Loading single neurons on a microsieve electrode array by passive pumping

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Recent advances in brain on a chip technology has led to the development of modified microelectrode arrays, for example, introducing mushroom shaped electrodes[1]. Here, we study a passive pumping mechanisms for the loading of cells into a microsieve technology platform by means of optical particle tracking (Fig 1). Previously, we have contributed to this exciting field of neuroscience by the development of a fabrication process for microsieves that contain 3D-micropores, with highly uniform apertures that allow us to capture hundreds of individual cells in parallel and directing the cells onto the electrode[2]. In brief, the fabrication process consist of a silicon sieving structure obtained by corner lithography with a patterned boron doped poly-silicon, connecting the contact electrodes with the 3D sensing electrodes in the pores. A LPCVD silicon-rich silicon nitride layer was used as insulation as described by Schurink et al[3] and this new technology platform for multi-site electrophysiology recordings was termed microsieve electrode array (µSEA) (Fig 2). However, trapping the neurons reproducibly under gentle, biocompatible conditions remains a challenge as the current set up involves the use of a hand-operated syringe assembled with a PDMS construct for sealing the syringe inlet to the back of the microsieve chip (Fig. 1). The latter, can lead to cell damage and deloading of cells when unplugging the syringe and PDMS constructs. Although, we achieved an efficient trapping of neurons within these types of sieves of 80-90%[4], cell culture performance varied significantly.

Due to simplifying the handling procedure, omitting the use of a syringe and PDMS constructs for this application with the goal to improve the µSEA performance, the set-up is compatible with real time microscopy techniques. Hence, we can visualise the particles (and subsequently the cells) and record particle velocities by video imaging. Analysing the particle velocities we can confirm a uniform particle flow towards the 3D pores of the microsieve. Here, we used software recognition tools to track the particle movements under passive pumping. Mosaic particle tracker was used with a setting of 1.5 µm, a cut-off of zero for non-particle discrimination and a percentile value of 0.6 for particle brightness. The displacement was kept at 2 (maximum pixel movement) and the link range at 10 frames. A typical tracking example is shown in Fig 3a. We show that passive pumping particle velocity can be tightly controlled (from 5 µm/s to 7.5 µm/s to 10.4 µm/s) simply by changing the droplet volume of the pumping droplets from 30 µL, 40 µL and 60 µL and keeping the reservoir drop constant (Fig 3b). This leads also to reproducible average particle speeds (+- 1 µm/s) and also guarantees that this gentle trapping procedure will benefit cell survival. Next we will characterize the reproducibility and efficiency of neuron capture onto the electrically functionalized 3D pores of the microsieve by applying this passive pumping mechanism.

References:

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3. B. Schurink et al: Fabrication and characterization of microsieve electrode array (µSEA) enabling cell positioning on 3D electrodes. J. Micromech. Microeng. 27 (2017).
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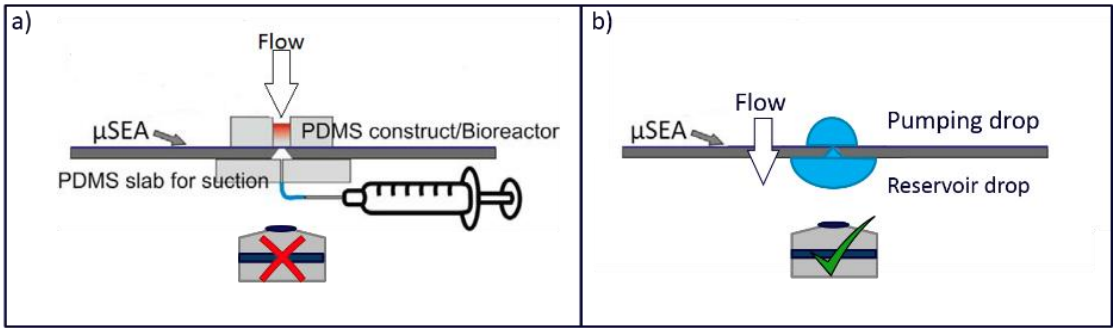

Figure 1: (a) The original trapping protocol showing the PDMS construct, direction of flow, the µSEA, the PDMS slab and syringe for suction which is not compatible with a microscopy. (b) The passive pumping trapping protocol showing the pumping and reservoir drops, direction of flow, the µSEA and the ability to visualize the whole process under a microscope.

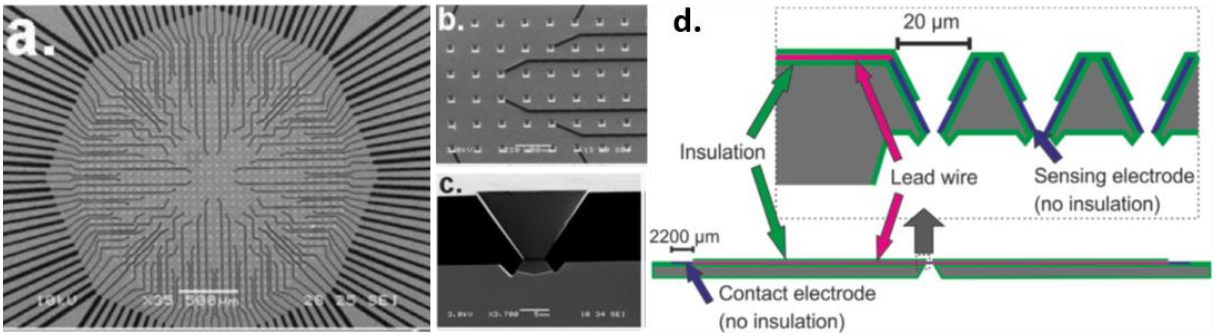

Figure 2: Scanning electron microscope images of the patterned and boron doped poly-silicon. The poly-silicon pattern forms the electrode layer (a) consisting of contact electrodes, lead wires (b) and sensing electrodes in the pyramidal shaped pores of the sieving structure (c). Schematic illustration of the cross section of the μSEA (d), presenting the layout including insulation layers, contact electrodes, sensing electrodes and the lead wires for connecting each contact electrode with a sensing electrode.

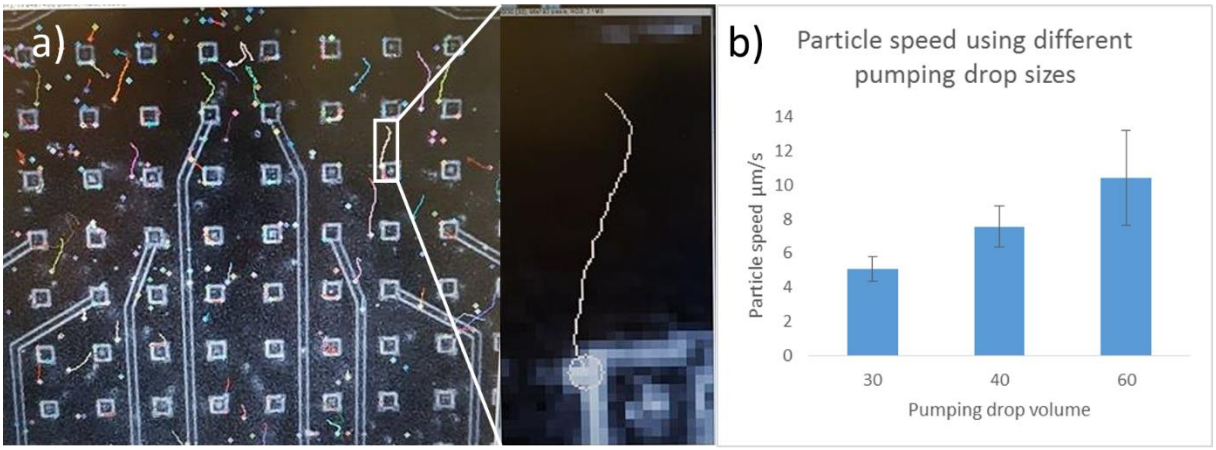

Figure 3: Determination of particle velocity using the mosaic particle tracker imageJ software (a). First the particles are identified and then they are tracked over time (insert). Using different pumping drop regime sizes, we calculated the average particle velocities (b) and found a direct correlation between increase in drop size and increase in particle velocity.