Researchers develop microscope that allows look at live muscle units in action

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Millions of people each year are diagnosed with diseases that result in the loss of neuromuscular function. One of the complications in treating these people has been an inability to track the progression of disease and provide the best possible therapeutics.

Now, a team of Stanford researchers has developed a microscope that can visualize and measure the force-generating contractions of these patients' individual motor units. This action has been studied for nearly 100 years, but this is the first time it has ever been observed in the muscles of a living human.

"When it comes to muscle microstructure and dynamics, we have not been able to visualize normal muscle, and we don't know how it changes with disease," said co-author Scott Delp, a Stanford professor of bioengineering, of mechanical engineering and, by courtesy, of orthopaedic surgery. "With this microscope, we have opened up a new window to how muscles change with strokes and diseases like ALS or muscular dystrophy. We can immediately use it in humans; it's very low risk, and it gives us a new way to examine muscle microstructure and dynamics."

The findings are published in today's issue of *Neuron*.

Measuring muscle

Every move you make is made possible by an electrical handshake between a motor neuron and muscle fibers. When the signal from the brain activates the neuron, it triggers the fibers, which contain many force-generating units called "sarcomeres," which contract.

At the tissue level, the microstructure of skeletal muscle is striped – hence its name striated muscle – and the distance between sarcomeres dictates how much force can be generated. Too long or too short, and they generate very little force. Scientists believe that in certain neuromuscular disorders, the stripes gradually fall out of the sweet spot for maximum force generation, becoming either too long or too short, and that weakens the muscle.

"We stand to gain important insights by visualizing the contractions of individual motor units in live patients," said co-author Mark Schnitzer, an associate professor of biology and of applied physics at Stanford. "The structure at the level of individual sarcomeres relates to how much force the muscles can generate. We can also observe their contractions to see if the fiber is slow or fast twitch. If we can track the relative preponderance of fast versus slow twitch motor units, and how that changes in various disease states, we may be able to monitor how quickly a particular disease is progressing. This could provide useful insights for diagnostic tracking in individual patients, and tailoring therapeutics in a way that best treats their current condition."

The group's microscope makes this possible and clinically feasible for the first time.

Shrinking a microscope

The microscope is based on technology that existed for two decades and has previously returned promising results in terms of providing insights into human physiology. The format of previous devices has been

cumbersome, however, consisting of an entire table of bulky optics and laser systems that could be operated only in total darkness, and were limited to analyzing tissue samples.

The new microscope consists of several small components that all fit neatly on a bedside pushcart. An ultrafast laser light source beams infrared light in 100-femtosecond pulses along an optical fiber from the cart to a handheld unit, which contains the miniaturized optics. This unit connects to an optical needle that is inserted into the patient's muscle. The light travels through the needle and sweeps over the sarcomere.

Through a process called second harmonic generation, the muscle fiber's striated structure converts the infrared light into a green light, which is returned up the needle, through the handset and to a detector and computer on the cart that interprets variations in the green return signal. These green images are pieced together to form an image of a sarcomere activation, and can provide precise measurement of the duration of a muscle twitch.

"In modern medicine, we're still taking pieces of people to microscopes, but now you can take a microscope to the living tissue and translate science into something that can be used clinically for a variety of diseases," said co-author Gabriel Sanchez, who prototyped the device while earning his doctorate at Stanford. "The size of the needle is similar to a flu shot, but it has optics in it, and produces the same optical performance as the table-size systems with an equivalent objective."

The needle probe can also act as a conductor and stimulate muscle to produce a contraction. This is particularly useful for gauging the muscle reactions in patients who have lost partial control of muscle function. For instance, in test subjects who had experienced partial loss of muscle function on one half of their body due to a stroke, the researchers were able to observe anatomic and physiological abnormalities in the affected

muscle.

"In the affected muscle, we could see differences in the sarcomere lengths as opposed to unaffected muscle on other side of body," Schnitzer said. "We could also see ongoing fluctuations in sarcomere length in the affected muscle at a microscopic scale that have been never seen previously."

The authors are fine-tuning the device, and exploring applications to other tissues, such as skin and cartilage. Currently it has tremendous value for research, but their ultimate goal is to make it available in a clinical setting to help tailor therapies to patients' specific needs and physiology.

"We see this as a very useful companion diagnostic to track disease progression and, in the future, help personalize medicine by gauging how a person responds to a drug," Sanchez said.

More information: In Vivo Imaging of Human Sarcomere Twitch Dynamics in Individual Motor Units. *Neuron*, DOI: dx.doi.org/10.1016/j.neuron.2015.11.022

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