interchim

BioLamina
REVOLUTIONIZING CELL CULTURE

INSTRUCTIONS FOR USE 011

BIOSILK 521 3D SCAFFOLDS

FOR PROLIFERATION AND DIFFERENTIATION OF hPSC

BIOSILK 521 IS A BIOMATERIAL MADE OF RECOMBINANT SILK, FUNCTIONALIZED WITH HUMAN RECOMBINANT LAMININ 521 PROTEIN (BIOLAMININ™ 521) FOR 3D CELL CULTURE. LAMININ 521 IS A KEY CELL ADHESION PROTEIN OF THE NATURAL STEM CELL NICHE, PROVIDING THE BIOSILK 521 MATERIAL WITH UNIQUE, FUNCTIONAL PROPERTIES, IDEAL FOR INTEGRATION, PROLIFERATION AND SUBSEQUENT LINEAGE-SPECIFIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS (hPSC) IN A 3D FORMAT. THE BIOSILK 521 IS A BIORELEVANT AND BIOCOMPATIBLE 3D SYSTEM THAT IS BIODEGRADABLE AND NON-IMMUNOGENIC. THE EXTENDABLE BIOSILK 521 MICROFIBRE MATERIAL IS COMPATIBLE WITH ANY CELL CULTURE MEDIA AND CAN SELF-ASSEMBLE INTO DIFFERENT FORMATS, LIKE FOAM, FIBER BUNDLES AND THIN FILMS. THE FOLLOWING PROTOCOL IS FOR FORMATION AND CULTURE IN BIOSIK 521 FOAM.

MATERIAL

Material	Comment
24-well hydrophobic surface plate	Validated surface is Sarstedt cat no 83.3922.500. Not all hydrophobic surface for suspension cell culture are suitable for foam attachment
Culture media	Pluripotent culture medium and/or differentiation medium of choice
ROCK inhibitor	Use e.g. Y27632
Pipettes	One 1-20 µL pipette is needed for transferring Biosilk 521 solution droplet to the plate One 200 µL pipette is needed for formation of the foam scaffold One 1-10 µL pipette is needed for adding the cell suspension One 1000 µL pipette is needed for adding the culture media
General lab ware for cell culturing	E.g. sterile tubes and pipette tips

IMPORTANT NOTES

- **All steps must be carried out under aseptic conditions**
- **Biosilk 521 should be stored at -80°C**
- **Thaw the Biosilk 521 solution at RT without moving the vial**
- **Do not vortex or shake the vial and be careful when mixing to avoid introduction of air bubbles**
- **Thawed Biosilk 521 solution has to be used within 1 hour. Re-freezing or storage in fridge is not recommended and will result in decreased foaming efficacy**
- **For research use only**

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PROCEDURE IN SUMMARY

A 20 µL droplet of the Biosilk 521 is placed in the center of a hydrophobic culture well. The foam scaffold is created by rapid introduction of air bubbles into the Biosilk 521 solution by quickly pipetting of air up and down using a pipette set at 40µl. A suspension of human pluripotent cell in culture media with 10 µM ROCK inhibitor added (typically at 10.000-15.000 cell/µL concentration) is immediately mixed into the foam by an additional 5 gentle strokes. The plate with cell-containing foams is stabilized for 20 min at 37°C in a cell incubator before addition of 0.7-1 mL pre-warmed cell medium. The culture medium without ROCK inhibitor is renewed daily until the desired confluence is reached.

PROTOCOL

1. **Preparation of Biosilk 521 solution**

   1.1. Thaw the Biosilk 521 solution at RT without moving the vial.

   1.2. Add Rock inhibitor to the thawed solution to final concentration of 10 µM. Gently pipette 3 times.

   Note:

   ! *Do not vortex or shake the vial and be careful when mixing to avoid introduction of air bubbles.*

   ! *It will take around 10 min for the frozen Biosilk 521 solution to thaw at ambient temperature. The thawed solution should be used as soon as possible, within1 hour at the latest. The thawed Biosilk 521 will gradually turn milky in ambient room temperature.*

2. **Preparation of a concentrated cell suspension**

   2.1. Prepare a concentrated single cell suspension according to "Instruction For Use BL003", roughly 10.000-15.000 cells/µL, diluted in warm media supplemented with 10 µM ROCK inhibitor.

   Note:

   ! *The cell suspension should be prepared freshly for foam seeding (use within 20 min after detachment from plate). Cell suspension standing too long in RT will result in reduced cell amplification rate in the foam.*

3. **Integration and expansion of hPSC in Biosilk 521 foam**

   3.1. Transfer 20 µL of the prepared Biosilk 521 solution from step 1 to the center of one culture well.

   3.2. Use a pipette with a tip for 200 µL and set at 40 µL. Push air bubbles into the droplet by quickly pipetting up and down 20 strokes, thereby creating a dense foam. Spread out the foam in circular motions with the pipette tip during pipetting to an area covering 0.7-1 cm in diameter.

   3.3. Immediately add 1–5 µL (typically 30.000-60.000 hPSCs/foam) of the cell suspension from step 2 (volume ratio of cell to Biosilk 521 ≤0.25). Use the pipette set at 40 µL with a new tip and disperse the cells throughout the 3D structure by 5 additional strokes.

   3.4. Repeat step 3.1 to 3.3 to create the desired number of foams. One vial of Biosilk 521 (270 µL) is sufficient for 12 foams.

   3.5. Place the plate with the cell-containing foams in an incubator at 37°C for 20 min. During this time, the 3D structure is stabilized.

   3.6. Gently add 0.7-1 mL of the pre-warmed medium containing 10 µM ROCK inhibitor per well, enough to cover the foam.

   3.7. Place the plate back into the incubator.

   3.8. Feed the cells daily with fresh culture media without ROCK inhibitor.

   Note:

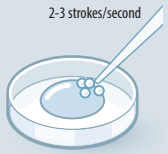
   ! *For best foam stability, cell suspension for foam seeding could be prepared beforehand or during the Biosilk 521 thawing time.*

   ! *It takes approximately 1 min to generate each cell-containing foam. It's good to plan the timing for best cell and foam quality.*

4. **Differentiation**

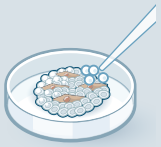
   Differentiation protocol of interest can be used when desired confluency has been reached.

TO GENERATE A BIOSILK FOAM



Add the Biosilk 521 solution to the plate and pipett air bubbles to obtain a 3D foam.

Add the cell suspension to the Biosilk 521 foam.



Mix by pipetting 5 times. Incubate at 37°C for 20 min.

Add pre-warmed medium to cover the foam. Incubate at 37°C.

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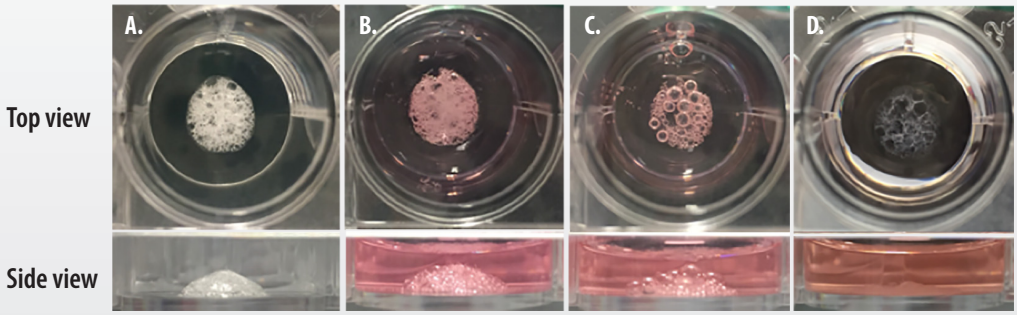
FOAM MORPHOLOGY DURING CULTURE

Representative pictures of the Biosilk 521 foam taken from above and from the side of the cell culture well, day 0 to day 3 after formation. Note that the initial small bubbles merge into larger bubbles that disperse after a few days, resulting in a 3D scaffold with cells integrated between the microfibers.



A. Day 0. Biosilk 521 foam without medium after 20 min stabilization at 37°C.

B. Day 0. Biosilk 521 foam with medium.

C. Day 1. The small bubbles start to merge into bigger bubbles.

D. Day 3. The bubbles have dispersed and the Biosilk 3D scaffold has formed with cells organized between the microfibers (approx. 10 cell layer thick).

TROUBLE SHOOTING

1. **The 3D foam does not shape properly or is very flat.**

   - Make sure a hydrophobic culture plate is being used (e.g. Sarstedt 83.3922.500). If the plate is not hydrophobic, the Biosilk 521 solution will be difficult to pipette and the foam will become flat.

2. **The stabilized 3D foam detaches from the bottom.**

   - Make sure a hydrophobic culture plate is being used (e.g. Sarstedt 83.3922.500).
   - The Biosilk 521 solution has been standing for too long before use or was roughly handled. Thaw the Biosilk 521 solution without moving the vial and use the solution as soon as possible (within 1 hour) after thawing for best result. Never centrifuge the Biosilk 521 solution, not even if it turns milky.
   - The generated foam diameter was too small. Too small and thick scaffold will bear more "lifting force" when medium is added. Make sure that the foam covers a diameter of 0.7-1 cm.
   - The media was added too fast. Start adding the media dropwise around the foam and also on top of it before filling up.

3. **The stabilized 3D foam partially disperses while adding medium.**

   - The Biosilk 521 solution has been standing for too long before use or was roughly handled (see point 2).
   - The ratio of cell suspension volume to the Biosilk 521 volume was higher than 0.25. The Biosilk 521 will then be too diluted to generate a stable scaffold. Do not use more than 5µL of cell suspension to 20 µL of Biosilk 521, or 3 µL of cell suspension for 15 µL Biosilk 521.
   - The bubbles of the 3D scaffold are too big. During foam formation, try to generate small and evenly distributed bubbles.
   - The temperature for stabilization was lower than 37°C. Calibrate incubator to be sure. Seed the foams in the outer wells of the plate. Alternatively, increase the stabilization time to 25 min.

4. **The cells do not amplify properly or grow unevenly in the Biosilk 521 scaffold.**

   - The cell suspension used for foam seeding has been standing for too long before use. Work in a way so that the concentrated cell suspension is ready as soon as the Biosilk 521 solution is ready to be used. Supplement both the Biosilk 521 solution, the cell suspension and the seeding media with a final concentration of 10 µM ROCK inhibitor.
   - For good cell integration, the cell suspension should be added immediately to the freshly generated foam.

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