Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary information

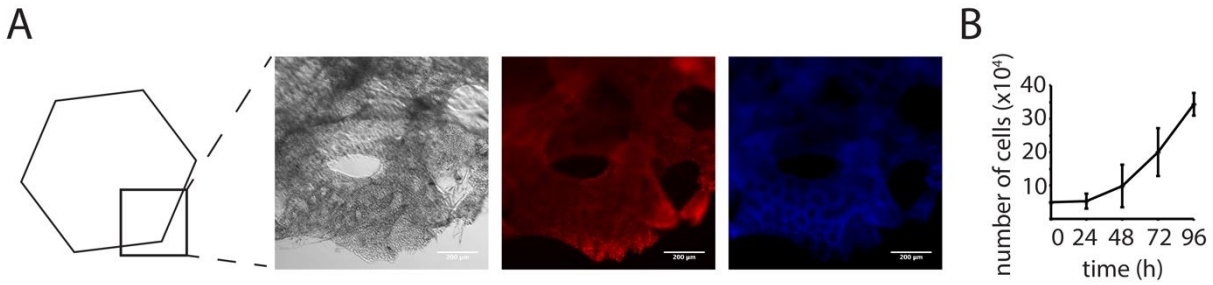

Fig S1. hESC growth in FN-silk/LN-521. (A) Representative section of FN-silk/LN521 at 72h after seeding 50 000 cells, visualized in bright field (left). Immunofluorescence analysis of pluripotency marker NANOG (red, middle) and DAPI (blue, right). Scale bar = 200µm. (B) Cell expansion quantified using Hoechst-based DNA quantification (Sigma Aldrich) and plotted as number of cells/foam over time. The data represents the mean ± SD for three cultivations (n=3).

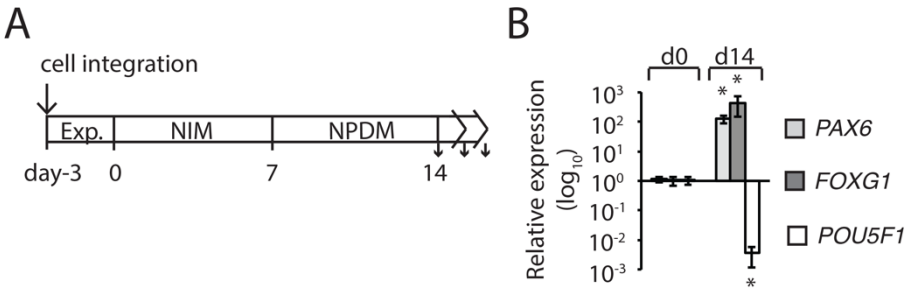

Fig S2. Gene expression of developing neuroectoderm in FN-silk/LN-521 foam. (A) Schematic illustration of the protocol for neuronal differentiation in FN-silk/LN-521 foam. hPSCs were integrated into the foam (large arrow), expanded (Exp.) in medium supporting pluripotency for 72h and cultured in neural induction medium (NIM). The induction medium was replaced at day 7 by a neuronal progenitor differentiation medium (NPDM). Cell samples were harvested for analysis at day 14 or later (small arrows). (B) Relative gene expression of *FOXG1*, *PAX6* and pluripotency marker *POU5f1* at day 0 and day 14, analysed by RT-qPCR. The data represents the mean ± SD for four or five cultivations (n=4,5), * p<0.05 compared to day 0 before induction.

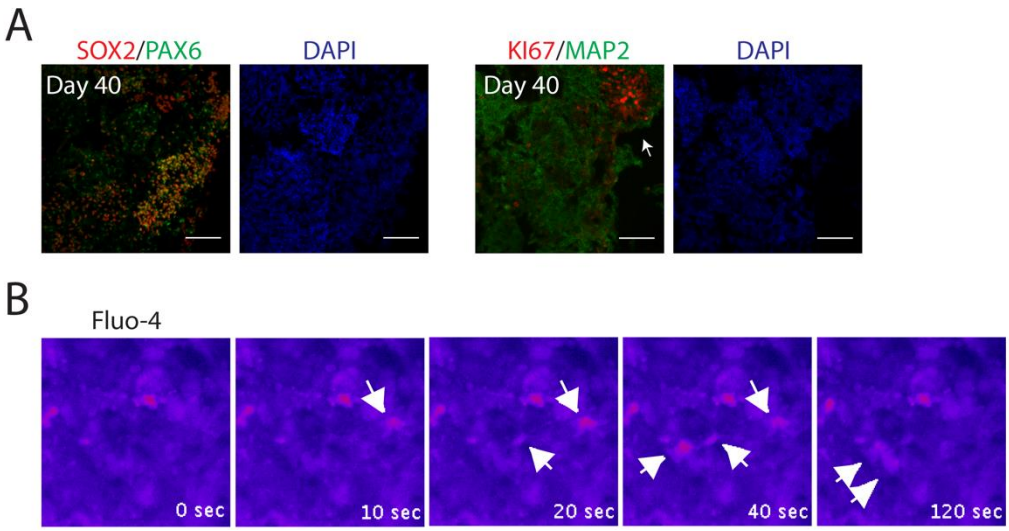

Fig S3. Dividing neural progenitors within the inner parts of the FN-silk/LN-521 supported culture at day 40. (A) Immunofluorescence analysis of cells within FN-silk/LN-521 foam after 40 days of differentiation in static culture. Left: Interspersed PAX6⁺ and SOX2⁺ cells in horizontal sections. Right: Zone of packed KI67⁺ cells (red), indicated by white arrow surrounded by post-mitotic MAP2⁺ cells (green) residing in the FN-silk foam. DAPI in blue. Scale bars = 100µm. (B) Time course

of live calcium imaging (Fluo-4) of progenitors differentiated for 40 days in FN-silk/LN-521 foam. Sequential calcium releases were visualized by spectra (imageJ). Arrowheads points at active cells. Time in seconds is shown below.

Supplementary movie1.avi

Supplementary movie 1. Calcium signalling in neurons within a FN-silk/LN-521 construct.
Live imaging of Fluo-4 in floating cultures (day 60), detecting spontaneous calcium signalling in individual neurons (white arrows). Frames taken every 2.1 s for 71 frames (over totally 2min 30 sec).