BioLamina
REVOLUTIONIZING CELL CULTURE

APPLICATION NOTE 016

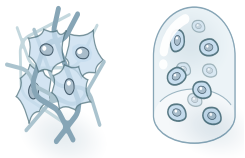
BIOSILK 3D BIOMATERIAL

MORE PHYSIOLOGICAL RELEVANT 3D STRUCTURES FOR ORGANOID RESEARCH

AN OPTIMAL 3D CULTURE ENVIRONMENT FOR EXPANSION AND DIFFERENTIATION OF PRIMARY CELLS

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 adhesion points. The cells rapidly proliferative, and spread within the silk scaffold, with a clear directional alignment in the fibers. Contrary, 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 521 Hydrogel

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 pluripotency markers as the 2D control (Åstrand, 2020) and after a few days, sufficient cell density of a homogenous, pluripotent starting cell population has been achieved for initiation of long-term, in situ differentiation towards neural lineages or other cell types.

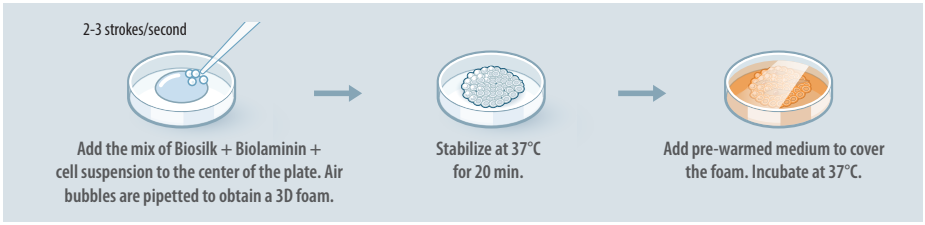
FEATURES AND SPECIFICATIONS:

- Biosilk™ is a natural biomaterial made of recombinant silk that easily can be biofunctionalized with different ECM proteins, such as laminins
- 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
- Boisilk mixed with laminin 521, Biosilk 521, is ideal for integration, rapid proliferation, and efficient long-term *in situ* differentiation of human PSCs and progenitor cells
- hPSC seeded in Biosilk are highly viable, expand and form shapeable, macro-sized 3D constructs. Cellular self-organization and morphogenesis
- 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
- Organoids can be generated from a variety of tissues
- Elastic material that can be formed into different structures
- Can be sterilized through autoclaving
- Biocompatible, non-immunogenic, and biodegradable
- Defined and animal origin-free

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 recombinant spider silk is biodegradable, possibly by macrophages by endocytosis and subsequent intracellular proteolysis, further facilitating the use in clinical applications (Fredriksson, 2009).

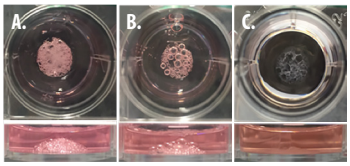
ID: AN-016-06. Valid from 2020-10-15

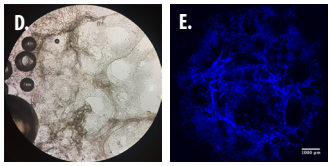
HOW TO GENERATE A BIOSILK 3D NETWORK WITH EVENLY INTEGRATED CELLS



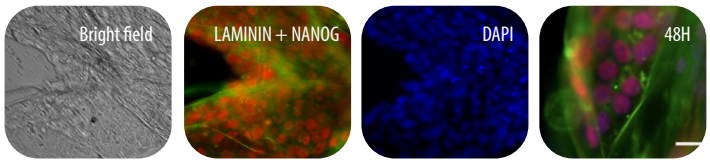
A 3D foam structure can easily be generated is by the gentle introduction of air bubbles into the Biosilk solution. The cell suspension is mixed into the foam, and the silk with cells assembles into a thin film around each bubble. The bubbles disperse and the foam transforms into a stabilized 3D network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

BIOSILK 521 FOAM MORPHOLOGY WITH hPSCs ATTACHED AND EFFICIENTLY AND EVENLY INTEGRATED





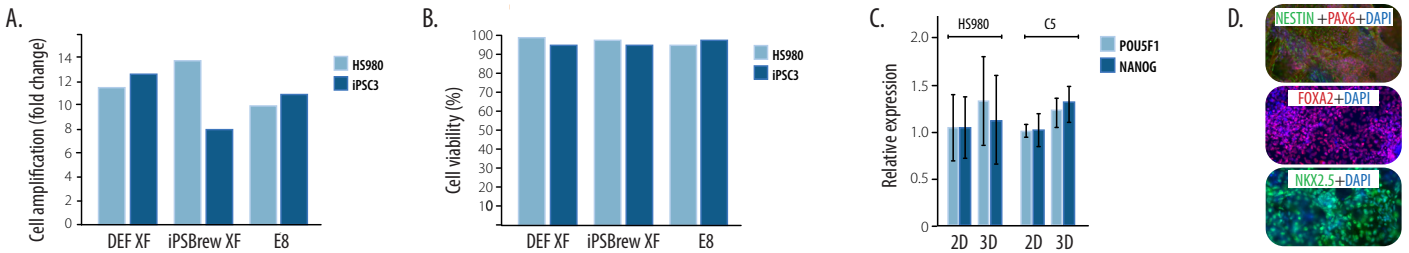
Representative pictures of the Biosilk 521 foam taken from above and from the side at A) day 0, B) day 1 and C) day 3 after cell seeding. The bubbles have dispersed after 3-5 days, transforming the foam into a Biosilk network (D) with evenly integrated cells (nuclei staining; DAPI) attached to the microfibers (E).



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.

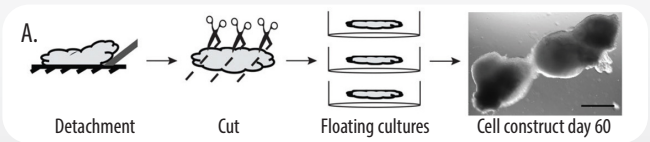
hPSCs SURVIVE, RAPIDLY PROLIFERATIVE AND CAN EFFICIENTLY BE IN SITU DIFFERENTIATED IN BIOSILK 521

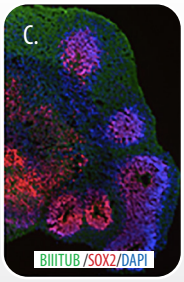
Human ES cells (HS980) and iPS cells (iPSC3 or C5) seeded at 50 000 cells/foam were cultured for 4 days in three different pluripotent cell culture media. The cells were detached with TrypLE solution and A) cell amount and B) viability was measured. C) Relative gene expression of POU5F1 and NANOG for HS980 and C5 were measured 72 h after Biosilk 521 integration as compared to culture on Biolaminin 521 coated plates. D) 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.



SELF-ORGANIZED, FUNCTIONAL NEURAL TUBE-LIKE STRUCTURES IN FREE-FLOATING BIOSILK 521 CONSTRUCTS

A) Schematic illustration of the procedure used for detaching Biosilk 521 foams for increased flexibility to allow cellular self-organization. After neuroectoderm 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. (B) Immunostaining images. The apical surface of radially arranged cells stains for N-cad (green), the presence of neural progenitor zones visualized with NESTIN (green) and SOX2 (red), and PAX6+ cells (green) surrounded by layers of DCX+ cells (red). C) 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.





REFERENCES

- 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
EMAIL: INFO@BIOLAMINA.COM

LÖFSTRÖMS ALLÉ 5A
172 66 SUNDBYBERG (STOCKHOLM)
SWEDEN

Direct link to Biosilk information online

www.biolamina.com

BIOLAMINA - REVOLUTIONIZING CELL CULTURE

ID: AN-016-06. Valid from 2020-10-15