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FRESH BIOPRINTING PROTOCOL

TeloCol-10

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®-10 (Type I Collagen from 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 Temperature-controlled printhead using the BIO X.

Materials needed

- TeloCol®-10 (10 mg/mL)*
- 1X PBS and 10X PBS
- Sodium hydroxide, 1M NaOH
- LifeSupport™*
- Well plate or Petri dish*
- Temperature-controlled printhead*
- BIO X* or BIO X6* bioprinter
- Positive displacement pipette
- Eppendorf tube 1.5 mL
- Ice bath
- Cell suspension in cell culture medium
- Cartridge, 3cc*
- 22G Conical bioprinting needle (1-inch length)*

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

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 premature collagen self-assembly, we recommend keeping the collagen, all reagents (including cell culture medium) 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. Preparing 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 400 *g*.
- 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 400 *g*.
- 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. Preparing for printing

MATERIAL

Temperature-controlled printhead

BIO X or BIO X6 bioprinter

TeloCol®-10 (10 mg/mL)

Eppendorf tube 1.5 mL

Positive displacement pipette

Ice bath

10X PBS

1M NaOH sterile

Cell suspension in cell culture medium of choice

Cartridge, 3cc

22G Conical bioprinting needle (1-inch length)

DESCRIPTION

- Place the Temperature-controlled printhead into the fridge at least 30 minutes before printing.
- Prepare the cell suspension, place in the fridge while preparing the TeloCol® solution.
- 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. Keep the Eppendorf tube on ice.
- 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.

- Take the Temperature-controlled printhead from the fridge and mount on the BIO X or BIO X6, set the temperature to 5°C and for the print bed 10°C, this is to guarantee the 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 to prevent the printing area from overheating.

- Load a cartridge with the bioink using a pipette. Cap the cartridge with a printing needle and place it in the printhead.

3. Printing

MATERIAL

BIO X or BIO X6 bioprinter

Well plate or Petri dish previously filled with LifeSupport™ bath

Cartridge with the TeloCol® bioink

DESCRIPTION

- Place the well plate or Petri dish previously filled with LifeSupport™ bath and place it on the print bed. Print constructs according to application. Suggested starting parameters are 3.5 mm/s printing speed and 8-10 kPa pressure.

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

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.