Electronic Supplementary Material (ESI) for Lab on a Chip

Microfluidic Construction of Minimalistic Neuronal Co-Cultures

Electronic Supplementary Information

Materials and Methods

Neuron Culture

SH-SY5Y Human Neuroblastoma Cells

Differentiated SH-SY5Y human neuroblastoma cells (DSMZ, Germany) were predominantly used in this study. These cells adhere to PL coatings and are rejected by PLL-*g*-PEG adlayers and therefore suitably mimic the adhesion characteristics of primary neurons and neuronal precursor cell types. Cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) foetal bovine serum (FBS), and 1% (v/v) penicillin and streptomycin at 37°C in a 5% CO_2 humidified atmosphere. Differentiation was achieved by the addition of 10 μM *all trans* retinoic acid (Sigma-Aldrich) for 4-5 days. This also reduces cell clustering to favour single cell arraying. Cells were harvested using a mixture of 0.5% (w/v, PANBiotech) trypsin and 1 mM EDTA, and resuspended in media with 10 μM retinoic acid to a density of 1-2 x 10^6 cells/mL for microfluidic arraying and cultured at 37°C in a 5% CO_2 humidified atmosphere.

Lund Human Mesencephalic (LUHMES) Cells

LUHMES cells were cultured on surfaces coated by incubation with 50 μg/mL poly-*L*-ornithine (PLO, Sigma Aldrich) and 1 μg/mL fibronectin (Sigma Aldrich) for ≥3 hours at 37°C followed by a distilled water wash step. LUHMES cells were cultured in advanced DMEM/Ham's F12 (Gibco Invitrogen) supplemented with 2 mM *N*-acetly-*L*-anayl-*L*-glutamine, 1% (v/v) N2, 40 ng/mL FGF and 1% penicillin/streptomycin at 37°C in a 5% CO_2 humidified atmosphere. Differentiation was achieved by replacing the media and culturing for 2 days in differentiation media (advanced DMEM/F12 supplemented with 2 mM *L*-glutamine (PANBiotech), 1% (v/v) N2, 1 mM cAMP (Sigma-Aldrich), 2 ng/mL glial cell line derived neurotrophic factor, 1 μg/mL tetracycline (Sigma-

Aldrich) and 1% penicillin/streptomycin. Following the pre-differentiation period cells were harvested using 0.125% trypsin (w/v) before resuspension in differentiation media to a concentration of 1.5-2.0 x 10^6 cells/mL for arraying on CNA microfluidic devices coated with PLO and fibronectin. Arrayed neurons were cultured at 37°C in a 5% CO_2 humidified atmosphere. An additional 3 days in chip culture was required for complete differentiation.

Mouse Cortical Neurons

All experiments were conducted in accordance with national laws for the use of animals in research and approved by the local ethics committee. Pregnant C57BL/6N mice were anesthetized with CO_2 and sacrificed by cervical dislocation 16 days after conception. Mouse embryos (E16) were harvested and put on ice in Hank's balanced salt solution (HBSS, Life Technologies), decapitated. After removing the meninges, cortices were minced and transferred to fresh HBSS buffer. Material was disaggregated into single cells using 0.25% (w/v) trypsin in HBSS buffer and incubated for 10 minutes at 37°C, followed by reaction arrest using 0.25 mg/mL (final concentration) soybean trypsin inhibitor (Life Technologies) along with 0.01% (w/v) DNase (Sigma-Aldrich). A cell suspension was prepared by sequential trituration (15-20 times) using three fire-polished Pasteur pipettes with decreasing diameters. Cells were then centrifuged at 200 *g* for 5 minutes and the pellet was resuspended in neurobasal medium containing 2% B-27 serum free supplement, 0.1% gentamicin (PANBiotech) and 0.5 mM stabilized glutamine (PANBiotech). A 2 x 10^6 cells/mL cell suspension was used for microfluidic arraying, followed by culture at 37°C in a humidified 5% CO_2 atmosphere.

Electronic Supplementary Material (ESI) for Lab on a Chip

Figure Legends

ESI Figure 1. Alternative CNA circuits with continuous neurite guidance channels. A 2-compartment circuit (A): The path 0→1 has a lower fluidic resistance than the serpentine path, 0→2, to direct neuron arraying. Following cellular valving (adhesion and flattening) of the first population, a second cell population can be arrayed, from top to bottom, using path 1→0, with path 1→3 serving as the bypass channel. A 3-compartment system (B): Neurons can be arrayed in the flanking compartments using paths 0→1, with the paths 0→2 serving as bypass channels. Following cellular valving neurons can be arrayed in the central compartment using paths 1→0, with path 1→3 serving as the bypass channel. Neuron arraying can also be achieved in the reverse order.

ESI Figure 2. A syringe was interfaced to the upper three ports using a cross union to trifurcate tubing sections of equal length for uniform neuron arraying.

ESI Video 1. Time lapse video of the development of a water mask by evaporation. A red dye was used to visualize the water mask.

ESI Figure 3. Undifferentiated (A) and 4 day RA-differentiated (B) SH-SY5Y neurons cultured on planar patterns (inset) of FITC-labelled PLL (green) and TRITC-labelled PLL-*g*-PEG (red). Images recorded following 5 days of patterned culture. Undifferentiated SH-SY5Y cells had an E_{patt} of 94% and differentiated SH-SY5Y cells had an E_{patt} of 87%. Patterns were prepared on cleaned and plasma-treated glass substrates incubated with fluorescein-conjugated PL (PL–FITC, 100 μg/mL, Sigma-Aldrich) in PBS buffer for 5 minutes and rinsed in water. A PDMS stencil with 250-μm-wide and spaced microchannel features was used for 1 minute oxygen plasma patterning, followed by a 5 minute incubation for back-filling with rhodamine-conjugated PLL-*g*-PEG (PLL-*g*-PEG–TRITC, 100 μg/mL in PBS, Surface Solutions, Switzerland) followed by a PBS and water wash sequence. In this experiment, a 1 mL suspension of 2 x 10^5 SH-SY5Y cells (passage 18) were seeded overnight, and the following day washed with PBS and replenished with fresh media.

ESI Video 2. SH-SY5Y neuron arraying using the CNA microfluidic circuit.

ESI Figure 4. Larger images of arrayed SH-SY5Y neuron-like cells in the CNA device (A,B). The meniscus-pinning micropillars have been outlined to distinguish these from the single neurons. Higher magnification image of optimally arrayed SH-SY5Y cells (C) and LUHMES cells (D) imaged 4 hours after arraying.

ESI Figure 5. Cell body deformation resulting from prolonged (30 minutes), low pressure microfluidic arraying by gravity-driven feed. All neurons are deformed with notable examples highlighted.

ESI Figure 6. Sub-optimal poly-lysine patterning by plasma stencilling with a water mask (A). PL-FITC remains intact on the outer wall of the microchannel, which prevents coating with PLL-*g*-PEG-TRITC (B).

ESI Figure 7. Examples of unguided neurite outgrowths bridging the central channel.

ESI Figure 8. Simulation of pressure (A) and velocity (B) distribution during balanced hydrostatic driven flow. Although the pressure difference between central and flanking channels is small (<2 Pa) this generates outward flows through the neurite outgrowth channels (~5 μm/s).

ESI Figure 9. HDF treatment of the central channel contaminates the flanking channels within 1 minute (A). The flanking channels remain fluidically isolated for ≥24 hours (B) when using a hydrostatic-driven flow. The boundaries of the central channel are highlighted using white lines. The treatment flow was doped with 10 μM FITC.