude more/less material at different speeds.

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

4. Thermal self-assembly

MATERIAL

Well plate or Petri dish with printed constructs

DESCRIPTION

- After printing, place the well plate or Petri dish with constructs in an incubator at 37°C (5% CO_2 and 95% relative humidity) for thermal gelation of collagen. 15-30 min of incubation is recommended but depend on the construct size. Confirm that the bioink is crosslinked before moving to Step 5.

5. Incubation

MATERIAL

Well plate or Petri dish with crosslinked constructs

Cell culture medium

DESCRIPTION

- Take the well plate or Petri dish with crosslinked constructs from the incubator. Submerge the constructs in the cell medium and place them back in the incubator for 3D cell culturing.
- 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.