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BIO ONE BIOPRINTING PROTOCOL

TeloCol®-10

This is a suggested procedure, please adjust it according to your experimental needs. Work under aseptic conditions.

Protocol aim

The aim of this protocol is to provide instructions for dispensing droplets and printing constructs with TeloCol®-10 at concentrations ranging from 4 - 8 mg/mL using the BIO ONE. This document covers collagen preparation and procedures for printing with cells including bioprinting parameters. Changing the neutralization procedure or the collagen concentration may change the bioprinting parameters.

Materials

- TeloCol®-10*
- Ice bath
- 1 M NaOH
- Collagen Buffer*
- Positive displacement pipette and tips
- Eppendorf tube
- Cells + cell culture medium
- 3 mL BD Plastipak™ Syringes with Luer-Lok™ Tip (Ref#309658)
- Female/female Luer lock adaptor*
- Conical bioprinting nozzles, 22-25G recommended*
- BIO ONE 3D bioprinter*
- Well plate

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

Protocol

The protocol has been optimized for use with the BIO ONE which has one cooled printhead. Clogging at the nozzle tip may still occur. Pre-set the printhead temperature to 2°C before beginning this procedure. Pre-chill all plastics in contact with the collagen solution and keep all solutions on ice. Be aware of the temperature of the collagen and keep it cooled on ice until loaded into the pre-cooled BIO ONE printhead.

1. Bioink preparation

MATERIAL

TeloCol-10 solution

Ice bath

Eppendorf tube

Collagen Buffer

1M NaOH

Positive displacement pipette and tips

3 mL BD syringes with Luer lock connections

DESCRIPTION

Refer to preparation protocol below for neutralizing the TeloCol®-10 using Collagen Buffer with phenol red or use the Directions for Use provided by Advanced BioMatrix for description on how to neutralize the collagen solution using PBS and 0.1M NaOH. For neutralizing TeloCol®-10 to a final concentration of 4, 6 or 8 mg/mL see volumes in Table 1.

- Keep the collagen and all other materials cool on ice.
- To prepare the collagen solution, transfer TeloCol®-10 and chilled Collagen Buffer according to volumes Table 1, to an Eppendorf tube and mix gently via pipetting up and down using a cool positive displacement pipette.
- Adjust the pH by adding 1M NaOH according to Table 1. Mix gently via pipetting up and down using a cool positive displacement pipette. Monitor pH of the mixture to 7.0-7.5 using the phenol red indicator, adjust if needed.
- After neutralization, transfer the neutralized solution using the positive displacement pipette to a cool 3 mL BD syringe. Keep on ice and begin the next step immediately.
- If not mixing with cells adjust to the desired concentration using PBS.

Table 1. Suggested volumes for neutralizing TeloCol®-10 to final concentration of 4, 6 and 8 mg/mL.

Final collagen concentration	4 mg/mL	6 mg/mL	8 mg/mL
Total volume (µL)	1000	1000	1000
TeloCol®-10 volume (µL)	400	600	800
Collagen Buffer volume (µL)	57	91	114
NaOH volume (µL)	6	9	12
Cell suspension (µL)	537	300	74

2. Mixing the bioink with cells

MATERIAL

Neutralized collagen solution

Cell suspension

3 mL BD syringes with Luer lock connections

Female/female Luer lock adaptor

DESCRIPTION

- At this point, mix the collagen solution with cell suspension according to Table 1, taking care not to introduce air bubbles to the mixture.
- In brief, prepare a cell suspension with the desired number of cells. It is recommended to connect two cooled 3 mL syringes with the Luer lock and divide the collagen between the two syringes. Disconnect the two syringes and pipette the cell suspension into one of the syringes very gently while pulling on the plunger to create room for the cell suspension in the syringe. Remove any air introduced into the syringe and connect the two syringes again. Gently mix back and forth between the syringes until the mixture is homogeneous. If detecting any air bubbles during mixing, disconnect the syringes and evacuate the air. Mix until homogeneous. The number of mixing cycles will depend on the cell type and bioink volume, optimize depending on application. Work fast and avoid heating the collagen with your hands to avoid self-assembly of the collagen.

Note:

- The maximum filling level of the 3 mL syringes is 2.7 mL.

3. Preparation for bioprinting

MATERIAL

Collagen solution mixed with cells in 3 mL BD syringe.

Conical bioprinting nozzles, 22-25G recommended.

DESCRIPTION

- Cap the syringe with a bioprinting nozzle of choice, 22-25G recommended.
- Attach the thermal insulator to the cooling block, by inserting it from below and rotating counterclockwise.
- Place the syringe into the pre-cooled printhead pre-set to 2°C. Rotate the syringe plunger holder arm over the plunger and twist the syringe by the tabs counterclockwise to ensure it is locked in place.

4. Calibration and nozzle priming

MATERIAL

BIO ONE

Well plate

DESCRIPTION

- Place a well plate on the printbed and perform either manual- or automatic calibration. Perform calibration each time a new syringe is placed in the printhead. If needed perform Autobed levelling.
- Right before each print, prime the nozzle by extruding a couple of drops. If any material has gelled at the tip of the nozzle, ensure it is fully extruded prior to starting a print.

Notes:

- Before starting the print, test the flow of the bioink using the Test extrude button with the recommended starting parameters in Table 2 or 3.
- If the system has been idle for an extended period, the collagen in the nozzle can dry or gel causing it to clog. If this occurs purge the nozzle by extruding 10 to 50 µL of the collagen, or until the gelated part is extruded. If the clog cannot be removed, replace with a new nozzle. Always ensure the nozzle is fully primed with liquid collagen prior to printing. Cells may sediment in the collagen if idle for extended periods. Remove the syringe from the printhead and flip back and forth a few times. Place back in the printhead and repeat Step 4.

5. Printing

MATERIAL

BIO ONE

Well plate

DESCRIPTION

- Dispense droplets or print single layer structures with parameters according to Table 2 or Table 3, in a well plate of choice.

Notes:

- The values are only a reference of starting parameters. The actual values needed to print will vary depending on the preparation procedures (amount of collagen and actual temperature of the material) as well as the print surface. If printing does not begin right away, it is most likely because the printhead and/or printbed has not yet reached the temperature set-point.

Table 2. Recommended settings in DNA Studio Core used for dispensing 10 µL collagen droplets at 4, 6 and 8 mg/mL through a 22G nozzle in a 96-well plate using the Droplet Print function on the BIO ONE. The density of the cell suspension may alter the flow rate. Do not heat the printbed for smaller droplets to minimize evaporation.

Parameters	4 mg/mL	6 mg/mL	8 mg/mL
Well plate	96-well plate	96-well plate	96-well plate
Printbed temperature	Disabled	Disabled	Disabled
Printhead temperature	2°C	2°C	2°C
Extrusion rate	20 µL/s	20 µL/s	20 µL/s
Extrusion volume	10 µL	10 µL	15 µL
Retract volume	0 µL	0 µL	5 µL
Z-offset	0.5 mm	0.5 mm	0.5 mm
Extra preflow volume	0 µL	0 µL	0 µL
Retract rate	20 µL/s	20 µL/s	20 µL/s
Postflow stop time	0.5 s	0.5 s	0.5 s
Z-lift between wells	30.0 mm	30.0 mm	30.0 mm

Table 3. Recommended settings in DNA Studio Core used for printing single layered structures using TeloCol®-10 at 4, 6 and 8 mg/mL.

Parameters	4 mg/mL	6 mg/mL	8 mg/mL
Well plate	6-well plate	6-well plate	6-well plate
Printbed temperature	25°C	25°C	25°C
Nozzle	0.25 mm (25G)	0.25 mm (25G)	0.25 mm (25G)
Speed	10 mm/s	10 mm/s	10 mm/s
Printhead temperature	2°C	2°C	2°C
Preflow volume	3.5 µL	3.5 µL	3.5 µL
Extrusion rate	0.9 µL/s	1.2 µL/s	1.0 µL/s
Retract volume	3.2 µL	3.2 µL	3.2 µL
Z-offset	0.2 mm	0.2 mm	0.2 mm
Extra preflow volume	2.5 µL	2.5 µL	2.5 µL
Infill extrusion multiplier	100%	100%	100%
Retract rate	5.0 µL/s	5.0 µL/s	10.0 µL/s
Extra retract	0 µL	0 µL	0 µL
Postflow stop time	0.5 s	0.3 s	0.3 s
Z-lift	2.0 mm	2.0 mm	2.0 mm

6. Crosslinking

MATERIAL

Incubator

Cell culture medium

DESCRIPTION

- Collagen is crosslinked through thermal crosslinking: place the well plate on the heated printbed or in a 37°C humidified incubator for approximately 20-30 min based on droplet or construct size. Check periodically if sufficiently crosslinked.
- After crosslinking, add the desired medium to the constructs and place in incubator.
- 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.