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CONTRACTILE FUNCTION OF IN VITRO TISSUE-ENGINEERED SKELETAL MUSCLE CONSTRUCTS

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Introduction: The objectives of the project were to develop methods for (1) engineering functional 3-dimensional skeletal muscle structures *in vitro* from primary explants without employing a synthetic matrix or scaffold, and (2) measuring the excitability and contractile function. We term these structures *myooids*, because they are muscle-like.

Methods: Myooids are produced by the co-culture of mammalian satellite cells and fibroblasts from adult rat soleus muscles on a laminin-coated SYLGARD substrate. Primary cultures were prepared by presoaking muscle strips for 50 hours in DMEM with 0.005% NaN3, dissociating in a solution of 4 u/ml dispase and 100 u/ml type IV collagenase, and plating at a density of ~ 12 mg of dissociated tissue per plate. Each culture dish contained a pair of laminin-coated size 0 silk suture anchors, 6 mm in length, pinned to the SYLGARD with 0.10 mm minutien pins, 12 mm apart. Cultures were fed every 72 hours, using HAM F12 with 20% FBS until confluent, followed by DMEM with 7% HS until evaluation. Within 3 to 4 weeks, myooids formed by progressive delamination of the cell monolayer from the substrate, with concurrent cell ingrowth into the suture anchors. The myooids remodeled into parallel-fibered cylindrical structures, 0.1 to 1.0 mm in diameter, suspended between the suture anchors. Excitability and isometric contractile function were assessed after one month in culture by attaching one of the anchors to a force transducer and electrically stimulating the myooid between parallel electrodes.

Results: The myooids began to contract spontaneously two days after formation, at ~ 1 Hz with peak-to-peak force amplitudes in the range of 3 to 30 μN. After 30 days in culture the myooids had an average diameter of 0.49 mm, a mean twitch tension of 215 μN, a mean peak tetanus of 440 μN, a resting baseline force of 181 μN, a rheobase of 1.0 V/mm, a chronaxie of 0.45 ms, and a specific force of 2.9 kN/m2. The mean specific force was approximately 1% of the force generated by control adult rat muscle of the same cross-sectional area.

Conclusions: Functional skeletal muscle constructs may be engineered and evaluated *in vitro* from primary mammalian cells. We hypothesize that the myooids remain arrested at an early developmental level because the necessary signals for full differentiation are not present.

Funding: Pilot Award, Ctr Biomed Engineering Research, Univ of Michigan