

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 size¹

Slic3r Software (v1.2.9)

CELLINK bioink^{1,2}

Human primary nasal chondrocytes (hNC), at a concentration of 20×10^6 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 20×10^6 cell/ml. First, prepare a cell suspension of 72×10^6 hNCs in a volume of 600µL were mixed

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with 3mL of CELLINK bioink using the [CELLMIXER](#) to obtain a final concentration of 20×10^6 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>. 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 CaCl_2 for 10 minutes. The constructs were then rinsed and incubated in culture medium in standard culture conditions (37°C, 5% CO_2 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

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Further Information:

human nose.stl

References:

1. 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.
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. *Biomacromolecules* **2015** 16 (5), 1489-1496.
[dx.doi.org/10.1021/acs.biomac.5b00188](https://doi.org/10.1021/acs.biomac.5b00188)