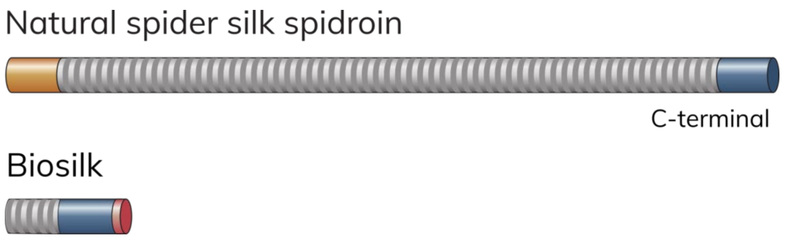
Biosilk: 3D recombinant matrix for biorelevant cell culture

BioLamina

Theresa Mader, Noah Fereydouni, Zhijie Xiao, Boris Eleuteri, and Therése Kallur

Biosilk - a recombinant spider silk protein 3D scaffold

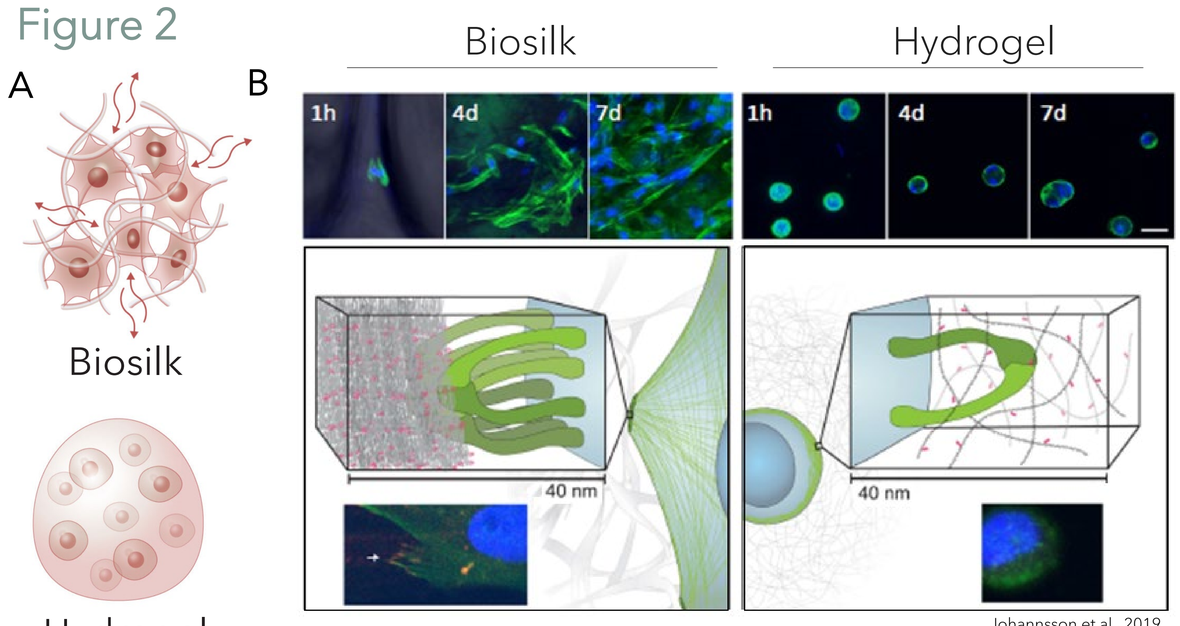
Figure 1



Biosilk is a unique biomaterial made of recombinant spider silk (Fig.1), which is easily functionalized with human recombinant laminin proteins, Biolaminin®. Laminins are tissue-specific key cell adhesion proteins of the natural cell microenvironment, providing the microfibrillar Biosilk network with functional properties for the integration, proliferation and lineage-specific differentiation of human pluripotent stem cells (hPSCs) in a 3D format.

More integrin binding sites on Biosilk vs. Hydrogels

Figure 2



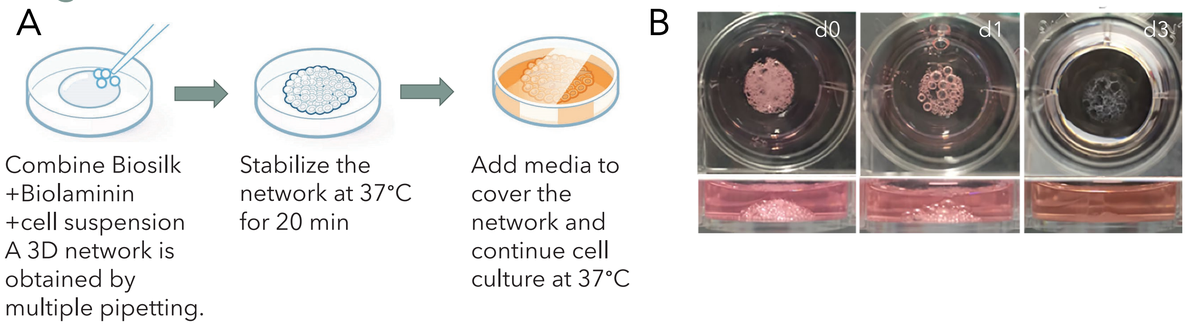
Johannsson et al., 2019

Biosilk forms a microfibrillar network for cells to adhere, proliferate and migrate more efficiently (Fig.2 A). Human mesenchymal stem cells showed physiological relevant spindle-like morphology and enhanced proliferation on Biosilk over 7 days, compared to hydrogel. This is likely a consequence of several integrin pairs to adhere to the fibers forming more local adhesion points (Fig.2 B).

Actin filaments (Phalloidin, green), focal adhesion (Vinculin, red) and cell nuclei (DAPI, blue) staining. After Johannsson et al. 2019.

Biosilk+Biolaminin network formation

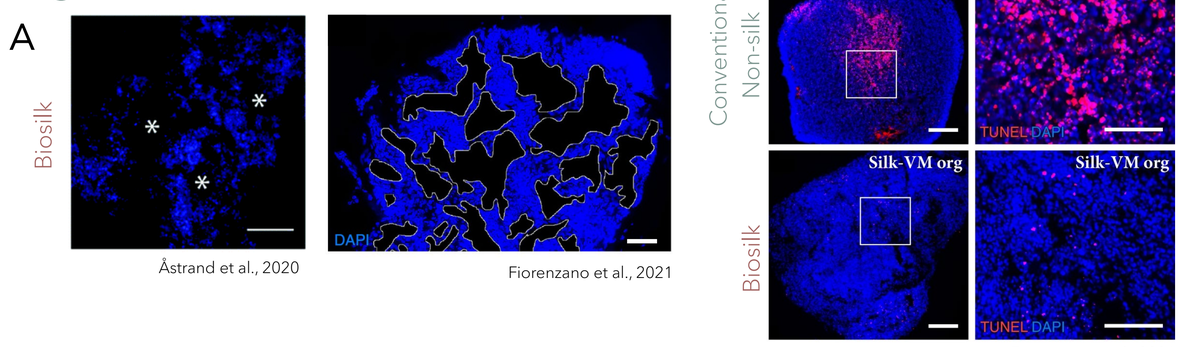
Figure 3



Graphical protocol for the creation of Biosilk+Biolaminin networks (Fig.3 A). The choice of the Biolaminin isoform depends on the tissue relevance. Representative images of the Biosilk521 networks taken on days 0, 1 and 3 after cell seeding. Over time, the bubbles will first merge and later disperse, resulting in a 3D scaffold with evenly integrated cells (Fig. 3 B).

No necrotic core - Microporous structure serve as nutrient-and oxygen distributing channels on Biosilk111

Figure 4



Åstrand et al., 2020

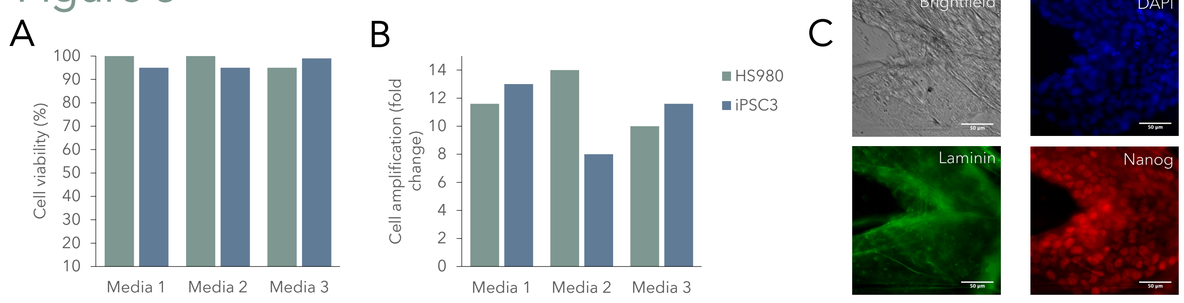
Fiorenzano et al., 2021

Biosilk networks consist of a porous microarchitecture, allowing for oxygen, nutrients and pattering factors to reach all cells (Fig.4 A). TUNEL staining of ventral midbrain (VM) organoids, at 6 months revealed less interior cell death in organoids generated with Biosilk+Biolaminin 111 (Biosilk111), compared to conventional non-silk organoids (Fig.4 B).

TUNEL (pink), DAPI (blue) staining. Scale bars, 100 µm. After Åstrand et al., 2020 and Fiorenzano et al., 2021.

hPSC proliferate and maintain pluripotency on Biosilk521

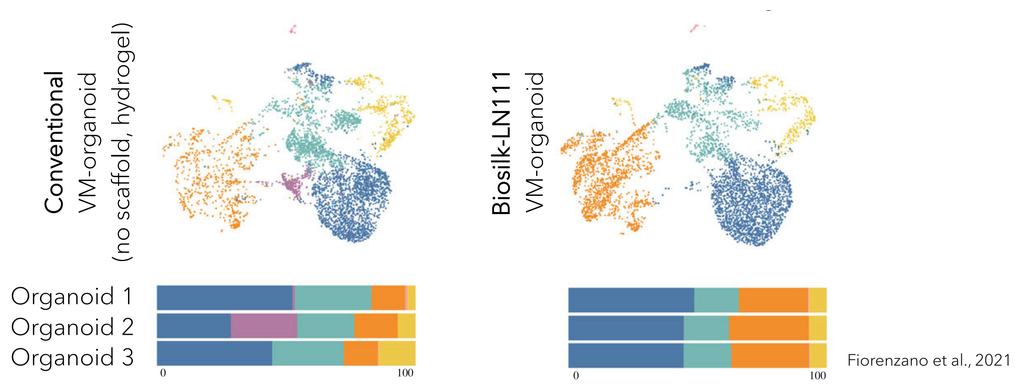
Figure 5



Human ES cells (HS980) and iPS cells (iPSC3) on Biosilk+Biolaminin 521 (Biosilk521) showed the same cell viability and amplification when cultured in different cell culture media (Fig. 5 A,B). hESC on Biosilk521 formed colonies and proliferated along the microfibers (LAMININ, green) with maintained expression of stemness marker (NANOG, red; Fig.5 C).

Less inter-and intra-organoid variation on Biosilk111

Figure 6



Fiorenzano et al., 2021

Single-cell transcriptomics identified lower variability and high reproducibility of VM organoids generated with Biosilk111, compared to conventional non-silk organoids at 1 month (Fig.6).

UMAP plots show cell clusters and bars show percentage of cells belonging to each cell cluster from individual organoids. After Fiorenzano et al. 2021.

Key advantages

- Defined and animal origin-free extracellular matrix-like network
- Efficient diffusion of oxygen, nutrients, and patterning factors
- Improved functionality and biorelevance in a 3D network
- Biocompatible, non-immunogenic, and biodegradable

References

Silk scaffolding drives self-assembly of functional and mature human brain organoids. Sozzi et al. Front Cell Dev Biol. 2022

Single-cell transcriptomics captures features of human midbrain development and dopamine neuron diversity in brain organoids. Fiorenzano et al. Nat Commun. 2021

Assembly of FN-silk with laminin-521 to integrate hPSCs into a three-dimensional culture for neural differentiation. Åstrand et al. Biomater Sci. 2020

Assembly of functionalized silk together with cells to obtain proliferative 3D cultures integrated in a network of ECM-like microfibers. Johansson et al. Sci Rep. 2019

Keep in touch
Email: **sales@biolamina.com**

BioLamina AB
Löfströms Allé 5
Stockholm, Sweden

For more information and publications, visit
www.biolamina.com