A fully automated system for 3D scale-up of Human Neural Stem Cells

Annette Pusch[1], Andreas Elanzew[1], Simone Haupt[1], Daniel Caminada[2], Brad Justice[3], Oliver Brüstle[1,4]
[1] Life&Brain GmbH, Bonn, Germany; [2] Hamilton Bonaduz AG, Bonaduz, Switzerland;
[3] Global Cell Solutions,Charlottesville, USA; [4] Institute of Reconstructive Neurobiology, University of Bonn, Germany

Introduction: Fully automated Microcarrier based Suspension Cultivation

The increased demand for cells that more closely mimic in vivo functions require novel cell culture approaches. We developed a complete automated 3D cell culture system that we used to streamline the cell culture process of human Neural Stem Cells (hNSC) (Fig. 1):
GEMs (Global Eucaryotic Microcarriers) are microcarriers uniquely designed for cell culture. The BioLevitator supports the complete cell culture process from inoculation to harvesting. The 3D CellHOST automates parallel scale-up of different cultures at a medium-scale level.

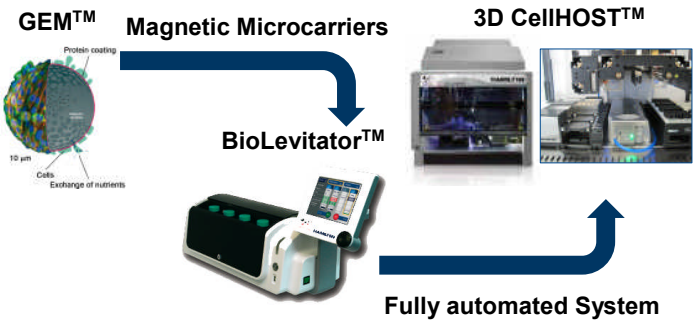

Fig.1: The 3D CellHost System. The novel fully automated 3D scale-up system (**3D CellHOST™**) employs magnetic microcarriers (**GEM™**), an integrated incubator (**BioLevitator™**) and a liquid handling Workstation (STARlet™) for automated sampling and media changes. The technology is based on levitation of microcarriers in rotating tubes (LeviTubes™) and provides optimal access to nutrients and oxygen under low sheer forces. The integrated BioLevitator ensures homogenisation of the cells / GEM suspension and provides all functions of a cell culture incubator.

Results: Fully automated 3D CellHOST mediated Expansion of Human Neural Stem Cells

1. Evaluation of 3D CellHOST mediated Expansion of human ES cell-derived Neural Stem Cultures (hESNSC)

✓ **Cultivated cells: hESNSC**

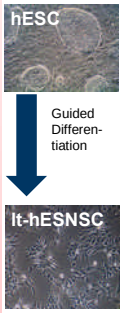

Fig.2: Long-term self renewing human ES cell derived Neural Stem Cells (lt-hESNSC). Recently we derived a neural stem cell population from H9.2 human Embryonic Stem Cells (hESC), which exhibit extensive self-renewal, clonogenicity, and stable neuro-genesis. Proliferating cells express high levels of the neural stem cell markers Nestin, Sox2, Sox1 and Pax6. Under optimal conditions only few cells differentiate spontaneously (Koch *et al.*, 2009).

✓ **3D CellHOST cultivated hESNSC yielded higher cell numbers per area than their 2D counterparts**

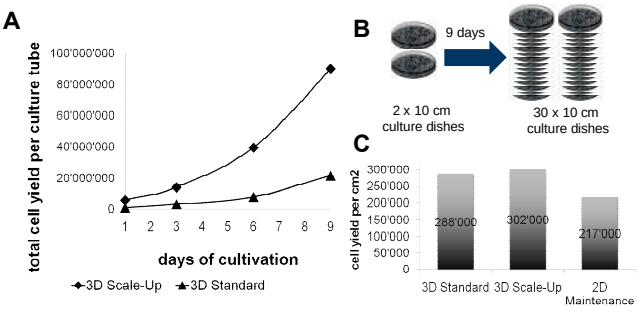

Fig.3: Growth analysis of lt-hESNSC after 3D CellHOST-mediated expansion. 2 x 10^4 / cm^2 lt-hESNSCs were inoculated and subsequently cultured for 9 days either with 0,5 ml (3D Standard) or 2 ml (3D Scale-Up) of GEM slurry per culture tube. (A) Cultures were automatically sampled at day 1, 3, 6, 9 of cultivation and total cell number per tube were calculated from those samples. (B) Inoculation of 24 x 10^6 lt-hESNSC (equivalent to two confluent 10 cm culture dishes) with 2 ml of GEM slurry per LeviTube and subsequent cultivation for 9 days within the 3D CellHOST (4 LeviTubes) yielded a 15-fold increase in cell number (equivalent to thirty 10 cm dishes). (C) The cell yield per cm^2 in the 3D CellHOST was compared to conventional 2D maintenance cultures. The determined cell number per cm^2 after 3D CellHOST mediated expansion was comparable higher as indicated.

2. Characterization of lt-hESNSCs after fully automated 3D CellHOST-mediated expansion

✓ **lt-hESNSC maintain their typical morphology and metabolic activity. Their proliferation rate under scale-up conditions is enhanced.**

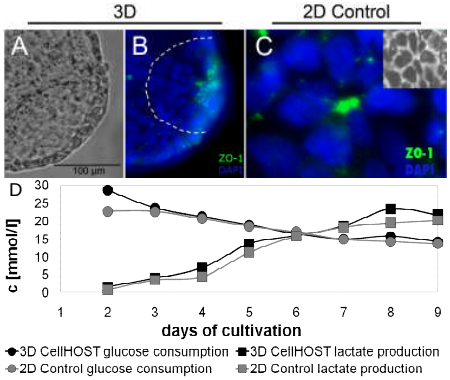

Fig. 4: lt-hESNSC maintain their typical rosette-like growth-pattern and metabolic activity. (A, B) lt-hESNSC cultured for 9 days on laminin coated GEMs maintained their typical rosette-like morphology (C) like in a conventional 2D culture centred around tight-junction protein ZO-1^+ lumens. (D) Glucose consumption and lactate production rates of lt-hESNSCs cultured by the 3D CellHOST system are comparable to those of manually cultured cells.

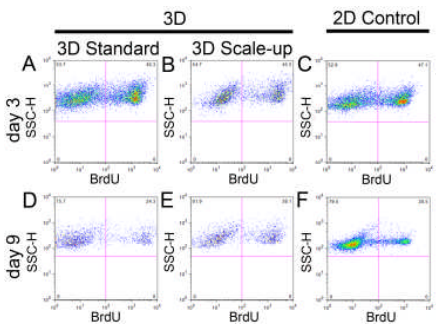

Fig.5: BrdU incorporation analysis in lt-hESNSC during 3D CellHOST-mediated expansion. lt-hESNSC were cultured under 3D standard, 3D scale-up and 2D conditions as indicated. Cell cultures were analysed at day 3 and day 9 as displayed. lt-hESNSCs were pulsed for 2 h with 10 µM BrdU. The analysis revealed comparable percentages at day two of cultivation for all cultures. Determination of BrdU positive cells at day 9 revealed more BrdU positive cells under scale-up conditions (E: 38,1 %) compared to 3D standard (D: 24,3 %) and 2D control (F: 20,5 %).

✓ **lt-hESNSC remain undifferentiated after fully automated 3D CellHOST-mediated expansion.**

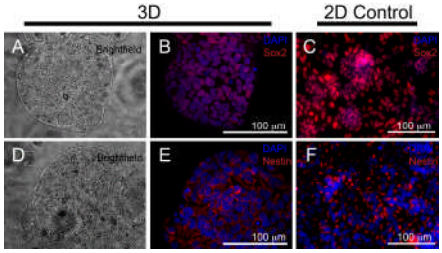

Fig. 6: Expression of the pan-neural markers Sox2 and Nestin. (A-B, D-E) According to immmuno-cytochemical analysis lt-hESNSC grown on laminin coated GEMs for 9 days maintain neural stem cell marker expression as indicated. Virtually all cells were positive for the pan-neural markers Nestin and Sox2 (C, F) like their 2D cultured counterparts.

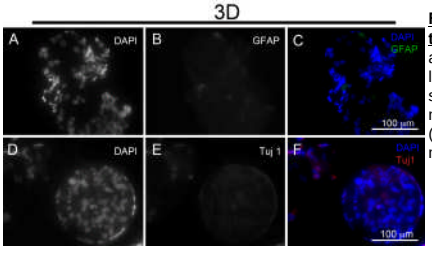

Fig. 7: Abundance of differentiated cells. Immunocytological analysis of lt-hESNSC, grown on laminin coated GEMs for 9 days showed no expression of (A-C) neuronal (TUJ1) or (D-F) glial (GFAP) markers after 3D CellHOST–mediated expansion.

Conclusion and Outlook

Conclusion: Fully automated 3D CellHOST-mediated cultivation of lt-hESNSC:

- Facilitated higher cell yields per cm^2 (Figure 3).
- Was shown to maintain typical morphological characteristics and metabolic activity of lt-hESNSC, thus demonstrating its biological compatibility (Figure 4).
- Enhanced the proliferation abilities of high-density cultures (Figure 5).
- Supported undifferentiated growth of lt-hESNSC (Figure 6 and Figure 7).

Outlook : Automated 3D cell culture opens the path towards using cells as reagents.

- Uncoupled cell culture from down stream assays.
- From freezer to assay in one step.

➔ Automated 3D CellHOST mediated culture could facilitate parallel scale-up of pluripotent Stem Cells and their differentiated progeny.

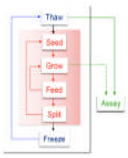

Fig. 8: Flowchart of the cells as reagent model. Automated 3-D cell culture eliminates the need for seed-split cycles and streamlines the cell culture process. It also enables the uncoupling of cell culture from down-stream assays and supports the direct use of cells from the freezer into an assay in a single step.

Reference: Koch P., Opitz T., Steinbeck J. A., Ladewig J. and Brüstle O.(2009) A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. PNAS USA *106(9)*:3225-30