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Biobased Elastomer Nanofibers for Guiding Skeletal Muscle Regeneration

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Extended Abstract

Skeletal muscle is the most abundant tissue in the human body. It is of high clinical importance because of the crucial role it plays in respiration, locomotion, and behaviour. Skeletal muscle is a highly organised tissue composed of aligned muscle fiber bundles, muscle connective tissue, blood vessels and other extracellular structures. Innervation by excitatory motor neurons enables muscle contractions, while support from other tissues such as the tendons, provide anchorage to contractile myofibers. Skeletal muscle can alter its' structural and functional properties in response to a range of environmental signals, including motor input, exercise, and disease. Genetic, metabolic, and age-related disease can affect its' regenerative potential, and can lead to debilitating muscle weakness and dysfunction.

To fully understand these mechanisms, physiologically and functionally relevant tissue engineered in vitro models are required [1] . Animal models are both cost- and time-intensive, whilst 2D cell culture assays fail to capture the 3D arrangement of cells and surrounding extracellular matrix (ECM) [2]. This project combines nano-engineered elastomer nanofiber sheets with human induced pluripotent stem cell (iPSC) derived myofibers in the establishment of an in vitro model of skeletal muscle function.

To do this, an elastomeric co-polymer was synthesised from renewable molecular building blocks and formed into aligned nanofibrous sheets using electrospinning technology. These nanofiber sheets were suspended across custom-built micro-wells. The nano-fabricated sheets were shown to provide an ECM-like substrate for supporting contractile skeletal muscle fibers in vitro. The nanofibrous sheets had hyper-elastic properties matched to that of skeletal muscle tissue and could be formed with controllable alignment to guide myotube orientation, and promote cell attachment, proliferation, and differentiation in skeletal muscle myofibers.

The chemical properties of the polymer were characterised by NMR and FTIR, and the orientation of molecular chains was compared between randomly oriented and aligned electrospun nanofibers by polarised-FTIR. The mechanical properties of the nanofiber sheets were characterised by static tensile loading, whilst morphological properties were characterised by SEM and AFM. Studies of cell survival, toxicity and metabolic activity confirmed that the nanofiber sheets enhance proliferation and differentiation of iPSC-derived skeletal muscle fibers. Myoblast fusion index and maturation index were found to be significantly greater in cells grown on elastomeric nanofibers than when compared to regular tissue culture plastic.

Functional studies of muscle contractility were made possible through incorporation of a light-gated ion channel, Channelrhodopsin-2 (ChR-2), into the cells, such that myofiber contractions could be controlled by light [3]. The nanofibrous sheets were shown to anchor contractile myofibers, promoting culture longevity, addressing a prominent and ongoing challenge in the field of skeletal muscle tissue engineering.

Finally, the nanofiber sheets were incorporated into a standard 96-well format suitable for automated high-content imaging analysis. To demonstrate the use of this technology, the system was adapted for modelling neuromuscular diseases such as Amyotrophic Lateral Sclerosis and Duchenne Muscular Dystrophy. Stabilised neuro-muscular co-cultures were achieved by incorporating human iPSC-derived myofibers and iPSC-derived motor neurons and astrocytes on 96-well arrays of elastomer nanofibers, providing a potential new system for high-throughput phenotypic disease modelling and drug screening.

References

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