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Bioprinting Skin Tissue Model Protocol

GelXA SKIN

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

Protocol aim

The aim of this protocol is to provide instructions for printing a skin tissue model using GelXA SKIN bioink, primary human fibroblast cells and primary human keratinocyte cells. The model has a fibroblast gradient mimicking papillary and reticular dermal compartments, with a higher fibroblast concentration in the papillary layer, see Figure 1. For a general protocol of bioprinting GelXA SKIN see *Bioprinting Protocol GelXA SKIN*.

Material needed

- CAD software
- Slic3r software
- USB Flash Drive
- Sterile Conical Bioprinting nozzles, 22G*
- 3 cartridges, 3cc*
- BIO X*
- GelXA SKIN*
- Primary human fibroblasts, HDF
- Primary human keratinocytes, HEK
- Nutritious solution for respectively cell type, e.g. FBS or growth supplement mixtures
- Cell culture medium
- Syringes and female/female luer lock adaptor
- Empty Cartridges with End and Tip cap, 3cc*
- (CELLMIXER*)
- 24-well plate
- Transwell inserts
- $CaCl_2$ Crosslinking Solution (included with the bioink purchase)
- Thrombin, 100 U (included with the bioink purchase)

*The product can be purchased in the CELLINK shop at *www.cellink.com/store/*.

Arvid Wallgrens Backe 20
413 46 Gothenburg
SWEDEN

100 Franklin St,
Boston, MA 02110
USA

Med-Pharm Collaboration
Building, 46-29 Yoshida-Shimo
Kyoto, JAPAN

www.cellink.com

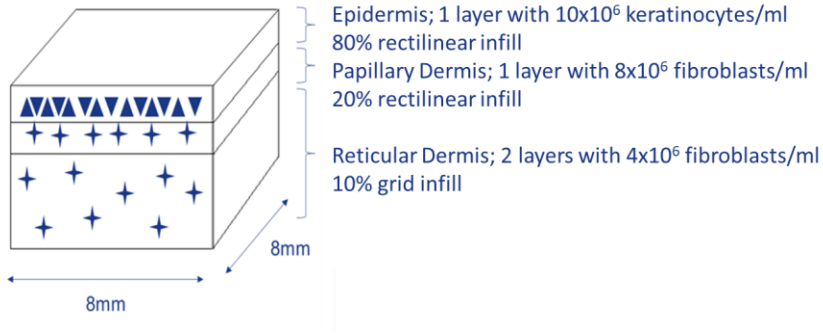

Figure 1. Blueprint of Skin model.

KEEP THE BIOINK PROTECTED FROM LIGHT IF TRANSFERRED FROM THE ORANGE UV PROTECTED CARTRIDGES TO AVOID CROSSLINKING BEFORE PRINTING. WORK WITH 3D PRINTERS IN DARK MODE. THE PHOTOINITIATOR IS SENSITIVE TO REPEATED OR PROLONGED EXPOSURE TO HEAT.

Protocol

This protocol can be performed with standard pneumatic printheads at room temperature and printbed cooled to 15°C, where room temperature is between 20-25°C.

Step	Title	Material	Description
1	Design	- CAD software	Open your CAD software and create following: - Top part (reticular dermis); Draw the tissue model with following dimensions: • Size = 8 x 8 x 0.8 mm • Layer Height = 0.4 mm - Middle part (papillary dermis); Draw the tissue model with following dimensions: • Size = 8 x 8 x 0.4 mm • Layer Height = 0.4 mm - Bottom part (epidermis); Draw the tissue model with following dimensions: • Size = 8 x 8 x 0.4 mm • Layer Height = 0.4 mm Save the parts as three separate stl files. Note: The model will be printed upside down and flipped after crosslinking to maintain grid structure.

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413 46 Gothenburg
SWEDEN

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USA

Med-Pharm Collaboration
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Kyoto, JAPAN

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 = 10 mm/s - Middle part (papillary dermis): • Print head = 2 • Infill pattern = rectilinear • Infill density = 20% • Printing speed, F = 10 mm/s - Top part (reticular dermis): • Print head = 3 • Infill pattern = grid • Infill density = 10% • Printing speed, F = 10 mm/s - Export G-code and save to a USB flash drive.
3	Prepare printing	- Sterile Conical Bioprinting nozzles - BIO X	- Make sure G-code is working and that sterile nozzles, 22G, sterile cartridges, BIO X and other equipment needed for printing are in place before proceeding to next step.
4	Prepare bioink	- GelXA SKIN bioink	- Prepare and pre-warm at least 3 ml of GelXA SKIN bioink according to the *Mixing Cells Protocol GelXA Series*. Note: Distribute the bioink into 3 syringes with at least 1 ml in each syringe. To maintain a full 24 well plate it's recommended to use 1.5 ml bioink for the reticular dermis layer.
5	Prepare the cell suspension	- HDF - HEK - Nutritious solution for respectively cell type - 3 pcs 3 ml syringes with luer lock connection	- Detach the cells according to protocol for your cells. - Prepare three cell suspensions: • HEK $10x10^6$ cells/ml bioink for epidermis • HDF $8x10^6$ cells/ml bioink for papillary dermis • HDF $4x10^6$ cells/ml bioink for reticular dermis - Spin down the cell suspensions and reconstitute the cell pellet in nutritious solution so that the total volume is 100 µl per 1 ml bioink.

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SWEDEN

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Med-Pharm Collaboration
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Kyoto, JAPAN

www.cellink.com

			- Add cell suspension to the syringes by pipetting. Remove excess air and seal with a tip cap. Keep at 37°C until mixing with the GelXA SKIN bioink.
6	Set up of the Bioprinter	- BIO X - 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, printhead 1, 2 & 3: Conical tip, 410 µm (22G) • Printing pressure: 9 kPa* • Printing speed = 10 mm/s* • Printhead temperature: - • Printbed temperature: 15°C *This is recommended starting parameters for the print. The pressure needed and optimal speed depends on the temperature of the surroundings and preparation procedures (amount of bioink and actual temperature of the bioink). ALWAYS test the pressure before starting the print and be prepared to adjust the speed and pressure during the print, especially for long printing sessions. Note: Since choice of printheads and layer parameters is set in G-code, these functions are disabled.
7	Mix the GelXA SKIN bioink with the cells	- Syringes with pre-warmed GelXA SKIN bioink - Syringes with cell suspensions - (CELLMIXER)	- Take the syringes with pre-warmed GelXA SKIN bioink. Attach it to the syringe with cell suspension using a Female/Female luer lock adaptor. Note: Make sure to connect epidermal cell suspension with GelXA SKIN bioink prepared for the epidermis etc. - Carefully mix the GelXA SKIN bioink with the cell suspension by gently pushing the bioink back and forth. Repeat for all 3 cell suspensions. - Place cartridges on counter for 20 min to reach room temperature. Note: To avoid an air gap when mixing the bioink and the cell suspension carefully pre-fill the luer lock adaptor with the bioink before attaching the syringe with the cell. If preparing for big quantities of GelXA SKIN we recommend using the CELLMIXER.

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Med-Pharm Collaboration
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www.cellink.com

8	Load the cartridges and print	- The 3 cartridges of GelXA SKIN with cells - Sterile Conical Bioprinting nozzles	- Mount cartridges with keratinocytes into printhead 1, fibroblasts $8x10^6$ cells/ml into printhead 2 and fibroblast $4x10^6$ cells/ml into printhead 3 with 22G nozzles. - Calibrate the printheads and start bioprinting.
9	Crosslinking	- $CaCl_2$ Crosslinking Solution - Thrombin	- Reconstitute the thrombin in the crosslinking solution to a concentration of 10 U/ml. - Submerge the cell-laden constructs in the crosslinking solution with thrombin for 5 minutes. - Remove crosslinking solution and rinse constructs with culture media once.
10	Incubation	- 3D culture medium	- Incubate the constructs in 3D cell culture medium in standard culture conditions (37°C, 5% CO_2 and 95% relative humidity). - When air-liquid interface culture is desired; flip the constructs around so the top part (reticular dermis) becomes the bottom and transfer them to transwell inserts. Adjust the 3D cell culture medium so the epidermis is exposed to air. Time recommendations: Submerge constructs for at least 5 days before initiating air- liquid interface culture. Incubation for at least 14 days to analyse the cell viability and morphology.

Want to see our talented Biologist proceed to this protocol? Feel free to find the video here:

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.

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

References:

N/A

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