NIRF, nodes and night-vision

Dr Eva M Sevick, Professor and Cullen Chair of Molecular Medicine at UTHSC's Brown Foundation Institute of Molecular Medicine, explains her work on near-infrared imaging and lymphedema

Can you describe some of the imaging devices that you have designed to overcome the lack of naturally-occurring molecules that can be excited in the NIR range? What advantages do these devices afford?

Firstly, this is a combinational drug and device product, so optimal imaging is achieved by both the device and the drug. The first step in overcoming fluorescence from naturally-occurring substances is to illuminate tissues using well-defined light sources with wavelengths above 780 nm, which produces virtually no fluorescence from naturally-occurring fluorophores. Secondly, we need to very efficiently collect the weak fluorescence generated, using the night-vision goggle technology (ie. NIR sensitive image intensifiers). Coupled with efficient optics, this enables us to collect the weak signal and reject all other light.

What are the limitations of the indocyanine green (ICG) dye that has been utilised to date? Are there any other dyes that would afford similarly advantageous qualities?

Because these devices are so sensitive to NIR fluorescence, we are able to use a very poor NIR dye – indocyanine green (ICG). ICG is a dark green-coloured dye which has been used safely in humans for the past 50 years for hepatic clearance and ophthalmic applications, and it has worked well with our highly-sensitive instrumentation. However, ICG is a very dim and unstable NIRF dye which has no functional group for conjugation to attach targeting imaging agents to.

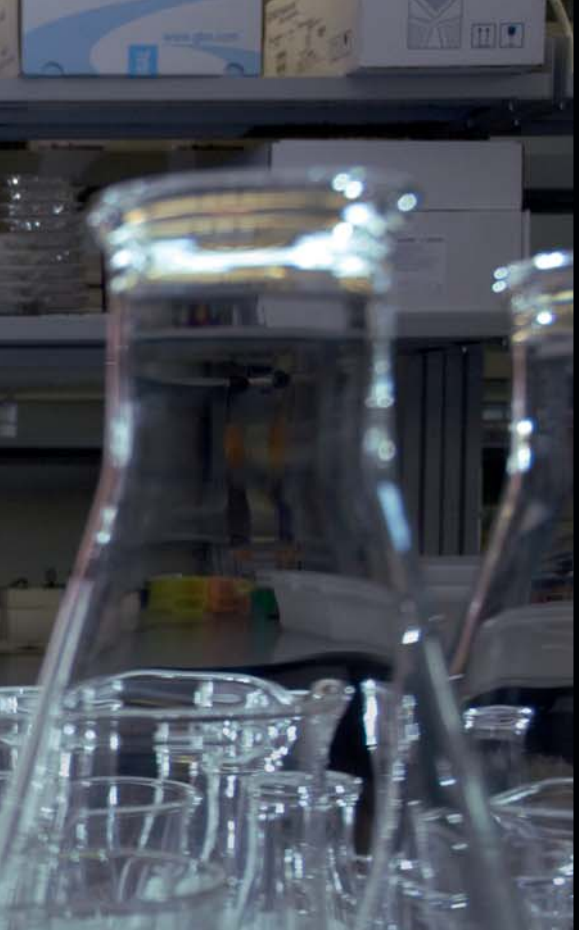
There are several emerging NIRF dyes which are brighter and more stable. Recently we have developed new agents that fluoresce brightly in the near-infrared wavelength region, promising even better lymphatic imaging. Yet more agents promise to detect cancer cells in lymph node basins drained by tumour lymphatics.

What are the medical upshots of chemically-conjugating NIRF dyes to target peptides, antibodies and sugars?

At present, only the nuclear imaging techniques (ie. those that employ a radioactive label) are clinically-suitable for molecular imaging. By conjugating a NIRF dye onto targeting moieties, we can perform clinical molecular imaging without radioactivity, and much more easily than using radioactive agents. Since radio isotopes have half-lives, it is truly phenomenal but difficult work to design agents for nuclear imaging that have the correct pharmacokinetics and target-to-background ratio within the timeframe of the radio isotope half-life. In addition, chemical preparation of these agents must be done immediately prior to administration. NIRF dyes have no half-life and therefore enable medicinal chemists to relax the pharmacokinetic requirements in designing new agents. The development of NIRF imaging will enable clinicians to implement a simple reconstitution of a molecular imaging agent prior to administration, without the need for chemical conjugation and purification in a radiochemistry facility. The upshot is more efficient, economical clinical use and enhanced opportunities to develop diagnostic imaging approaches impacting a greater number of patients.

What are the potential applications of this technology for those at risk of developing lymphatic diseases, such as cancer patients? Could this technique provide better screening opportunities?

In our studies of cancer patients, we have observed striking lymphatic abnormalities before clinical symptoms of lymphedema occur. This is important, because it is thought that early treatment can ameliorate or even prevent the disease. If we can detect the



Light fantastic

A multidisciplinary research project at The University of Texas Health Science Center is pioneering new methods of imaging the human lymphatic system using fluorescence-enhanced optical tomography

LYMPHEDEMA, OR LYMPHATIC obstruction, is an incurable disease resulting from a compromised lymphatic system, and is characterised by fluid retention and tissue swelling that can lead to skin discolouration and disfigurement. The primary form of the disease is governed by genetic factors, but the secondary, acquired form usually follows damage to – or removal of – lymph nodes, which often occurs during the treatment of cancer. In the U.S. alone, it is estimated that there are 7-9 million cancer survivors at increased risk of secondary lymphedema as a result of their treatments.

Unlike human blood vasculature, which is visible by virtue of the red blood cells it transports, the lymphatic vasculature carries a near-colourless fluid and is difficult to visualise directly. Until recently, there has not been a satisfactory means of imaging this system, meaning that our understanding of it has been comparatively poor. With the blood vasculature, CT and MR angiography are used, which involve the administration of large volumes of contrast agent. In addition, the red blood cells enable flow assessment using laser Doppler ultrasound, by monitoring ultrasound waves reflected by circulating red blood cells. In the case of lymphatics, there are very few cells, rendering Doppler ultrasound ineffective. It is also difficult to locate and cannulate lymphatic vessels in order to administer CT or MR contrast agents. Lymphoscintigraphy provides some insight, but its low temporal and spatial resolution does not yield a great deal of useful information which could lead toward new understandings of the lymphatic system.

SUPERIOR IMAGING

Dr Eva M Sevick is Cullen Chair in Molecular Medicine at the University of Texas Health Science Center's Institute of Molecular Medicine and Director of the Center for Molecular Imaging. She is currently researching near-infrared fluorescence-enhanced optical tomography – a technology which provides a new approach to imaging lymphatic function and is superior to lymphoscintigraphy in offering improved sensitivity and higher resolution with compact instrumentation, and without the need to introduce a radioactive agent into the patient.

"Our overarching goal is to develop instrumentation, imaging agents and algorithms for a brand new molecular imaging modality, based upon near-infrared light and near-infrared excitable dyes," outlines Sevick. "The first application of near-infrared fluorescence imaging and tomography we have focused upon is lymphatic imaging, since there is a significant unmet clinical need to diagnose lymphatic disorders."

NIGHT -VISION

The method involves injecting a microdose of NIR fluorescent dye into the subject intradermally, just under the skin, which is rapidly taken up by the initial lymphatics, transiting through the vessels to the lymph nodes and from there into the bloodstream. Surface tissues are then illuminated with a dim, near-infrared laser diode, the light from which is harmless and propagates deeply through the tissues, exciting the ICG within the lymphatic vessels and causing it to fluoresce. A military-grade night-vision image intensifier coupled to a CCD camera is then used to capture the fluorescence, rapidly collecting images and sequencing them to create a video which shows the NIR dye pumping through the lymphatic vessels, and sometimes in the case of disease, a dysfunctional, 'reverse' flow. For the first time, this has made it possible to quantify the frequency of lymphatic pumping action as well as the apparent velocity of the pulses, granting researchers and healthcare providers a near-real-time, non-invasive visualisation of the lymphatic system of any living subject.

onset of the condition prior to the occurrence of symptoms, perhaps we can prevent an otherwise incurable disease. We believe that the technique could provide the best means for detecting lymphedema and could personalise treatments for those who are diagnosed with the disease.

Can you elaborate on your work to develop highly specific NIR imaging agents for intraoperative guidance to remove only cancer-positive lymph nodes and what this could mean for long-term recovery?

Because we are able to see lymph nodes non-invasively using the NIRF and the dim ICG, we believe we can conjugate a brighter NIRF dye onto a molecule that targets cancer cells and use this to image only cancer-positive lymph nodes. So instead of removing all the draining lymph nodes, surgeons could remove only those that are cancer-positive. With the advent of better therapeutics, one could image whether malignant lymph nodes respond to systemic drug treatments simply by imaging their cancer status. This would obviate the need to remove the nodes, and possibly ameliorate lymphedema.

Considering the prevalence of lymphedema in developing nations, have you actively tried to reduce costs so that these imaging devices may be used in poorer nations where they are sorely needed?

Yes. Our device is currently an 'at point of care' technology and therefore amendable for use in developing nations. We have recently begun a commercialisation effort that is economising the manufacture of the systems.

FLUORESCENCE ENHANCED OPTICAL TOMOGRAPHY

OBJECTIVES

This objective of Dr Sevick's interdisciplinary research programme is to develop instrumentation, imaging agents, and algorithms for near-infrared fluorescence imaging and tomography within the Center for Molecular Imaging and to translate them in the context of preclinical drug discovery and unmet clinical needs.

KEY COLLABORATORS

Caroline Fife, MD; Erik A Maus, MD, The Memorial Hermann Hospital Lymphedema and Wound Care Clinic • **Anne Smith, PhD**, Siemens Medical Solutions, Inc • **Christopher Contag, PhD; Lihong Wang, PhD; Thomas Wang, MD**, PIs of the NCI Network for Translational Research • **Anu Godavarty, PhD**, Former student and Associate Professor Biomedical Engineering, Florida International University • **John C Rasmussen, PhD; Ali Azhdarinia, PhD, Sunkuk Kwon, PhD**, Assistant Professors of Molecular Medicine • **Yujie Lu, PhD; I-Chih Tan, PhD; Chinmay Darne, PhD; Mary Hall, PhD; Melissa Aldrich, PhD**, Research Engineers and Scientists in small animal imaging and tomography

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The method can be used to display images immediately at point of care within the clinic, so patients and clinicians can see the lymphatics directly, before, during, and after treatment. The technique has also been used to detect improved lymphatic pumping action after massage therapy. Previously, the stimulation of pumping action had been a hypothesised but unconfirmed therapeutic mechanism of manual lymphatic drainage techniques used to treat lymphedema subjects; near-infrared imaging shows that manual lymphatic drainage does indeed stimulate lymphatic pumping in some subjects.

BETTER IN 3D

The team's mathematical models use measurements of fluorescence and light excitation conducted at the tissue surface to reconstruct three-dimensional images of deep fluorescent tissues, and it is currently working to validate the NIRF tomography by labelling targeting agents with both a radioactive and NIRF dye. A collaboration with Siemens Medical Systems has resulted in the incorporation of the miniaturised NIRF system into the gantry of a small animal hybrid PET/CT system, and it is now imaging animals and phantoms. The imaging agent being dual-labelled should enable NIRF tomography to be displayed along with PET images, facilitating a comparison of the accuracy of NIRF relative to the clinical standard of PET.

The researchers are currently using ICG in FDA-approved investigational studies and Sevick hopes it will be soon approved for NIRF lymphatic imaging. "Because our imaging devices enable us to use microdose administrations of ICG, a very dim NIRF dye, we believe that new 'first-in-humans' imaging agents will likewise be enabled to be given in microdose administrations," she explains. These new agents would require a comparable or smaller dose – less than 100 micrograms – and the imaging studies suggest they will enable detection of deeper structures within the tissues, as well as faster image acquisition than the existing rate of 200 milliseconds.

COOPERATIVE NETWORKING

Another tool being developed with Siemens Corporate Research is SciPort – a unique and flexible database system where researchers can integrate the various images and research data in a format that will facilitate translation and commercialisation. The team is also working with The National Cancer Institute, which funds the Network for Translational Research – a network of four centres at the University of Texas, University of Michigan, Stanford, and Washington University at St Louis, and Sevick is enthusiastic about the cooperative nature of the network. "The communication and 'bouncing off' of ideas around more effective translation of optical technologies, enabled by the NCI Network for Translational Research, has been extremely useful," she recalls. "Each of these centres is focused on translating an optical imaging modality and validating it with a second, usually-conventional modality."

This coupling assists in translation, providing a means by which researchers can compare optical imaging results, and as each of the centres translates its optical technologies, it is developing consensus tools that others will use. The result is a set of tools and recommendations developed in the process of translation which will help other researchers accelerate the implementation of these technologies in standard-of-care practices. NIRF lymphatic imaging is showing significant clinical utility in diagnosing lymphedema, and directing and evaluating treatment. The initial stages of commercialisation are underway so that systems can be made available nationwide as well as internationally.

FUTURE APPLICATIONS

Since lymphatics play a critical role in the function of vascular circulatory system, NIRF lymphatic imaging may shed light on some blood vascular disorders. Sevick's team is working with Dr Philip King at the University of Michigan, who has developed an animal model of a human genetic condition known in humans to impact the blood vasculature, but in animals has been shown to impact dramatically the lymphatic vasculature. This suggests there may be exciting new applications of NIRF lymphatic imaging which, in tandem with the genetic research into primary lymphedema also being conducted by Sevick and her collaborators, are for the first time in almost a century creating the exciting possibility of significantly raising the bar in the standard of care for lymphedema sufferers.


DR SEVICK'S TEAM