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Optical microscopy of artificially isolated axons on microelectronic measurement platform

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To study the cell communication in different animal tissues extracellular recordings are formidable to monitor the dynamics of formation and wiring of populations of neurons. The extensive and complex wiring in cell cultures makes it very difficult to simultaneously observe and analyse neuronal electrical behaviour of multiple cells at the same time. For a comprehensive analysis multi-electrode recordings have proven to be a powerful tool. Yet, optical microscopy plays a crucial role in verifying the position and morphology of nerve cells. Especially the neuronal processes (dendrites, axons) play an essential role for subcellular processes. With a microfluidic setup these neuronal processes could be confined to predefined microchannels which allow for isolated optical investigation of these cell compartments. In this work we present an approach to optically verify the neuronal electrical activity measurements of isolated axons on a self-constructed biochip.

The presented biochip is a versatile platform that allows (i) direct electrical measurement of extracellular potentials originating from isolated axons and (ii) optical inspection of the neuronal network using confocal laser microscopy (Figure 1). The measurement platform consists of a microstructured axon isolating device (AID), which is precisely mounted on top of a microelectrode array (MEA, Figure 2). For this reason specific electrical recordings of neuronal activity of axons can be performed. The AI-device is a transparent PDMS microfluidic device and was fabricated by a soft lithographic process using a SU-8 structured master template with 35 microchannels for axon isolation [1]. The MEA was fabricated by microstructuring techniques and equipped with 60 electrodes [2]. Electrical recordings and analysis were performed using the MEA1060-Inv-BC (Multi Channel Systems).

The proof of isolated axon growth was performed with sympathetic neurons from the superior cervical ganglion of P5 WT mice [3] grown on the AI-MEA platform. 3-days post seeding, neuronal activity (Figure 3) was recorded and analysed with the fabricated platform as shown in Figure 1.

The transparent microelectronic platform enabled optical monitoring of the cells and even of the isolated neurites in the microchannel using confocal laser microscopy. Optical investigations were accomplished by immunocytochemistry. In a first step the cells in the device were fixed using paraformaldehyde. A staining recipe for neurites in microchannels was optimized for simultaneously staining using MAP2 and SMI31 as primary antibodies as well as Alexa 488 and Alexa 568 as secondary antibodies. Optical recordings were performed using a Leica TCS SP5 X (Leica Microsystems GmbH, Wetzlar, Germany) of the cultured cells (Figure 4).

The presented platform will facilitate studies where axons and somata can be treated independently of each other. Optical microscopy is enabled by design and will remain essential to verify the neuronal electrical activity measurements.

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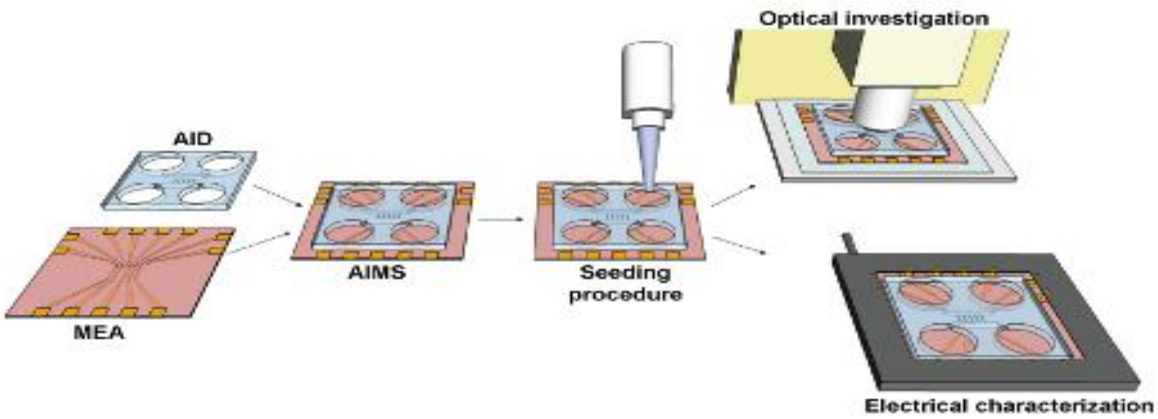

Figure 1. Schematic image of the developed platform with the possible applications.

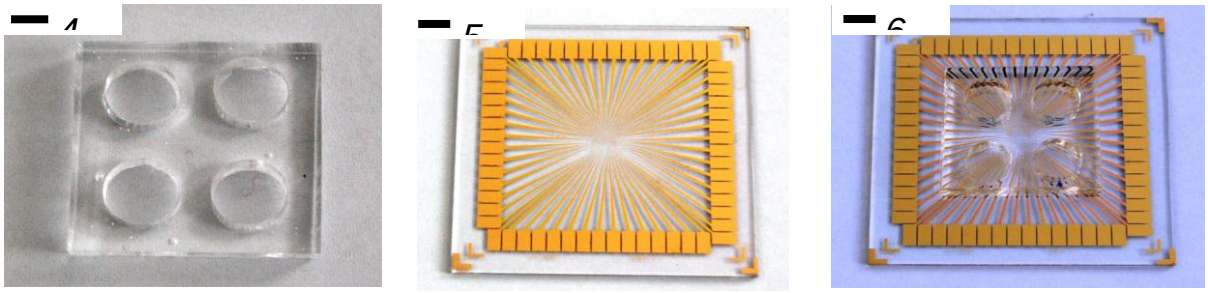

Figure 2. Images of AID, MEA, and AI-MEA.

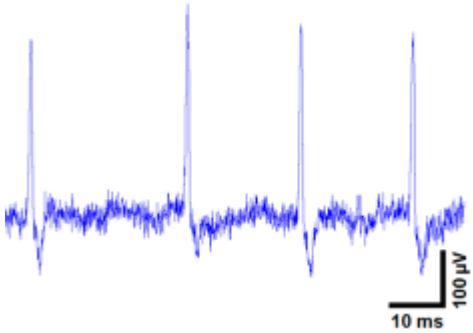

Figure 3. Action potential of a SCG neurite, recorded by an electrode placed in a microchannel

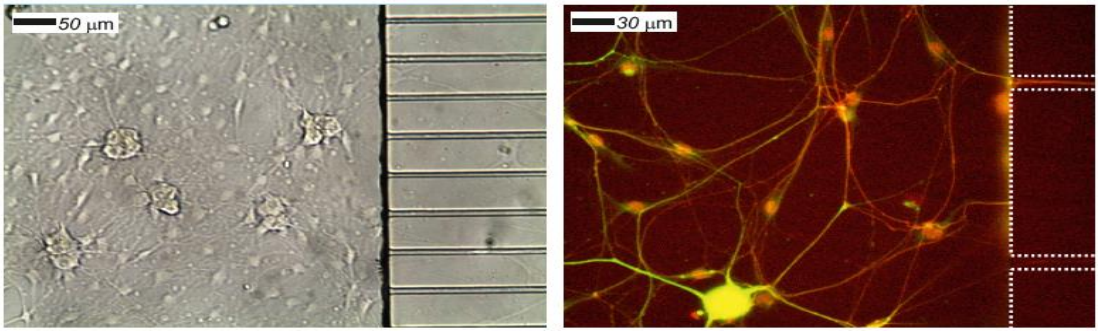

Figure 4. Left: Bright field microscopy of cultured neurons. The cell bodies adhere in the macrochannel of the fabricated platform. Right: Fluorescence microscopy of cultured neurons (SMI31 and MAP2). Only neurites can enter the microchannel.