BioLamina
REVOLUTIONIZING CELL CULTURE

APPLICATION NOTE 016

BIOSILK 521 3D SCAFFOLDS

3D EXPANSION AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

AN OPTIMAL 3D CULTURE ENVIRONMENT FOR EXPANSION AND DIFFERENTIATION OF HUMAN PSCs

Biosilk 521 is a natural biomaterial made of recombinant spider silk protein, functionalized with human recombinant laminin 521 protein (BiolamininTM 521), designed 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 that are ideal for integration, proliferation and subsequent lineage-specific differentiation of human pluripotent stem cells in a 3D format.

Biosilk 521 is a biocompatible, biodegradable and non-immunogenic biomaterial, which facilitates the use in clinical applications. The extendable Biosilk 521 macrofibre material can self-assemble into several formats; foams, fiber bundles, and thin films.

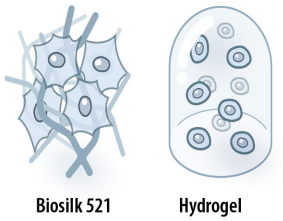
A MORE BIOLOGICALLY RELEVANT CULTURE ENVIRONMENT

Compared to 2D cultures, a 3D culture is a more biologically relevant cell culture environment with a higher degree of architectural complexity that retains homeostasis for longer.

FEATURES AND SPECIFICATIONS:

- **Flexible 3D culture system that can be assembled into different formats**
- **A fibrous network of strong and elastic microfibers that mimick the ECM**
- **A biologically relevant 3D culture environment for the expansion and differentiation of human ES and iPS cells.**
- **More authentic cell attachment and polarization compared to gels**
- **Uniform integration of the cells between the microfibers. Effective proliferation and organization along the microfibers.**
- **More perfused organoid structures**
- **Biocompatible and non-immunogenic**
- **Biodegradable**
- **Defined and animal origin-free to the primary level***

Direct link to Biosilk information online



Contrary to many other 3D scaffolds, the cells can be added to the Biosilk 521 solution before the network stabilizes, resulting in a uniform integration of the cells between the microfibers. Unlike cells cultured in a hydrogel, cells seeded in the Biosilk scaffold attach along the microfibers and get a more authentic polarization and are in contact with other cells. The cells proliferate efficiently and migrate along the fibers to organize themselves. The Biosilk scaffold gives more perfused organoid structures where the cells get better access to the medium, giving more homogenous structures compared to when encapsulated in a gel.

* ISCT guidance document "ISCT Animal-Free Origin Survey Results-Summary"

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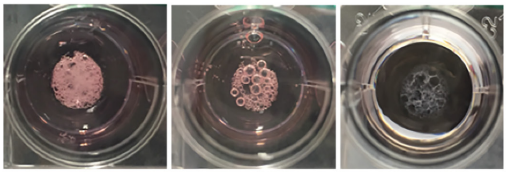
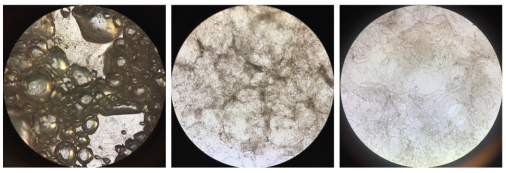
BIOSILK 521 FOAM MORPHOLOGY



Representative pictures of the Biosilk 521 foam taken from above day 0, 1 and 3 after cell seeding. The initial small bubbles merge into bigger bubbles that disperse after a few days, resulting in a 3D scaffold with integrated cells.

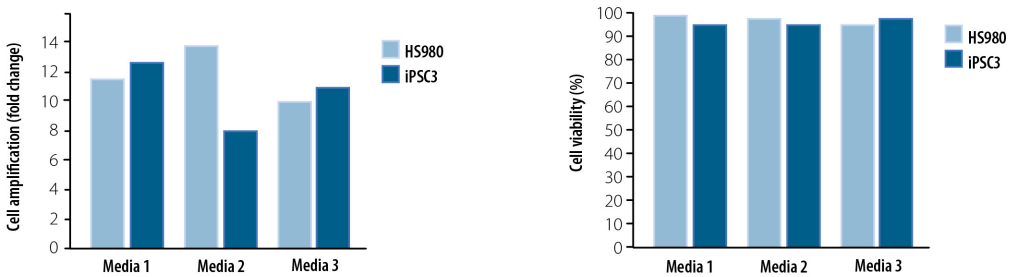
CELLS ATTACHED TO BIOSILK 521



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

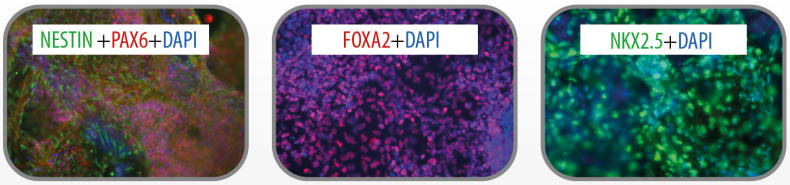
PLURIPOTENT CELLS EFFICIENTLY INTEGRATE AND PROLIFERATE IN BIOSILK 521 WITH MAINTAINED STEMNESS MARKER EXPRESSION

Human ES cells cultured in Biosilk 521 in media for culture of pluripotent cells form colonies and proliferate along the microfibres with a maintained expression of stemness marker (NANOG; red). Scale bar = 50 µm.



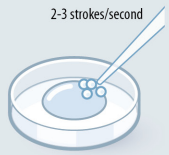
Human ES cells (HS980) and iPS cells (iPSC3) seeded at 50.000 cells/foam were cultured for 4 days in three different pluripotent cell culture media. The cells were detached with TrypLE solution and cell amount and viability was measured.

PLURIPOTENT CELLS CAN DIFFERENTIATE INTO ALL THREE LINEAGES



Human pluripotent stem cells were cultured in the Biosilk 521 scaffold for 2-3 days before lineage-specific differentiation to ectoderm (7 days, NESTIN and PAX6), endoderm (3 days, FOXA2) and mesoderm (12 days, NKX2.5). DAPI was used as a nuclear counterstain.

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.

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

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