Femtosecond laser technique opens new opportunities for research on nerve regeneration

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In a breakthrough for research on nerve regeneration, a team of scientists has reported using femtosecond laser pulses to precisely cut individual axons of nerves in the roundworm Caenorhabditis elegans, one of the most versatile and widely used experimental organisms for genetic and biomedical research. The nerves severed by this precision technique regrew within 24 hours, often with complete recovery of function.
The project was a collaboration between applied physics researchers at Stanford University led by Adela Ben-Yakar and biologists at the University of California, Santa Cruz, led by Yishi Jin and Andrew Chisholm.

The team's findings give researchers an experimental system in which they will be able to investigate in great detail the genetic and molecular factors that control whether or not damaged nerves can regrow, said Chisholm, an associate professor of molecular, cell, and developmental biology at UCSC.

"This technique will enable us to find the genes that are important in allowing an axon to regenerate. In the worm, we can do systematic screening of large numbers of genes, and of drugs and other small molecules as well, to ask how they affect the process of regeneration," Chisholm said.

The researchers reported their findings in a paper published in the December 16 issue of the journal Nature. The first author of the paper is Mehmet Fatih Yanik, a Stanford graduate student in applied physics, who worked with Ben-Yakar in her femtosecond laser nanosurgery project. Ben-Yakar initiated the project two years ago at Stanford and is continuing it as an assistant professor of mechanical engineering at the University of Texas at Austin. The other coauthors include Jin, a professor of molecular, cell, and developmental biology at UCSC and a Howard Hughes Medical Institute investigator; Hulusi Cinar, a postdoctoral researcher in Jin's lab; and Hediye Nese Cinar, a postdoc in Chisholm's lab.

The Cinars met Yanik through personal connections and initially discussed possible collaboration about a year ago. Yanik later told them about the femtosecond laser and how other researchers had begun using it in biological systems to surgically destroy extremely tiny structures.

"When Yanik described to me what this instrument can do, I immediately thought of my work on the nervous system of C. elegans and came up with a nerve regeneration experiment we could do together, and I designed the experiments," said Hulusi Cinar.

Yanik performed the nanosurgery procedure at Stanford. The technique uses extremely short pulses of intense laser light to focus energy in a very small volume. When properly focused, the energy delivered by the laser pulses breaks down chemical bonds at the targeted site, vaporizing the tissue within a tiny volume without causing side effects such as heating of surrounding tissue, Yanik said.

The duration of the laser pulses used in the study was 200 femtoseconds (a femtosecond is a millionth of a billionth of a second), and the pulses were delivered at a rate of one thousand per second. The delicate axons severed by the procedure, with no apparent damage to surrounding tissue, were on average just 0.3 microns, or 300 nanometers, in diameter (a nanometer is one billionth of a meter).

"I am very excited about the merging of this new technology into biological research. I didn't know anything about femtosecond laser technology until the physicists explained it to me," said Jin, who has spent years investigating the development of the worm's nervous system. "Now there is a lot to do--it has opened up the potential to address questions that we have never been able to address before."

In their experiments, the researchers cut a nerve that runs from one side of the worm's body to the other. The nerve inhibits contraction of muscles on one side of the body while the muscles on the other side are contracting. It functions during the alternating contractions of muscles on either side of the body that enable the worm to wiggle backward with a smooth wavelike motion. Hulusi Cinar and Jin have been studying so-called "shrinker" mutants that lack this ability due to a genetic defect.

"A normal worm, when you touch its head, will move backward in a smooth motion. In the shrinker mutants, the muscles on both sides contract simultaneously, so they don't move back," Cinar said. "So these neurons were a good target for surgery because we knew that when they are knocked out you get a well-defined behavioral effect, and it's straightforward to see if their function has been recovered through regeneration."

Nese Cinar designed a movement assay to evaluate the behavioral effects in the worms and evaluated the worms in the experiments in a "blinded" manner, not knowing which ones had received the surgery.

"Without such functional assays, any anatomically observed regeneration could be explained in various ways, such as bleaching and recovery of the marker protein used to label the neurons," she said.

The nanosurgery, performed on anesthetized juvenile worms, could be completed in about 10 minutes per worm once the equipment was set up. Although regeneration of peripheral nerves is nothing new, Chisholm said he was still surprised by the rapid recovery of function in the worms. Most of the severed axons regrew within 12 to 24 hours after the laser surgery. Preliminary observations indicated that after an axon is cut, the nerve cell sprouts a new axon from the severed end that regrows to reach the target muscle. In some cases, however, it appears that the two severed ends reattach, Chisholm said.

"Clearly there is a lot more biology going on here that we need to explore. This just opens up a lot of exciting things to study," he said.

C. elegans, an almost microscopic nematode or roundworm, has become an extremely important system for biomedical research. Geneticists have identified thousands of genes in the worm that have counterparts in humans. A relatively simple organism with a short generation time, reproducing in just three days, it is easy to study in the laboratory. It has many of the same basic physiological and anatomical features, such as muscles and nerves, found in more complex animals. And it is even transparent, making it easy to see things like nerves inside its body. The researchers who pioneered the use of C. elegans for biomedical research received the 2002 Nobel Prize for physiology and medicine.

Now this excellent model system for biomedical research is available for studying nerve regeneration. One of the fundamental questions researchers want to answer is why nerve damage in the central nervous system--the brain and spinal cord--is usually permanent.

"In humans, peripheral nerves will regrow, but in the central nervous system the regrowth of axons is inhibited by the local environment. That's why spinal cord injuries are so serious. We want to find out why a severed axon will regrow in some situations and not in others," Chisholm said.

Source: UC Santa Cruz