Generation of Human Motor Units with Functional Neuromuscular Junctions in Microfluidic Devices

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
This method makes use of	Human derived cells / tissues / organs
Specify the type of cells/tissues/organs	human induced pluripotent stem cell-derived motor neurons and human primary mesoangioblast-derived myotubes

DESCRIPTION

Method keywords

Microfluidic device

Human iPSC-derived motor neuron

Human primary mesoangioblast-derived myotube
Motor-unit
Neuromuscular junction
Compartmentalized
live-cell imaging
Immunocytochemistry
Scanning electron microscopy

Scientific area keywords

Amyotrophic lateral sclerosis
FUS
Neurite outgrowth
Neurite regrowth
HDAC6
Tubastatin A

Method description

This study aimed to create a versatile and reproducible *in vitro* model of a human motor unit with functional neuromuscular junctions (NMJs). Therefore, human induced pluripotent stem cell (hiPSC)-derived motor neurons and human primary mesoangioblast (MAB)-derived myotubes were co-cultured in commercially available microfluidic devices. The use of fluidically isolated micro-compartments allows for the maintenance of cell-specific microenvironments while permitting cell-to-cell contact through microgrooves. By applying a chemotactic and volumetric gradient, the growth of motor neuron-neurites through the microgrooves promoting myotube interaction and the formation of NMJs were stimulated. These NMJs were identified immunocytochemically through co-localization of motor neuron presynaptic marker synaptophysin (SYP) and postsynaptic acetylcholine receptor (AChR) marker -bungarotoxin (Btx) on myotubes and characterized morphologically using scanning electron microscopy (SEM). The functionality of the NMJs was confirmed by

measuring calcium responses in myotubes upon depolarization of the motor neurons. The motor unit generated using standard microfluidic devices and stem cell technology can aid future research focusing on NMJs in health and disease.

Lab equipment

Laminar flow cabinet
Cell-culture incubator

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

This method uses commercially available microfluidic devices and standard stem cell technology, which increases reproducibility.

Future & Other applications

The model is validated for research in amyotrophic lateral sclerosis, but can also be used in other fields where motor units or neuromuscular junctions are of interest.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

- Stoklund Dittlau K et al.. Human motor units in microfluidic devices are impaired by FUS mutations and improved by HDAC6 inhibition. Stem Cell Reports, 2021 Sep 14;16(9):2213-2227. doi: 10.1016/j.stemcr.2021.03.029

- Stoklund Dittlau K et al. Generation of Human Motor Units with Functional Neuromuscular Junctions in Microfluidic Devices. J Vis Exp. 2021 Sep 7;(175). doi: 10.3791/62959. Protocol includes a professional instruction video.

Associated documents

Human motor units in microfluidic devices are impaired by FUS mutations and improved by HDAC6 inhibition.pdf
jove-protocol-62959-generation-human-motor-units-with-functional-neuromuscular-junctions.pdf

Links

JoVE protocol instruction video

PARTNERS AND COLLABORATIONS

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