BIOPRINTING PROTOCOL FOR MUSCLE TISSUE MODEL

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

Materials:
BioCAD software
CELLINK RA
Primary human skeletal muscle cells, hSMCs, at a concentration of $10x10^6$ cells/ml
3D Cell Culture Media
CELLMIXER Kit
$CaCl_2$ Crosslinking Solution

Protocol:

1. The first step is to design a blue print for the structure. Using BioCAD software, draw the tissue model with a rectilinear pattern and the following dimensions:
    - Size = 6x6x1.2mm
    - Line space = 1.5mm
    - Layer Height = 0.4mm
    - Printing speed, F = 10mm/s

    Upload the bioprinting protocol with the following name: *"grid_6x6_LS15_Th04.iso"*

2. The primary human skeletal muscle cells are originally at a concentration of $10x10^6$ cell/ml. First, prepare a cell suspension of $33x10^6$ hSMCs in a volume of 300µL of culture media. This cell suspension will then be mixed with 3mL of CELLINK RA[1,2] bioink using the CELLMIXER to obtain a final concentration of $10x10^6$ cells/ml.

3. Once the cell suspension is ready in the correct volume of medium (eg. $33x10^6$ hSMCs in a volume of 300µL of culture media), mix it with the bioink using the CELLMIXER kit as indicated in the application note. 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

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4. The following bioprinting parameters can be used with the RegenHu 3D Discovery bioprinter using the inkjet piezoelectric printhead.

Bioink	F_R [mm/s]	L_T [mm]	Needle D [mm]	D_D [mm]	T_{VO} [µs]	P_{avg} [kPa]
CELLINK RA10	10	0.40	0.3	0.05	400	41 ± 2
CELLINK RA10**	10	0.40	0.3	0.025	400	32 ± 0

F_R: Feed rate, L_T: Layer Thickness, Needle D: needle internal diameter, D_D: Dosing distance, T_{VO}: Valve opening time, P_{avg}: average printing pressure.

****Note:** When the micro-valve is not entirely cleaned, you will need to decrease the Dosing Distance (D_D) and printing pressure.

Printhead temperature: Room temperature (22°C)
Printbed temperature: Room temperature (22°C)

5. Open the iso file *"grid_6x6_LS15_Th04.iso"* in the HMI Discovery software and set the bioprinting parameters as shown in above (D_D, T_{VO} and Pressure). Once the cells are mixed in the bioink and transferred to a cartridge, load the cartridge into the printhead holder for tool #1, calibrate the printhead and start bioprinting.

6. After the bioprinting process, the cell-laden constructs are crosslinked by submerging in an ionic solution of 100mM $CaCl_2$ for 5 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: 19 seconds per construct
    b. Volume of bioink per construct: 70 µL
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 14 days to analyze the cell viability and morphology.

G-codes:
N/A

Further Information:
grid 6x6 LS15_Th04.iso

References:

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2. 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.
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