BIOPRINTING PROTOCOL FOR HUMAN NOSE WITH CELLS

Overview: This protocol is a specific way to a human nose from a CT scan using CELLINK bioink and human primary nasal chondrocytes (hNC).

Materials:
Human nose CT Scan at a scale of 50% of original size
Slic3r Software (v1.2.9)
CELLINK bioink

Human primary nasal chondrocytes (hNC), at a concentration of 20x10^6 cells/ml
Cell Culture Medium, as described below
Differentiation Medium, as described below
CELLMIXER Kit
INKREDIBLE+ 3D Bioprinter by CELLINK
Conical tip, 250µm ID
CaCl₂ Crosslinking Solution

Protocol:

1. The first step is to upload the human nose CT scan to the Slic3r software to create an STL file. Using Slic3r (v1.2.9), convert the 3D model to a bioprinting protocol and toolpath with the following parameters:
   - Layer height = 0.40mm
   - External perimeters extrusion width = 0.45mm
   - Perimeters = 1
   - Infill density = 40%
   - Infill Pattern = Rectilinear
   - Printing speed, F = 600mm/min

   Upload the bioprinting protocol with the following name:
   “Human_Nose_Scale50_LH04_Infill40_F600.gcode”

2. The human primary nasal chondrocytes must be prepared at a concentration of 20x10^6 cell/ml. First, prepare a cell suspension of 72x10^6 hNCs in a volume of 600µL were mixed
with 3mL of CELLINK bioink using the **CELLMIXER** to obtain a final concentration of 20x10⁶ cells/ml.

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](https://www.youtube.com/watch?v=CmSYL1-oltI). This gives a final ratio of 10:1 (bioink to cell suspension).

3. The following materials are used in the culture medium: DMEM/Ham's F-12 (1:1, Biochrom), supplemented with 10% fetal bovine serum (Biochrom) and 1% penicillin–streptomycin.

The following materials are used in the differentiation medium: StemMACS ChondroDiff media (Miltenyi Biotec GmbH) supplemented with 1% penicillin–streptomycin.

4. 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: 15-18 kPa
   - Printing speed: 600 mm/min
   - Printhead temperature: Room temperature (22°C)
   - Printbed temperature: Room temperature (22°C)

5. After the bioprinting process, the hNC-laden nose constructs were crosslinked by submerging in an ionic solution of 100mM CaCl₂ for 10 minutes. The constructs were then rinsed and incubated in culture medium in standard culture conditions (37°C, 5% CO₂ and 95% relative humidity).

6. Bioprinting metrics
   a. Time for bioprinting: 6 minutes per construct
   b. Volume of bioink per construct: 1 mL

7. After the bioprinting process, cells were in 3D culture in vitro for 14 days.

8. The stability of constructs in vitro using macroscopic evaluation showed no change in shape after 7 days of *in vitro* culture.

**G-codes:**

*Human_Nose_Scale50_LH04_Infill40_F600.gcode*
Further Information:
human nose.stl

References: