Mechanistic models of lung disease

**Professor Greg Forest** reveals the interdisciplinary nature of his work in mathematics and lung biology, as well as his endeavour to train graduate students in the most inspiring and effective ways.

*Can you summarise the aims of your work in lung biology and mechanics?*

My previous research projects were all inspired by novel, unexplained phenomena and the aesthetics of mathematics. But in biology, once your intellectual curiosity is captured, and a judgement is made that the problems require original mathematics, you must put the biology first and have patience that the flow back to mathematics will happen, but on unpredictable timescales. So far, this has indeed worked out, so at least I feel as though I have made smart judgements in that these projects are worthy pursuits for my students, postdocs and junior faculty collaborators. Choosing problems that are career-builders is a critical element of my position as a mentor and promoter of interdisciplinary applied mathematics.

Scientific aims in biology are always rooted in an understanding of the underlying mechanisms. We build mechanistic models that help us interpret and explain experimental observations, test hypotheses and afford insights into dysfunction, as well as potentially predict or evaluate remedies or treatments. In pursuing these aims, we routinely meet open challenges, which our group happily takes on and often overcomes in highly interactive collaborations with our biological colleagues.

*You have recently been awarded a National Institutes of Health (NIH) training grant to co-direct the Big Data to Knowledge Training Program (BD2K) at the University of North Carolina (UNC) at Chapel Hill. What can students on this multidisciplinary course expect?*

This NIH BD2K graduate training grant is one of the most exciting developments of my career as an educator and mentor. Michael Kosorok, who chairs the Department of Biostatistics and I are co-principal investigators. We have well over 50 faculty mentors (48 in the original application) from the College of Arts & Sciences and the Schools of Medicine, Pharmacy, Public Health, and Information and Library Science. All graduate students across the University will be able to participate in the curriculum to develop advanced data analytics skills and to integrate those skills into fundamental biological or biomedical understanding.

*Why was the BD2K project launched?*

It responds to a fundamental question: how can we capture all the lessons we have learned about the best way to train graduate students to do research at the nexus of biomedical data, computer science and informatics, statistics and mathematics? Across the community of mentors, we built new curricula that we weave into the existing graduate programmes. The students will take a series of short courses and modules, co-taught by a team of at least three faculty representing domains in the aforementioned subjects. From these short courses and modules, students then apply for research rotations in the same collaborative research groups that develop and deliver the modules. In a sense, we are accelerating and formalising the very process that all of us use to incorporate new graduate students (and postdocs) into these groups. The lessons learned from 15 years of the Virtual Lung Project, and from my several years working in cell biology with Kerry Bloom, Ken Jacobson and Tim Elston, and in virology with Sam Lai, have all been integrated into this training grant. We know that by putting students from different disciplines together, they learn from one another while building multilingual as well as multidisciplinary skills necessary to undertake data-driven biology and biomedicine of the future.

*With previous collaborative ventures in areas such as physics, polymer science and biology, where do you plan to steer your studies in the future?*

The platform built by this training grant is equally, and perhaps more importantly, an engine for new collaborations that combine the biological and biomedical domain, the computer and information science domain, and the statistics and mathematics domain. So I already see myself migrating into many of these new directions on the drawing board, to address some of the critical issues of our time surrounding cancer, genetics and the brain. At the very least, I hope this platform for research collaborations will lead to the next Virtual Lung Project in a new area, even if I am not one of the key investigators.

*Could you outline the inspiration behind your love of mathematics?*

I didn’t choose that my brain is wired to understand the world around me through mathematics. I happily accept this reality though.

My joy in mathematical science flows from my joy of family, friends and life. I get inspiration from my wife Barbara, sons Scott, Mark and Alex, my daughter-in-law Julie, my grandkids Scotty and Lexi, my brother Lee, sister Robin, and our wonderful extended family and friends. Many of my professional colleagues are also my family; those relationships mean the world to me, and give me the energy to wake up every morning ready to face the next challenge.
THE ABILITY TO breathe without struggle is an inherent function for healthy individuals that many take for granted. Few also realise the price we pay for oxygen exchange: the inhaled particle and pathogen load must be trapped and cleared to the trachea, where it is swallowed into the gut. This is where mucus enters, lining the airways with over a tennis court of air-mucus interface. Mucus traps the inhaled load while the mucus layer with the trapped cargo is continuously transported to the trachea by coordinated cilia and breathing-induced air drag. Cystic fibrosis (CF) is a genetic disease that disrupts the lung’s ability to efficiently regulate the balance of salts and water between the epithelium and the airway mucus layer. As a result, individuals with CF develop dehydrated (thicker) mucus that cannot be efficiently transported by cilia and tidal breathing. The body responds with cough — individuals with CF spend their entire lives coughing, even in their sleep. Even so, mucus transport is compromised, raising the incidence and duration of lung infections and airway obstructions.

VIRTUAL LUNG PROJECT

In 2000, the Virtual Lung Project (VLP) at the University of North Carolina (UNC) at Chapel Hill began as an interdisciplinary response to the mucus transport problem for CF patients, integrating basic and medical science toward an understanding of disease and the potential for science-based solutions. Lung biologists Drs Ric Boucher, John Sheehan (deceased) and Bill Davis of the UNC CF Center, now the Marsico Lung Institute, teamed with physicists Professors Richard Superfine and Michael Rubinstein, applied mathematicians Professors Greg Forest, Roberto Camassa, Tim Elston, Rich McLaughlin and Sorin Mitran, and computer scientist Russ Taylor to devise scientific understanding and predictive solutions.

Forest and colleagues saw the opportunity to formulate many open questions from applied mathematics and materials science in one remarkable biological system – lung transport of mucus. For instance, how do hundreds of tiny cilia (8 μm long at full extension) that beat individually at 10–15 Hz manage to coordinate in the form of waves that propel mucus gels? What exactly is mucus, in the language and principles of materials science and fluid dynamics? What experiments could one do to test hypothetical answers to these questions? Furthermore, the driving force behind the people committed to the VLP was larger than science. How do answers to these challenging questions change during CF disease progression? Are there detectable signatures of failure to transport mucus prior to onset of symptoms? Are there science-based guides to treatments, if not cures, at any stage of disease progression? Each question presented new challenges and opportunities to ‘model’ this complex lung system, to lay down governing equations that one could solve, and thereby predict healthy versus unhealthy mucus transport in lung airways and simulate the impact of physical and drug treatments. This was the rallying cry of all the original participants in the VLP.

Cystic fibrosis: the challenge of clearing mucus from the airways

At the University of North Carolina at Chapel Hill, USA, a cross-disciplinary conversation about a fluid dynamics problem regarding mucus in humans with cystic fibrosis has blossomed into a 15 year-long collaboration known as the Virtual Lung Project.

Over the years, the VLP collaboration expanded to include many students, postdocs and faculties across the Chemistry, Computer Science, Mathematics, Pharmacology and Physics Departments, joining forces with colleagues in the Marsico Lung Institute.

TRAPPING AND TRANSPORT FUNCTIONS OF MUCUS

During the 15 years since the founding of the VLP, the researchers have been able to gain insight into mucus and its dual function to trap inhaled particles and pathogens and to be transported by coordinated cilia and air drag from breathing or cough. Mucus presents an especially intriguing challenge because it is a non-Newtonian or viscoelastic fluid, which means it behaves both like a solid and a fluid. Furthermore, the relative fluid-like or solid-like behaviour of mucus varies dramatically with the frequency and amplitude of forcing. In the lung, mucus is a mixture of water, mucins, salts, many other proteins, dead cells, immune response agents, inhaled particulates and pathogens. Healthy mucus serves as a diffusive barrier to prevent insults from reaching lung tissue and vasculature, while simultaneously responding to cilia and air drag forcing for its own clearance from the airway. A simple solid or liquid would be unable to perform these remarkable functions simultaneously.

MICORHEOLOGY

Rheology is the study of the way matter deforms or flows in response to external forcing. In their studies, Forest and his applied mathematics team, together with experimentalist collaborator, Professor David Hill of the Physics Department, and the Marsico Lung Institute, have established that micro rheology is one of the best techniques for not only assessing the baseline biophysical properties of mucus, but also for detecting differences between mucus samples. In their 2014 study, Forest and collaborators showed that a simple biomarker, the per cent weight of solids (salts, mucins, proteins) versus water, in human bronchial epithelial cell culture mucus reveals clear trends in viscous and elastic properties across a wide physiological frequency range. It has long been noted that the per cent weight of solids in mucus increases in correlation with the severity of lung diseases; but an understanding of the impact of increased solids relative to water on the viscoelasticity of mucus across a broad frequency range was enlightening, and served as a candidate baseline clinical biomarker.

Current projects aim to understand how living factors (immune response, inhaled particulates and pathogens) couple with weight per cent of salts and mucins to further modify viscoelasticity of in vivo mucus. Passive microbead rheology is potentially transformative for mucus biology and biomedicine. It gives a robust, repeatable metric of the equilibrium diffusive and viscoelastic properties of mucus and similar soft biomaterials. Furthermore, compared to any macro rheology experiment, micro rheology only requires micro litre volumes, and the results are not sensitive to instrument settings on imposed stress or strain,” Forest explains.

In passive particle tracking micro rheology, the typical goal is the bulk linear viscoelasticity of a sample. Forest’s team, however, aims to learn much more: the diffusive mobility of particles that are trapped in mucus, the averaged linear viscoelasticity of the sample, and the variability (heterogeneity) in both properties within each mucus sample. To do so, it is critical to study the diffusive paths of individual particles in addition to ensemble averaging, to assess whether the distribution of paths is Gaussian, and if not, to cluster the ensemble into distinct Gaussian sets of paths. These same metrics allow one to discern differences between mucus samples as well as modifications in mucus properties due to physical or drug therapies. One academic publication of these methods is in the article entitled ‘Micro-heterogeneity metrics for diffusion in soft matter’, in which the researchers adopted methods from machine learning and cluster analysis to detect heterogeneity in the way microbeads diffuse through mucus.

Heterogeneity in mucus diffusive properties has an impact on drug delivery, since it can predict the likelihood of outlier drug carrier particles that rapidly diffuse through the mucus barrier. This also impacts mucus clearance, since different viscoelastic patches will respond differently to physiological stresses – mucus plugs, for example, might be preferentially affected by cough. Macrorheometers cannot detect such heterogeneity and it is not clear how bulk rheology measurements average over these heterogeneities. This leads to further interesting questions for mathematical modelling, namely whether it makes sense to homogenise mucus properties for flow transport modelling, or whether one must resolve local variability.

BEYOND THE VLP

The decade and a half during which the VLP has been conducting research has proved incredibly fruitful for all involved. The ability to pull from the strengths of such a wide variety of disciplines and harness the power of experimental and clinical data has provided tangible results in lung biology and biomedicine, as well as advances in applied mathematics, physics and computational science. In addition, new research collaborations inspired by the success of the VLP are having impacts in other areas, as noted earlier in cell biology and in new antibody-based vaccines for viral diseases like HIV. Forest and his colleagues across UNC and outside UNC continue to build a research community founded on interdisciplinary cooperation and data-driven research that will impact numerous disciplines and improve understanding and treatment of disease.