– CUSTOMER SUCCESS STORY –

Australian Institute for Bioengineering and Nanotechnology (AIBN)

Shearing is believing!

The University of Queensland's Australian Institute for Bioengineering and Nanotechnology (AIBN) is an integrated multi-disciplinary research institute bringing together world-class researchers and cutting-edge technology with a focus on translational research. Based at The University of Queensland (St. Lucia campus, Brisbane, Australia), their work is underpinned by AIBN's "Five Pillars of Research", which cover stem cell ageing and regenerative engineering, precision nanomedicine, advanced materials, agriculture nanotechnology, and advanced biomanufacturing.

Professor Alan Rowan is performing his research at the interface of chemistry and biology with seminal and pioneering work on processive catalysis and functional self-assembly. His latest scientific achievement has been the development of the first truly biomimetic hydrogel which mimics the mechanical and functional properties of the extracellular membrane. This work has in part been made possible with the construction of a novel high-resolution instrument, the Anton Paar MCR 502 WESP confocal-rheometer (Figure 1).

Ashish Kumar (Market Manager for Characterization) and Laurie Obadia (Marketing Coordinator) at Anton Paar Australia sat down with Prof. Alan Rowan, Dr. Jan Lauko, and Dr. Petri Turunen of the AIBN to discuss this new setup and how it enables the group to understand the interface of extracellular matrices (ECMs) and cells in more detail.

What was the research challenge?

Reconstituted networks of natural extracellular matrices (ECMs), such as collagen or fibrin, show a large increase in stiffness upon externally applied stress or deformation. Recently, a new biomimetic hydrogel was developed by the group. The application of these materials in cell growth and drug therapeutics revealed the importance of polymer non-linear mechanics .
The group wanted to understand how to control the polyisocyanopeptide hydrogel properties, and their detailed microrheology, and therefore needed an instrument with the capability to measure, and simultaneously visualize, both synthetic and biological systems under different applied stimuli, i.e. shear strain, temperature, or compression.

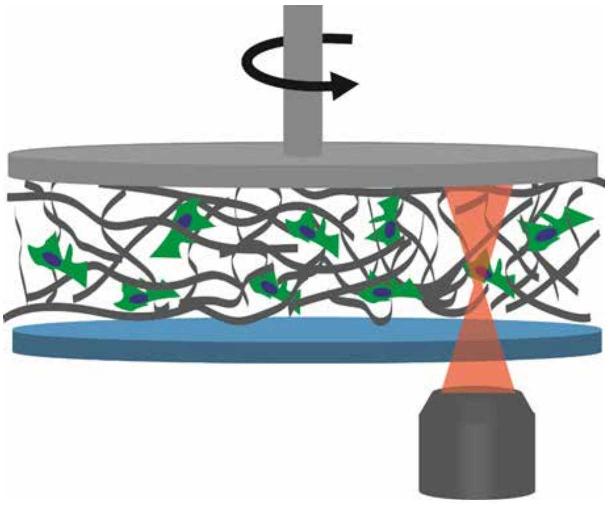

Figure 1. Schematics of a confocal-rheology experiment, in which a soft polymer (in this case containing cells) is visualized by fluorescence microscope while simultaneously applying strain to the sample by the rheometer plate on top.

Why combine rheometry and optical techniques?

Confocal Laser Scanning Microscopy (CLSM) is a well-established tool for studying nanoscopic features and processes in cells and materials. However, this technique is often focused on fixed cell samples and in cases of live cell imaging is limited to a static environment without the ability to dynamically change the matrix properties surrounding the cells. The confocal-rheometer's fine measurement capabilities enable applications in mechano-optical studies of synthetic ECM materials, in which the influence of mechanical force applied on the material by the rheometer component can be concurrently visualized by the confocal microscope.

"In our field of research, shearing is believing," says Dr. Jan Lauko, who built the setup along with Dr. Petri Turunen. "Pushing the boundaries of what this confocal-rheometer platform could do was a collaborative process. After all, real problems which impact society as a whole require multiple disciplines and engaged partners. Anton Paar worked with us throughout the process to design a customized sample stage that would better accommodate a large, motorized confocal objective – and they kept us informed of the progress along the way."

Dr. Turunen, who runs most of the experiments on the system, goes on to say: "I believe the instrument exceeded our expectations within the first year!" Dr. Lauko adds: "It's not just the instrument or the measurement parameters of the machine, it's also the technical support from Anton Paar, and service support as well, that really helped us push forward as quickly as we did. Also, Anton Paar put us in touch with other WESP users around the world and this allowed us to design the customized stage that we have now which works perfectly for our purpose and configuration."
Prof. Alan Rowan goes on: "You cannot do cutting-edge science without cutting-edge equipment. What we know in the university is that if you want to be world-leading, you've got to have the next generation of equipment."

Can you tell us about the customized confocal-rheometer system?

In collaboration with the groups of Prof. Egelhaaf (Heinrich Heine University, Düsseldorf, Germany) and groups from ETH, Zürich (Switzerland) the research group at University of Queensland constructed a novel high-resolution instrument, the confocal-rheometer, the first and only one in Australia.

Prof. Alan Rowan

"Currently, our resolution is 60 nm in diameter, which is extremely good!"

The MCR 502 WESP with confocal microscope setup gives a real-time rheology image. It is comprised of a rheometer 'tower' that houses the measuring drive and support plate to connect the lower optical Peltier plate. This tower is separated from the electronics box, allowing a much greater level of flexibility for integration into complex optical platforms, like the confocal microscope.

"Currently, our resolution is 60 nm in diameter, which is extremely good!" says Prof. Rowan. "And of course, we want to go down lower. If we look at a matrix around a cell, with that resolution we can investigate the cell shape as a function of the rheological test. The measurements at these scales are made possible by the nanometer-level accuracy and sensitivity of the MCR 502 WESP, and it's helped tremendously by the rigidity and weight of the tower unit, as this dampens a lot of vibrations that would otherwise ruin the nanometer imaging. Visualization of smaller samples and integration with different techniques is really important. It's not been trivial for us."

Dr. Lauko continues: "Measurements are performed at gaps of 100 microns, and we can zoom in on the image of a single cell under shear, in three-dimensions. In real-time, we can see ligands on the surface of cells 'sensing' their environment, or understand the rheology of the fibrin-matrices in 3D."

What has the response been in the research community after hearing about this system?

“It has been overwhelming! The rheometer could be running 24 hours a day to try and keep up with the requests. And I’m fairly certain other groups around Australia, New Zealand, and the world will be approaching Anton Paar to install similar systems in their departments. I genuinely believe that in our community the confocal-rheometer will become a standard piece of kit in many cases. Just the quality of the images and data we are getting out of it is amazing. We didn’t think this was possible,” reports Prof. Rowan.

Dr. Jan Lauko adds: “I’ve been at many conferences now, presenting the findings from this rheometer, and it’s just sold itself. People are really interested in the possibilities and what the technique brings to their research.”

Prof. Rowan reports: “The confocal-rheometer is a unique piece of kit, which undoubtedly would not have happened without Anton Paar’s help. It allows researchers to ask different questions, and that’s driving the science. It’s the ability to ask new questions that is opening up completely new topics and ideas we didn’t think about beforehand. I think we’ve got a lot more out of the instrument than we thought we would, but we knew what we were trying to achieve and we wanted to have one of the first [novel MCR WESP combined with a high-end confocal microscope] in the world because that allows us to do things other people cannot do.”

“I have to be honest,” Prof. Rowan continues, “it has been much easier than we thought, due to the collaborative nature between UQ [University of Queensland] and Anton Paar! I know how these things can go wrong from previous experience. We have now started to do things that all my colleagues around Australia are looking at and going ‘Wow! Can we come and do this on it? Can we come and do that?’ It’s phenomenal, especially because we are fairly new to Australia – so we are starting from scratch and this is the biggest magnet.”

Have you been approached with any ‘left-field’ request for measurements with the new instrument?

“The modularity of the MCR system should be highlighted more,” says Prof. Rowan. “There is another group here at UQ interested in understanding how the influence of shear changes the emission of light in fiber-optic-type applications. This would be an excellent application for the di-electric rheological device (DRD) Anton Paar offers. Soft matter scientists would be particularly interested in how the brain di-electrics and electrical pathways change under different forces, to see what happens in concussions, for example.

Because of the conferences we have been presenting it at, we have focused mainly on soft matter, where we have a short time-scale to complete a measurement. However, we have since been approached by people in biomimetic research, i.e. flexible electronics or wearables, and they are interested in understanding how the stretching of films also causes changes in di-electrics. These are more solid materials, but the inclusion of the linear drive for dynamic mechanical analysis (DMA) would tie in really well with this work. So again, the modularity of the system allows different departments and different universities to work more closely together. Unfortunately it doesn’t help Anton Paar so much, since everyone wants to share equipment rather than buy a new one!”

Why did you choose Anton Paar?

“For us, the challenge was to find an instrument that would be capable of being incorporated into a confocal-microscope system, but luckily Anton Paar already had an ‘off-the-shelf’ solution. This solution was an improved version of older confocal-rheometer systems by Anton Paar that we had seen in other countries,” summarizes Dr. Lauko. “Knowing that some customization was inevitably going to be required, the willingness of Anton Paar to adapt their solution to customer requirements made the decision even easier, as we knew that we could have custom-designed cells specially made to accommodate our motorized objective and lowered sample position.”

What are your expectations of an instrument supplier, especially when it comes to support for time-critical research?

“The ongoing technical support has been most important,” says Dr. Turunen. “For example, with the optimization of temperature control for the customized lower stage. These kind of queries and back-and-forth will continue, but Anton Paar’s response times have been commendable, especially in the early stages of setting up the right measurement profiles, normal force control, sampling rates, and remote triggers for the confocal microscope.”

Prof. Rowan continues: “When it comes to high-end research, we expect Anton Paar to be part of the scene. It requires solving problems relatively quickly and secondly, Jan [Lauko] made this interesting comment that we perceive it as very valuable to be part of the Anton Paar family now. I know it seems a bit clichéd but we are in the area where we start off with having a network of people and Anton Paar has helped to expand our network by bringing different people together, an aspect which should not be underestimated. It is really powerful to tap into Anton Paar's network to share ideas and knowledge from different parts of research around the world, which helps us to overcome many of the challenges we face. And now, UQ and Anton Paar can work together to solve new problems – it's a team effort and collaboration, not just an instrument sale. That relationship means customers keep coming back to Anton Paar for more support and more equipment. That knock-on effect is based on a mutually beneficial partnership. That's what we want from a supplier."

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> *Prof. Alan Rowan*
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What's next for the group?

"We need to promote the setup and features a lot more," says Dr. Lauko. "Sometimes people look at the instrument and don't realize what it is capable of. It's quite compact, doesn't announce itself, and is tucked away in a quiet, clean room. Once we talk to people more, then they are quite excited about the possibilities. But now, with all the conferences and publicity, we have enough interest that we can pick and choose the best ideas to be associated with."

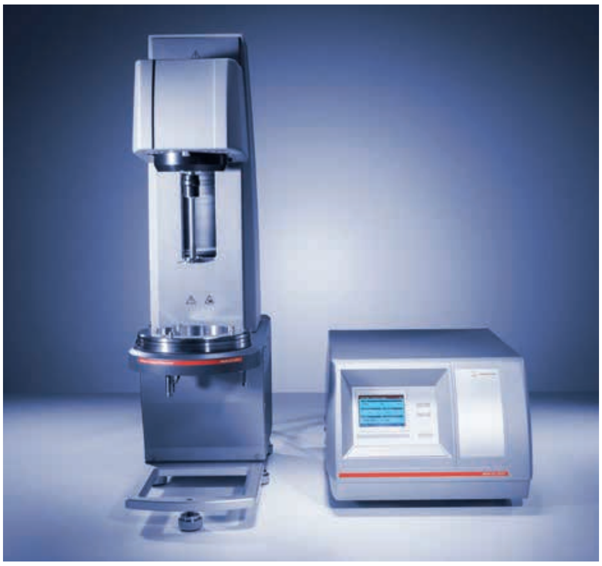
Prof. Rowan adds: "I am a very open scientist, so for me the more people that join in, the more problems can be solved. I'm not interested in keeping this instrument to myself exclusively. If there were fifty people out there doing the same thing, it would be better science! We have more than enough ideas to keep us going for the next hundred years, but the point is that if more people are working on a problem, we can have really good debates at the next conference leveraging on each other's research. Also, microrheology is a future area of interest that we would be keen to co-develop with Anton Paar. There are some very interesting opportunities in this field requiring a whole series of experiments."

Where will the research direction go from here?

"Being able to connect a microfluidic device to your smartphone – this is what everybody wants. Then you can sample immediately, just like a blood-glucose sensor. And it's not so far-fetched: a hand-held device that could measure rheology across a surface, sort of an indirect method," posits Prof. Rowan. "Using rapid screening, you could map a surface to a certain depth, for example a patient's back, and say that this pattern indicates that this area is more cancerous than the other, and warrants further consideration."

“Rheology on blood samples is already quite common, but we are now looking into rheology on single cells. We have a project on blood clots and we use snake venom to induce blood clotting. This is one of the experiments we would like to do on the rheometer. For this application bulk rheology is sufficient, but ultimately miniaturizing the system would be beneficial.”

Prof. Rowan continues: “If you have a look at the current workflow in hospitals, samples have to go to a pathology lab and it takes two or three days for the results to come back. Instead, you want to test it there and then! Miniaturized microrheology kits would be interesting to offer and commercialize. Soft matter analysis is also an interesting area. Ideally, you should be able to do a brain biopsy and then perform a quick rheology measurement in the operating theater. We are not sure how this would be done, but we anticipate this would become a standard tool or protocol. There is a worldwide push to find a solution like this, especially in China.”



The special design of the MCR 502 WESP, with its separate electronics box and isolated measurement tower, opens up new possibilities and situations that could benefit from simultaneous rheometer and optical measurements, including integration into synchrotrons and even neutron beamlines.

[1]R. K. Das, et al. Nature Materials 2016, 15, 318.; R. Hammink, et al. ACS Omega, 2017, 2, 937; S. M. Bruekers, et al. Cell Adh. Migr. 2016, 10, 495; P. de Almeida, et al. Nat. Commun. 2019, 10, 609.

Acknowledgements
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Text and interview by Dr. Ashish Kumar | Photos by Laurie Obadia