William Drennan
wcd2@illinois.edu
Graduate Student, University of Illinois Urbana-Champaign
Advisor: M Taher A. Saif

All Abstract Authors and Affiliations
William Drennan (1,2), Alexandra Barishman (1,3), Yelim Kim (1), M Taher A Saif (1,2) (1) Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign; Urbana, USA. (2) CZ Biohub Chicago, LLC; Chicago, USA. (3) Cullen College of Engineering, Biomedical Engineering, University of Houston; Houston, USA

Force-induced cross-talk between muscle and neurons in developing motor units

The formation of muscle in living systems depends on mechanical tension and spontaneous twitching activity, as demonstrated in studies of Drosophila flight muscles. Additionally, the outgrowth of motor neurons towards muscle during neuromuscular junction (NMJ) formation has been observed to increase muscle activity and sarcomere maturity. Resting tension in networks of hippocampal neurons has been shown to modulate network bursting activity. We hypothesize that similar mechanisms occur in motor units, where resting tension in motor neurons, along axons or at innervation sites, modulates activity.

In order to characterize the role of mechanical tension in signal transduction between motor neurons and innervated skeletal muscle, we developed a micro-molded, integrated force sensing platform capable of simultaneously applying mechanical forces and measuring muscle and neurite forces with 10 nN and 1 μN force resolution, respectively. Our sensors are produced by casting and molding PDMS off stereolithography (SLA) 3D-printed resin masters, which allow for 3-dimensional and overhanging features. Individual sensors are made in an array for high throughput studies. The height of the array is chosen to allow for second harmonic generation imaging of collagen structures and calcium imaging of neuronal bursting and muscle twitching. Each individual sensor consists of a pair of PDMS grips across which a muscle tissue is grown and a third grip which is aligned perpendicular to the muscle tissue for culturing motor neurons. The PDMS is functionalized through treatment with the organosilane APTES followed by glutaraldehyde, which coats the surface with reactive amine groups which form covalent bonds with collagen. Protrusions on the grips increase the surface area available for adhesion with the collagen. A μm-resolution programmable manipulator allows us to apply dynamic tension or compression to the tissues.

We used our sensor platform to study the evolution of motor units formed between embryonic stem cell-derived optogenetic motor neurons expressing ChR2 and C2C12-derived muscle tissue. We observe neurite outgrowth toward the muscle around the same time that the muscle is observed to twitch spontaneously. After 12 days in culture, the muscle exhibits strong twitching synchronous with our stimulation of the neurons with blue light. This synchronous activity confirms formation of mature NMJs, supporting this platform's potential for the study of motor unit mechano sensitivity and cross-talk. The role of tension in muscle and the neurites in the emergence and function of the motor units will be discussed.