3D *in-vitro* Neuromuscular Junction Modeling

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Introduction: The neuromuscular junction (NMJ) is a specialized synapse where motor neurons innervate muscle fibers in order to induce muscle contraction. Aging is associated with the alteration and decline of the neuromuscular synapse morphology and function, leading to musculoskeletal impairment and reduction in muscle strength. In recent years, the influence of the mechanical environment on cellular behavior, especially in the context of aging and fibrosis, has come into the spotlight. However, few studies have focused on the impact of extracellular matrix (ECM) mechanobiology on the neuromuscular junction, especially in a three-dimensional (3D) context[1,2]. Therefore, incorporating modified ECM-like proteins with precisely controlled mechanical properties is essential for the tissue engineering of physiologically relevant NMJs[3]. In this study, we propose to incorporate synthetic protein hydrogels into a 3D NMJ co-culture platform. Specifically, by incorporating induced pluripotent stem cell (iPSC)-derived motor neuron spheroids with muscle microtissues, we aim to study the formation and maintenance of NMJs under different mechanical conditions. Successful in vitro modeling will enable functional assays of NMJs under conditions mimicking aging and neuromuscular diseases, such as amyotrophic lateral sclerosis (ALS).

Materials and Methods: Motor neurons were differentiated from iPSCs (GM25256) following a protocol adapted from Castellanos-Montiel et al.(4) Spheroids were formed using 5000 cells plated on 96-well ultra-low attachment plates. Muscle microtissues were formed in a polydimethylsiloxane (PDMS) platform based on Afshar *et al* (2020), with some modifications.(5) 16-well culture plates were made with Sylgard-184 PDMS mixed with curing agent at a ratio of 15:1 and cured at 60˚C for at least 6 hours with a negative PU mold. 3D in-vitro co-culture is performed by suspending human myoblasts together with 5 MN spheroids per tissue in a fibrin/Geltrex hydrogel (3D), induced by thrombin. The 3D skeletal muscle tissue media is supplemented with neurotrophic factors (BDNF, GDNF) to support MN viability.

Results and Discussion: We demonstrated the successful generation of iPSC-derived motor neuron spheroids, confirming expression of motor neuron differentiation markers (SOX1, OLIG2, MNX1) (Figure 1a,b). Cell morphology progressively changed throughout the differentiation protocol, exhibiting neurite-like extensions in later stages of differentiation. Microtissues were formed using fibrin/Geltrex through co-encapsulation of myoblasts and motor neuron spheroids (Figure 1c). A PDMS 16-well microtissue platform was fabricated, enabling the support of microtissues on cantilever supports to enable muscle contractile force readouts (Figure 1d). Incorporation of motor neuron spheroids increased the metabolic profile of the co-culture relative to mono-cultured myoblasts.

Conclusion: We demonstrated generation of an in vitro NMJ model using iPSC-derived motor neurons. Further optimization of the system using on-demand tunable hydrogels with precise mechanical properties will enable investigation of NMJ mechanobiology.

References

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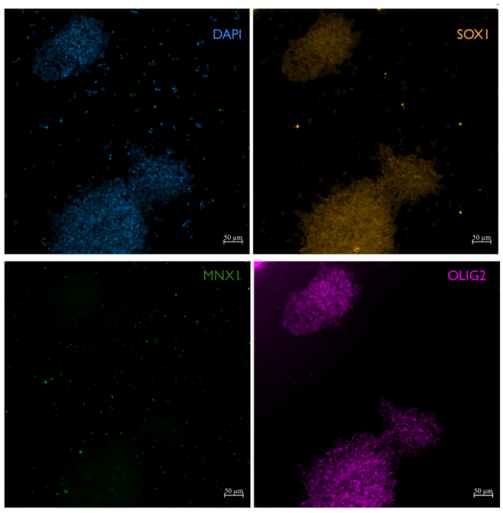
a)


DAPI
SOX1
MNX1
OLIG2
50 µm
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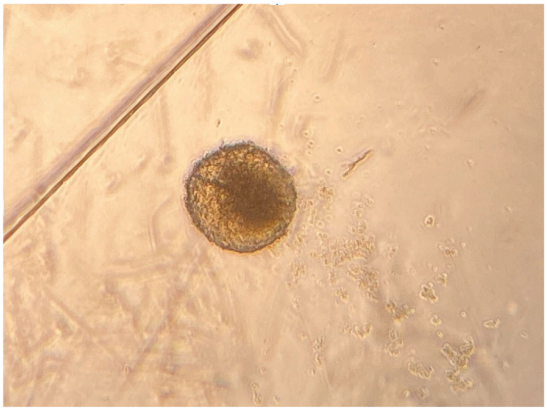
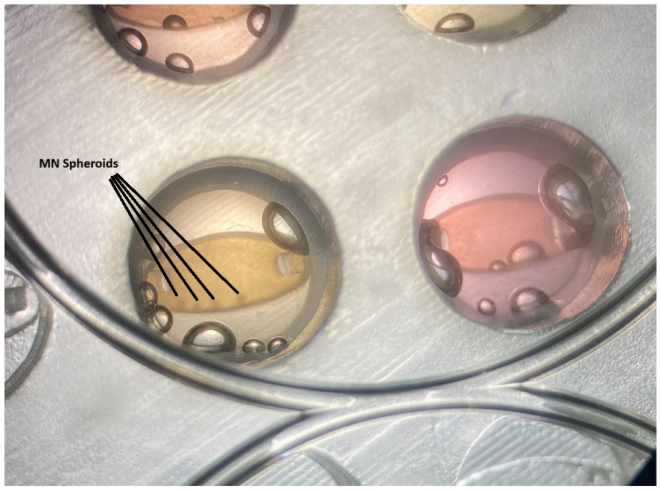
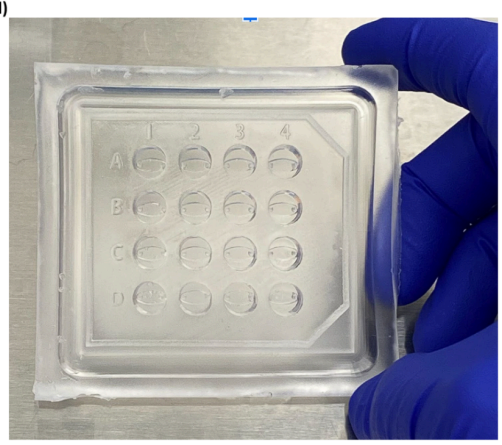
b)



c)


MN Spheroids

d)


1 2 3 4
A
B
C
D