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PROTOCOL FOR PRINTING SKIN TISSUE WITH **CELLINK FIBRINOGEN**

This is a suggested procedure, please adjust according to your experimental needs.

Aim of the protocol:
To create a skin tissue model with a fibroblast gradient using **CELLINK FIBRINOGEN** bioink, primary human fibroblast cells and primary human keratinocyte cells. Gradient mimic papillary and reticular dermal compartments with a higher fibroblast concentration in the papillary layer, see Figure 1.

Materials needed:

- CAD software
- Slic3r software
- USB
- **CELLINK FIBRINOGEN** bioink
- Primary human fibroblasts, HDF
- Primary human keratinocytes, HEK
- 3D Cell Culture Media
- 3 Cartridges
- Syringes and Female/Female luer lock adaptor
- CELLMIXER
- 24 well plate
- Transwell inserts
- CaCl2 Crosslinking Solution

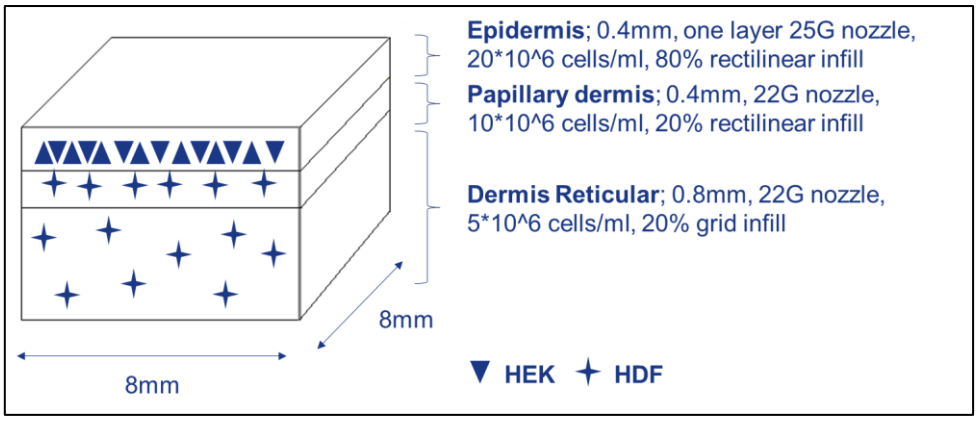

Figure 1. Blue print of Skin model.

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Protocol:

Step n°	Title	Material	Description
1	Design*	▪ CAD software	❖ Top part (reticular dermis); Draw the tissue model with following dimensions: • Size = 8x8x0.8mm • Layer Height = 0.4mm ❖ Middle part (papillary dermis); Draw the tissue model with following dimensions: • Size = 8x8x0.4mm • Layer Height = 0.4mm ❖ Bottom part (epidermis); Draw the tissue model with following dimensions: • Size = 8x8x0.4mm • Layer Height = 0.4mm ❖ Save the parts as three separate stl files
2	Create g-code	▪ Slic3r ▪ USB	❖ Add the bottom part into Slic3r. Open the object settings, load the middle part and the top part in named order. Make sure the parts are aligned and on top of each other. Adjust to following slicing parameters: ❖ Bottom part (epidermis): • Print head = 1 • Infill pattern = rectilinear • Infill density = 80% • Printing speed, F = 10mm/s ❖ Middle part (papillary dermis): • Print head = 2 • Infill pattern = rectilinear • Infill density = 20% • Printing speed, F = 10mm/s ❖ Top part (reticular dermis): • Print head = 3 • Infill pattern = grid • Infill density = 20% • Printing speed, F = 10mm/s ❖ Export g-code and save to an USB.
3	Prepare the cell suspension	▪ HDF ▪ HEK ▪ Cell culture medium	❖ Prepare two cell suspension, one of $30x10^6$ HDF and one of $15x10^6$, each in a volume of 300µL of culture media. The lower cell concentration is for the top part (reticular dermis) and the higher cell concentration for the middle part (papillary dermis).

			❖ Prepare one cell suspension of 40x10^6 HEK and in a volume of 200µL of culture media.
4	Mix the **CELLINK FIBRONOGEN** bioink with the cells	▪ Syringes ▪ Female/Female luer lock adaptor	❖ Mix the HDF cell suspensions (step 3) with 3ml of **CELLINK FIBRINOGEN** using the CELLMIXER to obtain a final concentration of 10x10^6 cells/ml respectively 5x10^6 cells/ml. ❖ Mix the HEK cell suspension (step 3) with 2ml of **CELLINK FIBRINOGEN** using the CELLMIXER to obtain a final concentration of 20x10^6 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
5	Set up of the Bioprinter	▪ CELLINK BioX ▪ USB with g-code	❖ Open the g-code on the USB stick and set the bioprinting parameters as shown below: ❖ • Pneumatic-driven microextrusion • Nozzle type and size: PH1: Conical tip, 410µm ID (22 GA) PH2: Conical tip, 410µm ID (22 GA) PH3: Conical tip, 410µm ID (22 GA) • Printing pressure: 9 kPa • Printing speed = 10 mm/s • Printhead temperature: Room temperature (22°C) • Printbed temperature: Room temperature (22°C) ❖ Note: Since choice of printhead is set in g-code this function is disabled.
6	Loading the cartridge and printing	▪ The 3 cartridges	❖ Once the cells are mixed in the bioink and transferred to a cartridge, load the cartridge with HEK into the printhead holder for tool #1, HDF 10x10^6 cells/ml ml into the printhead holder for tool #2 and the cartridge with HDF 5x10^6 cells/ml into the printhead holder for tool #3. ❖ Calibrate the printhead and start bioprinting.
7	Crosslinking	▪ CaCl2 Crosslinking Solution	❖ Submerge the cell-laden constructs in an ionic solution of 50mM CaCl2 for 5 minutes. ❖ Rinsed the constructs with culture media and flip them around so the top part (reticular dermis) becomes the bottom.
7	Incubation	▪ Incubator	❖ Incubate the constructs in 3D cell culture medium in standard culture conditions (37°C, 5% CO2 and 95% relative humidity) over night. ❖ Move to transwell inserts and adjust 3D cell culture medium so the epidermis is exposed to air.

			❖ Time: incubation for at least 14 days to analyze the cell viability and morphology.

*The model will be printed upside down and flipped after cross-linking to maintain grid structure.

Want to see our talented Biologist proceed to this protocol? Feel free to find the video here: https://www.youtube.com/...

Applications:

➔ Videos / photos of the kind of result you can obtain

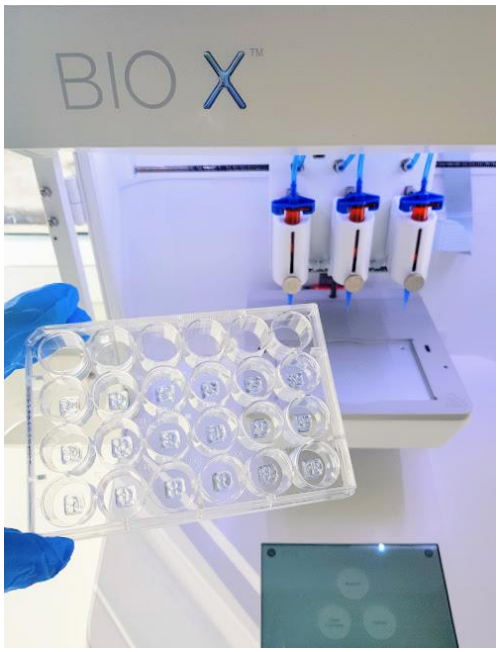

Figure 2. Skin model printed in CELLINK START.

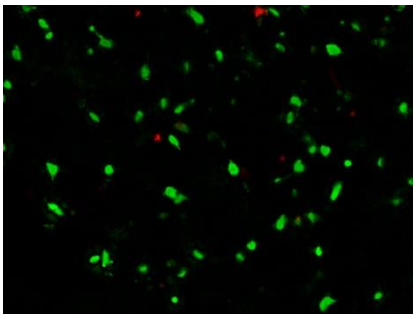

Figure 3. HEK after 14 days culture.

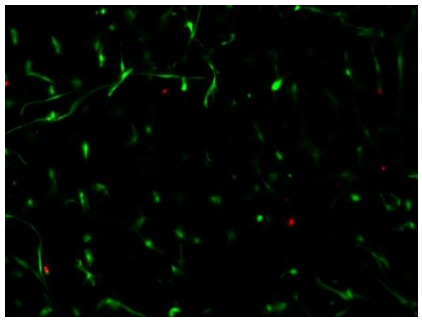

Figure 4. HDF after 14 days culture.

Want to see our existing tissue model?
Just go to http://bioverse.co/ and discover a whole library of CAD files especially created for sharing 3D Bioprinting models.

References:
N/A

➢ This protocol is compatible with the use of other bioprinters. For more information, please contact: info@cellink.com

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