

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

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## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo

**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](#)

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