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REVOLUTIONIZING CELL CULTURE

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APPLICATIONS

- Expansion of human PSC
- Mesenchymal stem cells
- Clonal cell culture applications
- Eye cells
- Cardiac cells
- Neural cells
- Skeletal muscle cells
- Kidney cells
- Hepatic cells
- Cancer cells
- Lung cells
- Animal stem cells
- Endothelial cells
- Pancreatic cells
- Intestinal cells
- Normal and cancerous mammary cells
- Epithelial cells

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Product	Prod.no	Amount	Size	Price	Qty
BIOLAMININ 521 CTG Human recombinant laminin 521	CT521-0501	500 ug	1 x 5 ml	€325	1

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SIZE GUIDE

Not sure how much laminin you need? To make it easy, we have created a tool where you can calculate the amount needed for your experiments. Just choose culture well format and fill in the desired coating concentration to see the amount required.

FORMAT	CONC. (ug/cm2).	NO OF WELLS	AMOUNT (uq)
6-w	0.5	1	

Product	Prod.no	Amount	Size	Price	Qty
BIOLAMININ 521 MX Human recombinant laminin 521	MX521-0501	500 ug	1 x 5 ml	€258	1

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SIZE GUIDE

Product	Prod.no	Amount	Size	Price	Qty
BIOLAMININ 521 LN Human recombinant laminin 521	LN521-02	100 µg	1 x 1 ml	€57	1
	LN521-05	500 ug	1 x 5 ml	€216	1

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SIZE GUIDE

Product	Prod.no	Amount	Size	Price	Qty
BIOLAMININ 511 LN Human recombinant laminin 511	LN511-0202	100 ug	1 x 1 ml	€73	1

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Product	Prod.no	Amount	Size	Price	Oty
BIOLAMININ 421 LN Human recombinant laminin 421	LN421-02	100 µg	1 x 1 ml	€73	1
	LN421-0501	500 µg	1 x 5 ml	€319	1
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SIZE GUIDE

Product	Prod.no	Amount	Size	Price	Oty
BIOLAMININ 411 LN Human recombinant laminin 411	LN411-02	100 µg	1 x 1 ml	€73	1
	LN411-0501	500 µg	1 x 5 ml	€319	1
	For larger quantities contact us				

SIZE GUIDE

Product	Prod.no	Amount	Size	Price	Oty
BIOLAMININ 332 LN Human recombinant laminin 332	LN332-0202	100 µg	1 x 1 ml	€73	1
	LN332-0502	500 µg	1 x 5 ml	€319	1
	For larger quantities contact us				

SIZE GUIDE

Product	Prod.no	Amount	Size	Price	Oty
BIOLAMININ 221 LN Human recombinant laminin 221	LN221-02	100 µg	1 x 1 ml	€73	1
	LN221-0501	500 µg	1 x 5 ml	€319	1
	For larger quantities contact us				

SIZE GUIDE

Product Prod.no

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SIZE GUIDE

Product	Prod.no	Amount	Size	Price	Qty
BIOLAMININ 121 LN Human recombinant laminin 121	LN121-02	100 µg	1 x1 ml	€73	1
	LN121-0501	500 µg	1 x1 ml	€319	1

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SIZE GUIDE

Product	Prod.no	Amount	Size	Price	Qty
BIOLAMININ 111 LN Human recombinant laminin 111	LN111-02	100 µg	1 x 1 ml	€73	1
	LN111-0501	500 µg	1 x 5 ml	€319	1

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SIZE GUIDE

Product	Prod.no	Amount	Size	Price	Qty
LAMSCREEN Laminin isoform kit	LNKT-0201	400 µg	4 x 100 ug	€219	1

For larger quantities contact us

Product	Prod.no	Amount	Size	Price	Qty
BIOSILK 3D culture substrate	BS-0101	750 µg	1 x 250 µL	€247	1

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CHECKOUT

Product	Product Number	Quantity	Size	Cost	Total	
Biosilk	BS-0101	1	1 x 250 µL	$278	$278	x
Biosilk 521	BS521-0101	1	1 x 270 µL	$278	$278	x
					$556	

Freight will be added to your invoice according to the list below. Click here for full terms and conditions.

	Standard shipping	Premium shipping
Europe	€110	Upon request
North America	$145	Upon request
ROW	€210	Upon request
Sweden	300SEK	Upon request

Delivery terms

BioLamina's Terms of Shipment is always ExWorks (Incoterms 2010) unless otherwise agreed.

Premium shipping

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Biosilk is a natural biomaterial made from recombinant spider silk protein, a useful tool for a wide range of 3D culture applications, such as organoid culture and other tissue engineering applications. Biosilk can easily be biofunctionalized with different ECM proteins, such as laminin proteins, to better replicate the authentic cell environment. The functional properties of this unique biomaterial make it ideal for organoid research with both human pluripotent stem cells or adult progenitor cells as starting material. The mild assembly process of the silk and cells enables an even cell integration between the Biosilk microfibers. Biosilk promotes long-term cell survival without the need for encapsulation. The Biosilk biomaterial creates a fibrous network and contrary to cells encapsulated in a hydrogel, the cells are highly proliferative, migrate initiate cell-cell contact and the Biosilk attached cells become more elongated and contain filamentous actin and defined focal adhesion points. The scaffold enables efficient diffusion of oxygen, media, and patterning factors which enables long-term differentiation protocols and uniform differentiation. Biosilk is a biocompatible, biodegradable, and non-immunogenic biomaterial, which facilitates its use in clinical applications. The unique properties of Biosilk make it an excellent culture system for a wide variety of tissue applications, such as pancreas, skin, and brain.

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A NATURAL BIOMATERIAL THAT PROVIDES AN OPTIMAL 3D CULTURE ENVIRONMENT FOR EXPANSION AND DIFFERENTIATION OF HUMAN PRIMARY CELLS

The development of organoid culture techniques is one of the most impactful advances in stem cell research in the last decade. Compared to 2D cultures, a 3D culture is a more biologically relevant culture environment with a higher degree of architectural complexity that retains homeostasis for longer. The reasons for pursuing 3D cell culture models is simply to generate a higher degree of complexity by growing cells in a way that most closely resembles how they grow and interact with each other and

The fibrillar Biosilk network allows the formation of channels throughout the 3D culture, which facilitate diffusion of oxygen, medium, and patterning factors (Åstrand, 2020). This enables long-term differentiation protocols and makes it possible to generate larger organoids with uniform cellular specialization and organization, without an increased risk of getting necrotic centers.

Biosilk is an ideal 3D substrate for integration, expansion, and long-term differentiation of human primary cell types. The unique properties of the silk scaffolds combined with the ease of use and that does not require any specialized equipment, provide researchers with a platform that allows for the generation of any organoid

WHY USE BIOSILK ORGANOID RESEARCH?

- Biosilk™ is a natural biomaterial made of recombinant silk that easily can be biofunctionalized with different ECM proteins, such as laminins, to better mimic the natural, cell-specific environment
- Human primary cells seeded in Biosilk are highly viable, expand and form shapeable, macro-sized 3D constructs. Cellular self-organization and morphogenesis.
- The mild assembly process provides a 3D structure with instant and even integration and attachment of viable cells between the microfibers
- No encapsulation needed. Contrary to cells encapsulated in a hydrogel, the cells seeded in Biosilk survive, are highly proliferative and migrate to initiate cell-cell contact. The attached cells become more elongated and develop defined focal adhesion points

aggregates or spheroids. However, to permit culture of larger 3D cell arrangements, a scaffold support is needed.

Biosilk is a unique, natural biomaterial that has the ability to self-assemble into a network of microfibers in aqueous physiological-like buffers at room temperature. Biosilk can easily be biofunctionalized with different ECM proteins, such as laminin proteins, to better recapitulate the more physiologically relevant aspects of developing human tissue. The Biosilk microfibrillar network is elastic and flexible, can be formed into different 3D structures, and serve as a stationary scaffold both during early differentiation phases and as a floating scaffold for long-term organoid cultures. The mild assembly process, where the cells are included already during the assembly of the 3D construct, enables an instant and even cell integration and attachment between the Biosilk microfibers.

Biosilk promotes long-term cell survival without the need for encapsulation. A more tissue-like microenvironment is provided where the integrin-involved attachment to the Biosilk fibers gives the cells an elongated shape, with organized cytoskeleton and the formation of defined focal

properties, Biosilk is able to support extensive cellular remodeling, self-organization, and morphogenesis. Biosilk has successfully been used for many different cell types and applications (see examples below). It is possible to co-culture multiple cell types in the Biosilk, for example, include endothelial cells for *in vitro* vascularization. Different silk constructs can also be combined next to or on top of each other.

Biosilk is defined and animal-origin free and importantly, it's biocompatible and this type of recombinant spider silk fibers can be implanted subcutaneously in rats without any negative systemic or local reactions. After implantation, newly formed capillaries and fibroblast-like cells have been identified which indicates the formation of vascularized tissue. In addition, this type of recombinant spider silk is biodegradable, possibly by macrophages by endocytosis and subsequent intracellular proteolysis, further facilitating the use in clinical applications (Fredriksson, 2009).

PRIMARY CELLS

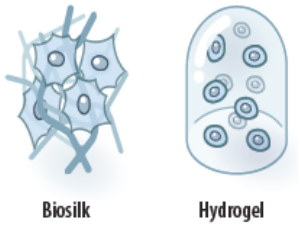
HSkMSC - human skeletal muscle satellite cells
HDMEC - human dermal microvascular endothelial cells
Schwann - human Schwann cells
HMSC - human mesenchymal

organoids with more effective and uniform cellular specialization and organization, without increased risk of necrotic centers

- Elastic material that can be formed into different structures
- Organoids can be generated from a variety of tissues
- Can be sterilized through autoclaving with retained morphology, structure, and properties
- Biocompatible & non-immunogenic
- Biodegradable
- Defined and animal origin-free

APPLICATION NOTE

encapsulating cells within a hydrogel prohibits the formation of focal adhesion points which result in more rounded cell morphology, limited spreading, and the cells become static with an almost steady metabolic state (Johansson, 2019).



Biosilk Hydrogel

stem cells
HUVECs - human umbilical vein endothelial cells

CELL LINES

HaCaT - Human keratinocyte cell line
MIN6m9 - insulin-secreting mouse pancreatic β-cell line
MCF7 - human brest cancer cell line

PLURIPOTENT STEM CELL LINES

HS980 - human embryonic stem cell line
HS975 - human embryonic stem cell line
C5 - human induced pluripotent cell line
iPSC3 - human induced pluripotent cell line

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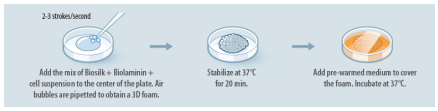
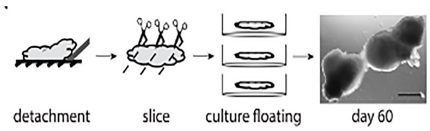
HOW TO GENERATE A BISILK 3D NETWORK WITH EVENLY INTEGRATED CELLS

A 3D foam structure can easily be generated is by the gentle introduction of air bubbles into the Biosilk solution. The cell suspension is mixed into the foam, and the silk with cells assembles into a thin film around each bubble. The bubbles disperse and the foam transforms into a stabilized 3D network with uniformly integrated cells between the microfibers.



PROCEDURE USED FOR ORGANOID FORMATION

Schematic illustration of the procedure used for detaching Biosilk foams for increased flexibility to allow cellular self-organization. After initial differentiation, the foams with integrated progenitors were detached from the bottom of the well, cut into ≈2 mm thick slices, and further cultured in low-attachment plates. Representative image of floating cell constructs at day 60.

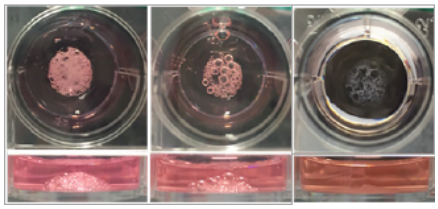


BIOSILK FOAM MORPHOLOGY

Representative pictures of the Biosilk foam taken from above and from the side at day 0, 1, and 3 after cell seeding. During the assembly process, the Biosilk solution is transformed into a foam by rapidly pipetting air bubbles. The cell suspension is mixed into the

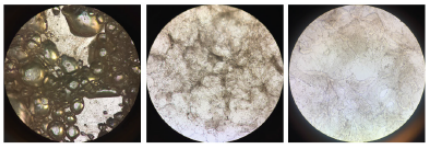
Biosilk can easily be fuktionlized with different laminin isoforms. Recombinant human laminin 521, Biolaminin™ 521 has been shown advantageous in promoting self-renewal and pluripotency of hPSCs. To create a 3D niche suitable for hPSCs, Biosilk 521 (Biosilk pre-mixed with Biolaminin 521)

of culture, these films will burst, thereby transforming the foam into a 3D network of microfibrillar silk with evenly integrated cells.

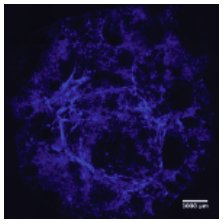


CELLS ATTACHED TO BIOSILK MIXED WITH LAMININ 521

Micrographs of hPSCs attached to Biosilk 521 days 2, 3, and 5 after seeding., The cells are evenly integrated between the Biosilk microfibers. When the bubbles have dispersed, the Biosilk scaffold with cells organized between the microfibers can be visualized.



The mild assembly process enables an instant and even cell integration and attachment between the Biosilk microfibers (nuclei staining; DAPI).



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PROTOCOL

Preparation of Biosilk solution

1. Thaw the Biosilk solution at RT without moving the vial.
2. Add Rock inhibitor to the thawed solution to a final concentration of 10 µM. Gently pipette 3 times.

Note:
! *Do not vortex or shake the vial and be careful when mixing to avoid the introduction of air bubbles.*
! *It will take around 10 min for the frozen Biosilk solution to thaw at ambient temperature. The thawed solution should be used as soon as possible, within1 hour at the latest. The thawed Biosilk will gradually turn milky in ambient room temperature.*

Preparation of a concentrated cell suspension

1. Prepare a concentrated single-cell suspension

Integration and expansion of hPSC in Biosilk foam

1. Transfer 20 µL of the prepared Biosilk solution from step 1 to the center of one culture well.
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. Immediately add 1–5 µL (typically 30.000-60.000 hPSCs/foam) of the cell suspension from step 2 (volume ratio of the cell suspension to Biosilk ≤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.
4. Repeat steps 1 to 3 to create the desired number of foams. One vial of Biosilk (270 µL) is sufficient for 12 foams.
5. Place the plate with the cell-

IMPORTANT NOTES

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

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

pre-warmed medium containing 10 µM ROCK inhibitor per well, enough to cover the foam.

7. Place the plate back into the incubator.
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 thawing time.*
! *It takes approximately 1 min to generate each cell-containing foam. It's good to plan the timing for the best cell and foam quality.*

4. **Differentiation**

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

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PRODUCT NAME

Biosilk

PRODUCT CODE

BS-0101

DECLARATION

For research use only

PRODUCT DESCRIPTION

Recombinant spider silk protein for 3D culture applications

STORAGE

-80°C

STOCK CONCENTRATION

0.1mg/ml

STABILITY

12 months

SHIPPING CONDITION

Dry Ice

APPEARANCE

Clear, colorless

PRODUCT APPLICATION

Human PSC expansion and differentiation

CLASSIFICATION

Defined and animal origin-free, human recombinant protein

DOCUMENTS

SDS

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- Assembly of functionalized silk together with cells to obtain proliferative 3D cultures integrated in a network of ECM-like microfibers. Johansson U. et al. Scientific Reports, 2019.
- Tissue Response to Subcutaneously Implanted Recombinant Spider Silk: An in Vivo Study. Fredriksson C. et al. Materials, 2009.

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Biosilk is a natural biomaterial made from recombinant spider silk protein, a useful tool for a wide range of 3D culture applications, such as organoid culture and other tissue engineering applications. Laminin 521 is a key cell adhesion protein of the natural stem cell niche and Biosilk 521 (Biosilk pre-mixed with Biolaminin 521) is an ideal 3D substrate for integration, expansion, and long-term differentiation of human pluripotent stem cells (hPSCs) and different progenitor cells. The mild assembly process enables an instant and even cell integration and attachment between the Biosilk microfibers. hPSC efficiently proliferates, expand, and form shapeable, macro-sized 3D constructs with an even distribution of a homogenous pluripotent that can be long-term in situ differentiated. Biosilk promotes long-term cell survival without the need for encapsulation. The Biosilk biomaterial creates a fibrous network and contrary to cells encapsulated in a hydrogel, the cells are highly proliferative, migrate initiate cell-cell contact and the Biosilk attached cells become more elongated and contain filamentous actin and defined focal adhesion points. The scaffold enables efficient diffusion of oxygen, media, and patterning factors which enables long-term differentiation protocols and uniform differentiation. Biosilk 521 is a biocompatible, biodegradable, and non-immunogenic biomaterial, which facilitates its use in clinical applications.

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A NATURAL BIOMATERIAL THAT PROVIDES AN OPTIMAL 3D CULTURE ENVIRONMENT FOR EXPANSION AND DIFFERENTIATION OF HPSCS AND MANY PROGENITOR CELL TYPES

The development of organoid culture techniques is one of the most impactful advances in stem cell research in the last decade. Compared to 2D cultures, a 3D culture is a more biologically relevant culture environment with a higher degree of architectural complexity that retains homeostasis for longer. The reasons for pursuing 3D cell culture models is simply to generate a higher degree of complexity by growing cells in a way that most closely resembles how they grow and interact with each other and their microenvironment in native

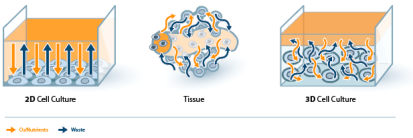
The fibrillar Biosilk network allows the formation of channels throughout the 3D culture, which facilitate diffusion of oxygen, medium, and patterning factors (Åstrand, 2020). This enables long-term differentiation protocols and makes it possible to generate larger organoids with uniform cellular specialization and organization, without an increased risk of getting necrotic centers.

Recombinant human laminin 521, Biolaminin™ 521 has been shown advantageous in promoting self-renewal of high-quality human pluripotent stem cells (hPSCs). Biosilk 521 (Biosilk pre-mixed with Biolaminin 521) is an ideal 3D substrate for integration, expansion, and long-term differentiation of hPSCs. The cells rapidly expand with the same expression of

WHY USE BIOSILK ORGANOID RESEARCH?

- Biosilk™ 521 is a natural biomaterial made of recombinant silk that has been biofunctionalized with human recombinant laminin 521 (Biolaminin™ 521). The biomaterial more authentically replicates the natural cell environment for hPSC and other primary cell types.
- Biosilk 521, is ideal for integration, expansion, and efficient long-term *in situ* differentiation of human PSCs and different progenitor cells
- hPSC seeded in Biosilk are highly viable, expand and form shapeable, macro-sized 3D constructs. Cellular self-organization and morphogenesis.
- The mild assembly process provides a 3D structure with instant and even integration and attachment of viable cells between the microfibers
- No encapsulation needed. Contrary to cells

However, to permit culture of larger 3D cell arrangements, a scaffold support is needed.



Biosilk is a unique, natural biomaterial that has the ability to self-assemble into a network of microfibers in aqueous physiological-like buffers at room temperature. The pure Biosilk can easily be biofunctionalized with different ECM proteins, such as laminin proteins, to better recapitulate the more physiologically relevant aspects of developing human tissue. The Biosilk microfibrillar network is elastic and flexible, can be formed into different 3D structures, and serve as a stationary scaffold both during early differentiation phases and as a floating scaffold for long-term organoid cultures. The mild assembly process, where the cells are included already during the assembly of the 3D construct, enables an instant and even cell integration and attachment between the Biosilk microfibers.

Biosilk promotes long-term cell survival without the need for encapsulation. A more tissue-like microenvironment is provided where the integrin-involved attachment to the Biosilk fibers gives the cells an elongated shape, with organized cytoskeleton and the formation of defined focal adhesion points. The cells

population has been achieved for initiation of long-term, in situ differentiation towards neural lineages or other cell types.

The unique properties of the silk scaffolds combined with the ease of use and that does not require any specialized equipment, provide researchers with a platform that allows for the generation of any organoid type in a reproducible and functional manner. Due to its favorable functional and mechanical properties, Biosilk 521 is able to support extensive cellular remodeling, self-organization, and morphogenesis. Biosilk 521 has successfully been used for hPSC 3D differentiation into many different neural applications (forebrain, midbrain, cerebral and glial organoids) but also from other tissues, such as pancreas and skin. It is possible to co-culture multiple cell types in the Biosilk, for example, include endothelial cells for *in vitro* vascularization. Different silk constructs can also be combined next to or on top of each other.

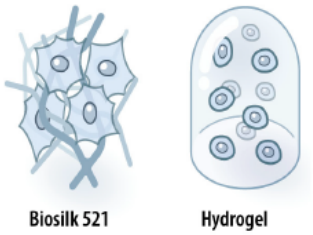
Biosilk is defined and animal-origin free and importantly, it's biocompatible and this type of recombinant spider silk fibers can be implanted subcutaneously in rats without any negative systemic or local reactions. After implantation, newly formed capillaries and fibroblast-like cells have been identified which indicates the formation of vascularized tissue. In addition, this type of

attached cells become more elongated and develop defined focal adhesion points

- Efficient diffusion of oxygen, nutrients, and patterning factors which make it possible to generate larger organoids with more effective and uniform cellular specialization and organization, without increased risk of necrotic centers
- Elastic material that can be formed into different structures
- Organoids can be generated from a variety of tissues
- Can be sterilized through autoclaving with retained morphology, structure, and properties
- Biocompatible & non-immunogenic
- Biodegradable
- Defined and animal origin-free

APPLICATION NOTE

hydrogel prohibits the formation of focal adhesion points which result in more rounded cell morphology, limited spreading, and the cells become static with an almost steady metabolic state (Johansson, 2019).



the use in clinical applications (Fredriksson, 2009).

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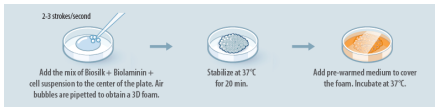
HOW TO GENERATE A BISILK 3D NETWORK WITH EVENLY INTEGRATED CELLS

A 3D foam structure can easily be generated is by the gentle introduction of air bubbles into the Biosilk solution. The cell suspension is mixed into the foam, and the silk with cells assembles into a thin film around each bubble. The bubbles disperse and the foam transforms into a stabilized 3D network with uniformly integrated cells between the microfibers.

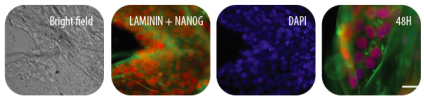


BIOSILK 521 FOAM MORPHOLOGY

Representative pictures of the Biosilk 521 foam taken from above and from the side at day 0, 1, and 3 after cell seeding. During the assembly process, the Biosilk solution is transformed into a foam by rapidly pipetting air bubbles. The cell suspension is mixed

PLURIPOTNET CELLS SURVIVE, PROLIFERATE AND CAN EFFICIENTLY BE IN SITU DIFFERENTIATED IN BIOSILK 521

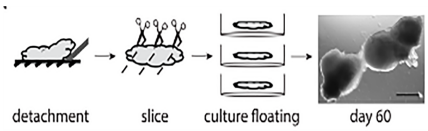
hES cells cultured in Biosilk 521 in hPSC culture media form colonies and proliferate along the microfibres (LAMININ, green) with the maintained expression of stemness marker (NANOG; red). DAPI was used as nuclear counterstain. Typical morphology of an hPSC colony 48 h after integration into Biosilk 521.



Human ES cells (HS980) and iPS cells (iPSC3) seeded at 50 000 cells/foam were cultured for 4 days in three different pluripotent cell culture media. The cells were detached with TrypLE solution and cell amount and viability was measured.

PROCEDURE USED FOR ORGANOID FORMATION

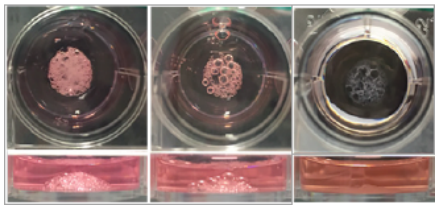
Schematic illustration of the procedure used for detaching Biosilk 521 foams for increased flexibility to allow cellular self-organization. After neuro-ectoderm formation, the foams with integrated neuronal progenitors were detached from the bottom of the well, cut into ≈2 mm thick slices, and further cultured in low-attachment plates. Representative image of floating cell constructs at day 60.



SELF-ORGANIZED NEURAL TUBE-LIKE STRUCTURES AND NEURAL FUNCTIONALITY IN FLOATING BIOSILK 521 CONSTRUCTS

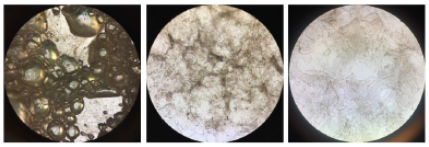
Immunostaining images. The apical surface of radially arranged cells stains for N-cad (green), the presence of neural

Within 1–3 days of culture, these films will burst, thereby transforming the foam into a 3D network of microfibrillar silk with evenly integrated cells.

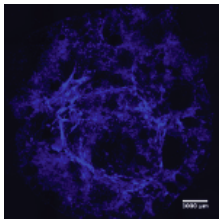


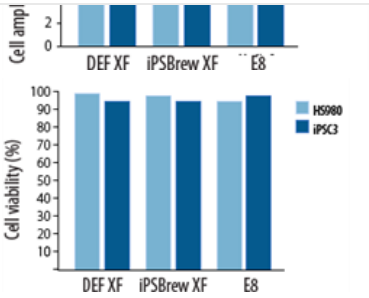
CELLS ATTACHED TO BIOSILK 521

Micrographs of human ES cells attached to Biosilk 521 days 2, 3, and 5 after seeding., The cells are evenly integrated between the Biosilk microfibers. When the bubbles have dispersed, the Biosilk scaffold with cells organized between the microfibers can be visualized.

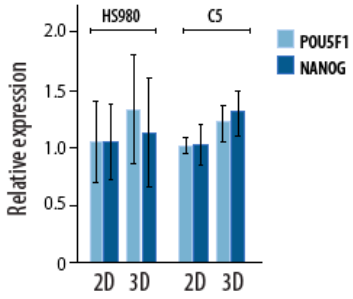


The mild assembly process enables an instant and even cell integration and attachment between the Biosilk microfibers (nuclei staining; DAPI).

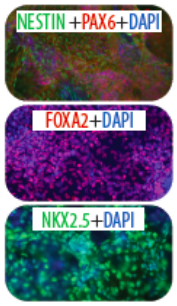




Relative gene expression of POU5F1 and NANOG for HS980 and C5 were measured 72 h after Biosilk 521 integration as compared to culture on Biolaminin 521 coated plates.

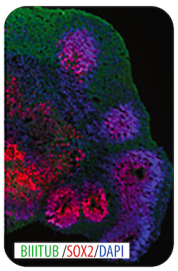


Linage specific differentiation to ectoderm (7 days, NESTIN and PAX6), endoderm (3 days, FOXA2), and mesoderm (12 days, NKX2.5) were initiated after 2-3 days of culture in the Biosilk 521 scaffold. DAPI was used as nuclear counterstain.



cells (red).

Section of a floating cell construct (height approx.1.5mm) stained for SOX2 (red) and DAPI (blue) revealed proliferative zones developing around multiple ventricular-like regions, surrounded by BIII-TUBULIN (green) at the basal surface.



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PROCEDURE IN SUMMARY

The protocol described here is for the expansion and differentiation of hPSCs in Biosilk mixed with Biolaminin 521 (alternatively Biosilk 521 can be used) in a 24-well plate format. Hydrophobic surface culture plates should be used. Prepare a mixture of Biosilk, Biolaminin, and cell suspension in medium containing ROCK inhibitor. The mixture should be prepared fresh before the foaming steps. For each well, add 22 µL of the mixture to the well center. The 3D scaffold is created by the rapid introduction of air bubbles into the mixture by quick pipetting of air, 22 to 25 times using a pipette set at 44 µL. Repeat the procedure until all wells have been prepared. Stabilize the cell-containing foams in a cell incubator at 37°C for 20 min. Gently add 0.7-1 mL pre-warmed cell medium to cover the culture (with ROCKi). Replace the culture medium daily (without ROCK inhibitor). When the desired cell confluence is reached, differentiation can be initiated by the addition of the

PROTOCOL

Preparation

- Thaw the Biosilk solution at RT without moving the vial.

Note:
! *Do not vortex or shake the vial and avoid the introduction of air bubbles.*

! *It will take around 12 min for the frozen Biosilk solution to thaw at ambient temperature. For the best result, the thawed solution should be used as soon as possible, the latest within 1 hour from removal from -80 °C. The thawed Biosilk solution will gradually turn milky in ambient RT.*

- Prepare a concentrated single-cell suspension according to “Instruction For Use BL003”. For a vial of Biosilk or Biosilk 521, prepare a concentrated cell suspension of a total of 7-14 x10^5 cells in 25 µL of medium (roughly 20 000 - 60 000 cells/µL) supplemented with 10 µM ROCK inhibitor.

Note:

Biosilk 521 organoid differentiation

- Pluripotent stem cells seeded in Biosilk-Biolaminin foam generally need to be cultured in medium supporting pluripotency for 3 to 4 days with daily feeding to reach the desired confluence before switching to differentiation medium. Depending on lineage differentiation and protocol used, culture for 1 to 2 weeks in a suitable medium with appropriate feeding frequency is needed.

- When cells have reached the desired confluency within the microfibrillar network, manually detach the foam from the bottom of the well using a cell scraper or a pipette tip. Cut the foam structure into 2 -4 pieces (approx. 2 mm thick) using a blade or a pair of small scissors and transfer to new low-attachment culture plates for culture as free-floating entities.

Note:

! *Before the foam can be detached from the culture*

lifted from the bottom of the well with a cell scraper around day 6-15, depending on the preferred differentiation protocol.

IMPORTANT NOTES

- All steps must be carried out under aseptic conditions
- Biosilk 521 should be stored at -80°C
- Gently 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 the introduction of air bubbles
- The Biosilk products have to be used within 45-50 min after thawing
- Re-freezing or long-term storage of thawed Biosilk solution in the fridge is not possible as this will affect product stability and functionality
- 1 vial of BioSilk or Biosilk 521 is enough material to generate 12-13 foams (24 well format)
- The cell suspension should be prepared fresh before mixing into the Biosilk to ensure high cell quality
- For research use only

INSTRUCTION FOR USE 011

foam stability, prepare the cell suspension for the foam incorporation beforehand or during the Biosilk thawing time.

! *Optimize the cell incorporating density for best amplification in the 3D scaffold, as this is cell type-dependent and needs to be adjusted accordingly.*

- Prepare a Biosilk-Biolaminin-Cell suspension mixture by adding Biolaminin 521 or isoform of choice (25 µL), cell suspension (25 µL), and 10mM ROCK inhibitor (0.27 µL) to the thawed Biosilk solution (250 µL). The final mixture will have a concentration of 10 ug/mL Biolaminin and 10 µM ROCK inhibitor. Mix by gently pipetting 3 times without introducing air bubbles. The Biosilk-Biolaminin-cell suspension mixture should be used within 10 min to ensure high cell viability.

Note:
! *If the Biosilk 521 (BS521-0101) pre-mixed product is used, the manual addition of Biolaminin can be omitted unless a mix with an additional Biolaminin isoform is desired.*

! *Be careful when mixing to avoid the introduction of air bubbles as this will cause premature fiber formation.*

3D scaffold formation and maintenance

desired confluency.

! *Embedding the organoid in Matrigel is not needed to maintain the organoid shape and cell phenotype. If embedding is preferred, a xenofree and defined material is recommended (e.g. HyStem™ available from Merck).*

! *If using another cell type than hPSCs, culture with appropriate culture medium and feeding frequency before detaching the foam for free-floating organoid culture.*

- Feed the free-floating organoid cultures at appropriate frequency until further analysis or desired applications.

- Use a pipette set at 44 µL, to push air bubbles into the droplet by quick pipetting up and down 22 times, 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. See the protocol described in Fig. 1.

Note:
! *Insufficient (<22) or excessive (> 25) pipetting for the foam formation will result in an unstable scaffold or low cell viability, respectively.*

! *If the cells are sensitive to pipetting, an increased cell seeding density could help to increase cell viability. Alternatively, the cells could be mixed in after the foam has been generated. In this case, add 20 uL Biosilk solution to the well and use a pipette, set at 40 uL, to push air bubbles by quickly pipetting up and down 20 times. Add 1-4 µL dense cell suspension (roughly 20 000 - 60 000 cells/µL) for each foam and mix by pipetting an additional 5 times. See the protocol described in Fig.2.*

! *It takes approximately half to one minute to generate each foam. We recommend to thoroughly plan the procedure for best cell- and foam quality.*

- Repeat step 4.1 to 4.2 to create the desired number of

the cell-containing foams in an incubator at 37°C for 20 min. During this time, the Biosilk product polymerizes and the 3D structure is stabilized.

- Remove the plate from the cell incubator. Gently add 0.7-1 mL per well of the pre-warmed medium containing 10 µM ROCK inhibitor, starting dropwise around the foam before slowly filling up to cover the foam.
- Place the plate back into the incubator.
- Feed the cells daily or at an appropriate frequency with fresh culture media without ROCK inhibitor.

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PRODUCT NAME

Biosilk 521

PRODUCT CODE

BS521-0101

DECLARATION

For research use only

PRODUCT DESCRIPTION

Recombinant spider silk protein, functionalized with human recombinant laminin 521 protein (Biolaminin 521) for 3D culture applications

STORAGE

-80°C

STOCK CONCENTRATION

0.1mg/ml

STABILITY

12 months

SHIPPING CONDITION

Dry Ice

APPEARANCE

Clear, colorless

PRODUCT APPLICATION

Human PSC and progenitor cell expansion and differentiation

CLASSIFICATION

Defined and animal origin-free, human recombinant protein

DOCUMENTS

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Biosilk is a natural biomaterial made from recombinant spider silk protein, a useful tool for a wide range of 3D culture applications, such as organoid culture and other tissue engineering applications. Laminin 521 is a key cell adhesion protein of the natural stem cell niche and Biosilk 521 (Biosilk pre-mixed with Biolaminin 521) is an ideal 3D substrate for integration, expansion, and long-term differentiation of human pluripotent stem cells (hPSCs) and different progenitor cells. The mild assembly process enables an instant and even cell integration and attachment between the Biosilk microfibers. hPSC efficiently proliferates, expand, and form shapeable, macro-sized 3D constructs with an even distribution of a homogenous pluripotent that can be long-term in situ differentiated. Biosilk promotes long-term cell survival without the need for encapsulation. The Biosilk biomaterial creates a fibrous network and contrary to cells encapsulated in a hydrogel, the cells are highly proliferative, migrate initiate cell-cell contact and the Biosilk attached cells become more elongated and contain filamentous actin and defined focal adhesion points. The scaffold enables efficient diffusion of oxygen, media, and patterning factors which enables long-term differentiation protocols and uniform differentiation. Biosilk 521 is a biocompatible, biodegradable, and non-immunogenic biomaterial, which facilitates its use in clinical applications.

BUY ONLINE!

AmountSize	Price	Qty
752 µg 1 x 270 µL	€247	1

For larger quantities contact us

Estimated shipping time
1-5 working days

Please select your currency.

EUR

SAVE MONEY

Buy larger quantities and save shipping cost.

- Assembly of functionalized silk together with cells to obtain proliferative 3D cultures integrated in a network of ECM-like microfibers. Johansson U. et al. Scientific Reports, 2019.
- Assembly of FN-silk with laminin-521 to integratehPSCs into a three-dimensional culture for neural differentiation. Åstrand C. et al. Biomaterials Science, 2020.
- Tissue Response to Subcutaneously Implanted Recombinant Spider Silk: An in Vivo Study. Fredriksson C. et al. Materials, 2009.

KEEP IN TOUCH
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BIOLAMINA AB - REVOLUTIONIZING CELL CULTURE