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CELLINK
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FRESH BIOPRINTING PROTOCOL

PhotoCol®

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 the bioprinting of complex 3D structures with PhotoCol® (Methacrylated Type I Collagen from Advanced BioMatrix) using FRESH printing method. It covers the steps of pre-print procedures, printing, and post-print crosslinking. Changing the parameters in the protocol might change printing conditions such as pressure and speed. This protocol was optimized for the pneumatic Temperature-controlled Printhead installed at the BIO X.

Materials needed

- PhotoCol® (Kit 2,3,4* – Methacrylated collagen, 20 mM acetic acid, Neutralization Solution, a photoinitiator)
- LifeSupport™*, 1X PBS
- BIO X*, BIO X6* 3D Bioprinter
- Cells in suspension and cell culture media
- Positive displacement pipette
- Eppendorf tube with a proper size
- 22G Conical Bioprinting needle (1-inch length)*
- Well plate or Petri dish
- 3 mL syringes and cartridges with Luer lock connections, female/female Luer lock adaptors*

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

Protocol

This protocol is adjusted for printing scaffolds at the final PhotoCol® concentration of 6 mg/mL. For other concentrations, recalculations need to be made but the same protocol can be followed. To avoid premature collagen self-assembly, we recommend keeping the collagen and all consumables in the fridge prior to printing.

Once compacted, LifeSupport™ should be kept in a fridge at 4 °C and used within 12 hours. The ambient temperature should not exceed 23°C during handling or printing.

1. Preparation of PhotoCol® 10 mg/mL stock solution

MATERIAL

PhotoCol® (lyophilized methacrylated Type I collagen)

20 mM acetic acid

DESCRIPTION

- In a separate container, weigh the desired mass of PhotoCol® for stock solution preparation. Store the remaining PhotoCol® material in a fridge.
- Add the corresponding volume of 20 mM acetic acid to the PhotoCol® container to achieve the target concentration of 10 mg/mL.

Note. For example, to prepare 3 mL of 10 mg/mL stock solution, add 3 mL of 20 mM acetic acid to 30 mg of lyophilized Photocol®.

- Mix on a shaker table or rotator plate at 2-10 °C overnight or until fully solubilized.

2. Preparation of LifeSupport™ bath

MATERIAL

LifeSupport™

1X PBS

Well plate or Petri dish

DESCRIPTION

- Add 40 mL of cold 1X PBS (4 °C) to LifeSupport™ tube (sterile powder).

Note: 2 g of sterile LifeSupport™ corresponding to 15 mL of LifeSupport™ bath. For detailed directions, visit https://www.cellink.com/wp-content/uploads/2022/03/FluidformDirectionsforUseVersion6-rev-Sep-2021.pdf.

- Vortex for 1 minute.
- Put the tube into the fridge (4 °C) for 15 minutes.
- Centrifuge for 5 minutes at 2000 rpm.
- Gently pour off or aspirate the liquid supernatant.
- Grab the tube by the cap, hold it horizontally and gently tap it against a palm 15 times.
- Shake the tube containing dislodged LifeSupport™ vigorously for 10 seconds. Shake along the length of the tube.
- Centrifuge for further 5 minutes at 2000 rpm.
- The LifeSupport™ should now be compacted at the bottom of the centrifuge tube. Gently pour off or aspirate any remaining liquid supernatant to leave only the compacted LifeSupport™ in the bottom of the tube.
- Transfer the resulting LifeSupport™ bath with a sterile spatula into well plates or Petri dish and store it in a fridge until use.

3. Preparation for printing

MATERIAL

PhotoCol® 10 mg/mL stock solution

Neutralization Solution (NS)

Photoinitiator (PI)

Ice bath

Bioprinter (BIO X or BIO X6)

3 mL syringes with Luer lock connections

Cartridge, 3cc

Luer lock adaptors

Positive displacement pipette

Eppendorf tube 1.5 mL

Cell suspension in cell culture medium of choice

22G Conical Bioprinting needle (1-inch length)

DESCRIPTION

- Place the Temperature-controlled Printhead into the freezer at least 30 minutes before printing.
- Set the BIO X printhead at 5 °C and print bed at 10 °C to guarantee LifeSupport™ bath stability.

Note: Make sure the ambient temperature in the lab is maintained at 21-23 °C, otherwise you may use ice packs inside the printing chamber and on the top of the printer to prevent the printing area from overheating.

- Weigh the necessary amount of PI to achieve a desired concentration of PI in the final bioink.

Note: In our bioinks, we commonly use a PI at 0.25% (w/v) concentration (see Table 1).

- Dissolve the PI in the Neutralization Solution.

Note: Always prepare some extra solution to compensate losses during filtration and transfer from one container to another. For example, dissolve 5 mg of a PI in 90 µL of NS for 1 mL of final bioink.

- Sterile filter the NS/LAP solution using a 0.22 µm filter.
- To prepare 1 mL of 6 mg/mL PhotoCol® solution for printing, transfer 600 uL of PhotoCol® 10 mg/mL stock solution into a sterile Eppendorf using a positive displacement pipette.
- Add 45 µL of NS/LAP solution and use the same pipette to homogenize the resulting solution.
- Add 355 µL of cell suspension with desired cell density and pipette the solution up and down until complete homogenization.

Table 1. Suggested values for the preparation of 1 mL of 6 mg/mL PhotoCol® bioink.

V_{bioink}	$C_{final\ bioink}$	$C_{stock\ solution}$	$V_{stock\ solution}$	V_{NS}	m_{PI}	$V_{cell\ suspension}$
1 mL	6 mg/mL	10 mg/mL	600 µL	45 µL	2.5 mg	355 µL

- Load a cartridge with the bioink using a pipette.
- Place the cartridge in the printhead and cap with a printing needle.

4. Printing

MATERIAL

Bioprinter (BIO X or BIO X6)

Well plate or Petri dish previously filled with LifeSupport™ bath

Cartridge with the PhotoCol® bioink

22G Conical Bioprinting needle (1-inch length)

DESCRIPTION

- Print constructs using suggested parameters:
    - pressure at 8-10 kPa.
    - speed at 3.5 mm/s.

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

5. Incubation and crosslinking

MATERIAL

Cell culture medium

Bioprinter (BIO X or BIO X6) with UV modules of choice for photocuring

DESCRIPTION

- Keep the constructs for 10 min at room temperature to ensure initial collagen self-assembly prior to the melting of the supporting bath.
- Incubate the constructs for 30 minutes at 37 °C (5% CO_2 and 95% relative humidity) for further self-assembly of PhotoCol® and LifeSupport™ melting.

Note: Large volumes may require longer times for the supporting bath to fully melt.

- Photocure each construct using the UV module. Time will depend on a chosen PI and its concentration.

Note: It is recommended to use the 405 nm photocuring module for 30 seconds instead of 365 nm if possible, when photocuring PhotoCol® with LAP photoinitiator. Overexposure at the 365 nm wavelength might damage the cells.

- Remove melted LifeSupport™ by replacing it with warm cell media to avoid handling the printed construct. For example, if you printed into a 6-well plate, this can be done by carefully aspirating 2 mL of melted LifeSupport™ out and adding 2 mL of warm cell media. Repeat this process until most of the support bath has been replaced by media.