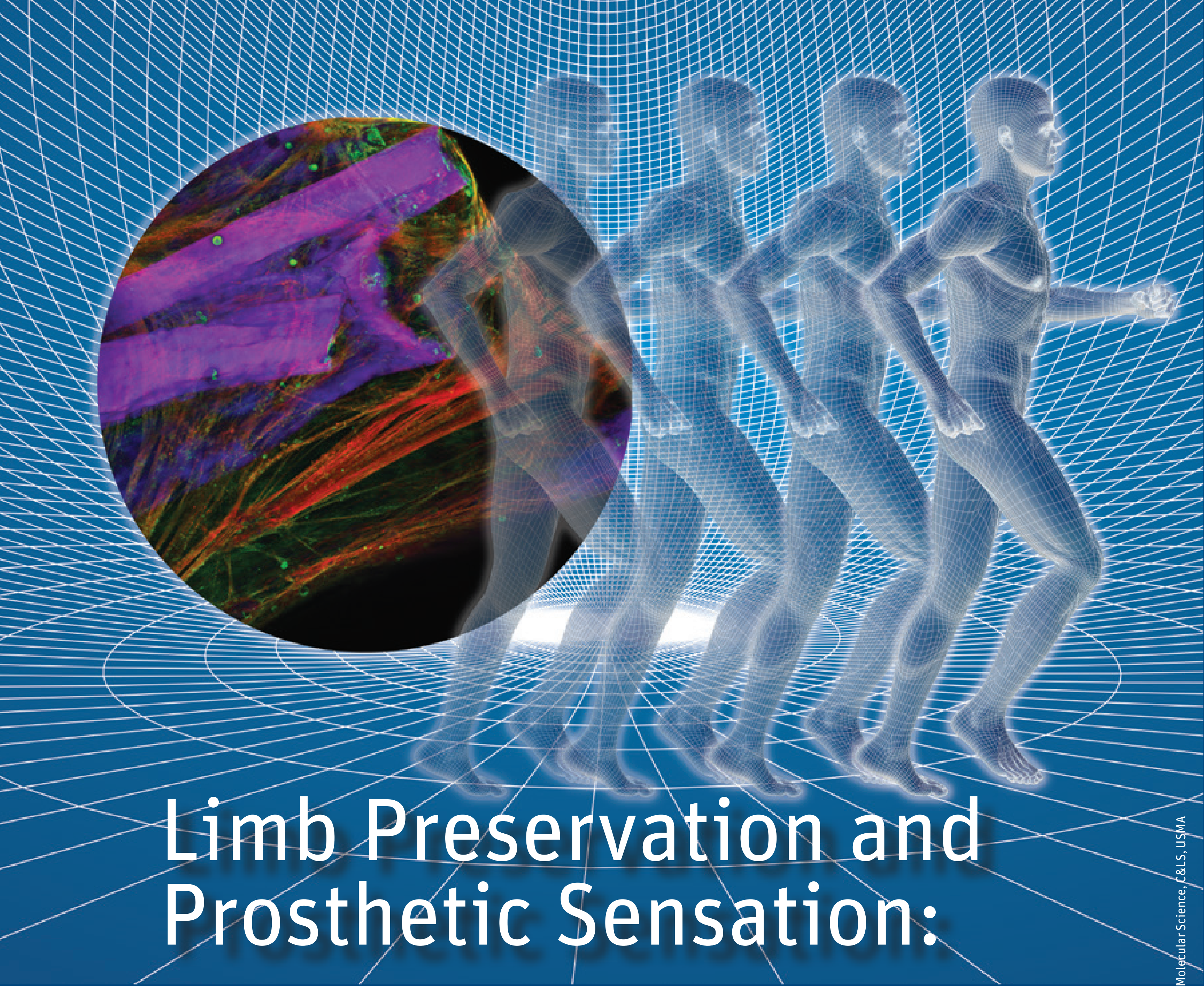

Limb Preservation and Prosthetic Sensation:

Two USMA Capstone Projects Advance Treatment for Severed Limbs

By **Keith J. Hamel,** WPAOG staff

Two capstone teams from the United States Military Academy's Department of Chemistry and Life Science (C&LS), supported by the Academy Scholars Program and led by Lieutenant Colonel Luis Alvarez '97, are working on different research projects to address a common problem: major limb amputations. Due to the prevalence of improvised explosive devices (IEDs) in Operations Iraqi Freedom or Enduring Freedom, approximately 1,600 Soldiers who fought in either Iraq or Afghanistan have undergone amputation procedures. Advancing the treatment of wounded and amputee Soldiers is a key research initiative for the Department of Defense and U.S. Army.

One of the cadet teams, working on what is known as the "Biostasis" Project, is investigating a new low-temperature preservation method that could keep limbs lost to blunt force combat trauma viable for at least 24 hours, which would be enough time to medevac the casualty to a vascular surgery team that could reattach the limb. The other team, working on the "SENSA" Project, aims to create a neuro-sensory interface that could give prosthetic limbs the sense of touch.

Above, inset: A confocal micrograph shows 0.05mm collagen fibers (violet) guiding sensory neuron axons (red).

Photo: Courtesy of Dr. Kevin O'Donovan and Dr. Joseph Loverde, Center for Molecular Science, C&LS, USMA

The Biostasis Project

When a Soldier loses a limb in combat, time is the main enemy. Due to the typically remote location of the battlefield, there is usually not enough time to move the wounded Soldier from the front lines to a hospital with the resources to reattach the limb. For example, it takes more than six hours to fly a casualty from Afghanistan to Landstuhl Regional Medical Center in Germany, but the optimal time window for reattachment is under three hours. The goal of the Biostasis Project is to "stop" biological time and preserve a lost limb for a long enough time period to allow advanced trauma intervention to occur. The key to this seemingly impossible endeavor is an isochoric chamber that the team received on loan from Dr. Boris Rubinsky of the University of California, Berkeley's Mechanical Engineering Department. As explained by the Biostasis Project's team leader, Cadet Marco Dela Cruz '16, the chamber follows Boyle's Law and uses pressure to maintain cooling in a liquid phase to preserve living tissue without damage. "Unlike what is seen in movies, you just can't place a severed limb on ice and expect to reattach it without problems," Dela Cruz says. "The temperature drop causes the water in the cells to freeze, freezing causes expansion, expansion causes the cell to rupture and rupture kills the tissue." He likens this effect to freezer-burned meat. By working at a high pressure, the chamber will prevent freezing and mitigate any tissue damage during the cooling process. This will significantly lengthen the time the limb can survive without blood circulation from the current 3 hours to beyond 24 hours, extending the crucial time window during which a limb can be successfully reattached.

Photo: Anthony DiNoto/WPAOG

CDTs Marco Dela Cruz '16 (foreground) and Sean McGivney '16 assemble the isochoric chamber for an experiment while LTC Luis Alvarez '97 observes.

Biostasis finished in the top three among all service academy teams at the DARPA SAIC competition and won an honorable mention from Dr. Steven H. Walker, Deputy Director of DARPA.

To test the chamber's effectiveness, Dela Cruz and two other team members (Cadets Sean McGivney '16 and Daniel Fullmer '16) performed a series of analytical techniques—including a basic live-dead assay, a metabolic function test and a cell stress protein analysis—to measure cell survival and function. With support from Dr. Joseph Loverde and Dr. Kevin O'Donovan, both neuroscientists in the C&LS's Center for Molecular Science, the team began the project by working with HeLa cells, an immortal line of cancer cells often used in research, in order to perfect their lab techniques: pipetting cells, growing cultures, working under a sterile hood and using microscopes. From there, the team worked with chick heart cells and finally beating human heart cells generated from induced pluripotent stem cells (iPSCs), which are adult, not embryonic, human stem cells. Their goal was to cryopreserve these cells in the isochoric chamber for varying periods of time and, in a first-in-the-field type of demonstration, successfully rewarm them to their functional beating state. "We just adjusted our independent variables—cooling temperature, cooling rate and the duration of cooling—to see which gave us the highest recovery by comparing the living cell count of the control group to our experimental group," Dela Cruz says. Team Biostasis worked closely with collaborators Dr. Gary Carlson and Mr. David Fisher from UC Berkeley on the experimental design and data collection.

The Biostasis Project's research opens the door to larger model isochoric chambers which could be permanently kept on medical Blackhawk helicopters or Armored Medical Evacuation Vehicles. For these larger chambers, Dela Cruz envisions a seamless canister/circular-refrigerator combo. "It would be low cost and low maintenance," he says, "and best of all it will greatly increase the successful reattachment rate of severed limbs." Because of its research and application potential, the Biostasis project was one of three projects chosen from more than two dozen to represent the United States Military Academy at the 2015 Defense Advanced Research Project Agency's Service Academy Innovation Challenge (DARPA SAIC), where it placed third out of nine teams. Now in its second year, the DARPA SAIC encourages service academy students to develop practical, transformative technologies that could successfully address the challenges facing the U.S. military. By allowing an injured Soldier to possibly retain his or her severed limb, the Biostasis project certainly qualifies in this regard.

The SENSA Project

Of course, not all severed limbs can be saved, and sometimes a prosthetic is the best option for injured Soldiers. In these cases, offering a prosthetic that replicates the lost real limb as closely as possible would be ideal. Thanks to advances in prosthetic technology, there are now natural-looking artificial limbs and even ones with motor capability that allow the user to pick up objects or lift his or her prosthetic arm. Yet, at this time, modern prosthetics are not able to confer a sense of touch to the user. This is where Team SENSA comes in.

Led by Cadets Minhee Pak '17 and Edmund Mullin '17, Team SENSA is creating a method for fiber-guided neuroregenesis, or the re-growing of nerves, which would be applied to develop sensation-generating prosthetics of the future. Conferring sensation through a prosthetic is entirely possible to achieve, but it requires separating sensory nerve cells from motor nerve cells at the site of amputation. The team's final product vision is a Y-shaped fiber array that contains the appropriate nerve growth factors on each of its branches so that it can bridge the gap between damaged neurons and either sensory input from prosthetic sensors or motor output to a myoelectric system.

Like the cadets of the Biostasis Project, members of Team SENSA began their project by learning how to work in the lab. They were in the lab two to three times per week refining their skills and learning how to perform complicated culturing techniques on sensory neurons from chick tissue. Afterwards, the team divided into three groups: one continued culturing nerve cells for experimentation, one performed growth factor testing and one focused on constructing the fiber array. Team SENSA experimented with five nerve factors (proteins that signal neurons to grow), three of which showed promise: 1) nerve growth factor, 2) brain-derived neurotropic factor and 3) pleiotrophin. In another important step, Team SENSA began growing type-I collagen fibers as a platform on which neurons will grow and assembled these into fiber "bundles." Lastly, the team tethered the

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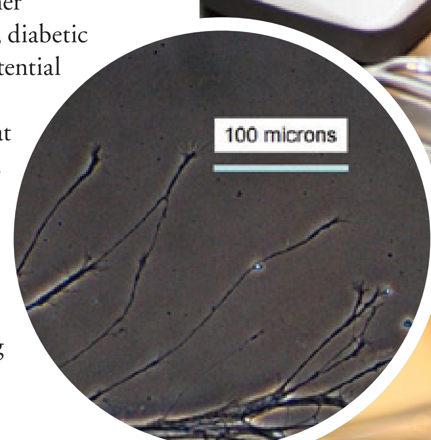
>
> —LTC Luis Alvarez '97

Left to right: CDTs Edward Mullin '17, Jerry McMurray '15, Haana Yu '17 and Minhee Pak '17 stand in front of a poster for the SENSA Project, along with Dr. Joseph Loverde, on Projects Day 2015.

Photo: Anthony Battista, West Point DPTMS



Clockwise: Isolating neurons (nerve cells) from a chick embryo. Looking at neurons through a microscope in the new Center for Molecular Science lab. A custom-made fiber bundle used to guide neuron growth along desired paths. **Inset:** Neurons growing in a culture.

fibers with the corresponding nerve factor. The hope is that their fiber array can be implanted wherever it is needed to allow a prosthetic with sensors to 'plug into' the sensory nerve branch. Such research is at the very cutting edge of the field of regenerative medicine, and Team SENSA believes its project will not only benefit veteran amputees, but could also help alleviate other medical conditions such as phantom limb pain, neuroma, diabetic neuropathy and more. Because of the groundbreaking potential of its research, Team SENSA was invited to present its findings at the 12th annual Soldier Design Competition at the Massachusetts Institute of Technology in Cambridge, Massachusetts, where it won the Gore Innovation Prize.

Participation in this rigorously high level of research as undergraduate students represents a unique opportunity for West Point cadets. Yet despite its academic benefits and potential for applied use in saving limbs or enhancing the function of prosthetic limbs, the research for the Biostasis and SENSA Projects did not come without cost. Alvarez estimates that the materials needed to conduct the research for these projects totaled more than $35,000. "Cell lines, cell culture media and nerve factors alone cost nearly $10,000," he says. Donors who would like to know more about contributing to the Academy Scholars Program, which benefits experiential learning opportunities for cadets, or the Cadet Pre-Medical Education Fund, which prepares cadets for medical school and careers as Army physicians, are welcome to contact the West Point Association of Graduates Development Office. Not only would they be supporting potentially groundbreaking advancements for Soldiers such as those offered by projects Biostasis and SENSA, but they would also be directly enhancing cadet education and leadership development. "While the final products are impressive, the overall process is a tremendous teaching opportunity," says Alvarez. "Having research that truly allows our cadets to learn through guided inquiry, now that is powerful." ★

Photos: Anthony DiNoto/WPAOG, Courtesy of LTC Alvarez, C&LS