Engineered Skeletal Muscle and Neuromuscular Tissues

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Primary satellite cells and fibroblasts from mammalian skeletal muscle tissue can be cultured *in vitro* under conditions that promote the self-organization of 3-dimensional skeletal muscle constructs, termed 'myooids'. Myooids are formed between laminin-coated silk suture anchors, which act as artificial tendons and allow the attachment of force transducers and servomotors. The contractile segment of the myooid is generally 12 mm in length, and ranges from 0.1 to 0.8 mm in diameter. Myooids can be readily engineered from any mouse or rat skeletal muscle and with tissue from animals aged from 1-day neonates to the oldest old (36 month old rats). In general, 10 to 15 mg of tissue is required to provide enough cells to form each myooid. Thus, the skeletal muscle tissue from a single rat can be used to make hundreds of myooids. Myooids can also be engineered from co-culture of cell lines (C2C12 and 10T1/2), but the resulting contractility is inferior to that of myooids from primary cells. The excitability and contractility of myooids can be readily measured by attaching a force transducer to one of the suture anchors and applying transverse electrical stimulation pulses. Representative data include forces generated during spontaneous contractions of myooids in culture, twitch and peak isometric force, force-frequency and length-tension curves. When normalized by the cross sectional area, the forces generated by myooids are ~1% of control values for adult skeletal muscle. The classical physiological parameters of excitability, chronaxie and rheobase, are modified to usefully describe the bulk excitability of engineered skeletal muscle. Myooids are cultured aneurally, and therefore have low excitability. Co-culture of myooids and spinal cord explants has resulted in axonal sprouting from the spinal explant to the myooid, but synaptogenesis and subsequent increased myooid excitability have not been detected. Future research in engineered functional mammalian skeletal muscle will focus on angiogenesis and perfusion, synaptogenesis and improved tissue excitability, and engineered tendons to permit mechanical impedance matching with other musculoskeletal tissues, for eventual incorporation of engineered skeletal muscle into a functional musculoskeletal system.