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14. ABSTRACT The high density electrophysiological data acquisition system obtained through this DURIP grant provides important state-of-the-art instrumentation to communicate with individual neurons in the brain and the peripheral nervous system. The major theme of the research is to develop rodent models for the central and peripheral nervous system using microfabrication and microfluidics technologies that can be used to advance our understanding of how the nervous system works at cellular and molecular levels and to improve the diagnosis and treatment of neurological illness and disability. With this support, we have the necessary capability to build a high density					
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Report Title

Final Report: A High Density Electrophysiological Data Analysis System for a Peripheral Nerve Interface
Communicating with Individual Neurons in the Brain

ABSTRACT

The high density electrophysiological data acquisition system obtained through this DURIP grant provides important state-of-the-art instrumentation to communicate with individual neurons in the brain and the peripheral nervous system. The major theme of the research is to develop rodent models for the central and peripheral nervous system using microfabrication and microfluidics technologies that can be used to advance our understanding of how the nervous system works at cellular and molecular levels and to improve the diagnosis and treatment of neurological illness and disability. With this support, we have the necessary capability to build a high density communication highway between BCI and the brain.

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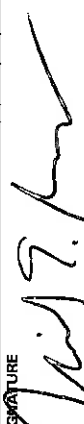
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A High Density Electrophysiological Data Analysis System for a Peripheral Nerve Interface Communicating with Individual Neurons in the Brain

ARO DURIP Final Report

**Contract: W911NF-15-1-0343
U.S. Army Research Office
P.O. Box 12211**

November 11, 2016

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1. Summary

The high density electrophysiological data acquisition system obtained through this DURIP grant provides important state-of-the-art instrumentation to communicate with individual neurons in the brain and the peripheral nervous system. The major theme of the research is to develop rodent models for the central and peripheral nervous system using microfabrication and microfluidics technologies that can be used to advance our understanding of how the nervous system works at cellular and molecular levels and to improve the diagnosis and treatment of neurological illness and disability. The fundamental functional units of the nervous system, neurons, have their dimensions at the micron scale and are extremely sensitive to their microenvironment interacting continuously with neighboring neurons, surrounding matrix, and supporting fluids. Microfabrication and microfluidics technologies enable us to generate neural system animal models that accurately address the human nervous system. With the high density data acquisition system, we will have the necessary capability to build a high density communication highway between 86 billion brain neurons and intelligent vehicles or robots.

With this DURIP support, we were able to install one of the most advanced electrophysiological data acquisition system. TDT 512-channel recording/stimulation system (Tucker-Davis Technologies Inc, Alachua, FL) allows us to capture biological signals from the body with unprecedented detail and further process them to decode invaluable behavioral data. Figure 1 shows TDT high density electrophysiological data acquisition system which was used to measure ECoG signals from *Lewis* rats. Currently, we have developed the second generation scalable μ PNI which can interact with individual brain neurons. The brain signals can be confirmed and matched between μ PNI and μ ECoG signals. The second generation INI will have the necessary capability to build a high density communication highway between brain neurons and intelligent vehicles or robots. The final outcome of the INI using TDT system will be beneficial to wounded warriors suffering from loss of limb function, so that, using sophisticated bidirectional robotic limbs, these individuals could be

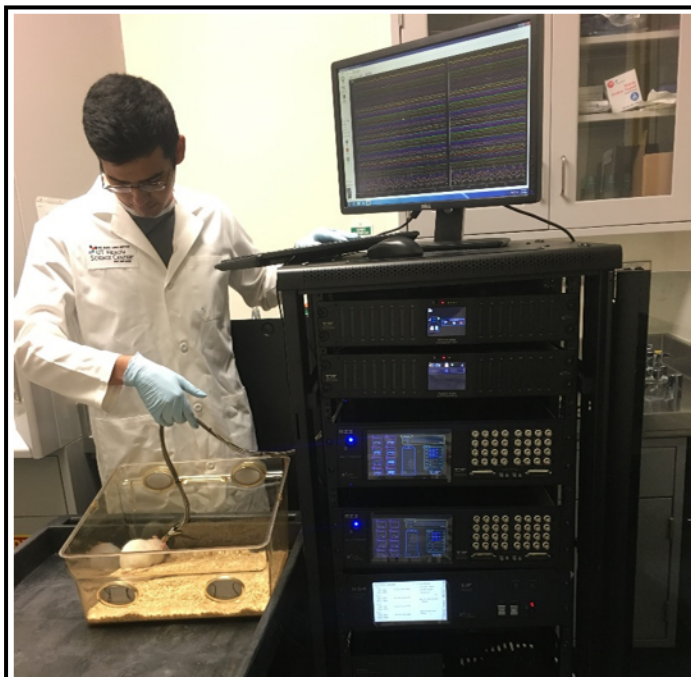


Figure 1. 512-channel high density electrophysiological data acquisition system. TDT data acquisition system has been used to measure neuronal signals from the sciatic nerve of *Lewis* rats.

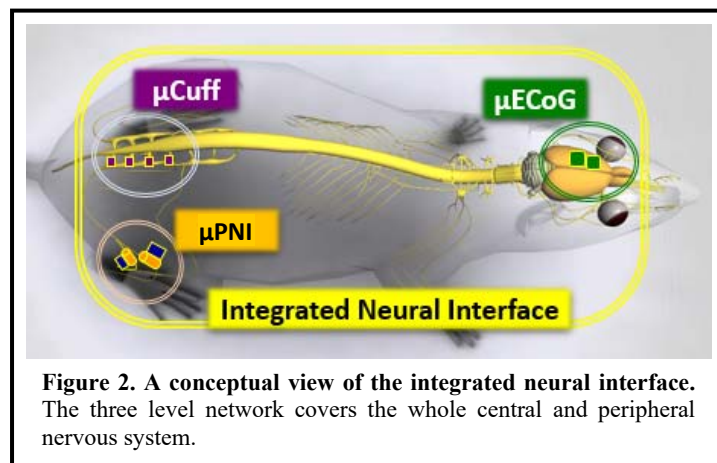
recruited again, allowing highly trained military personnel to continue their careers after what would normally be a career ending incidents. An amputee's decoded and reprogrammed sensory and motor system will be able to control real-time and/or remote intelligent vehicles or robots.

2. Major Research Pathways

We have pursued two frontiers of neuroscience: 1) Development of the single neuron peripheral nerve interface system. 2) Mapping of animal behavioral patterns with global neural network data throughout both the central and the peripheral nervous systems (CNS-PNS). These objectives are attainable using a three-level single neuron network that consists of a micro peripheral nerve interface (μ PNI) placed on the peripheral nervous system and custom-designed μ Cuff/ μ ECoG interfaces on the central nervous system. A neuron in the peripheral nervous system is connected to a neuron in the brain through a single spinal cord neuron in between. Single neuron stimulation then generates a repeatable discrete electrophysiological signal pattern throughout the CNS-PNS. We have developed an integrated neural interface (INI) with three discrete neural interfaces, covering the whole CNS-PNS, that can make a unique neural communication network via single neuron stimulation. Figure 2 shows the conceptual view of the INI. The μ PNI isolates individual neurons and communicates with them. It gives us the capability of both electrophysiological recording and stimulation to complete an integrated neural interface (INI) from the brain to the endings of peripheral nerves. Single neuron stimulation from one end of the peripheral nervous system initiates a neural signal pathway, whereupon the spinal cord μ Cuff is activated, and the immediate brain response is captured by the μ ECoG. A series of brain mapping patterns activated by a single neuron in a peripheral nerve is then confirmed by behavioral patterns observed in freely behaving animals. The primary goal of this research is to complete the INI and make a single neuron electrophysiological map match the specific behavioral pattern of awake, freely behaving animals.

3. Progress and results

Dr. Choi himself has performed all animal surgeries to maintain the highest quality and the consistency of surgery outcomes possible (Figure 3) and have allowed graduate students gain valuable experiences in the lab while focusing on the development of micro devices and



analysis of the data acquired from their devices which were implanted inside animals. For the first generation INI, the μ PNI (32 electrodes), the μ Cuff (26 electrodes), and the μ ECoG (128 electrodes) were integrated. A μ PNI was implanted first on the right hind limb of a *Lewis* rat with two electromyography (EMG) electrodes and two cuff electrodes. Electrophysiological signals coming from the sciatic nerve controlled both the soleus muscle (SOL) and the tibial anterior muscle (TA). The large data concurrently captured from three devices have been analyzed in detail matching the behavioral video data. We also utilize a specialized animal treadmill which is a total behavioral analysis system combining a treadmill and a sophisticated imaging technique. We have used its gait analysis technique to match the behavioral patterns and acquired electrophysiological data. After the termination of the animal study, the harvested tissues have been analyzed comparing the electrophysiological data with the individual electrodes of the μ PNI.



Figure 3. Animal surgery performed by Dr. Choi. All procedures were reviewed and approved by the UTRGV Institutional Animal Care and Use Committee.

Uphill and downhill walking on a treadmill were chosen as target behavioral models. We controlled the slope 5° increment which was not detectable using the standard sciatic nerve model cuff electrodes and EMG electrodes setting. We have used two different treadmill systems. One has the slope angle control capability (760306, Harvard Apparatus, South Natick, Massachusetts). The slope can be changed in the range of -25° to 25° as shown in Figure 4. The other treadmill in our lab has the capability of gait analysis (KinemaScan, Cleversys, Reston, VA). It is the most advanced video analysis system for behavioral studies which can determine various characteristic parameters that are related to the pathophysiological conditions. These parameters include traditional ventral view gait parameters including stance time, swing time, stride length, foot contact area size, and also joint movement data using markers placed on joints that traces joints over time. While we have developed the INI and its specific maps for uphill and downhill walking on an inclined treadmill, we also analyzed the recovery progress of the damaged nerves from the injuries using the details of KinemaScan parameters. Figure 5(A) shows the experimental setup of the KinemaScan treadmill locomotion analysis which was installed in a procedure room at the animal facility at UTRGV. Figure 5(B) shows the joint movement study by tracing the markers

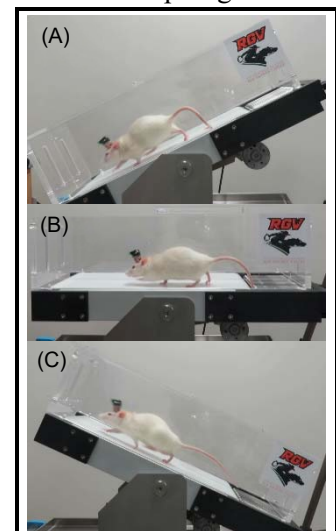


Figure 4. Video analysis of Uphill and downhill walking locomotion. 32-channel TBSI wireless recording device was attached on the headplug (A) 25° downhill (B) 0° degree slope (C) 25° uphill walking

placed on the hind leg joints. Ventral view gait movement studies have been performed at the same time as shown in Figure 5(C).

Figure 6 shows the electrophysiological signals acquired through TDT data acquisition system.

The individual devices of the μ PNI, the μ Cuff, and the μ ECoG have been implanted in the animal separately and neural signal recordings were obtained while the animal was running on both treadmills. Our lab is the third in the world to acquire this state-of-the-art automatic visual analysis system for rodent behavioral studies. Currently the recorded neural signals have been analyzed to extract meaningful signal units which will be matched to the behavioral patterns. Through this research pathways, we were able to implement the whole integrated system with all three-level

single neuron network in an animal model. The completion of the work leads profound outcomes that any behavioral patterns of freely behaving animals will be described with unprecedented accurate and detail information represented from both CNS-PNS together.

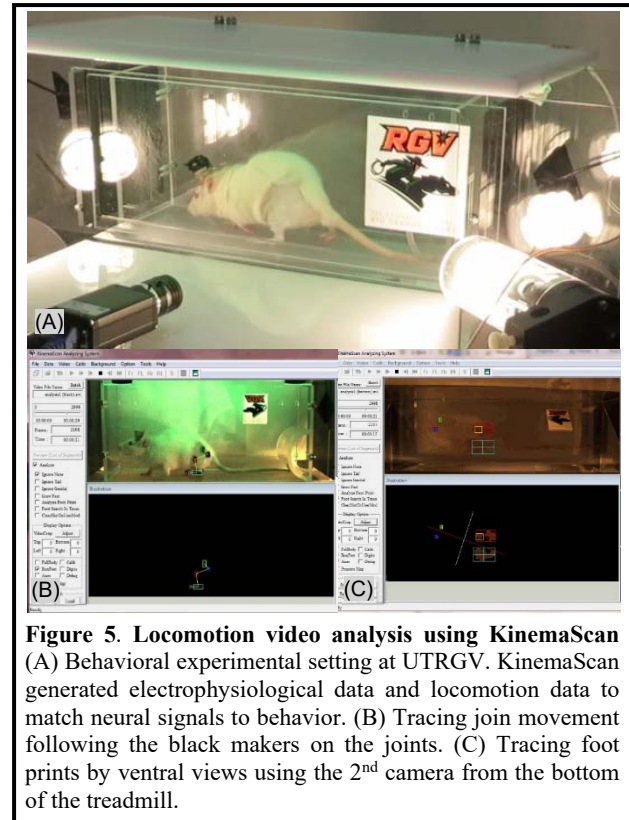


Figure 5. Locomotion video analysis using KinemaScan (A) Behavioral experimental setting at UTRGV. KinemaScan generated electrophysiological data and locomotion data to match neural signals to behavior. (B) Tracing joint movement following the black makers on the joints. (C) Tracing foot prints by ventral views using the 2nd camera from the bottom of the treadmill.

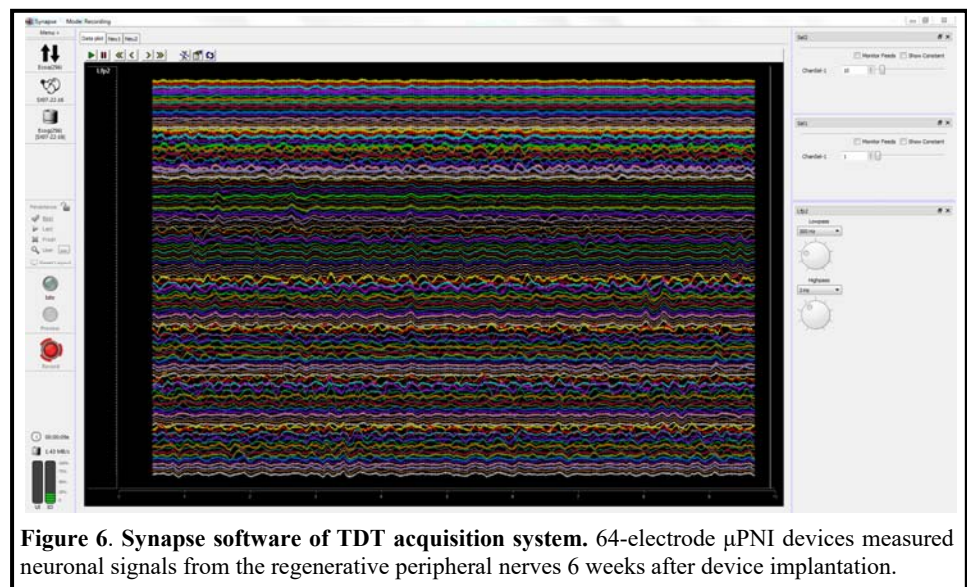


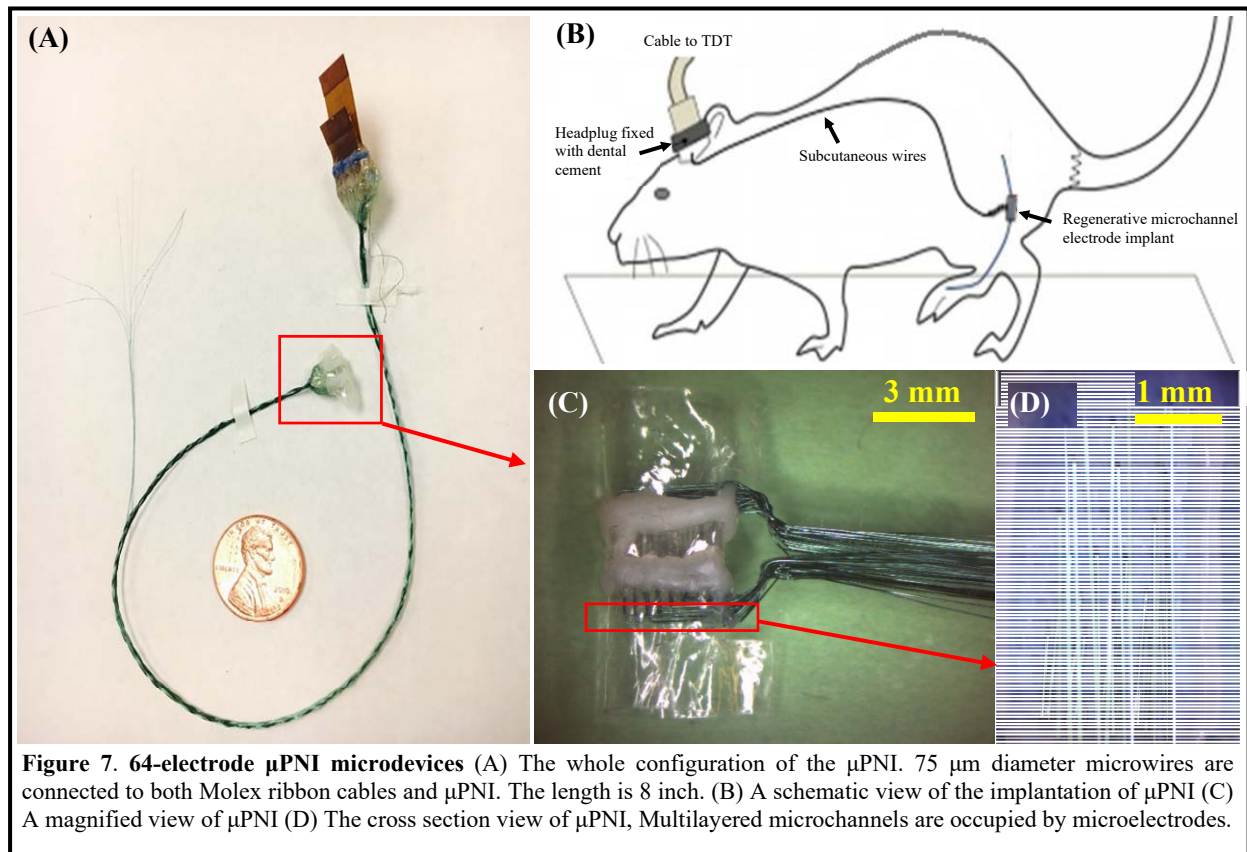
Figure 6. Synapse software of TDT acquisition system. 64-electrode μ PNI devices measured neuronal signals from the regenerative peripheral nerves 6 weeks after device implantation.

4. The next generation microdevices and the future work.

With this DURIP support, we were able to install one of the most advanced electrophysiological data acquisition system. TDT 512-channel recording/stimulation system allows us to capture biological signals

from the body with unprecedented detail and further process them to decode invaluable behavioral data. Currently, we have developed the second generation scalable μ ECoG and μ PNI which can interact with individual brain neurons. The brain signals captured by μ ECoG can be confirmed and matched by the signals acquired from μ PNI. μ ECoG is a minimally invasive neural recording method that has been extensively used for neuroscience applications. We have used μ ECoG devices not only for recording brain signals, but also for networking with the neuronal signals from the peripheral nervous system.

Recently, the second generation 256-electrode μ ECoG microdevices have been developed to acquire brain signals and 64-electrode μ PNI devices have also been developed to communicate with the peripheral nervous system. Thanks to the DURIP support, we have optimized our system to adjust up to 512 electrodes concurrent recording using the large-scale recording capability of TDT data acquisition system. We will take the best advantage of the 512-channel TDT system by performing a surgery with multiple devices in an animal up to 512 electrodes. The future work with fully functional 512 electrodes will give us unprecedented detail of neural signals matched with behavioral data. Figure 7 shows the latest 64-electrode μ PNI microdevices and Figure 8 shows 256-electrode μ ECoG microdevices.



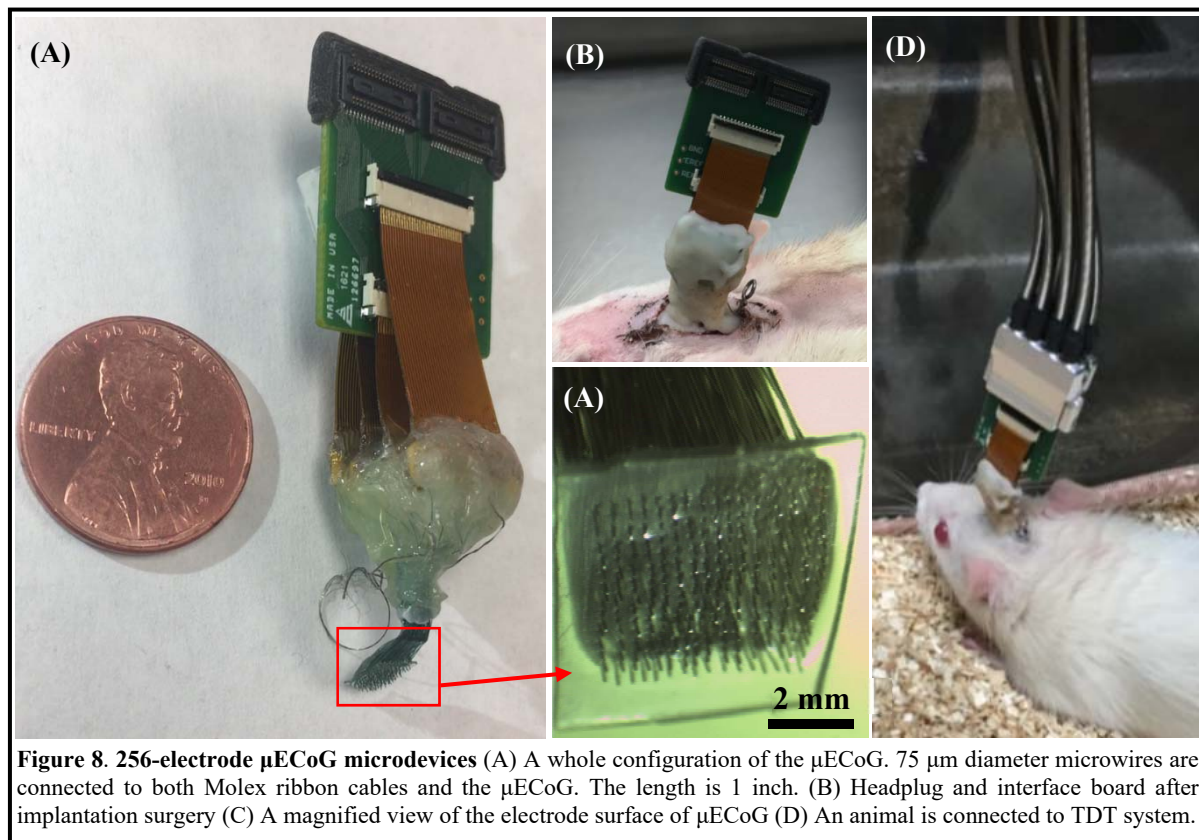


Figure 8. 256-electrode μ ECoG microdevices (A) A whole configuration of the μ ECoG. 75 μ m diameter microwires are connected to both Molex ribbon cables and the μ ECoG. The length is 1 inch. (B) Headplug and interface board after implantation surgery (C) A magnified view of the electrode surface of μ ECoG (D) An animal is connected to TDT system.

5. Publications

- 1 N. Tasnim, A. Ajam, R. Ramos, M.K. Koripalli, M. Chennamsetti, and Yoonsu Choi, "Handcrafted Electroencephalography Electrodes for a Rodent Behavioral Model," *Technologies* 2016, 4(3):23, <http://dx.doi.org/10.3390/technologies4030023>
- 2 A. Ajam, R. Hossain, N. Tasnim, L. Castanuela, R. Ramos, D. Kim, Yoonsu Choi, "Handcrafted Microwire Regenerative Peripheral Nerve Interfaces with Wireless Neural Recording and Stimulation Capabilities," *International Journal of Sensor Networks and Data Communications*, 2016; 5(133):1000133, <http://dx.doi.org/10.4172/2090-4886.1000133>
- 3 Yoonsu Choi, H.M. Noh, "Peripheral nerve regeneration monitoring using multilayer microchannel scaffolds" *Neural Regeneration Research*, 2016; 11(3):422, <http://dx.doi.org/10.4103/1673-5374.179052>
- 4 R. Hossain, B. Kim, R. Pankratz, A. Ajam, S. L. Biswal, and Yoonsu Choi, "Handcrafted Multilayer Microchannel Scaffolds for Peripheral Nerve Regeneration," *Biomedical Microdevices*, 2015; 17(6):109, <http://dx.doi.org/10.1007/s10544-015-0012-4>