BioLamina

Protocol: Biosilk® and Biolaminin® free-floating scaffolds

in 96-well plate for hPSC culture

hPSC integration into free-floating Biosilk scaffolds

Format: 96-well

Human pluripotent cells mixed with soluble Biosilk can be transformed into free floating Biosilk networks with integrated cells that are transformed into Biosilk spheroids for 3D culture. The following protocol is a guideline for human pluripotent stem cell integration into free-floating Biosilk scaffolds fitted into 96-well plates.

IMPORTANT NOTES

- All steps must be carried out under aseptic conditions
- Biosilk vial is thawed at RT without moving the vial. Handle with care.
- The Biosilk vial should never be vortexed or shaken, mixing should be done carefully to avoid the introduction of air bubbles
- Thawed Biosilk has to be used within 45 min, or as soon as possible. Re-freezing is not recommended and will result in decreased foaming efficiency
- For research use only

Material

Material	Comment
96-well cultivation plate	U-shaped ultra-low (ULA) attachment plate e.g. NunclonTM SpheraTM 96-well plate (#174925, Thermofisher), CellCarrier Spheroid (#605530, PerkinElmer)
Culture medium and solutions for making single cell suspensions	e.g. Essential 8 medium (Gibco) or mTeSR Plus supplemented with ROCK inhibitor, 10 µM
Biosilk	Stock solution 3 mg/mL (Biosilk, BioLamina)
Biosilk/Biolaminin - Biosilk521 - Biosilk/Biolaminin	- Biosilk pre-combined with LN521 (#BS521-0101) - Biosilk combined with any other LN-isoform by the researcher (#BS-0101)
Cells	hPSC cell line of choice
Pipettes	- A 100-200 µL pipette, for adding the Biosilk suspension and cell medium to stock mix - A single or multi-pipette set at 30 µL, for the formation of the Biosilk/Biolaminin foam scaffolds - A 1-10 µL pipette, for adding the cell suspension to the stock mix and aliquoting of the Biosilk solution
General lab ware	Sterile 1.5 mL tubes and sterile pipette tips

Summary of protocol

- The amount of Biosilk (or Biosilk521) and cells for each replicateis calculated for 2 mg/mL Biosilk and typically 10 000-30 000 cells per replicate are needed, for the total volume of 10 µL for each Biosilk/Biolaminin (or Biosilk521) foam scaffold replicate.
    - o Using Biosilk/Biolaminin: The stock mix is created by adding the cell suspension, Biolaminin and Biosilk
    - o Using Biosilk521: The stock mix is created by adding the cell suspension and Biosilk521
- Droplets of 10 µL Biosilk/Biolaminin/cell suspension (or Biosilk521/cell suspension) mix are placed at the bottom of each well. Repeat for all foam scaffold replicates.
- A dense foam scaffold is created by introducing air bubbles by rapid pipetting, up and down into the Biosilk/Biolaminin/cell droplets (or Biosilk521/cell) for 15 sec.
- The cell-containing Biosilk/Biolaminin (or Biosilk521) foam scaffolds are then stabilized in a cell incubator at least 15 min
- Media is added to each well to induce floating of the cell-containing Biosilk/ Biolaminin (or Biosilk521) foam scaffolds
- Floating Biosilk/Biolaminin/cell-foam scaffolds (or Biosilk521/cell-foam scaffolds) are transferred into new wells using a spoon for easy continuation and media exchange of the floating Biosilk/Biolaminin organoid culture

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Protocol

1 Preparation of plate and media

1.1. Place a U-shaped ultralow (ULA) attachment plate in the tissue culture hood (laminar flow cabinet) and prepare the pipettes needed.
1.2. Pre-warm cell culture medium for the cell splitting step.

2 Preparation of a concentrated cell solution

2.1. Prepare a concentrated single cell solution (around 10 000 cells/µL) diluted in warm media supplemented with 10 µM Rocki (typically 10 000-30 000 cell per foam scaffold replicate are needed). Needed cell number and cell suspension volume: See table 3.2.1.

3 Preparation of the Biosilk/Biolaminin/cell mix solution

NOTE: Do not shake, flip or vortex the tube with Biosilk solution.

3.1. Bring the vial of Biosilk from -80 °C to RT and thaw with the tube standing still. When thawed and in solution (usually takes 12-15 min) immediately proceed to step 4

3.2. Using Biosilk/Biolaminin: Prepare the Biosilk/cell mix: transfer 250 µL Biosilk solution from the concentrated stock vial to a new sterile 1.5 mL tube.
Gently add a 37.5 µL Biolaminin isoform at a final concentration of 0.01 mg/mL and prepared cell suspension (see 2.1. or 3.2.1.) to the Biosilk solution to a final concentration of 2 mg/mL Biosilk.
Mix slowly (no vortexing or shaking) without introducing any air bubbles.

Using Biosilk521: Prepare the Biosilk521/cell mix: transfer 250 µL Biosilk521 solution from the concentrate stock vial to a new sterile 1.5 mL tube. Gently add prepared cell suspension (see 2.1. or 3.2.1) to the Biosilk solution to a final concentration of 2 mg/mL Biosilk.
Mix slowly (no vortexing or shaking) without introducing any air bubbles.

3.2.1 Example calculation

Using Biosilk/Biolaminin

Biosilk stock	**3 mg/mL**
Volume (µL) of Biosilk (Final concentration: 2 mg/mL)	250
Volume (µL) of Biolaminin (Final concentration: 0.01 mg/mL)	37.5
Volume (µL) of cell suspension (containing 375 000 cells)	87.5
Total volume (µL) Biosilk/Biolaminin/cell suspension	375
Scaffold replicates	37

Using Biosilk521

Biosilk521 stock	**3 mg/mL**
Volume (µL) of Biosilk521 (Final concentration: 2 mg/ml)	250
Volume (µL) of cell suspension (containing 375 000 cells)	125
Total Volume (µL) Biosilk521/ cell suspension	375
Scaffold replicates	37

3.3. Immediately proceed with step 4 below

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4 Preparation

4.1. Transfer 10 µL of the prepared Biosilk/Biolaminin/cell (or Biosilk521/cell) solution to the center of each culture well. Do not touch the well walls with the pipette tip (see Fig.1A).

4.2. Use either a single or multi-pipette fitting 100 µL tips and set it to 30 µL. Air bubbles are pushed into the droplets by quickly pipetting up and down 30 time i.e. 15 seconds (see Fig.1B)., thereby creating a dense foam scaffold (see Fig.1C).

4.3. Place the plate with the cell-containing Biosilk/ Biolaminin (or Biosilk521) foam scaffold in an incubator at 37°C for at least 15 min. During this time the stabilization of the 3D structure occurs (see Fig.1D).

4.4. Bring the plate back to the tissue culture hood (laminar flow cabinet). Gently add 150 µL pre-warmed media at the side of the culture wells, with enough force to make the cell-containing Biosilk/ Biolaminin (or Biosilk521) foam scaffold loosen from the bottom (see Fig.1E). If the foam scaffolds are stuck at the bottom, flush the well with one more stroke to make the foam scaffold lift (see Fig.1F).

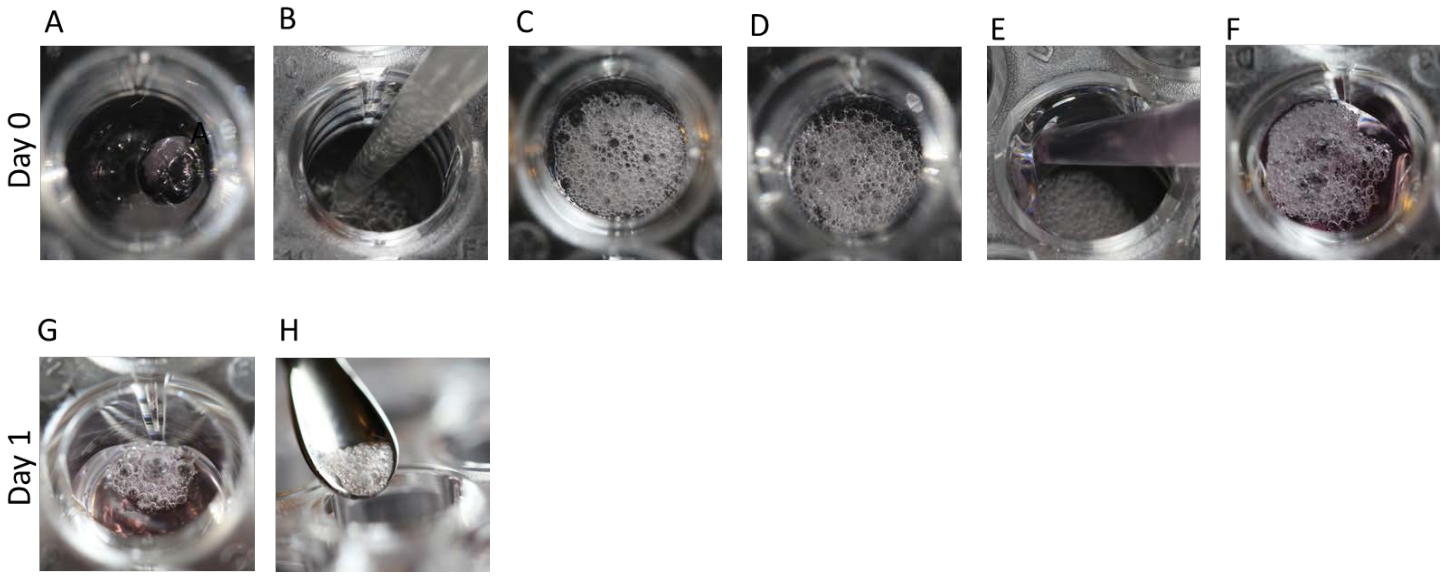
4.5. Place the plate back into the incubator for culture or continue with step 5.

5 Optional - Transfer scaffolds

5.1. Add pre-warmed media to new wells on a ULA-plate, either in the same plate or in a new plate. To facilitate medium changes, a larger plate format (e.g. 48-well) can be used.

5.2. After 1day of cultivation (see Fig.1G) use a small sterile metal spoon, wet in media before, for the transfer of the floating foam scaffolds to the new wells (see Fig.1H). Multiple cell-containing Biosilk/Biolaminin– (or Biosilk521) foam scaffolds can be pooled into 1 well.

FIGURE 1
Generation of Biosilk/Biolaminin/cell (or Biosilk521/cell) scaffolds



A: Droplet of Biosilk/Biolaminin/cell (or Biosilk521/cell) solution in culture medium in 96-well
B: Foaming procedure to create foam scaffold
C: Scaffolds* before stabilization
D: Scaffolds* after 15 min stabilization
E: Addition of culture medium (flush wall to lift scaffold)
F: Scaffolds* floating in culture medium
G: Scaffolds* with 10 000 hPSCs after one day in culture
H: Transfer of scaffolds* with wet sterile metal spoon

**Scaffold = Biosilk/Biolaminin/cell (or Biosilk521/cell) scaffold*

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