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Biomaterials to enhance the integration of grafted human iPSC-derived motor cortical neurons in a mice model of traumatic injury

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► To cite this version:

Oriane Rabesandratana, Anaïs Lainé, Marie-Laure Bonnet, Laure Belnoue, Sébastien Brot, et al.. Biomaterials to enhance the integration of grafted human iPSC-derived motor cortical neurons in a mice model of traumatic injury. FSSCR annual meeting 2021, Nov 2021, Montpellier, France. hal-03489108

HAL Id: hal-03489108

<https://hal.science/hal-03489108>

Submitted on 17 Dec 2021

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Abstract

The cerebral cortex is the most complex structure in the mammalian brain, organized in six specific layers. It is the target of many pathological conditions such as **Traumatic Brain Injuries (TBI)**. Cell transplantation is a potential strategy to repair the injured brain. One highly clinically relevant cortical tissue in the context of cortical lesions is the **motor cortex**, however to this date there is no reliable protocol to generate cortical neurons of motor identity. We have recently generated **motor cortical neurons from human induced pluripotent stem cells (hiPSC)**. These neurons grafted into the injured motor cortex allowed reestablishment of the damaged motor cortical pathways. To enhance graft survival, structural and functional recovery after TBI, **biomaterials** protecting grafted cells and/or supporting repair processes such as extracellular matrix substitutes are currently in development and could be a promising neurorestorative approach. To further improve *in vivo* grafts efficacy, motor cortical neurospheres were embedded with hyaluronic acid (HA)-based hydrogel complemented with maturation-enhancing factors (poly-L-ornithine, laminine, fibronectine), at day 18 of differentiation *in vitro*. In this project, we tested the impact of biomaterials on the maturation of cortical neurospheres over time *in vitro*, and on the survival of these neurons grafted into the motor cortex of a mice model of traumatic injury.

Differentiation of encapsulated neurospheres with HA-based hydrogels

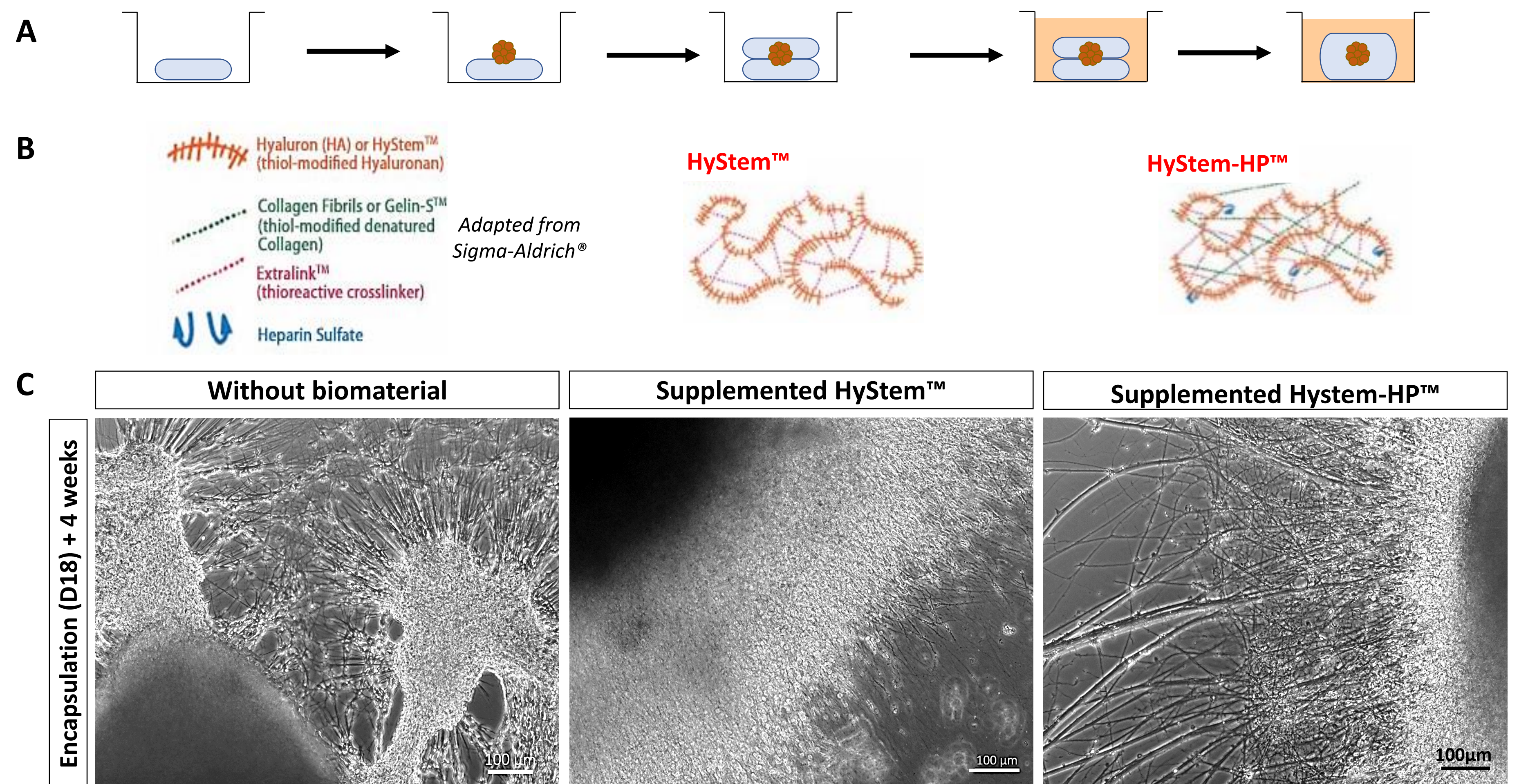


Figure 2:

(A) Schematic illustration of the encapsulation technique for hiPSC-derived neurospheres at day 18. Firstly, a liquid drop of hydrogel was seeded on the bottom of a well. One neurosphere was placed per well on the polymerized drop and immediately covered with the second liquid drop. Neural induction medium was added on top of the polymerized double-drop system for cortical maturation. (B) Representative schemes of HA-based hydrogels compositions. (C) hiPSC-derived neurospheres were encapsulated into supplemented HyStem™ or supplemented Hystem-HP™ at day 18. Cultures were observed 4 weeks after encapsulation. Encapsulated neurospheres with supplemented Hystem-HP™ showed longer distance neurite elongation and enhanced neurite fasciculation compared to supplemented HyStem™. Scale bars = 100µm.

Characterization of encapsulated hiPSC-derived neurospheres with HA-based hydrogels

2D immunofluorescence analysis

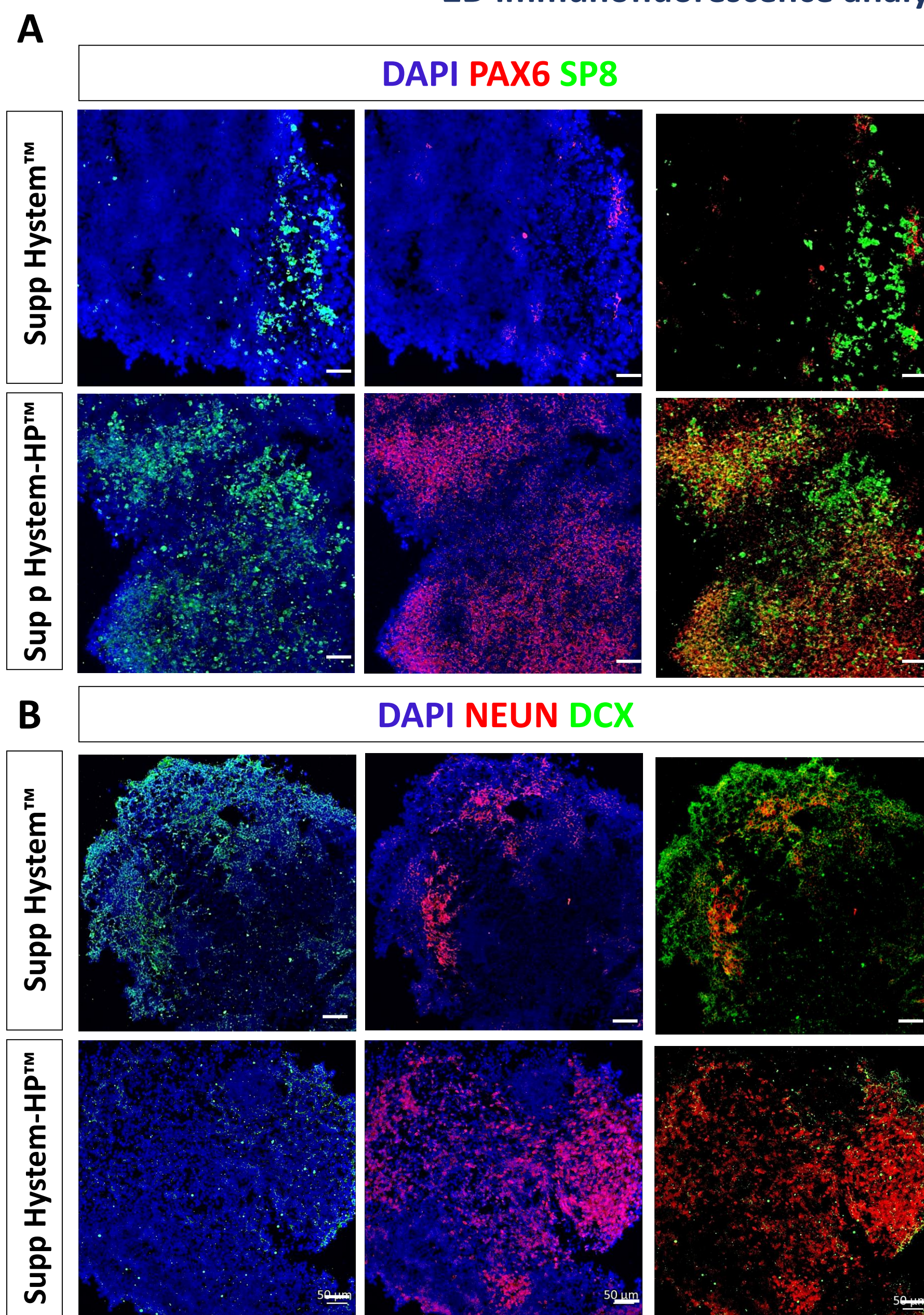


Figure 3:

Immunostaining on encapsulated hiPSC-derived neurospheres sections at day 46 of differentiation. (A) Encapsulated neurospheres in both hydrogels expressed anterior cortex identity and neuronal precursor cell identity, as assessed by SP8 and PAX6 expression patterns respectively. Preliminary results suggested an increased expression of SP8+/PAX6+ cells in supplemented HyStem-HP™ hydrogel condition. (B) In supplemented HyStem™ hydrogel condition, a majority of the neurons were still immature expressing doublecortin (DCX+) while few cells expressed NeuN (mature neuronal marker). Notably in supplemented HyStem-HP™ hydrogel condition, a majority of cells became mature, expressing NeuN marker, whereas few DCX+ immature cells still remained. Scale bars = 50µm.

3D iDISCO analysis

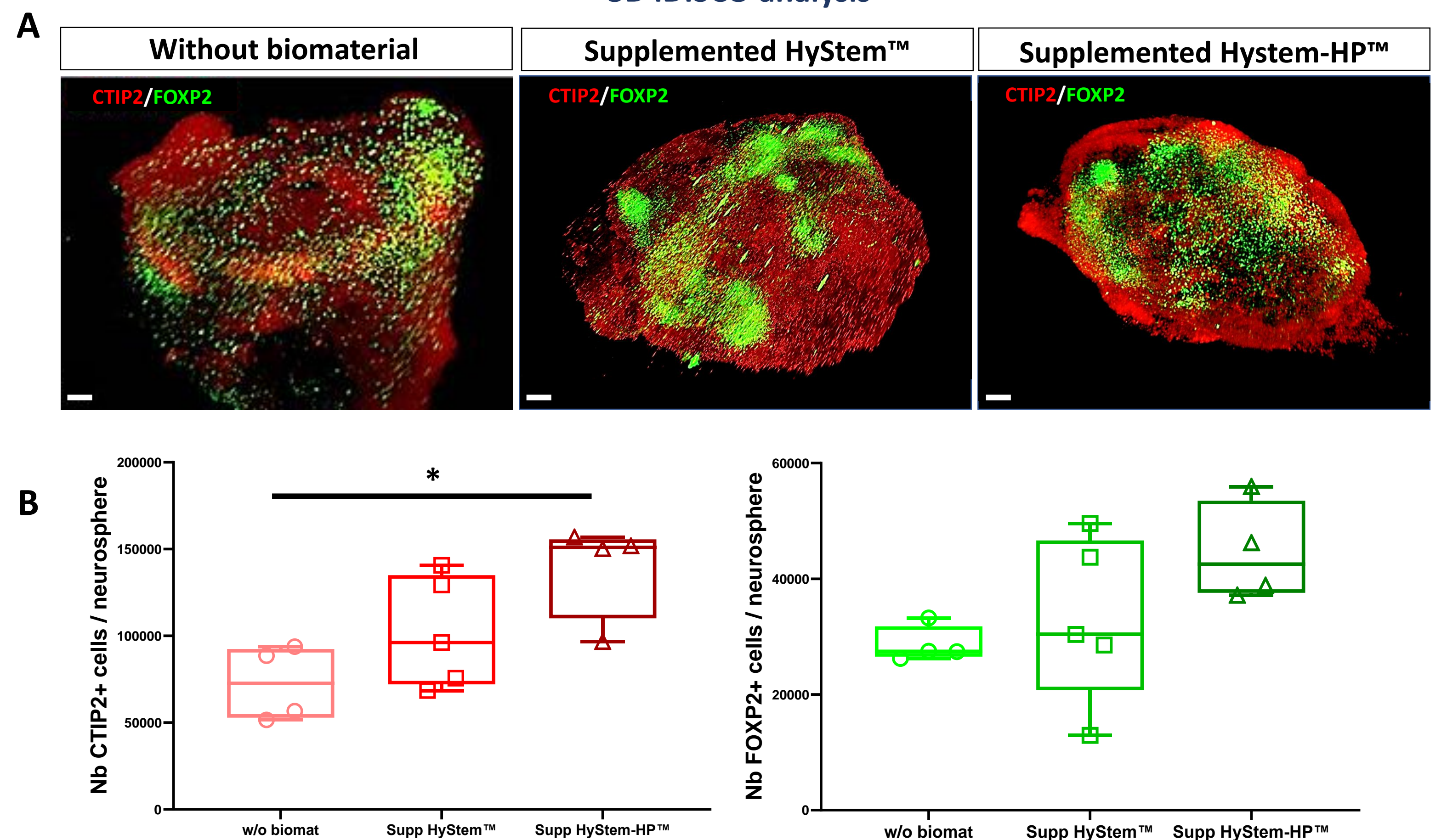


Figure 4:

(A) 3D Imaris® reconstruction of whole-immunostained neurospheres with deep layer cortical markers (CTIP2 (layer V) and FOXP2 (layer VI)), encapsulated without hydrogel, with supplemented HyStem™, or with supplemented HyStem-HP™ hydrogel at day 46. Scale bars = 100µm. (B) Preliminary quantifications of CTIP2+ (red) and FOXP2+ (green) cell populations in hiPSC-derived neurospheres in following conditions: without hydrogel, encapsulated into supplemented HyStem™, or supplemented HyStem-HP™. Neurospheres encapsulated with Supplemented HyStem-HP™ hydrogel displayed an increased number of CTIP2+ cells and of FOXP2+ cells, compared to the neurospheres without hydrogel and with supplemented HyStem™ (Ordinary one-way ANOVA test, n=13 neurospheres, *p* value = 0,0137). These findings suggest that encapsulation with supplemented HyStem-HP™ may improve the expression patterns of deep cortical layer markers in hiPSC-derived neurospheres at a mature stage of differentiation *in vitro*.

Graft integration of HyStem™-encapsulated neurospheres in lesioned mouse cortex

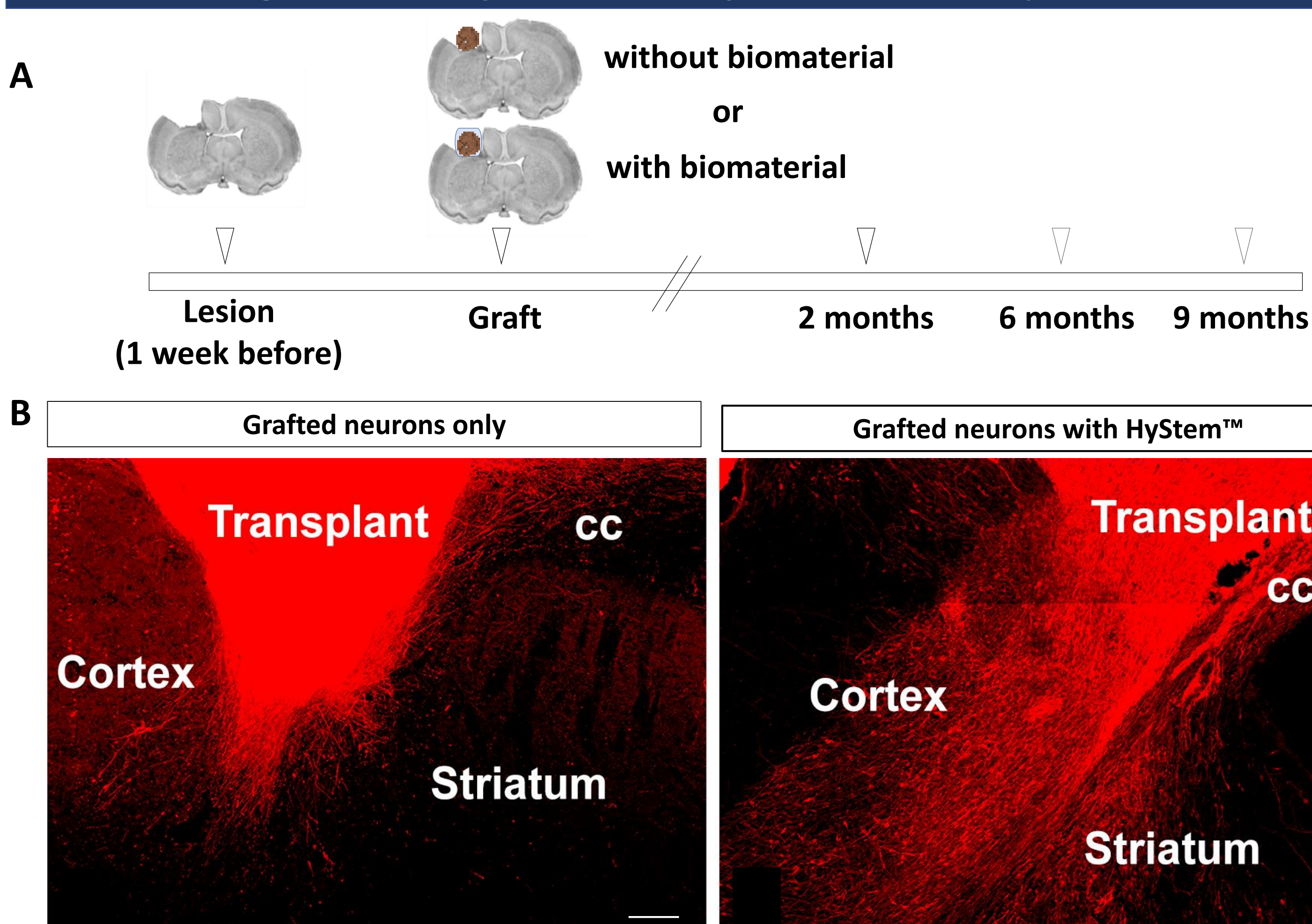


Figure 5:

(A) Representative scheme for grafting strategy of encapsulated neurospheres with or without supplemented HA-based hydrogels into lesioned mice motor cortex. Neurons are grafted a week after lesion (Péron S. et al., *Jneurosci.*, 2017). (B) Two months after transplantation, grafted neurons with hydrogel displayed an increased number of axonal prolongations, and longer-distance projections towards cortical and thalamic targets (the corpus callosum (CC) and the striatum respectively) compared to neurons grafted alone. Scale bars = 200µm.

Methods

Differentiation of hiPSC: hiPSC were cultured as floating embryoid body-like aggregates from day 0 to day 18 in the presence of WNT signaling pathway inhibitor (C59-Wnt) to induce anterior cortex (adapted from *Motono M et al, StemCellTransMed, 2016*).

Encapsulation of hiPSC-derived neurospheres: At day 18, neurospheres were encapsulated into HA-based hydrogels, using a double-drop encapsulation strategy (cf. figure 2A). Two HA-based hydrogels were tested: HyStem™ and Hystem-HP™ (Sigma-Aldrich) supplemented with poly-L-ornithine / fibronectine / laminine. Starting day 18, encapsulated neurospheres were transferred in cortical maturation adherent conditions.

2D and 3D immunostaining analysis: Encapsulated cortical motor neuron precursor cells were fixed. Immunofluorescence analysis were realized on neurospheres at day 46 for patterning and cortical markers, using either 2D cut sections, or either whole mount immunostaining (iDISCO) method. Whole mount neurospheres were acquired with 3D imaging light sheet microscopy.

Conclusion

Overall, our results show as early as two months after transplantation, an increased number of axonal projections in grafted neurons encapsulated with HyStem™. This increase could be explained by an acceleration of neuronal maturation due to the HA-based biomaterials. Experiments are underway, to study the long-term survival and functional integration of the grafted neurons.