Are engineered nanotubes harmful to health?

Drs Christopher J Wingard and Jared M Brown – experts in physiology and nanotoxicology, respectively – are working together to illuminate the toxic effects that some nanoparticles appear to have on the heart and lungs.

What initially inspired your research interests?

CJW: My inspiration for the study of smooth muscle regulation can be largely attributed to my PhD mentor who suggested I think about the variety of organs that contain smooth muscle and contemplate the role each plays in normal physiology. After working in cardiac muscle energetics for my dissertation, I found this a fascinating prospect, and I quickly recognised the diversity of the muscle responses each of those organs displayed. My postdoctoral studies into the relationship of calcium and myosin phosphorylation in controlling arterial mechanics and energetics was a logical extension, marking my first steps in exploring the diversity of cellular signalling process that influence smooth muscle in normal and pathological conditions.

JMB: My inspiration for the study of how xenobiotic exposures impact immune responses began with my undergraduate training in immunology and was solidified by my PhD mentor and training in immunotoxicology. My dissertation work focused on understanding mechanisms by which inhalation of particles could exacerbate or initiate autoimmune responses. Through these studies, I quickly recognised the sensitivity of the immune system to external stimuli such as particles and chemicals. Based on this interest, I pursued postdoctoral studies in allergy and immunology where I began studies on how nanomaterials may impact mast cell function and allergic disease.

Many of your research activities centre on nanomaterials. How prominent are engineered nanotubes, like buckminsterfullerene (C60) for example, in the everyday objects and the environment?

CJW: Engineered carbon nanomaterials play a significant role in the consumer market due to their wide diversity of forms and modifications. They are used in an array of consumer products spanning electronics to healthcare applications; for example, a variety of cosmetics utilise forms of C60 for their antioxidant capacity and ability to improve and rejuvenate the skin.

What do we know about the general human health impacts of engineered nanomaterials?

JMB: Very little is known about the impacts of exposure to engineered nanomaterials. While the use of engineered nanomaterials in biomedical applications or consumer products is rapidly expanding, there are minimal exposure data in the general human population. Current work is focusing on utilising in vitro and in vivo animal models to understand which engineered nanomaterials may pose a health hazard to the human population. Much of this work has focused on identifying properties of engineered nanomaterials that contribute to toxicity in these various models and the intention is to use those data to engineer nanomaterials that are non-toxic and can be readily used in biomedical applications and consumer products.

A large majority of your work has focused on how mast cells could contribute to cardiovascular and pulmonary toxicity of engineered nanomaterials. Can you describe your findings?

JMB: Mast cells are well known to mediate many allergic immune responses and are involved in allergies, anaphylaxis, asthma, urticarial and autoimmune disease. Our initial work focused on comparing pulmonary and cardiovascular responses in wild-type and mast cell deficient mice. Across several studies, we examined multi-walled carbon nanotubes and cerium oxide nanoparticles in these two animal models, and we found that adverse responses – pulmonary inflammation, fibrosis, vascular changes and myocardial infarction – only occurred in the wild-type mice. In contrast, mice that were mast cell deficient did not have significant adverse responses.

Based on these findings, we also utilised a model to reconstitute mast cells back into the mast cell deficient mice. In this set of experiments, we were able to restore the adverse toxicological response (eg. inflammation, fibrosis and cardiovascular events) to carbon nanotubes. In addition, we found a major role for the cytokine interleukin 33 (IL-33) – which activates mast cells – in these responses. Our overall findings were that certain engineered nanomaterials can elicit IL-33 production through damage to epithelium and endothelium of the lung and cardiovascular system, which then promotes mast cell activation and subsequent adverse pulmonary and cardiovascular responses.

Can you comment on the role collaboration has played in your investigations?

CJW: The efforts of our labs in investigating nanosafety has been very eye-opening and intriguing. Each lab focuses on its individual expertise and together we see the interaction of systems through the common link of exposure to the nanomaterials. I have continued to learn and develop a better understanding of the role of the immune system in overall cardiovascular functions. Keeping pace with the developments in each of our disciplines is challenging, but working together has proven to be invigorating and has opened new avenues of questions and experimental models.
A collaboration between researchers based at Skaggs School of Pharmacy and Pharmaceutical Sciences and Brody School of Medicine, both in the US, is showing the effects that nanomaterials are having on human health with the aim of mitigating their hazardous health effects.

**SINCE THEIR DAWN**, civilisations have looked for tools and materials to make their existence on this planet easier and more enjoyable. One material that has played a huge part in the advancement of society is asbestos.

Deemed by our ancestors as a material with ‘miraculous’ heat- and fire-resistant properties, asbestos utilisation dates back to 3000 BC when ancient Egyptians used the material to wrap their dead to preserve the bodies. As time progressed, so too did its applications; in fact, by the Industrial Revolution, asbestos production had grown worldwide to more than 30,000 tonnes annually. Humanity was surrounded by its presence as it was weaved into construction materials for houses, laced with asphalt and built into roads, and intertwined in the functioning of the most notable technological advances of the day, including ships, trains, electric turbines and steam boilers.

There was only one problem: the high toxicity of asbestos. Millions of people throughout history and across the globe succumbed to horrible diseases from interacting with it, ranging from asbestosis to mesothelioma or lung cancer. However, society did not realise how toxic the material was until it had pervaded every aspect of it. Drs Christopher Wingard and Jared Brown are determined to ensure that the stories told about asbestos’s crippling, disease-riddled past are not the same ones told about a newly emerging, human-made technology: nanomaterials.

**THE NEW MATERIAL ON THE BLOCK**

Nanomaterials are materials composed of a single unit between 1 and 100 nanometres in at least one dimension. The reasons for the recent explosion in the field’s popularity are vast: the materials are small and lightweight, and they can be structured at the nanoscale to imbue materials with different characteristics by creatively combining chemical and physical properties.

Because of the nearly innumerable ways nanomaterials can be structured, their applications are enormous. “Nanomaterials such as carbon nanotubes and fullerenes are being increasingly incorporated into many different aspects of society,” expands Brown, Associate Professor of Toxicology at the Skaggs School of Pharmacy and Pharmaceutical Sciences based at the University of Colorado. “They are being used in electronics and sensors due to their conductive properties and in biomedical applications due to their large surface area and ability to attach drugs or imaging agents.”

However, in recent years, voices in society have called the safety of these materials into
SAFETY OF NANOMATERIALS

OBJECTIVE
To illuminate the impact of the immune cell response following nanomaterial exposure on the cardiovascular system, particularly the establishment of a heightened sensitivity of an evocable injury associated with cardiac ischaemia and reperfusion.

KEY COLLABORATORS
Dr Robert M Lust, Department of Physiology, East Carolina University, USA • Dr Wayne Cascio, US Environmental Protection Agency (EPA), Research Triangle Park, USA • Dr Tim Fennell, RTI International, Research Triangle Park, USA • Dr Ramakrishna Podila, Clemson University, USA • Dr Apparao Rao, Clemson University, USA

PARTNERS
RTI International, Research Triangle Park, USA • The Hamner Institutes for Health Sciences, Research Triangle Park, USA

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CONTACT
Dr Christopher J Wingard
Professor of Physiology
Brody School of Medicine
600 Moye Boulevard
Department of Physiology, Brody 6N-98
Greenville, North Carolina 27834
USA
T +1 252 744 2804
E wingardc@ecu.edu

JARED M BROWN

CHRISTOPHER J WINGARD is Professor of Physiology and Director of the PhD Program in Physiology in the Brody School of Medicine at East Carolina University. His research examines the impact of xenobiotic exposure and cardiovascular risk factors on cardiovascular health with a focus on inflammatory signalling, vascular reactivity, myocardial response to ischaemia and erectile function.

JARED M BROWN is Associate Professor and Director of the PhD program in Toxicology in the Skaggs School of Pharmacy at the University of Colorado Anschutz Medical Campus. He examines the role of the innate immune system in the response to engineered nanomaterial exposure. He is interested in mechanisms by which nanomaterials and nanomedicines induce an allergic immune response.

question, especially when they are used in biomedical applications. “Remarkably little is known about the impact of engineered nanomaterials and human health,” states Wingard, Professor in the Department of Physiology at Brody School of Medicine. In response, governments, regulatory bodies and the producers of these materials throughout the world have started investigations to help clarify any potential human impact of various types of nanomaterials. Wingard and Brown are at the heart of these investigations, and they are paying close attention to the effect that purposely produced nanomaterials like carbon nanotubes might be having on our heart, lungs and immune systems.

THE DARK SIDE OF CARBON NANOTUBES
Carbon nanotubes are nanomaterials constructed entirely of carbon. They have extraordinary thermal conductivity and mechanical and electrical properties, and as such have applications as additives in various structural materials. Multi-walled carbon nanotubes (MWCNTs) are a special form of carbon nanotubes made up of multiple layers of graphite rolled into a tubular shape, and they have recently shown great promise in many medical applications. However, Wingard and Brown wanted to determine if these up-and-coming technological breakthroughs are as miraculous to human health as they seem, so they instilled Sprague-Dawley rats intratracheally with 1, 10 and 100 μg of MWCNTs and examined the effects. “In a series of investigations, we found that the pulmonary aspiration of MWCNTs time and dose-dependently promotes susceptibility of cardiac tissue to ischaemia/reperfusion injury without a significant pulmonary inflammatory response,” shares Wingard.

According to Wingard and Brown, the process through which this injury response occurs is not entirely straightforward. The instilled MWCNTs are shepherded into the lungs where they can inflame them and initiate pulmonary fibrosis. Over time, the physiological impact of inflammation and fibrosis results in decreased lung function. However, in the early events following exposure the endothelial cell integrity becomes compromised and local cytokine production increases. “We found that cardiac injury, as assessed by arrhythmia and infarction, was enhanced in isolated hearts 24 hours following intratracheal instillation of MWCNTs,” states Wingard. The pair have supported these findings in additional studies, noting that while the severity of myocardial injury varied with the form of nanomaterial used and the route of exposure, worryingly, cardiac injury effects were observed even at low concentrations of some of these materials.

THE ROLE OF MAST CELLS
Wingard and Brown believe that the one of the culprits responsible for driving these problems are mast cells. These cells are located in connective tissue and are responsible for regulating the body’s immune system. For example, if a harmful pathogen invades the body, mast cells will kick into action and take the pathogen down. However, with great power comes great responsibility, and mast cells are not always responsible with the power they exert. In many cases these cells overreact to innocuous body invaders; in fact, scientists have linked mast cells with asthma attacks, eczema and allergies.

In the case of carbon nanotube exposure in the lungs, the researchers found an increase in the cytokine interleukin 33 (IL-33), which has recently been discovered to elicit mast cell activation. In addition, they also found that the myocardial infarction response to carbon nanotube exposure was linked to similar immune responses as observed in the lung. “Our findings establish for the first time that mast cells and IL-33 orchestrate adverse pulmonary and cardiovascular responses to engineered nanomaterials, giving insight into a previously unknown mechanism of toxicity,” states Brown. “This mechanism could be used for assessing the safety of engineered nanomaterials and provides a realistic therapeutic target for potential nanoparticle induced toxicities.”

NANOMATERIALS THAT WORK FOR HUMAN HEALTH
Like asbestos, as nanomaterials become more ubiquitous in society, everyday interactions with these materials will undoubtedly increase. However, there is a notable difference between the history of asbestos and the future of nanomaterials: nanomaterials can be created to navigate around health concerns.

As for Wingard and Brown, they are looking forward to engaging in scientific activities that deliver a future where nanomaterials are useful and not harmful to health. “An area in nanotechnology that will be critical in creating this future is understanding how the chemistry of the materials can be modified to prevent both the priming of systems and exaggerated injury responses,” Wingard muses. “This will require continued investigations and close collaborations between labs like those of Brown and myself.”