UNRAVELING SELECTIVE SIGNALS OF NEURODEGENERATION WITH NEUROFLUIDIC DEVICES

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ABSTRACT

Over the last two decades, models of neurodegenerative diseases of the central nervous system (CNS) have emerged in microfluidic devices as a tool to better understand the underlying disease mechanism. Transient calcium influx and efflux events in combination with calcium dysregulation are highly accessible signals to determine drug efficacy against neurodegeneration. Furthermore, calcium signals regulate the secretion of exosomes, another essential cell-to-cell communicator. However, calcium events are often uniformly assessed across complex heterogeneous formations of neurite networks. Here, we present advancements to a previously established neurofluidic-based method that allows us to capture spatial differences in calcium signaling and exosome secretions selectively.

KEYWORDS: Calcium Signaling, Exosomes, Co-pathology, Primary Neuron Cultures, Neurofluidics, Phosphatase inhibition

INTRODUCTION

Calcium ions are important in eukaryotic cells and play a significant role in vital functions such as growth, apoptosis, survival, synaptic plasticity, and membrane excitability[1]. Lately, it has been suggested that calcium signals show abnormalities in the presence of common neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease in the central nervous system[1]. Calcium ions, however, are not the only messengers indicating the onset of neurodegenerative events. Mediated through calcium ions can be the release of exosomes[2]. Exosomes are nanometer-sized membrane-derived vesicles with an important role in clearing the cellular body of unwanted proteins and cell signals. However, this clearance process can also lead to the further spreading of neurodegenerative signals[3]. To unravel a link between calcium signaling and a potential onset of spreading of neurodegenerative signals through exosomes in a microfluidic model of neurodegenerative disease, we induced localized neurite networks to a phosphatase inhibitor called okadaic acid (Fig. 1A). Subsequently, we monitored calcium activity and exosomes secretion profiles within different compartments in an in-house designed neurofluidic device.

THEORY

Modeling neurodegeneration in microfluidic devices[4], referred to as neurofluidics, is a relatively

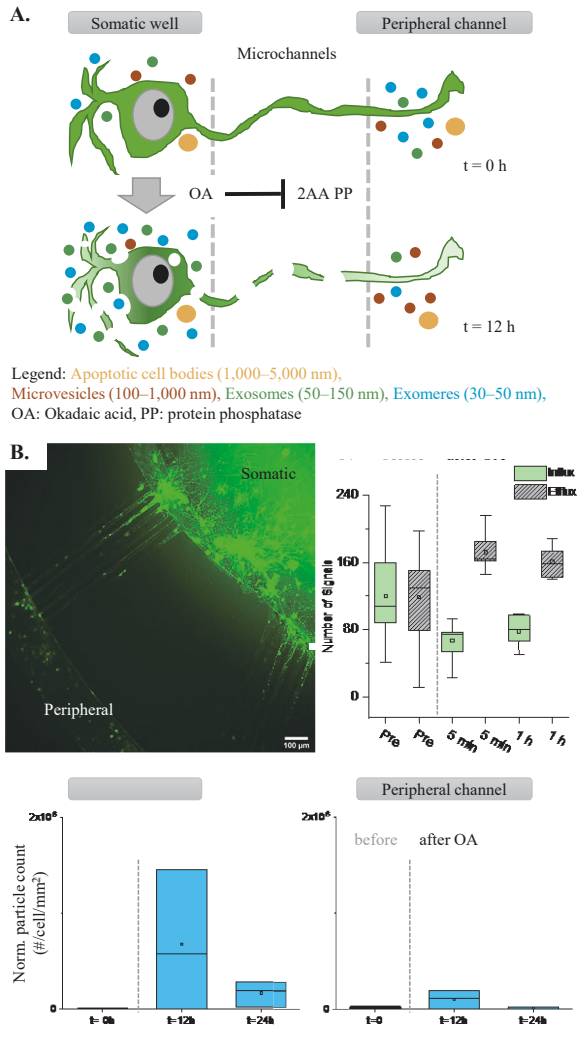

Figure 1: Profiling selective neurodegeneration signals in neurofluidics reveal a better understanding of disease progression. (A) Schematic of selective phosphatase inhibition through okadaic acid (OA) treatment leading to neurodegeneration. (B) Compartmentalized neurite network with calcium indicator (E18, rat, 9 DIV, green: Fluo-4 AM). (C) Selective calcium influx and efflux events. (D) Selective exosomes secretion before and after induced neurodegeneration.

new and convenient method to reveal differences in network vulnerability. The spatial separation of neuronal cells and networks grown in distinct areas allows us to control the chemical and biophysical environment locally. Based on millimeter-sized multiwells, with shallow and deep microchannels, axonal growth can be guided away from a somatic cell seeding area towards a peripheric collection area (Fig. 1B). It also enables us to perform localized toxicology testing and induce localized biochemical changes to the underlying neuronal signaling cascade. Combining compartmentalized and shallow channels establishes a barrier of convective transport between the somatic and the peripheric compartments, which can be used to locally expose cells to a phosphatase inhibitor which can induce a co-pathology of neurodegenerative events[4b]. This phosphatase inhibitor is called okadaic acid (OA), known to induce tauophaties[5]. These design features of neurofluidic devices make them ideal for collecting and analyzing spatial differences in cell segregation products in parallel to monitoring intracellular signals.

EXPERIMENTAL

To monitor calcium signaling and exosomes secretion profiles from neuronal cultures exposed to okadaic acid, we utilized a previously designed microfluidic device with three microwells, shallow microchannels (L: 0.5-1 mm, W: 20 μm) and a peripheral collection channel (W: 200 μm)[6]. The neurofluidic devices were coated with poly-l-lysine. Primary neuron cultures were grown from dissociated hippocampal brain tissue sections (E18, whole rat brains) following previously established protocols[4b] and seeded at a concentration of $3.5x10^4$ cells/ml. Hippocampal cells were then incubated for nine days (at 37 °C, 5% CO_2, 95% RH) and were imaged using brightfield and fluorescent imaging (Leica DMi-8, 20x, Fluo4). Time-lapse images were taken (1 min, 4 fps) in the three different compartments (peripheral channel, microchannels, and somatic microwells). After the first set of imaging, the neuronal cells were treated with okadaic acid (100 nM), only in the somatic microwells, and time-lapse imaging was repeated during the calcium activity was analyzed using ImageJ in all compartments based on calcium influx and efflux counts from ten randomly selected cells/neurites per region of interest (ROI). The ROI of each frame was kept constant over the entire image stack. Pre- and post OA treatment, we also collected and purified exosomes using the Total Exosome Isolation Reagent (Fisher Scientific) and Dynamic Light Scattering ($N_{measurements}$ = 7/sample).

RESULTS AND DISCUSSION

Figure 1C shows calcium events sorted based on influx and efflux before and after the OA treatment. Untreated, the number of calcium influx and efflux events appears balanced in cell bodies in the somatic multiwells. The induced phosphatase inhibition, however, already shows an imbalance between calcium influx and efflux events after 5 min. This trend gets further attenuated after 1 h with a decrease in influx events over the recording period of 1 min. After removing OA and washing the culture with pre-warmed media, and collecting exosomes 12 h later, hippocampus cells in the somatic compartment shed an increased number of exosomes (Fig. 1D).

CONCLUSION

Models of neurodegenerative diseases in microfluidic devices provide refined access to local differences in calcium network signaling, communication, and the simultaneous secretion of exosomes. These new insights from the neurofludic platform can be used further to better understand the calcium-signaling machinery and calcium dysregulation in neurodegenerative diseases.

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