NEURITE GUIDANCE THROUGH 3D HYDROGEL LAYERS IN A MICROFLUIDIC ENVIRONMENT

Anja Kunze, Robert Meissner and Philippe Renaud

Laboratory of Microsystems, Ecole Polytechique Federale de Lausanne, SWITZERLAND

ABSTRACT

Neuronal cell culture models *in vitro* are often restricted to 2D surfaces. Engineering the complexity of the neuronal microenvironment in microfluidic systems can help to generate more tissue like cultures. We have developed a new neuronal cell culturing system based on a microfluidic device that can culture primary neurons in a 3D patterned hydrogel based microenvironment. Perpendicular to the culture channel a chemical gradient was established to guide neurites. Neurons cultured under a 62.5 ng (ml mm)$^{-1}$ nerve growth factor (NGF) gradient up to 9 days *in vitro* (DIV), extended their neurites through the hydrogel in the direction of the higher concentration.

KEYWORDS: patterned 3D cell culture, neurons, hydrogel, microfluidic, chemical gradient, neurite guidance

INTRODUCTION

The network formation in our brain plays an important role for our learning capacity; however neuroscientists cannot fully describe the guiding mechanism for neurites. Today, we know that the guidance of neurites can be achieved by chemical cues or mechanical structures. For chemical neurite guidance, laminin [1], NGF or netrin [2 and 3] are well known molecules. However, the cited studies are showing only neurite guidance in microenvironments restricted to 2D surfaces with bounded or diffusive gradients, whereas the natural environment guides the neurites through 3D formations. In this abstract we present a new microfluidic device, which can be used to pattern a 3D hydrogel matrix with embedded primary cortical neurons and guides the neurite outgrowth through a concentration gradient of nerve growth factor.

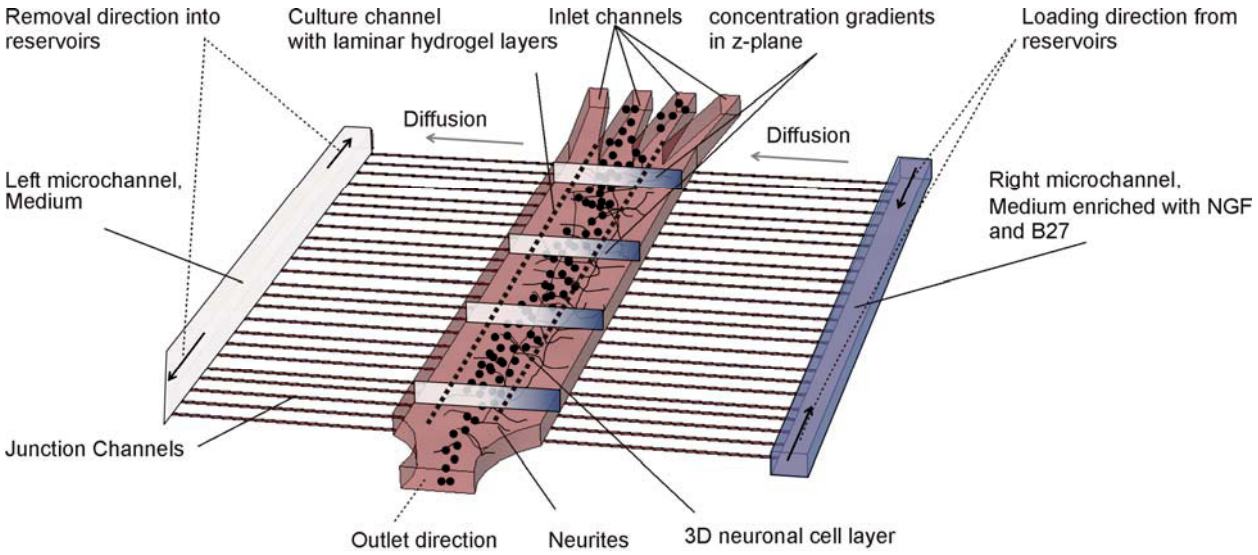

Figure 1: Drawing of the microfluidic device with embedded neuronal cell culture pattern in the cell culture chamber and the generation of a NGF gradient through the cell culture with reservoir channels

THEORY

The microfluidic device is fabricated by molding PDMS over a silicon master. The device, as demonstrated in figure 1, consists of four inlet channels, which are leading into a main culture channel. Parallel to the culture channel are two side microchannels. The microchannels are connected through junction channels perpendicular to the culture channel. The junction channels are 10 µm high and 1 mm long. The concentration difference δc between the left and the right microchannels generates transient chemical gradients across the junction channels and through the hydrogel layers with a distance W. To keep the handling simple for the cell culture no perfusion is used. The concentration gradient is transient, based on the 2nd fick's law (equ. 1) considering a different diffusion coefficient D_{HG} of nutrients in the culture channel through the hydrogel as described in equation 2.

$$\frac{\partial c}{\partial t} = D_{HG}\frac{\partial^2 c}{\partial w^2} \qquad (1)$$

$$D_{HG} = 0.5 \cdot D_{H_2O} \qquad (2)$$

To keep a diffusion flux as long as possible, the parallel microchannels are connected to 20 µl reservoirs. From experimental data shown in figure 2a, achieved with fluoresceine, we extracted the concentration profiles over time as plot-

ted in figure 2b. In the COMSOL model we adapted the diffusibility in the hydrogel area in the main channel according to equation 2 with D_{H2O} = 640 µm²/s [4]. In figure 2c the transient formation of the NGF concentration profile is simulated up to 5 days, assuming that the NGF is transported as fluoresceine through the hydrogel area.

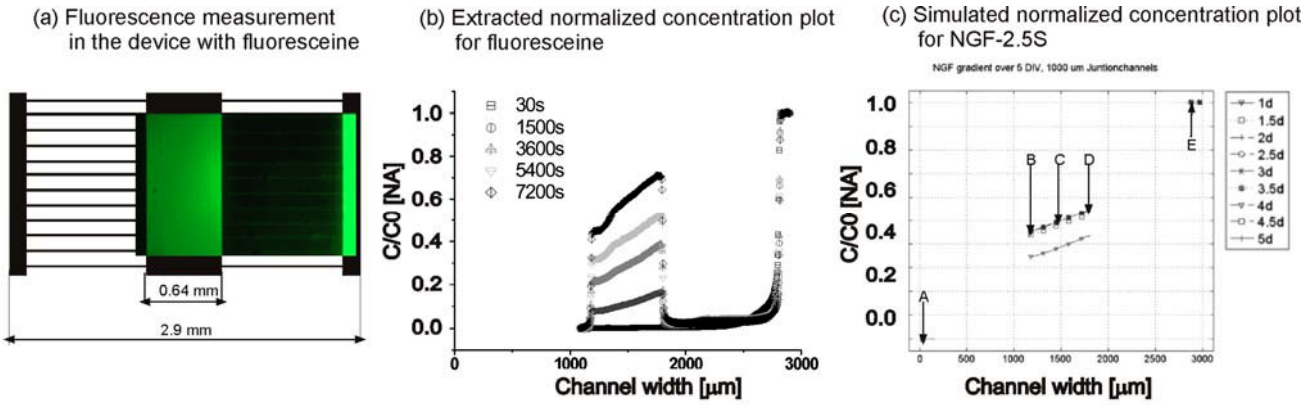

Figure 2: The chemical gradient in the microfluidic device. (a) Merge top view of channel design and fluorescence image after 2h. The gradient was generated with fluoresceine. (b) Concentration plots measured at different times, (c) Concentration plots for NGF-2.5S simulated with COMSOL for every half day up to 5 days.

EXPERIMENTAL

For the cell culture experiment the device was autoclaved and plasma bonded to a cover slip. The PDMS and glass walls were coated with poly(ethylenimine). Primary neuronal cells were extracted from E19 embryonic rats based on standard protocols with papain. The 3D patterned hydrogel matrix with embedded primary cortical neurons was generated by injecting the liquid hydrogel solution at 37 °C with either cells or just medium in the desired inlet channels. The hydrogel solution consisted of 0.5 % (w/v) agarose matrix with 0.3 % (w/v) unbounded alginate and was autoclaved before use. The parallel hydrogel layers were formed in the culture channel at a flowrate of 200 nl/s. The flow is stopped by cooling down the 3D patterned matrix under less than 26 °C. Before incubation, Neurobasal medium enriched with nutriments (12% (v/v) B-27) and 0.4 µg/ml NGF-2.5S) was injected in the right microchannel and only medium in the left microchannel. The medium in the reservoirs and the microchannels has been refreshed every other day. From the simulation plot in figure 2c we calculated for five different positions in the microfluidic device the following concentrations of ingredients in table 1. Where A and E are located in the microchannels, B - C are positions in the culture channel, where neuronal cells are cultured in the hydrogel pattern. According to table 1 neuronal cells have been exposed to a NGF gradient of 62.5 ng (ml mm)$^{-1}$ and a B27 gradient of 1.875 (v/v) % mm^{-1} through the patterned 3D neuronal cell culture.

Table 1. Composition of hydrogel layers and perfusion medium

Ingredients	**Channel positions**				
	A	**B**	**C**	**D**	**E**
Agarose (w/v %)	0	0.5	0.5	0.5	0
Alginate (w/v %)	0	0.3	0.3	0.3	0
Neurobasal (v/v %)	100	92	85.2	90.4	84
B27 (v/v %)	0	5.4	6	6.6	12
NGF-2.5S (ng/ml)	0	180	200	220	400
Neuronal cells (v/v %)	0	0	6	0	0

RESULTS AND DISCUSSION

The neuronal cells are cultured up to 9 DIV. The visibility of neurites indicates cell viability until the last culture day. Figure 4 shows the response of the neurite outgrowth with an enriched neurite density in the direction of the higher NGF and B27 concentration after 9 DIV. Analyzing the growth behavior at 2, 5, 7 and 9 DIV in figure 4, we observed a directional neurite growth initially after 5 DIV.

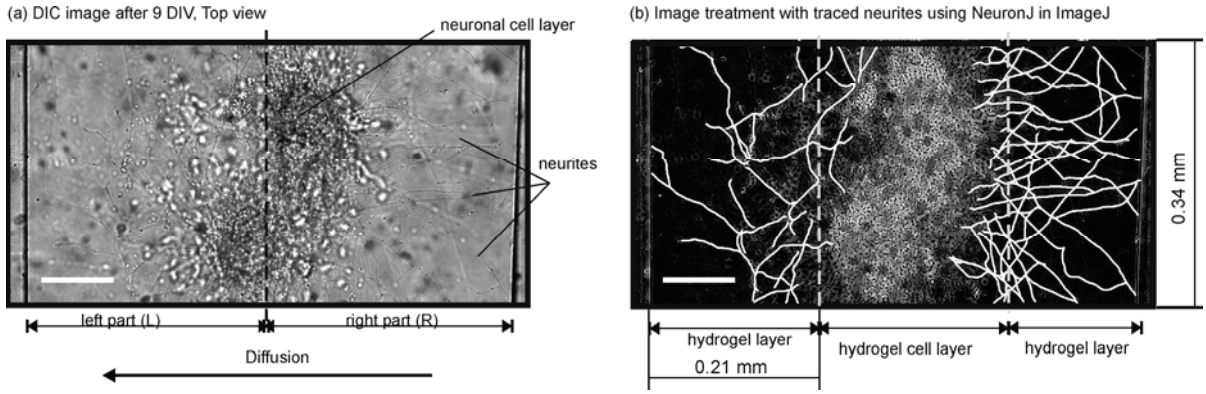

Figure 3: Neurite extension in the direction of a higher NGF gradient. (a) Top view image of primary neurons patterned in the middle of four laminar hydrogel layers in the culture channel after 9 DIV. The differential interference contrast (DIC) image shows more neurites extending in the right part of the cell culture than in the left part. (b) Image processing with FFT high pass filter in ImageJ to highlight neurites for post processing with NeuronJ.

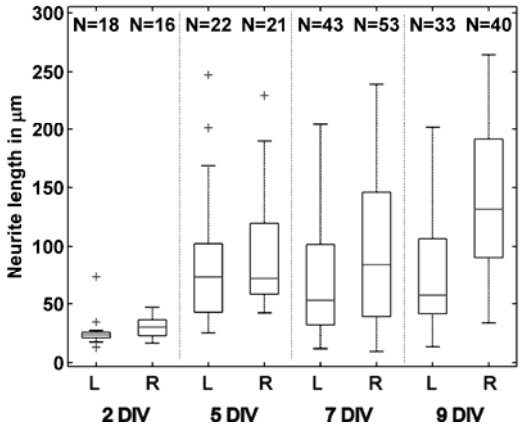

Figure 4: Box plots of neurite lengths after different days in vitro, extracted from the left (L) and the right (R) hydrogel layer. N is the number of neurites traced in a 340 µm x 320 µm (h x w) image area.

CONCLUSION

We have developed a new neuronal cell culturing system in a microfluidic device that can culture primary neuronal cells in a pattern 3D microenvironment. With this device we are able to control multiple parameters including the nutrient supply, the location of sources and sinks and the concentration of guidance cues. We think this device can have a great potential for engineering the complexity of neuronal tissues in microfluidic systems and can help to test drugs against neurodegenerating diseases on more tissue like cultures.

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CONTACT

*A. Kunze, tel: +41-21-6836839; anja.kunze@epfl.ch