Project Details	
Project Code	MRC23NMHEx Gielen
Title	Functional assembly of 3D neuromuscular junctions on a chip for neuromotor disease modelling
Research Theme	Neuroscience and Mental Health
Summary	The neuromuscular junction (NMJ) is an essential synapse for muscle contraction and movement. Understanding the functional establishment of an NMJ is crucial to studying neuromotor processes and associated diseases such as ALS. In this project, the student will generate 3D in-vitro models of NMJs by developing novel microfluidic organ-in-a-chip devices. They will subsequently assess their functional response to known therapeutics and validate their findings in-vivo.
Description	Motor neuron diseases such as Amyotrophic Lateral Sclerosis affect approximately one in every 100,000 people, typically in their 50s or 60s. ALS is characterized by the degeneration and death of motor neurons with five years life expectancy from diagnosis. ALS affects upper and lower motor neurons with no cure currently available. Neuromuscular junction (NMJ) defects are believed to play a crucial role in the onset of ALS. The neuromuscular junction is a specialized cholinergic synaptic interface between a motor neuron and a skeletal muscle fiber that translates presynaptic electrical impulses into motor function. To date, physiologically relevant human models for ALS are lacking, hampering the development of novel drugs and the early validation of therapies. The combination of induced pluripotent stem cell derived motor neurons and 3D cell cultures using organ-in-a-chip technologies is paving the way for novel strategies in generating functionally relevant 3D cell culture models for NMJs, which will be the main goal of the studentship. We will use and further develop cutting-edge microfluidic sorters developed in Gielen's lab (Anagnostidis et al, 2020; Howell et al., 2021) to rapidly screen large populations of multicellular spheroids made up of both motor neurons and muscle cells derived from myoblasts. Bhinge's lab has already developed 3D cell culture models for both types of cells and Gielen has shown model spheroid selections using machine learning approaches which will be extended in this work to more detailed morphological studies. The student will further develop this tool further to realise on-demand spheroid pairing and fusion, allowing real-time evaluation of cellular architecture dynamics and formation of NMJs for hundreds of spheroid pairs. We will leverage our ability to spatially pattern two different types (motoneuron and muscle cells) of spheroids while also having control over the cellular microenvironment. Physiological assessment of the fused spheroids will inform on cell self-organisation and establishment of synapses. We will form fused spheroids using patient-derived motor neurons of both healthy and ALS patients to contrast the functional differences seen. We will then use light-sheet microscopy of live spheroid cultures as well as confocal imaging of immune-stained fused spheroids. Functional assessments of muscle contraction will be done by integrating optogenetic elements to selectively activate motor neurons coupled to calcium influx fluorescence imaging upon light stimulation. Different drugs and combinations will be tested to quantify functional responses. These responses will be then validated in a zebrafish model of ALS in

	Subramanian's lab. Her lab has extensive expertise in studying zebrafish motor neurons function (Ferguson et al., 2019). In summary, this project has the potential to provide a new platform for the building of physiologically-relevant human NMJs and the early testing of novel drugs and therapies.
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