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

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 FIBRIN** 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 FIBRIN** 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
- Thrombin

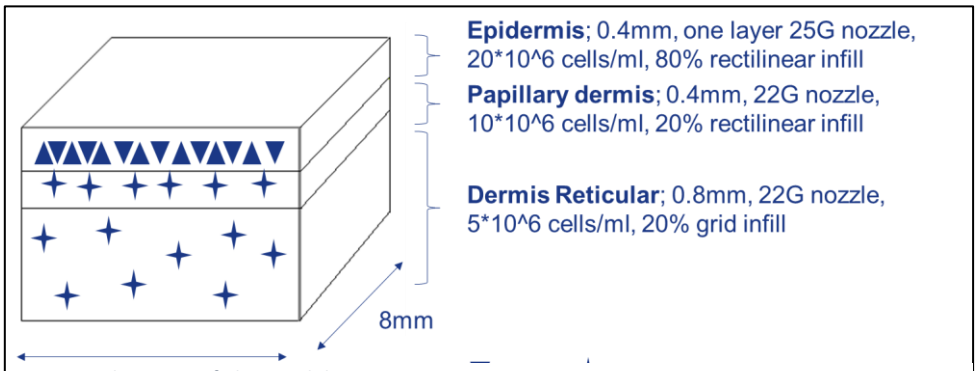

Figure 1. Blue print of Skin model.

CELLINK AB

Arvid Wallgrens Backe 20
41346 Gothenburg,
SWEDEN
CIN No.: 559050-5052

675 W Kendall St,
Cambridge,MA 02142
USA

Yoshida-honmachi,
Sakyo-ku, Kyoto 606-8501
JAPAN

www.cellink.com
info@cellink.com
+1(833) CELLINK

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 bioink with the cells	▪ Syringes ▪ CELLMIXER	❖ Mix the HDF cell suspensions (step 3) with 3ml of CELLINK FIBRIN 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 FIBRIN using the CELLMIXER to obtain a final concentration of $20x10^6$ cells/ml.
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 250UN Thrombin diluted in 25ml 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

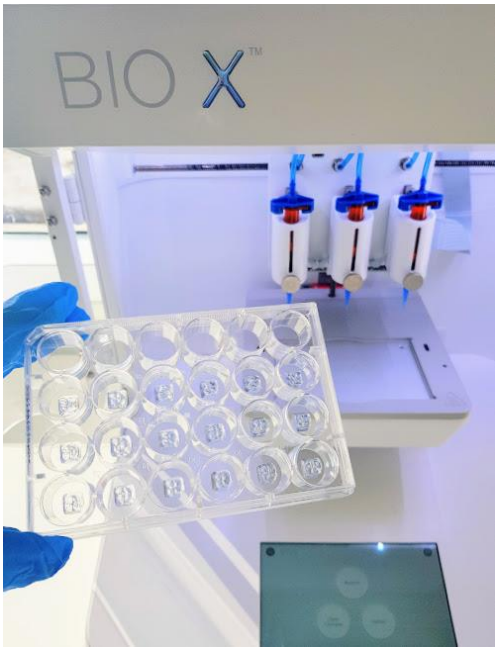

Figure 2. Skin model printed in CELLINK START.

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