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Fully human iPSC-derived neuromuscular assembloids to model myelination and neuromuscular features in CMT

B. Bekaert (1), T. van Wermeskerken (1), N. Donies (1), S. in't Groen (1), T. Vangansewinkel (1), Y. Lambrechts (2-4), K. Libberecht (2-5), E. Wolfs (2), V. Timmerman (1)

(1) Peripheral Neuropathy Research Group, Department of Biomedical Sciences, University of Antwerp, Belgium
(2) Functional Imaging & Research on Stem Cells (FIERCE), BIOMED Research Institute, Department of Cardio and Organ Systems, Hasselt University, Belgium
(3) Lab of Neurobiology, VIB-KU Leuven Center for Brain & Disease Research, Department of Neurosciences, KU Leuven, Belgium
(4) Experimental Neurology and Leuven Brain Institute (LBI), Department of Neurosciences, KU Leuven, Belgium
(5) Laboratory for Histology and Regeneration (HISTOREGEN), Department of Cardio and organ systems, Hasselt University, Belgium

Animal models for CMT often fail to translate into therapies, emphasizing the need for human-derived systems. We developed neuromuscular assembloids to study myelination and neuron-muscle interactions. Initially, we created hybrid models by fusing induced pluripotent stem cells (iPSC)-derived motor neuron spheres with immortalized muscle spheres. We are now advancing to complete iPSC-based models by fusing motor neuron spheres with skeletal muscle spheres, to which Schwann cell precursors can be added, all derived from iPSCs.

We assess myelination and neuromuscular characteristics through immunostaining (whole-mount and cryosections), Western blot, and RT-qPCR, with a focus on cellular maturation. Functional analyses using calcium imaging and multielectrode array assays are underway. We are currently introducing endogenously marked cell types to facilitate downstream analyses. Additionally, we are implementing optogenetics to create a more controllable experimental environment.

In response to the limited Schwann cell marker S100B signal observed in hybrid assembloids, Schwann cell precursors were incorporated into fully iPSC-derived assembloids. In these preliminary experiments, S100B-positive cells were initially detected in the 3D structure but declined over time, likely due to suboptimal culture conditions that are currently being optimized. While Myosin Heavy Chain (MyHC) signals were also detected in the hybrid assembloids, the fully iPSC-derived assembloids exhibited more widespread MyHC expression, suggesting enhanced muscle maturation. Further refinement of the muscle compartment is currently underway. Additionally, α-bungarotoxin signals were detected.

Overall, fully iPSC-derived neuromuscular assembloids offer a more suitable platform to study myelination and neuromuscular dynamics. We plan to extend this model to include CMT1A-derived cells in future studies.