Title: Human cardiac innervation-on-a-chip platform for disease modeling

Authors:
Emma Pesu, Elias Kuusela, Anna-Mari Moilanen, Andrey Vinogradov, Kaisa Tornberg, Henna Lappi, Lassi Sukki, Tomi Ryynänen, Timo Salpavaara, Fikret Emre Kapucu, Pasi Kallio, Katriina Aalto-Setälä, Susanna Narkilahti

Keywords:
cardiomyocytes, central nervous system, human induced pluripotent stem cells, microphysiological systems, organ-on-chip, peripheral nervous system

Abstract
The cardiac autonomic nervous system (cANS) regulates cardiac function through innervation. Dysfunction of neuronal and cardiovascular crosstalk has been linked to various pathologies. Completely human cell-based *in vitro* models for studying brain-heart interactions with the central nervous system (CNS), peripheral nervous system (PNS), and cardiac tissue are lacking in the field. Our aim is to model the function of cANS *in vitro* by combining CNS type neurons (CNs), PNS type neurons (PNs) and cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSC) in a compartmentalized microfluidic device called a 3D3C chip. The structure of the 3D3C chip allows for the culturing of each cell type separately while allowing axonal connections to form via microtunnels to the adjacent cell compartment. Integrated in-house produced microelectrode arrays (MEAs) enable repeatable measurements of cellular activity in the multiculture and investigation of cell-specific electrophysiological functionality development and responses. By stimulating neural activity in the neuronal compartments, axon-mediated functional responses between the cell types can be investigated. Here, hiPSC-derived CNs, PNs and CMs were successfully multicultured in the 3D3C chip for up to three weeks, allowing physical axonal connections to form via microtunnels from CNs to PNs and from PNs to CMs. All cell types developed cell-specific electrophysiological functionality in the platform over time and axonal connections were confirmed to be functional with pharmacological excitation of neuronal activity, indicating of successful innervation. This advanced, completely human cell-based cardiac innervation-on-a-chip provides a powerful platform for examining disease-related mechanisms in which the brain–heart axis is involved.