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FRESH BIOPRINTING PROTOCOL

TeloCol®

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 TeloCol® (Type I Collagen (Bovine) Solution 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

- TeloCol®-10 (10 mg/mL)*
- 1X PBS and 10X PBS
- Sodium hydroxide, 1M NaOH
- LifeSupport™*
- 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 TeloCol® concentration of 6 mg/mL. For other concentrations, recalculations need to be made but the same protocol can be followed. To avoid prematurity shelf 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 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™ powder 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 with 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.

2. Preparation for printing

MATERIAL

TeloCol®-10 (10 mg/mL)

10X PBS

1M NaOH sterile

Bioprinter (BIO X or BIO X6)

3 mL syringes with Luer lock connections

Cartridge, 3cc

Luer lock adaptors

Positive displacement pipette

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. All solutions should be kept on ice. TeloCol® starts self-assembly at temperatures above 5 °C.

- To prepare 1 mL of 6 mg/mL TeloCol® solution for printing, transfer 600 µL of TeloCol® 10 mg/mL solution into a sterile Eppendorf tube using a positive displacement pipette.
- Add 100 µL of 10X PBS and use the same pipette to homogenize the resulting solution.
- Add 7.0 µL of 1M NaOH sterile. Pipette the solution up and down to neutralize collagen.

Note: The amount of NaOH needed to neutralize the TeloCol® 10 mg/mL can slightly vary from batch to batch. We recommend the addition of it in small volume increments, adjusting the pH to 7.0-7.4.

- Add 293 µL of cell suspension and pipette the solution up and down until complete homogenization.

Note: The volume of cell suspension can change depending on the volume of NaOH needed for the neutralization. Adjust it to have a final volume of 1 mL bioink.

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

3. Printing

MATERIAL

Bioprinter (BIO X or BIO X6)

Well plate or Petri dish previously filled with LifeSupport™ bath

Cartridge with the TeloCol® 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.

4. Incubation and crosslinking

MATERIAL

Cell culture medium

Bioprinter (BIO X or BIO X6)

DESCRIPTION

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

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

- 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.