BIOPRINTING PROTOCOL FOR BLOOD VESSELS WITH CELLS

Overview: This protocol is a specific way to create blood vessels using CELLINK bioink and primary human skeletal muscle cells.

Materials:
Autodesk® 123D Design software
Slic3r Software (v1.2.9)
CELLINK bioink
Human smooth muscle cells, SMCs, at a concentration of $10x10^6$ cells/ml
3D Cell Culture Media
CELLMIXER Kit
INKREDIBLE 3D Bioprinter by CELLINK
Conical tip, 250µm ID
$CaCl_2$ Crosslinking Solution

Protocol:

1. The first step is to design a blue print for the structure. Using Autodesk® 123D Design software, create the 3D model of a tube geometry with the dimensions of 6mm x 10 mm (ODxH)

2. Using Slic3r (v1.2.9), convert the 3D model to a bioprinting protocol and toolpath with the following parameters:
    - Layer height = 0.30mm
    - External perimeters extrusion width = 0.30mm
    - Printing speed, F = 600mm/min

    Upload the bioprinting protocol with the following name: *"Blood Vessel_D6xH10_LH_F600.gcode"*

3. The human smooth muscle cells are originally at a concentration of $10x10^6$ cell/ml. First, prepare a cell suspension of $33x10^6$ SMCs in a volume of 300µL of culture media. See

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reference for culture media composition.
http://dx.doi.org/10.1016/j.biomaterials.2005.10.026

4. This cell suspension will then be mixed with 3mL of CELLINK[1,2] bioink using the CELLMIXER to obtain a final concentration of $10x10^6$ cells/ml. This gives a final ratio of 10:1 (bioink to cell suspension). Please watch the video in this link for a detailed illustration on how to do the mixing process:
https://www.youtube.com/watch?v=CmSYL1-oltI

5. The following bioprinting parameters can be used with the INKREDIBLE 3D Bioprinter by CELLINK using the pneumatic-driven micro-extrusion technology.

    - Conical tip, 250µm ID (25 G)
    - Pressure: 15-17 kPa
    - Printing speed: 600 mm/min
    - Printhead temperature: Room temperature (22°C)
    - Printbed temperature: Room temperature (22°C)

6. After the bioprinting process, the cell-laden constructs are crosslinked by submerging in an ionic solution of 100mM $CaCl_2$ for 6 minutes. The constructs are then rinsed with culture media and incubated in 3D cell culture medium in standard culture conditions (37°C, 5% CO_2 and 95% relative humidity).

7. Bioprinting metrics
    a. Time for bioprinting: 2 minutes per construct
    b. Dimensions of bioprinted construct: OD: *6.1mm,* H: *10mm*
8. Post-bioprinting, incubate the cell-laden constructs in 3D cell culture medium and standard culture conditions (37°C, 5% CO_2 and 95% relative humidity) for at least 7 days to analyze the cell viability and morphology.
9. The stability of constructs in vitro using macroscopic evaluation showed no change in shape after 7 days of *in vitro* culture.

G-codes:
Blood Vessel_D6xH10_LH03_F600.gcode

Further Information:
blood vessel.stl

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References:

1. 3D Bioprinting Human Chondrocytes with Nanocellulose–Alginate Bioink for Cartilage Tissue Engineering Applications. Kajsa Markstedt, Athanasios Mantas, Ivan Tournier, Héctor Martínez Ávila, Daniel Hägg, and Paul Gatenholm. *Biomacromolecules* **2015** *16* (5), 1489-1496.
dx.doi.org/10.1021/acs.biomac.5b00188
2. 3D bioprinting of human chondrocyte-laden nanocellulose hydrogels for patient-specific auricular cartilage regeneration. Héctor Martínez Ávila, Silke Schwarz, Nicole Rotter, Paul Gatenholm. *Bioprinting* **2016**, Volumes 1–2, 22-35.
dx.doi.org/10.1016/j.bprint.2016.08.003

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