Silk scaffolding drives self-assembly of functional and mature human brain organoids

Sozzi E.[1], Kajtez J.[1], Bruzelius A.[2], Wesseler MF.[3], Nilsson F.[1], Birtele M.[1], Larsen NB.[3], Ottosson Rylander D.[2], Storm P.[1], Parmar M.[1], Fiorenzano A.[1]

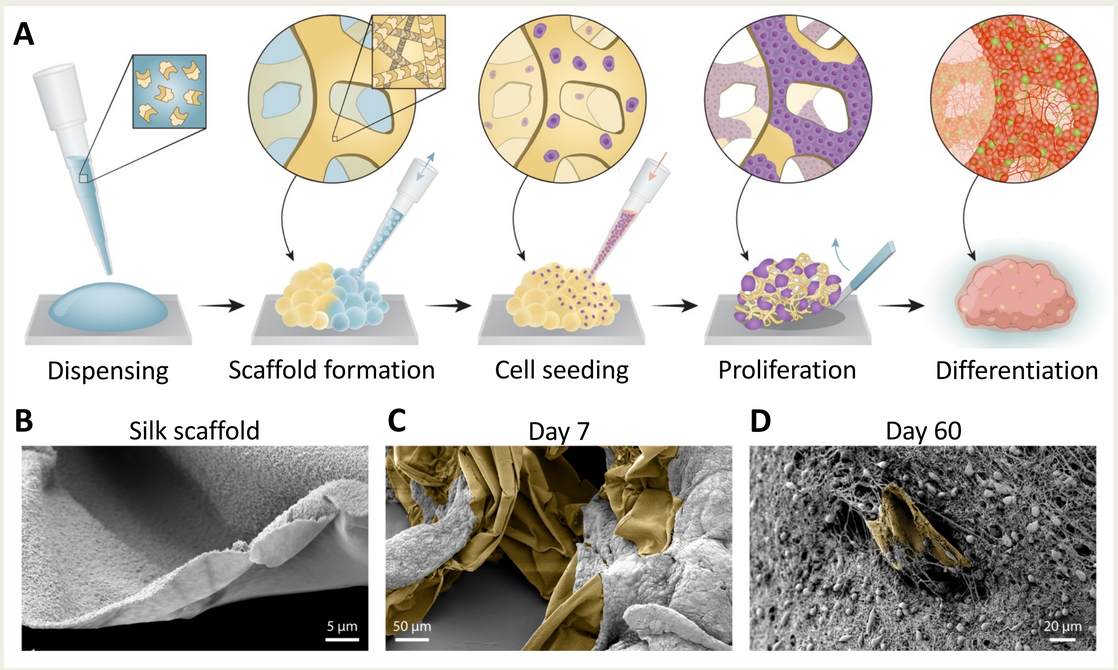
[1]Developmental and Regenerative Neurobiology, Lund Stem Cell Center, Lund University, Sweden; [2]Regenerative Neurophysiology, Lund Stem Cell Center, Lund University, Sweden; [3]Department of Health Technology (DTU Health Tech), Technical University of Denmark, Kongens Lyngby, Denmark

Background

Human brain **organoids** have rapidly become a widely used system to study brain development, thanks to their remarkable properties in modeling cytoarchitecture and cell-cell interactions that resemble the complexity of the human brain. However, current *in vitro* brain organoid methodologies often result in intra-organoid **variability** as well as incomplete maturation and **cell death** in the inner core due to cell stress and **hypoxia**.

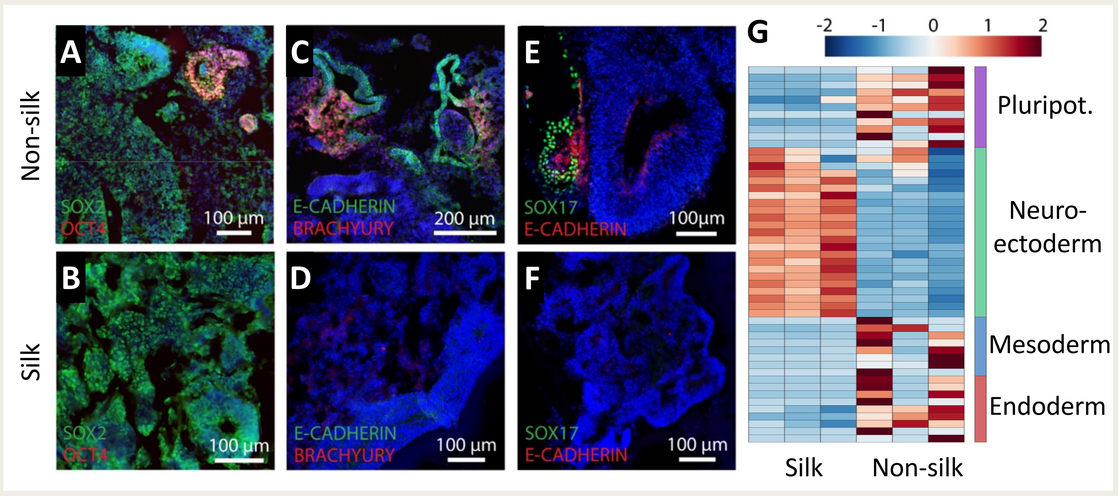
1.Generation of silk cerebral organoids

In this study we use **recombinant spider silk** proteins to generate a **3D bioengineered scaffold** that can supports human pluripotent stem cells (hPSCs) differentiation towards cerebral organoids. The aim is to create a more **favourable growth environment** by facilitating **oxygen diffusion** and promoting a more **homogeneous neural induction**, therefore reducing intra-organoid variability.



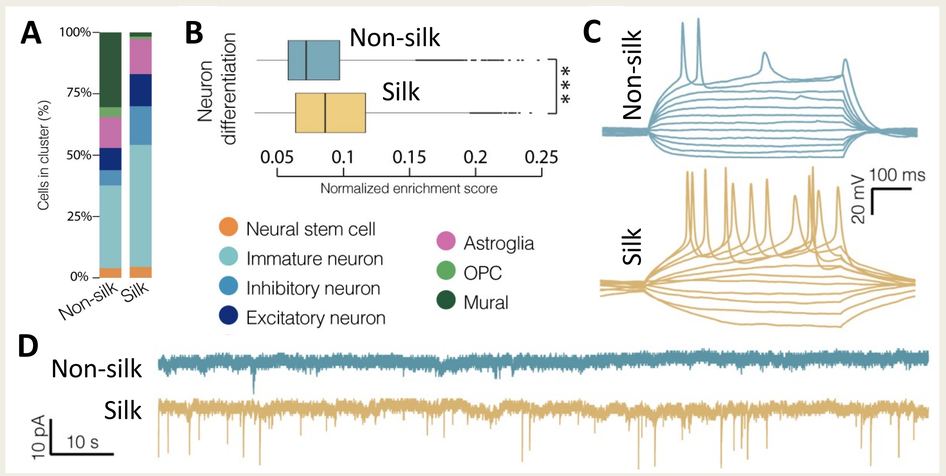
A) Overview of bioengineered silk organoid generation, including hPSCs seeding and differentiation (according to Lancaster et al., Nature Biotech, 2017). **B-D)** Scanning Electron Microscopy (SEM) pictures of silk scaffold (B) and developing silk organoids at C) day 7 and D) day60. Silk scaffold C,D) has been pseudo-colored in gold, cells in grey.

2.Increased neurogenesis in silk organoids



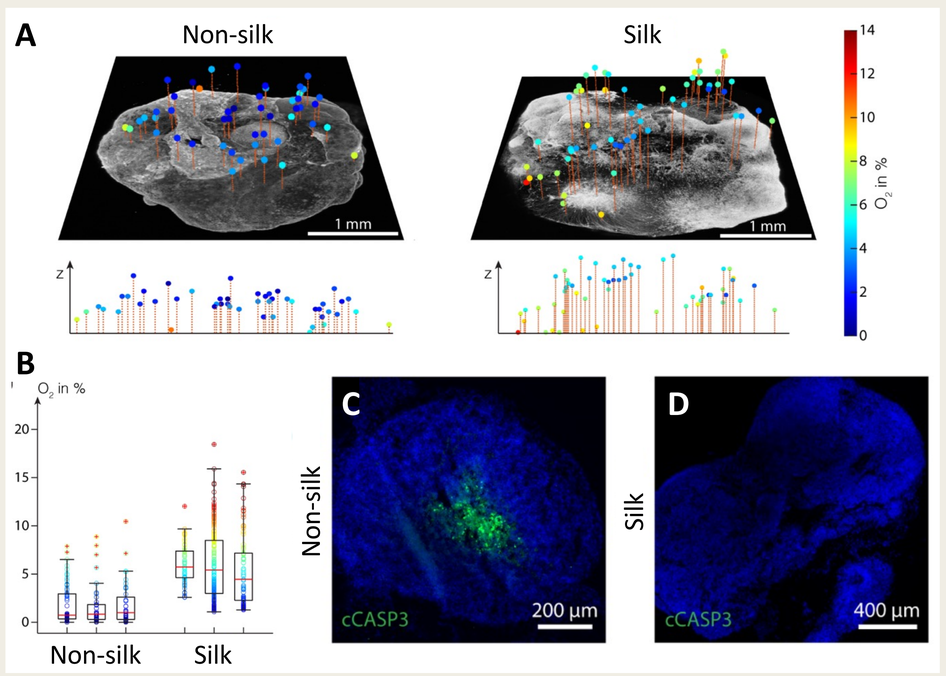
A,B) Immunohistochemistry of pluripotency markers Sox2/Oct4 in A) non-silk and B) silk organoid at day 20. **C-F)** Immunohistochemistry of C,D) E-Cadherin/Brachyury and E,F) E-Cadherin/Sox17 in organoids grown with or without silk scaffold at day 20. **G)** Heatmap of selected differentially expressed genes, considered markers of pluripotency, neuroectoderm, mesoderm and endoderm at day 20.

3.Enhanced neuronal maturation



A) Silk and non-silk organoids relative composition at day 120. **B)** Gene set enrichment analysis of the *Neuron differentiation* term in non-silk and silk cerebral organoids. **C,D)** Induced action potentials C) and spontaneous activity D) in silk and non-silk organoids after 3 months.

4.Hypoxia and cell death attenuation



A) 3D oxygen maps of non-silk and silk organoids with simultaneously acquired co-localized live cell images and their cross-sectional oxygen distribution. **B**) Oxygen distribution in 3 silk and non-silk organoids at day 60 showing average oxygenation (red line) and 25th and 75th percentile (box). **C,D**) Immunohistochemistry of cleaved caspase 3 in C) non-silk and D) silk organoids at day 120.

Conclusions

Our findings suggest that the introduction of a 3D silk scaffold in human cerebral organoids:

- Enhances neural induction and reduce the spontaneous differentiation of hPSCs towards non-neuronal cell populations.
- Sustains the generation of mature neurons
- Attenuates oxidative stress and hypoxia
- Increases cell survival in the inner core

In summary, our approach provides a novel platform based on spontaneous hPSC differentiation that can be used to better study key aspects of human brain physiology in a dish.

Contacts

Edoardo Sozzi , PhD student: edoardo.sozzi@med.lu.se
Dr. Alessandro Fiorenzano: alessandro.fiorenzano@med.lu.se
Prof. Malin Parmar: malin.parmar@med.lu.se