Title: Stroke-Heart Syndrome on-a-chip

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Keywords:

cardiomyocyte, electrophysiology, human induced pluripotent stem cell, innervation, microfluidics, neuron

Abstract

Cardiac autonomic nervous system regulates the heart function through innervation. In Stroke-Heart Syndrome (SHS), an acute stroke leads to elevated serum levels of cardiac troponin and arrhythmias, impacting heart function, leading to cardiovascular complications that are the second leading cause of post-stroke mortality. Completely human cell-based *in vitro* models studying functional neuron-cardiomyocyte connections with central nervous system (CNS), peripheral nervous system (PNS), and cardiac tissue are missing from the field.

Our aim is to model SHS *in vitro* by combining three cell types; CNS type neurons (CNs), PNS type neurons (PNs) and cardiomyocytes (CMs) all derived from human induced pluripotent stem cells (hiPSC), in compartmentalized microfluidic devices, so called 3D3C chips. The structure of 3D3C chip enables the culturing of each cell type separately while allowing axonal growth through microtunnels to the adjacent, e.g., cardiac compartment. Integrated in-house produced microelectrode arrays (MEA) enable the electrophysiological functionality measurements as shown previously in seizure-on-chip platform.

Here, hiPSC-derived CNs, PNs and CMs were successfully cocultured in the 3D3C chip up to three weeks. The physical axonal interactions were investigated with microscopy and immunocytochemistry, and the functionality of the cells with MEAs. The axonal elongations between CNs to PNs and PNs to CMs were detectable, suggesting successful innervation. All cell types developed cell-specific electrophysiological functionality in the platform.

As proof of concept, the compartmentalized microfluidic chip with integrated MEA allowed formation of physiologically relevant connections with neurons and cardiomyocytes. This opens a path for studying aspects of SHS on-a-chip after integration of oxygen control for simulating stroke within the CNs compartment.