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# Presentation PL1-05

## Bioengineering the neuromuscular junction to investigate CMT2-pathophysiology using hiPSC-derived cell models

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Charcot-Marie-Tooth disease (CMT), like most peripheral neuropathies, currently lacks effective therapeutic strategies. A major barrier to therapeutic development is the absence of suitable preclinical models that recapitulate the complex neuromuscular phenotypes observed in patients. Although animal models have significantly advanced our understanding of disease pathophysiology, they often fall short in translational relevance. Likewise, conventional 2D in vitro systems often lack the architecture and cell–cell interactions that influence disease pathology.

To address these challenges, we aim to develop in vitro platforms to investigate the human neuromuscular system using iPSC-derived motor neurons and skeletal muscle cells. First, we have utilized microfluidic devices to establish a 2D model containing the neuromuscular junction (NMJ), compatible with high-resolution microscopy and live-cell imaging. Second, we are optimizing a 3D-Innervated-Skeletal-Muscle-on-a-chip (3D-iSM) platform that allows for contractile force measurements and NMJ-mediated action potential transmission. Finally, we aim to establish a functional NMJ-on-a-microelectrode array (MEA) platform for electrophysiological assessment of neuromuscular activity.

Thus far, we have visualized and quantified axonal transport of mitochondrial and lysosomal cargo in healthy iPSC-derived motor neurons using the microfluidic system. We are currently investigating the impact of HSPB1 and HSPB8 mutations (CMT2F and CMT2L, respectively). In parallel, we have engineered healthy 3D-iSM tissues displaying aligned myofibers, myonuclear chains, and acetylcholine receptor clusters, representing motor endplates and potential NMJ sites.

Next, we will evaluate bioengineering strategies to promote guided axonal extension and muscle innervation. We also plan to adapt the 3D-iSM model for MEA-based assessment of neuromuscular activity and further investigation into CMT pathophysiology.