Generation of human PSCs-derived multi-tissue organoids

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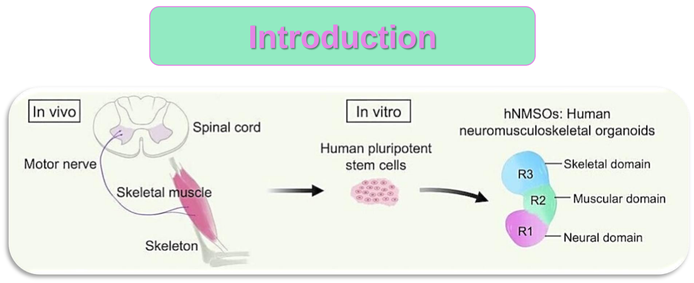
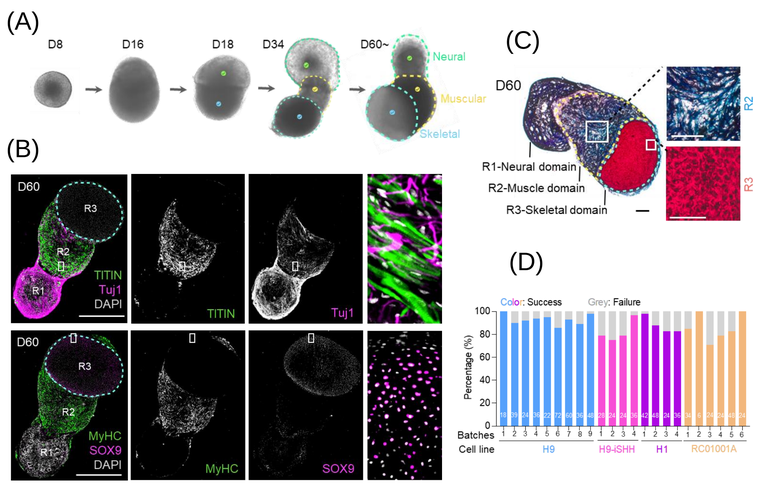
Introduction



The human body function requires the crosstalk between different tissues. One such essential crosstalk is in the neuromusculoskeletal axis, which is challenging to model with human cells. Here, we describe the generation of 3D neuromusculoskeletal tri-tissue organoids (**hNMSOs**) from human pluripotent stem cells through a co-development strategy. Staining, single-nucleus RNA sequencing, and spatial transcriptome profiling revealed the co-emergence and spatially self-organized domains of neural, muscular, and skeletal lineages relevant to human tissues within individual organoids, and the neural domains of hNMSOs obtained a ventral-specific identity and produced motor neurons innervating skeletal muscles. The three regions of hNMSOs exhibited maturation and established functional connections during development. Notably, structural, functional, and transcriptomic analyses revealed that the emergence of skeletal support in hNMSOs benefited human muscular development. Modeling with hNMSOs also unveiled the neuromuscular alterations following pathological skeletal degeneration. Together, our study provides an accessible experimental model for future studies of human neuromusculoskeletal crosstalk and abnormality.

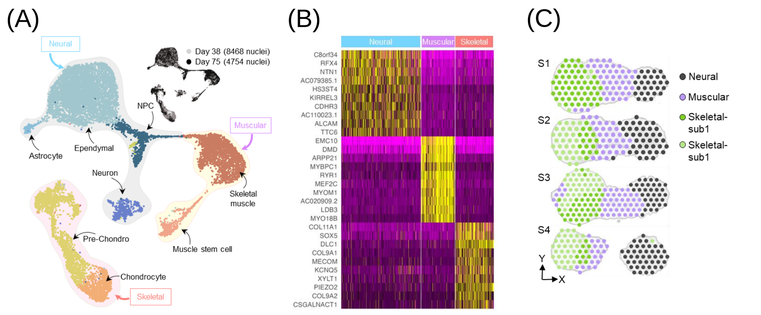
Result

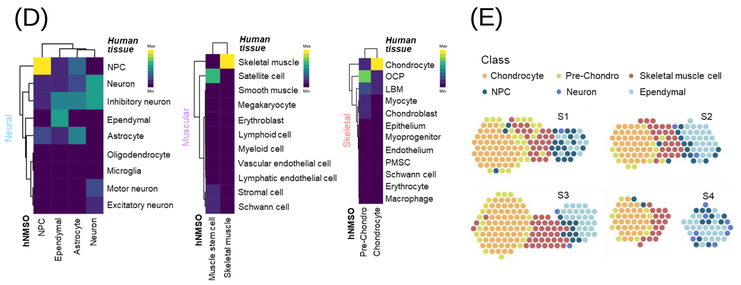
1. Generation of hNMSOs from human PSCs



(A) Morphology of hNMSOs at different developmental stages. (B) Immunostaining of hNMSOs for TITIN, Tuj1, MyHC and SOX9. (C) Safranin O and Fast Green staining of hNMSO. (D) Quantification of the successful rate of hNMSO differentiation.

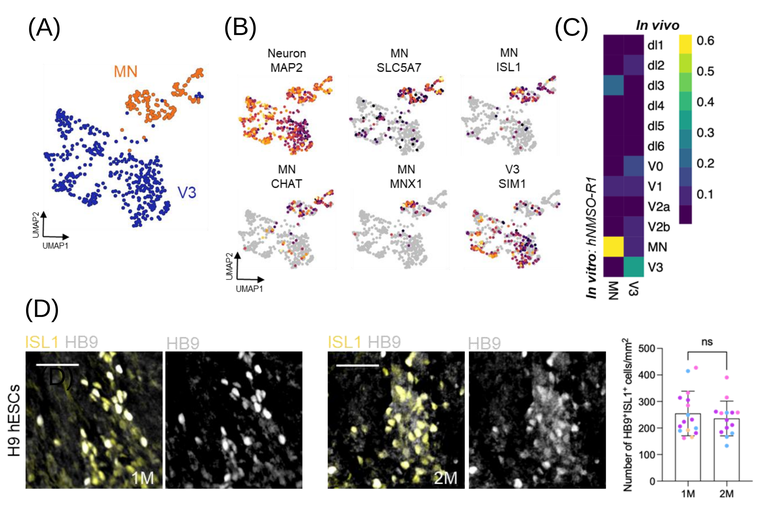
2. Single-nucleus and spatial transcriptomic analysis of hNMSOs





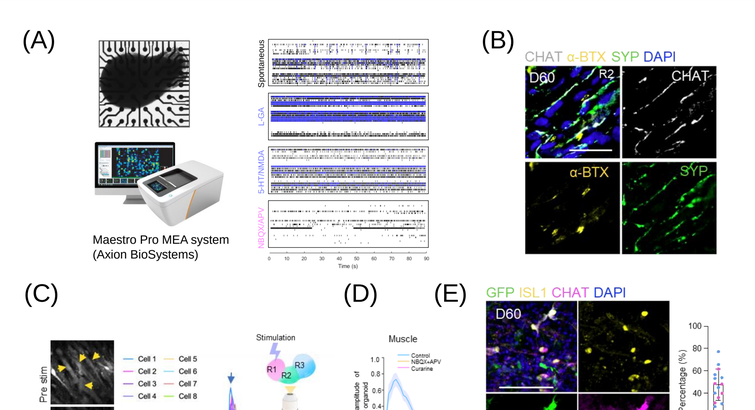
(A) UMAP plot of snRNA-seq profiles of hNMSOs. (B) Differential gene expressions in neural, muscular, and skeletal lineages. (C) Clustering of spatial spots in different hNMSO sections. (D) The similarities of hNMSOs-derived neural, muscular, and skeletal clusters compared to human tissues. (E) Spatial spots annotation using deconvolution from snRNA-seq of hNMSOs.

3. Neural domain contains motor neurons and V3 neurons from the ventral spinal cord



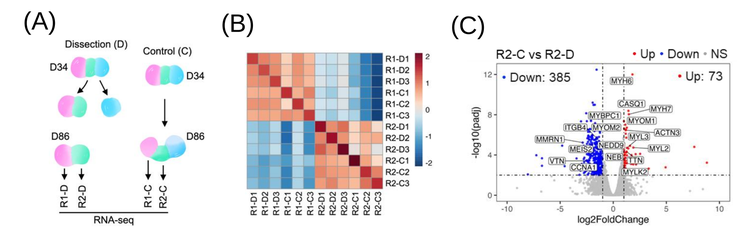
(A) UMAP embedding and cell annotation of neuron cluster subtype. (B) The expression of marker genes for MNs and V3 neurons. (C) The similarities between organoid neuron subtype and human fetal spinal cord scRNA-seq profiles. (D) Immunostaining and quantification for ISL1 and HB9 of 1- or 2 months-old hNMSOs.

4. Maturation and functional connections in hNMSOs



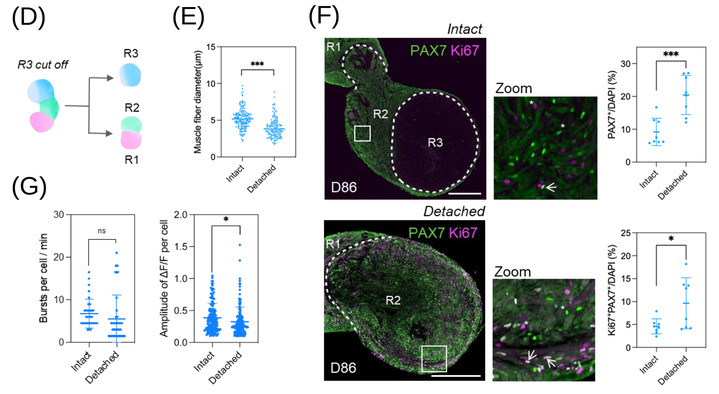
(A) MEA profiling of hNMSOs for spontaneous and modulated activities. (B) Immunostaining for α-BTX, CHAT and SYP in hNMSOs. (C) Photostimulation and calcium imaging in hNMSOs. (D) Quantification of responsive amplitude in control or treated hNMSOs. (E) rAAV2-retro retrograde tracing, immunostaining and quantification for CHAT and ISL1 in retrogradely-traced hNMSOs.

5. Skeletal support contributes to skeletal muscle development



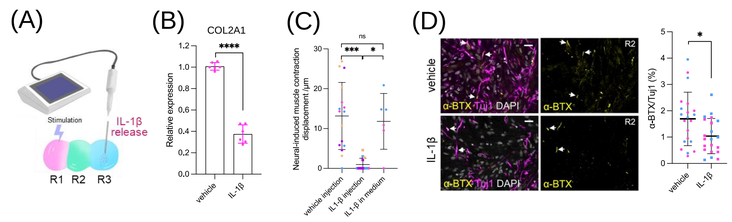
Conclusion

- A protocol for human neuromusculoskeletal (NMS) organoids (hNMSOs) from hPSCs
- Neural, muscular, and skeletal tissues self-organize and co-develop in hNMSOs.
- Motor neurons are produced in neural domains with ventral-specific identity.
- Functional connections establish between motor neurons and muscular cells through neuromuscular junctions in hNMSOs.
- Skeletal tissue benefits muscle fibers arrangement, skeletal muscle differentiation and maturation in hNMSOs.
- hNMSOs can be used to model neuromusculoskeletal diseases like arthritis.



(A) Schematic describing skeletal detachment and bulk RNA-seq analysis. (B) The Pearson correlation between bulk RNA-seq samples. (C) The DEGs between R2-C and R2-D samples. (D) Schematic describing the skeletal detachment analysis. (E) quantification of muscle fiber diameters. (F) Immunostaining and quantification of PAX7+Ki67+ cells in the muscular region. (G) Quantification of calcium spikes in intact and R3-detached hNMSOs.

6. Skeletal inflammation impairs neuromuscular function



(A) Schematic describing the IL-1β release assay. (B) qPCR analysis of the skeletal region. (C) Quantification of muscle contraction displacement in different conditions. (D) Immunostaining for α-BTX and Tuj1 in the muscular region and quantification of percent of α-BTX$^+$ area per Tuj1$^+$ area.

Acknowledgements

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