Transforming Research Into Practice

NCRR programs foster collaboration among researchers and clinicians to address unmet medical needs. **BY LAURA BONETTA**

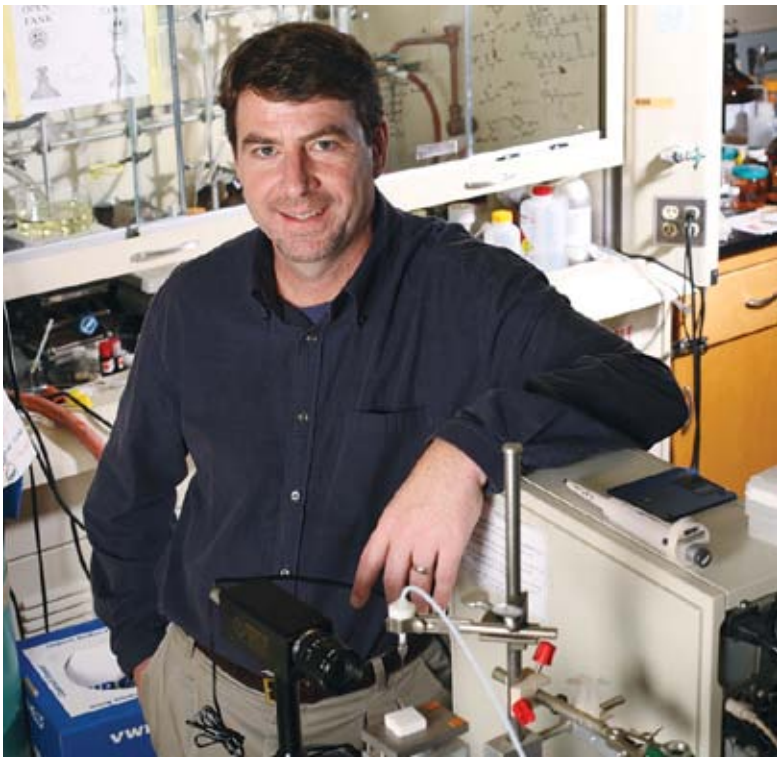
A sea mussel clinging to a rock would not make most people think of medical advances in the operating room. Yet research on the properties of the sticky substance that mussels produce to keep them from being tossed around by waves may someday result in a synthetic adhesive to repair tears in amniotic sacs.

Countless clinical advances have had their starts in such basic discoveries, while others are born out of medical problems in need of a solution. Regardless of their origin, advances require extensive dialogue and collaboration among basic researchers and clinicians from different backgrounds and the public.

Many NCRR-funded programs provide the infrastructure and support needed to bring researchers together to tackle the critical problems that lack obvious solutions and to facilitate the translation of the resulting knowledge to benefit patients — whether they be pregnant women, HIV-infected individuals, cancer patients or firefighters.

MIMICKING MUSSEL GLUE TO MEND MEMBRANES

More than a decade ago, Phillip Messersmith, currently a professor of biomedical engineering at Northwestern University in Chicago, came across scientific reports describing proteins produced by the foot of the common mussel (*Mytilus edulis*) that enable this organism to glue itself to rocks. "I thought that maybe those proteins could have significant applications," he said. "If we could better understand their properties, we could come up with new materials that are able to bind to different surfaces in a wet environment, which is no small feat."



In his laboratory at Northwestern University in Chicago, Phillip Messersmith has been developing synthetic materials that mimic proteins produced by mussels and can stick to different surfaces even in wet environments. With pilot funding from the Northwestern University Clinical and Translational Sciences Institute, supported through NCRR's Clinical and Translational Science Awards program, Messersmith has been testing these mussel-based "glues" to seal tears that occur in amniotic sacs, a complication of some pregnancies.

PHOTO COURTESY OF NORTHWESTERN UNIVERSITY

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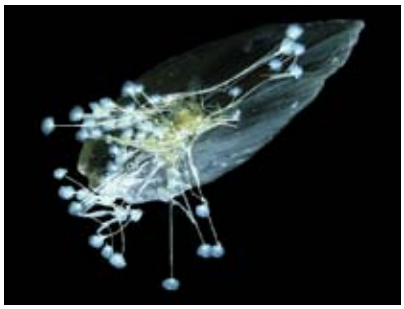
Messersmith, whose research team has laboratory space in the medical building at Northwestern, learned about the need for new materials from conversations with clinicians. "Based on what they see in their practice, they tell us what they need," he said. "There is no shortage of ideas."

Glue for fixing rips and tears in the membrane that surrounds a developing fetus during pregnancy is one such need. Such defects, which may arise naturally or as a result of surgery, can cause amniotic fluid to leak, resulting in premature labor or an end to the pregnancy. "There are some glues for closing superficial skin wounds," explained Messersmith. "But for the fetal membrane, there is no existing commercial product available. This is a totally unmet need."



■ The foot of the common mussel (*Mytilus edulis*) produces sticky proteins that allow the organism to glue itself onto rocks, keeping it from being tossed around by waves. Researchers at Northwestern University in Chicago are developing synthetic materials with properties similar to these mussel proteins for a variety of medical applications.

Messersmith and colleagues started to investigate the features that make mussel glue work so well in wet environments. In a paper published in *Proceedings of the National Academy of Sciences* in 2006, they showed that one of the key components of a family of unique mussel adhesive proteins, the amino acid dihydroxyphenylalanine (DOPA), was capable of forming strong bonds to organic surfaces in water. At the same time, the group has been developing synthetic materials for a variety of medical applications. With pilot funding from the Northwestern University Clinical and Translational Sciences (NUCATS) Institute, supported through NCRR's Clinical and Translational Science Awards (CTSA) program, Messersmith asked whether new materials synthesized to contain DOPA could seal fetal membranes.

In collaboration with a group of researchers in Switzerland, Messersmith's team punched holes in amniotic sac membrane to replicate what happens *in vivo*. They then applied their sealant and several candidates developed by other researchers to the holes. The mussel-based sealant was the only one that was effective in this system. Messersmith now plans to test the new sealant in animal models and is hopeful that it will move to clinical trials in patients within, approximately, the next five years.

The project is consistent with one of the main goals of the CTSA program — to provide the resources researchers need so the scientific discoveries they make in the laboratory can more quickly become treatments for patients. "We fund about eight to 10 pilot studies a year that seem to have high translational possibility," said Philip Greenland, NUCATS' director. "These are high-risk and potentially high-reward projects. We are probably the only source of this type of funding at Northwestern. Most funding is for projects that are already further down the line. That is one of the distinct roles that NUCATS serves."

In addition to identifying research projects worthy of support, NUCATS plays an active role in getting Northwestern faculty to interact, which is another goal of the CTSA program. "We encourage our researchers to connect outside of their primary areas of work," said Greenland. "In particular, we encourage basic scientists to establish stronger connections with the medical world and vice versa."

CURTAILING HIV'S ABILITY TO TRIGGER DEMENTIA

Bringing scientists together to create novel approaches to solve clinical problems is a goal shared by the NCRR-funded Institutional Development Award (IDeA) Centers of Biomedical Research Excellence (COBRE). The IDeA program fosters health-related research at institutions in states where the aggregate success rate for grant applications to NIH historically has been low. It does this by supporting multidisciplinary research centers through the creation of core facilities, the hiring of new faculty and the establishment of needed research infrastructure.

Now in its second phase of five-year funding from NCRR, the COBRE in Women's Health at the University of Kentucky's College of Medicine in Lexington funds five projects studying the impact of hormones and gender on heart disease, brain function, HIV, reproductive tract physiology and behavior. Each area brings together teams of two to four researchers, including more established investigators and newly hired faculty. The junior scientists are mentored in all areas of research and professional development, not only by the more senior scientists on their own team, but also by all COBRE investigators, to ensure their continued success.

"We have 15 investigators from different colleges and backgrounds who interact regularly within the infrastructure provided by the COBRE," said Thomas Curry, the center's principal investigator. The University of Kentucky always has had

PHOTO COURTESY OF NORTHWESTERN UNIVERSITY

■ Melinda Wilson (right), an investigator at the NCRR-funded COBRE in Women's Health at the University of Kentucky's College of Medicine, is trying to understand the mechanism by which estrogen protects women from the dementia caused by long-term HIV infection of brain cells. Wilson is shown with postdoctoral fellow Jenne Westberry. A major goal of the COBRE is the mentoring and training of junior investigators who are interested in pursuing research in women's health.

a core of researchers working on women's health. But "what the COBRE has achieved," explained Curry, "is that it has provided the infrastructure to formalize the interactions behind what was an informal group of investigators to promote and focus women's health research at Kentucky."

When Melinda Wilson was first hired, she teamed up with a more established COBRE investigator, Avi Nath, who is an expert in HIV. "My background is in physiology and, more specifically, neuroendocrinology — how hormones impact brain function," said Wilson, an associate professor at the University of Kentucky. The collaboration with Nath sparked Wilson's quest to understand how estrogen, an ovarian hormone, protects women from the dementia caused by long-term HIV infection in the brain.

HIV infects primarily immune cells but also certain cells of the nervous system called astrocytes. As a result, people with advanced HIV infection often have inflammation of the brain, behavioral changes, and trouble with concentration, memory and attention — some of the same cognitive problems associated with aging. "This is going to be particularly important now that people with HIV are living much longer. It is possible that many more people may suffer from some form of cognitive problem," said Wilson.

Researchers long have known that estrogen protects brain cells from inflammation and damage, but little is known about the molecular mechanisms involved. Taking advantage of COBRE funding and resources, Wilson and Nath discovered that estrogen attenuates HIV transcription — the first step in production of HIV-encoded proteins — in an astrocyte cell line, raising the possibility that the hormone might protect from HIV dementia.

The next question Wilson wanted to ask was, "How does estrogen do it?" She knew that estrogen mediates its effects through the estrogen receptor alpha (ERα), found on the surface of many cell types, including astrocytes. "Our hypothesis was that the estrogen-mediated suppression of HIV transcription in astrocytes was dependent on ERα," said Wilson.

When Wilson and her team administered estrogen to HIV-infected astrocytes, they found that, as expected, the treatment reduced HIV in the cells, but they could not detect any ERα protein in these cells, suggesting that this receptor did not play a major role. Surprisingly, when they engineered astrocytes to produce high amounts of ERα, estrogen was no longer able to inhibit HIV transcription in those cells. Thus, ERα actually blocks estrogen's action in reducing HIV. "The mechanism of action is obviously more complicated than we envisioned," said Wilson, who published this work in *AIDS Research and Human Retroviruses* in 2009.

Although the project started as a mentoring relationship with COBRE funding, Wilson has since been able to obtain independent R01 NIH and National Science Foundation grants to support her work and in the process was promoted from assistant to associate professor. Grant independence and mentoring are key goals of the IDeA COBRE program. With the funds, she plans to look for other possible receptors responsible for mediating estrogen's function. "If we could figure out the molecular mechanism by which estrogen works in normal tissues and disease, we could make designer estrogen that protects neurons from damage in aging and HIV infection," she said.

DECODING CANCER'S PROTEIN SIGNATURE FOR IMPROVED DIAGNOSIS

The opportunity for intensive collaboration with clinical and biomedical researchers motivated Catherine Costello to move from Massachusetts Institute of Technology to Boston University School of Medicine (BUSM), when she transitioned from co-principal investigator of a long-standing NCRR-supported mass spectrometry center to principal investigator of a new one. That decision has benefited a host of researchers who use this NCRR-funded resource.

Costello long has been using mass spectrometry, a technique for determining the chemical structures of molecules, to catalog the types and quantities of proteins produced by different cells in the body. This approach is referred to as proteomics. In one of her more recent projects, she used the technique to identify a group of proteins that are uniquely produced in high amounts in a particular type of blood cell cancer, providing its molecular signature.

Cancer is a disease in which cells gain an enhanced ability to multiply, survive and spread. Many types and even

PHOTO COURTESY OF THE UNIVERSITY OF KENTUCKY

subtypes of cancer exist, depending on the tissue of origin and the molecular events that lead a particular cell in that tissue to become a cancer cell. That is one reason why patients diagnosed with the same type of cancer often respond differently to treatment. Thus, researchers have been looking for ways to classify cancer more accurately.

Costello joined forces with BUSM cancer researcher Gerald Denis to find a protein signature for a type of non-Hodgkin's lymphoma, one of the most common and deadly malignancies in adults. "There are many forms of non-Hodgkin's lymphoma, and they are all very similar histologically, but depending on which type is present, the patient will have a very different outcome," explained Denis.

Working with mouse cells at first, Costello and Denis analyzed the protein profiles of a subset of lymphomas called diffuse large B-cell lymphoma. They then compared these profiles to those of normal cells — as is typically done in proteomics studies like this one — as well as to those of cells that were not cancerous but were growing rapidly and multiplying. This last comparison allowed Costello and Denis to distinguish the unique cancer signatures from changes in proteins that occur simply as a result of normal cell proliferation, narrowing the focus of their search.

Using this procedure, the researchers identified a specific cluster of proteins that appear to constitute lymphoma-associated markers, as reported in a paper published in the *American Journal of Pathology* in July 2009. "This example shows the power of the proteomic approach and also the importance of proper long-range planning and experimental design that involves all partners in the collaboration," said Costello.

In addition to clever design, the project required the development of new bioinformatics tools to analyze the vast amount of protein data generated. "This particular application has to do with lymphoma, but the general approaches that we developed using this technology and the biological methods we describe are much more widely applicable," said Denis. To which Costello added, "These tools can be applied to other malignancies, but also noncancerous mechanisms such as cardiovascular disease."

The approach will have to be repeated in human cells and eventually refined to provide a diagnostic test. "One of the advantages of being here is that at this institution, interactions among researchers are very open and strong," said Costello. "As we move forward, we plan to get clinical people involved."

HELPING FIREFIGHTERS BEAT THE HEAT

The medical application of tests like the one Costello and Denis are working on is perhaps far in the future, but some NCRR-supported research collaborations are focusing on solutions that quickly can be translated to practice.

The NCRR-funded Clinical and Translational Science Institute (CTSI) at the University of Pittsburgh (Pitt), another CTSA-supported institution, provided technical and clinical support for a clinical trial that involved collaboration among some unlikely partners — university researchers, nearly 100 firefighters, Allegheny County Emergency Services and Allegheny County Fire Academy staff — to find a way to tackle the number one killer of firefighters.

Contrary to common perceptions, the greatest harm to firefighters does not come from falls or burns, but rather from sudden cardiac arrest. "There is a paucity of information about why firefighters are at such high risk for cardiovascular events," said CTSI director and volunteer firefighter Steven Reis.

There are some clues; firefighters get extremely hot and dehydrated and must work under high stress. The gear they wear, designed to protect them, also places a significant weight on their bodies and traps internal heat. All these factors are thought to contribute to heatstroke and sudden cardiac arrest and also may lead to long-term complications.

With funding from the Federal Emergency Management Agency (FEMA), the CTSI helped design the Fireground Rehab Evaluation (FIRE) trial, led by volunteer firefighter David Hostler, an assistant professor of emergency medicine at Pitt and director of the Emergency Responder Human Performance Lab (ERHPL), and co-investigator Joe Suyama, also an assistant professor at Pitt and the medical director of ERHPL.

As a first aim, FIRE set out to assess cooling and rehydration methods to counteract firefighters' increased body temperatures, which can climb as high as 104 degrees

■ The NCRR-funded Mass Spectrometry Resource at Boston University School of Medicine (BUSM) provides the latest instruments and approaches of mass spectrometry, a technique for determining the chemical structures of molecules, which researchers apply to address a wide range of medical research questions. Resource director Catherine Costello (shown), working with BUSM collaborator Gerald Denis, recently completed a project to identify a group of proteins that serve as markers for a particular type of blood cell cancer.

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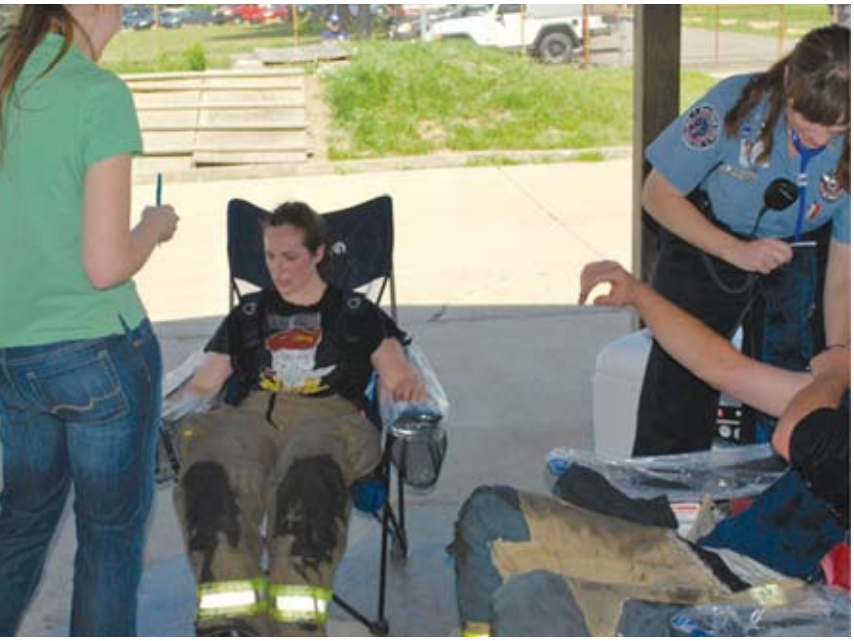

■ When working to put out fires, firefighters' body temperatures can reach a staggering 104 degrees Fahrenheit, placing them at risk for heatstroke and sudden cardiac arrest. The NCRR-funded Clinical and Translational Science Institute at the University of Pittsburgh helped design the Fireground Rehab Evaluation trial to assess cooling and rehydration methods to counteract firefighters' increased body temperatures. In this photo, study participants sit in chairs with arms specially designed to hold bags of cool water, in which they immerse their forearms.

Fahrenheit (40 degrees Celsius). "I've seen some guys sit on the bumper of the fire truck and chug water to cool. That really is the extent of existing cooling methods," explained Hostler. "Fire rehabilitation, if done at all, is inconsistent at best."

The first phase of the FIRE trial enrolled 18 firefighters to complete 50 minutes of exercise in a hot room while wearing their protective gear. After the exercise, participants were randomly assigned to receive water, a sports drink or an intravenous infusion of normal saline. After rehydration, the firefighters performed a second round of exercise while the researchers assessed vital signs. In a report published in the journal *Prehospital Emergency Care* in April 2010, the researchers showed that performances in the second round did not differ among groups, regardless of the method of rehydration. Furthermore, none of the subjects was able to complete both rounds of exercise.

To find a better approach to lowering body temperatures, Hostler, Suyama and Reis investigated three other cooling methods in a live burn drill at the Allegheny County Fire Academy's "burn building," which is set ablaze for training simulations. During the drill, 25 firefighters spent 20 minutes extinguishing a controlled structural fire. After the fire was suppressed, the firefighters were randomized to use one of three cooling techniques. The first group sat in chairs with arms specially designed to hold bags of cool water, into which the firefighters immersed their forearms. A second group put on cooling vests that pump water from a cooler into tubing sewn into the vests. These vests also are used by NASCAR drivers to help them maintain a healthy body temperature during races. A third group sat or stood in air-conditioned vehicles parked at the scene of the fire. During the treatments, researchers monitored firefighters' core temperatures, lactate levels, and heart and circulatory functioning.

Reis, Hostler and Suyama are now finalizing the data analysis and publishing their study results. They also are investigating further how firefighters respond physiologically to heat stress and how preventive measures, including aspirin, can be used to decrease firefighters' risk of sudden cardiac arrest and other cardiac events. "Our hope is to move the results of our research into practice at fire departments across the United States," said Reis.

Reis credits NCRR and similar funding sources that helped accelerate translation of his research into practice. "Traditionally, when we think of NCRR's CTSA program, we think of leveraging other NIH support to reach into a broader community," he said. "But, in the case of the FIRE trial, we were able to use NCRR's resources to set up a unique collaboration with FEMA that has allowed this line of work to go well beyond where it could have otherwise."

The studies highlighted here show the breadth of research spurred by NCRR programs and resources. From very basic studies in the laboratory to high-level investigations that involve sophisticated equipment and technologies to develop better tests for cancer diagnosis, NCRR-supported research aims to accelerate the speed with which scientific discoveries improve preventions, treatments and cures for disease. ■

ADDITIONAL READING:

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