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KERNEL

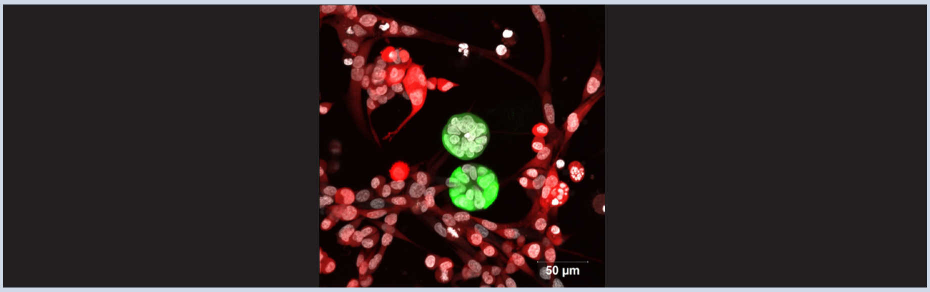
Editorial

The human body is a complex machine. Recreating even a fraction of its functioning is challenging. But scientists studying cancer are now able to mimic how cells and tissues grow and move even in a lab setting. We focus on their efforts in this issue of *Kernel.*

We also feature the work of a mechanical engineer who studies diverse topics, from desalination and heat management to food safety.

Read about how removing grazing animals can affect soil carbon stability, how bacterial vesicles coated on gold nanoparticles can help combat TB, and more.

TRACKING CANCER WITHOUT CULTURE



Breast tumour microenvironment reconstructed using normal breast cells (green) and invading breast cancer cells (red) within the native extracellular matrix (black) (Image: Dharma Pally)

3D REPLICAS OF TISSUES IN THE LAB ARE ENABLING SCIENTISTS TO GET BETTER INSIGHT INTO CELL ORGANISATION AND BEHAVIOUR

In September 2022, the US Senate unanimously passed the FDA modernisation Act 2.0 bill, which suggests reforms to the archaic Food, Drug, and Cosmetic Act that mandated testing drugs and cosmetics on animals for toxicity studies. Under the new bill, animal testing is no longer compulsory. Besides hoping to put an end to the ethical controversies surrounding animal testing, the bill encourages the development of better alternatives to animal testing as well.

But even before the bill was introduced, scientists had been working on alternative strategies to study cell and tissue behaviour in the lab without animal models. Biologists and engineers have joined forces to design systems that can recreate tissues as they are found inside the human body. However, one of the key challenges they face is that not all tissues have simple geometries. The intestinal lining, for example, is not a flat sheet – it has multiple protruding folds like a creased blanket, which increases the surface area available for food absorption.

Such complexities in tissue architecture cannot be recreated in the lab by using traditional cell culture techniques, which involve growing cells in flasks or dishes supplied with nutrient media. In these dishes, the cells attach to the surface and

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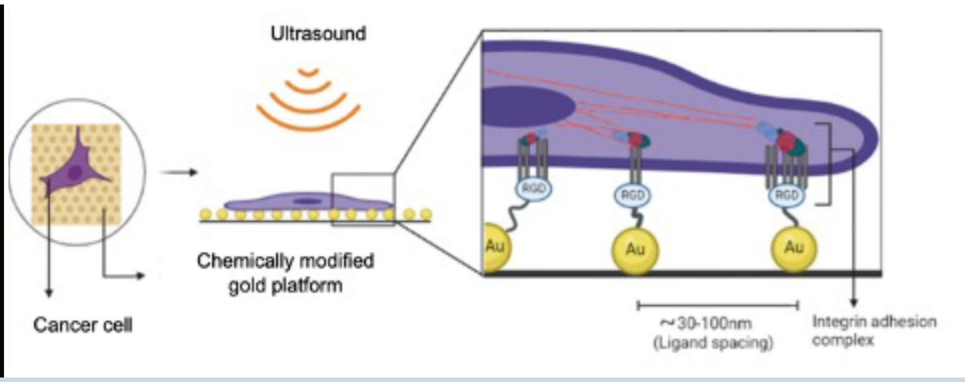
Gold nanopatterned platform to assess the effect of ligand spacing on cancer cell killing upon ultrasound treatment (Image courtesy: Ajay Tijore)



form a flat sheet on which biologists can perform experiments. However, what scientists see happening in these 2D cell sheets may not reflect what actually happens inside the body, because most tissues have complex 3D architectures, behaviours and properties. Cancer cells, for example, migrate very differently in a lab dish compared to how they move inside the human body. This is why many drug studies that show promising results in 2D cell cultures in the lab fail in subsequent clinical trials. And this is why researchers are rapidly moving towards organoids or 3D cell culture systems.

One example of such an organoid is a mammosphere. Mammospheres are breast tissue-derived cells arranged as dense spheres floating in a nutrient solution. Annapoorni Rangarajan, Professor at the Department of Molecular Reproduction, Development and Genetics (MRDG), uses these mammospheres to study breast cancer in her lab. To prompt the formation of these 3D structures, her team grew cells in specialised flasks in which cells cannot stick to the bottom. While most cells died due to the lack of a surface to attach to, the remaining proliferated to form floating mammospheres, enriched in stem cells.

Unlike the floating mammospheres in the lab, both cancer and healthy cells inside the human body actually attach to a substrate in the breast. Different tissues of the body have substrates with varying stiffness and protein composition. This substratum, onto which the cells latch and grow, is called the extracellular matrix (ECM). To mimic the ECM in 3D, Anu's team collaborated with Kaushik Chatterjee, Associate Professor at the Department of Materials Engineering. In place of the substrate found in the breast tissue, they used porous PCL (Poly-Caprolactone) substrates synthesised in the lab that mimicked breast cancer stiffness. However, they found that the patient-derived cells could not latch onto and survive for long on the PCL scaffold on their own. So, they first cultured fibroblasts – cells which secrete ECM components such as proteins – on the PCL scaffold. After the fibroblasts had secreted ECM components on the PCL scaffold, they were killed. Then, breast cancer cells from human patient samples could attach to the scaffold and form 3D organoids, similar to how tissues are arranged inside the body.

Organoids are also the model system used to study cancer in the lab of Ramray Bhat, Associate Professor at MRDG. To study ovarian cancer, the group uses spheroids – cell clusters found in ovarian cancer patients. They grow spheroids in combination with cells of the gut lining to study how cancer cells colonise and penetrate the tissue to reach other organs. They also grow 3D cultures of breast cancer cells embedded in substrates containing fibronectin, collagen, hyaluronic acid and matrigel – important components of the breast ECM.

Besides the ECM, the tissues in our body are also in contact with bodily fluids and other cell types. The local environment around a tissue is called the tissue microenvironment (TME). The group mimics the TME found in diseased conditions by altering the composition of the fluid in which cells are cultured in the lab. "These are complex cell systems where we can look at the intersection of cancer and non-communicable chronic diseases such as diabetes," explains Ramray.

While most cells in tissues are attached to the ECM, some cells, such as red blood cells and white blood cells, flow with blood in blood vessels. Blood vessels are also the highway for cancer cells to migrate from one organ to another, a process called metastasis. During metastasis, some cancer cells may also be found flowing inside the blood vessels. To isolate cancer cells from blood vessels, Ramray's group collaborated with Prosenjit Sen, Associate Professor at the Centre for Nano Science and Engineering (CeNSE). Using microfluidics, they have devised narrow tubes that mimic blood vessels and by tweaking the tube size, are able to replicate how blood and cancer cells flow inside our bodies.

To enter blood vessels from the primary tumour site, cancer cells usually bore their way through the ECM of the tissue. An alternative route weaves through free spaces in the ECM, called interstitial spaces. "Cells of sizes of a few 100 micrometres can squeeze through channels of about ten micrometres, which changes the mechanical properties of cells. We try to understand whether we can use this change in mechanical properties to kill cancer cells using external mechanical forces without damaging normal cells," explains Ajay Tijore, Assistant Professor at the Centre for BioSystems Science and Engineering (BSSE). To mimic the journey of cancer cells via narrow interstitial spaces, the group uses a combination of microfabrication and soft lithography techniques to construct artificial microchannels. Cancer cells are passed through these channels and are subjected to ultrasound-generated mechanical forces to see if they can be killed.

Ajay's lab is also working on mimicking the ECM at the nanometer scale. Cells use a transmembrane protein called integrin to attach to the ECM. One end of the protein lies inside the cell, and the other end latches onto specific proteins at the ECM which repeat at regular intervals. To mimic these proteins, the researchers use gold nanoparticles to which integrins bind. These nanoparticles also control the spacing between consecutive integrin molecules – an important property that differs in normal and cancer cells – providing a system to study how receptors contribute to mechanical weaknesses in cancer cells.

"What we have done until now has been static 3D organoids. Moving forward, we would like to bring together multiple organs at homeostasis on a single chip," says Ramray. Such efforts tie into the emerging personalised medicine revolution: soon, scientists may be able to tailor treatments and drugs to suit each individual by replicating entire human organs or organ systems – a mini "you" in the lab.

- Sindhu M

Long-term field experiment in the high-altitude Himalayan desert ecosystem in Spiti, Himachal Pradesh (Photo: Dilip GT Naidu)

GRAZING ANIMALS KEY TO LONG-TERM SOIL CARBON STABILITY

Large mammalian herbivores like the yak and ibex play a crucial role in stabilising the pool of soil carbon in grazing ecosystems such as the Spiti region in the Himalayas, a 16-year-long study reports. It was carried out by researchers at the Centre for Ecological Sciences (CES) and the Divecha Centre for Climate Change (DCCC), IISc.

Experimental removal of grazing by herbivores from such ecosystems was found to increase the fluctuations in the level of soil carbon, which can have unintended negative consequences for the global carbon cycle.

As soil contains more carbon than all plants and the atmosphere combined, it is important to ensure its persistence. When plants and animals die, dead organic matter remains in the soil for a long duration before microbes break it down and release carbon into the atmosphere as carbon dioxide. “The soil pool is a reliable sink for trapping carbon,” explains Sumanta Bagchi, Associate Professor at CES and senior author of the study published in the *Proceedings of the National Academy of Sciences.* Maintaining stable levels of carbon in the soil is therefore key to offsetting the effects of climate change.

Bagchi began studying the impact of grazing animals on Himalayan ecosystems during his PhD back in 2005. With support from the Himachal Pradesh state government, local authorities, and the people of the Kibber village in Spiti, he and his team established fenced plots (where animals were excluded) as well as plots in which animals like yak and ibex grazed. Over the following decade, he and his students collected soil samples from the region and analysed their chemical composition, tracking and comparing the levels of carbon and nitrogen in each plot year after year.

From one year to the next, soil carbon was found to fluctuate 30-40% more in the fenced plots where animals were absent, compared to the grazed plots where it remained more stable each year. A key factor underlying these fluctuations was nitrogen. Depending on the soil conditions, nitrogen can either stabilise or destabilise the carbon pool. Grazing by herbivores, however, changes their interactions in ways that tip the balance in favour of the former, the researchers found.

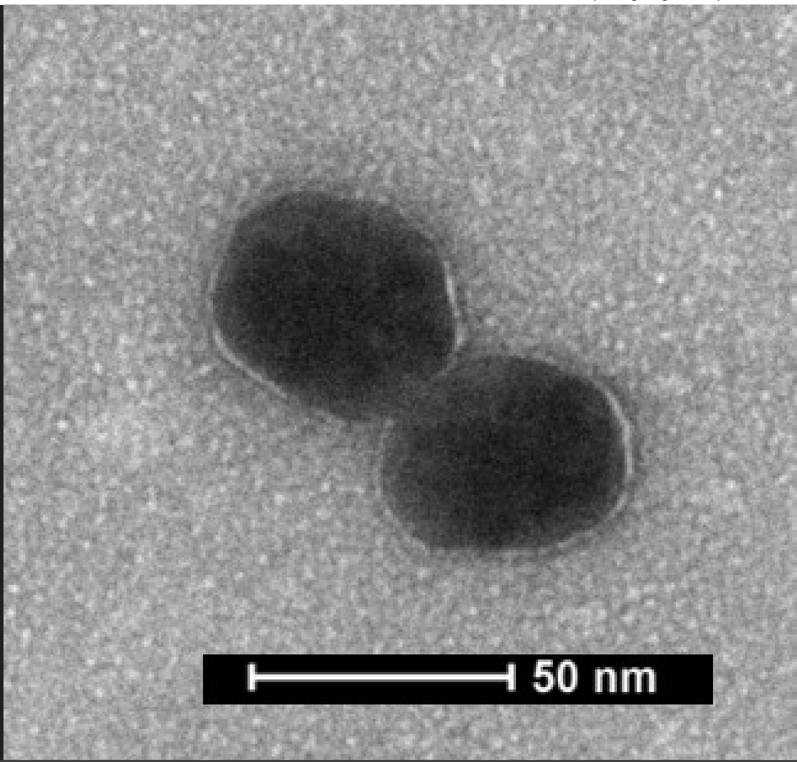
Many previous studies have focused on measuring carbon and nitrogen levels at long time intervals, assuming that the accumulation or loss of carbon is a slow process, explains Dilip GT Naidu, PhD student at DCCC and first author of the study. But the interannual fluctuations they noticed in their data paint a very different picture, he adds. These fluctuations can be consequential for climate as they are linked to how large mammalian herbivores influence soil.

Because grazing ecosystems make up about 40% of the Earth’s land surface, protecting the herbivores that keep the soil carbon stable should remain a key priority for mitigating climate change, the researchers suggest.

“Both domestic and wild herbivores influence climate via their effects on soil carbon,” explains Shamik Roy, a former PhD student at CES and another author of the study. In ongoing research, Bagchi and his team are also assessing why domestic herbivores such as goats and sheep differ from their wild relatives in how they impact ecosystems. “Domestic and wild herbivores are very similar in many respects, but they differ in how they influence plants and soil. Understanding why they are not alike can lead us toward more effective stewardship of soil carbon,” he adds.

- Ranjini Raghunath

Transmission electron microscope image of gold nanoparticles coated with bacterial Outer Membrane Vesicles (Image: Edna George)



BACTERIAL VESICLES COATED ON GOLD NANOPARTICLES TO COMBAT TB

Researchers from IISc have designed a new method to deliver a vaccine candidate for tuberculosis (TB). It involves using spherical vesicles secreted by bacteria coated on gold nanoparticles which can then be delivered to immune cells. This can potentially trigger an immune response and offer protection against the disease.

Caused by the bacterium *Mycobacterium tuberculosis,* TB kills over a million people worldwide every year. The only effective vaccine currently in use is the BCG vaccine. It contains a weakened form of the disease-causing bacterium. When injected into our bloodstream, it triggers the production of antibodies that can help fight the disease.

While the BCG vaccine works well in children, it is not as effective at protecting adolescents and adults. This prompted Rachit Agarwal, Assistant Professor at the Centre for BioSystems Science and Engineering (BSSE), IISc, and his group to develop a potential subunit vaccine candidate that contains only parts of the infectious bacterium to stimulate an immune response.

Scientists have earlier developed subunit vaccines based on just a handful of proteins from the disease-causing bacteria, but none of them have been effective so far. Instead, Agarwal's group decided to use Outer Membrane Vesicles (OMVs). OMVs are spherical membrane-bound particles released by some bacteria, and contain an assortment of proteins and lipids which could induce an immune response against the pathogen.

"They're safer compared to a live bacterium, and since they are membrane-derived, they contain all kinds of antigens," explains Agarwal, the senior author of the paper published in *Biomaterials Advances.* Subunit vaccines typically only contain a limited number of antigens – bacterial proteins that can elicit an immune response in the host. In contrast, OMVs contain a variety of antigens and can induce a better immune response, according to the researchers.

Mycobacterium-derived OMVs are usually unstable and come in different sizes, making them unsuitable for vaccine applications. But the OMVs coated on gold nanoparticles (OMV-AuNPs) by the IISc team were found to be uniform in size and stable. The researchers also found that human immune cells showed a higher uptake of OMV-AuNPs than of OMVs or gold nanoparticles alone.

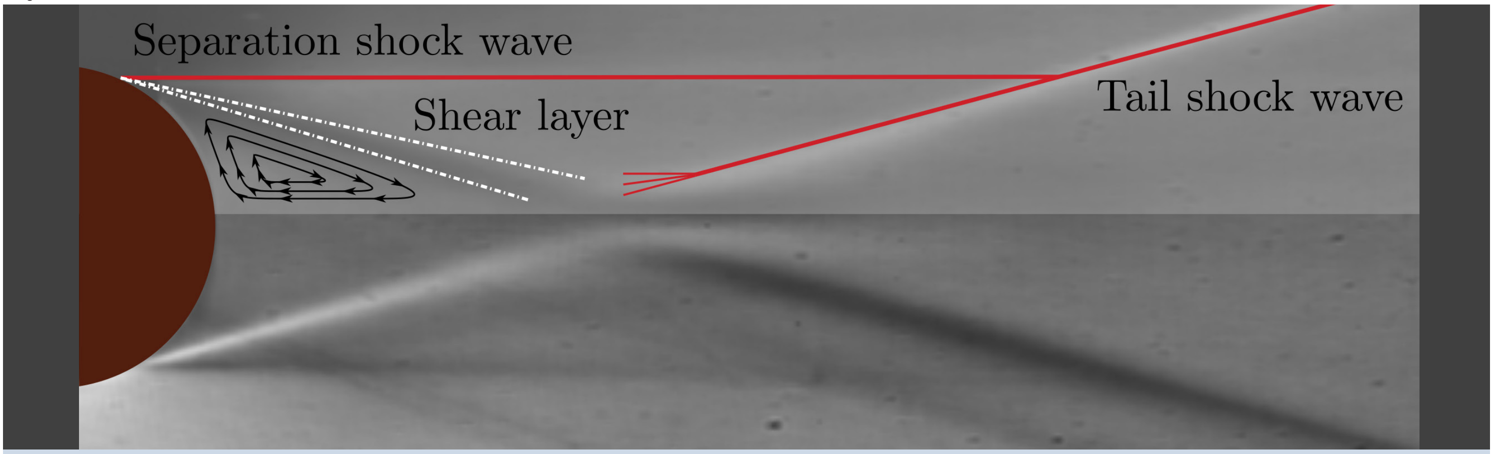
"Producing the OMVs is a complex process, and scaling it up was challenging," says Avijit Goswami, a former postdoctoral fellow at BSSE and one of the first authors of the study.

"To synthesise OMV-AuNPs, the OMVs and the gold nanoparticles are forced together through a 100 nm filter. The OMVs break up in the process and encapsulate the gold nanoparticles," explains Edna George, a former postdoctoral fellow at BSSE, and co-first author of the study.

In the study, immune cells cultured in the lab were treated with OMVs derived from *Mycobacterium smegmatis,* a related bacterial species that does not cause disease in humans. In future studies, the team plans to develop gold-coated OMVs derived directly from *Mycobacterium tuberculosis* and test them on animal models to take the results forward for clinical applications. Such efforts could open up new avenues for the development of vaccines for other bacterial diseases as well.

- Sindhu M

Image: Premika S Thasu



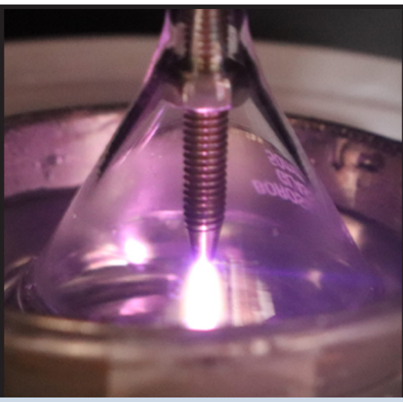
IN THE WAKE OF A CYLINDER AT MACH 6

Air gusts or currents in the wake of a fast-moving object like a car or truck is a common phenomenon that many among us have experienced. In such ‘wake flows’, air typically moves around in an unsteady and whirling manner. This motion of air presents very interesting patterns in space, with a certain associated periodicity in time.

Premika S Thasu and Duvvuri Subrahmanyam from the Department of Aerospace Engineering attempted to answer the following question: what do wake patterns for an object that is moving at a very high speed look like? They experimentally studied the wake generated by a simple object — a circular cylinder — moving at Mach 6 (six times the speed of sound).

The wake flow was found to have a strong characteristic frequency, and interestingly, the frequency exhibited ‘universal scaling’. This means that once the frequency for a given cylinder size and flow speed is known (measured), the frequency for any cylinder of a different size and/or moving at a different speed can be readily predicted. The researchers were therefore able to obtain new fundamental insights into the physical mechanisms that give rise to the characteristic frequency by analysing the wake flow patterns.

Photo courtesy: Lakshminarayana N Rao



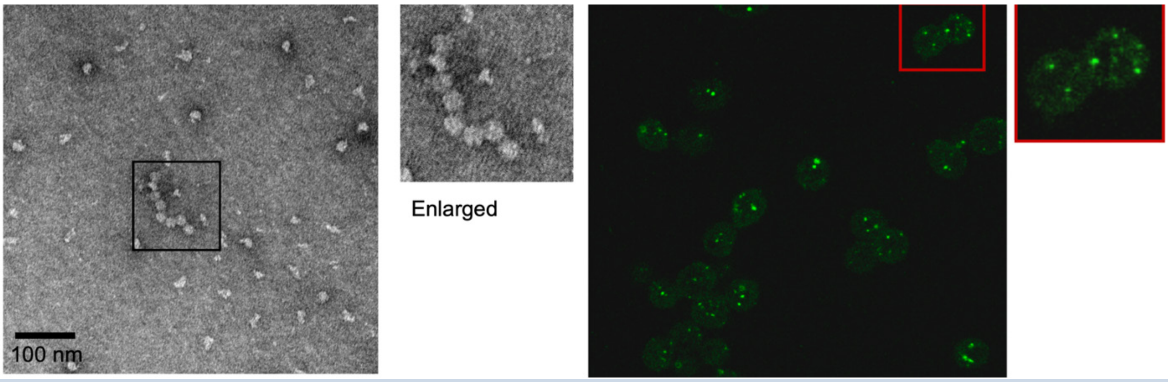
PLASMA-ACTIVATED-WATER TO COMBAT MULTIDRUG-RESISTANT PATHOGENS

Researchers from IISc have demonstrated the generation of plasma-activated water containing reactive oxygen and nitrogen species such as H_2O_2, NO_2^-, and NO_3^- in high strengths (hs-PAW). This hs-PAW was found to be capable of inactivating even hypervirulent multidrug resistant pathogens. The study, led by Lakshminarayana N Rao and Dipshikha Chakravortty, also shows that plasma-activated water is neutral, making it suitable for real-world biomedical applications.

The team generated hs-PAW using pin-to-water discharge with a funnel enclosure. Simulations showed that the funnel enclosure increased the velocity and circulation without allowing the reactive species to escape far away from the water surface, leading to their increased concentration in the plasma-activated water. The team found that the reactive species in hs-PAW disintegrates the bacteria’s outer cell membrane by perforation, arresting the metabolic activity, eventually inactivating or killing the bacteria.

The team also found that the hs-PAW could retain its bactericidal activity against pathogens even after being stored for up to 15 days. They suggest that such high strength plasma-activated water can be used in several medical applications, such as wound healing, apart from tackling hard-to-treat multidrug-resistant pathogens.

Image: Sangeetha Devi Kumar



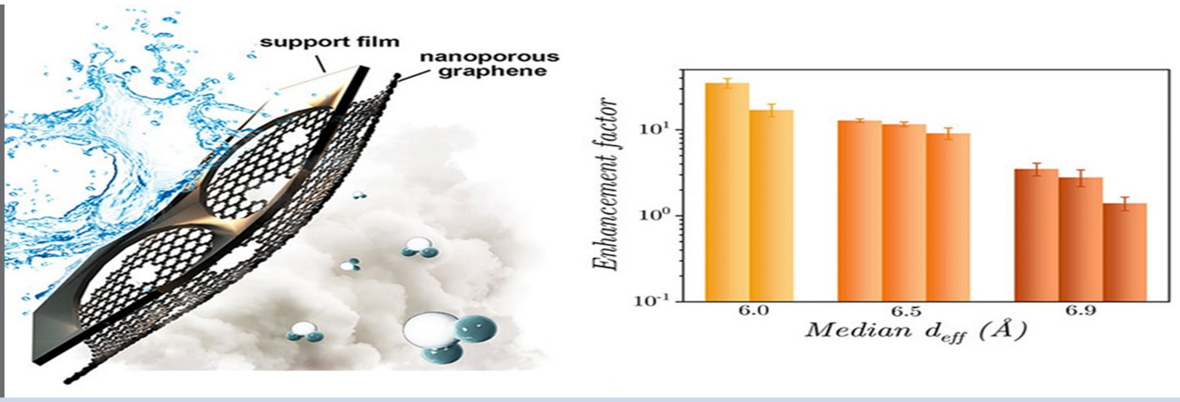
PROTEIN SYNTHESIS IN CELLS WITHOUT A NUCLEUS

Translation, or protein synthesis, is the final step in the central dogma of molecular biology, where an mRNA carrying information from DNA synthesises proteins. It is an indispensable process that is performed by almost all living cells. An exception where translation was believed to be absent is red blood cells (RBCs). Now, a team led by Sandeep M Eswarappa in the Department of Biochemistry has shown that mature human red blood cells can also make their own proteins.

Although initially thought to be mere 'bags of proteins', RBCs are metabolically active and harbour a wide repertoire of mRNAs, microRNAs, and other long non-coding RNAs. Using advanced labelling and imaging techniques, the researchers have discovered proteins being actively synthesised in these cells too.

This study opens up new avenues to treat diseases caused by abnormal haemoglobin, called haemoglobinopathies. Currently, the treatment for these disorders includes targeting blood cells from the bone marrow, which can be very challenging. By focusing on the translation process in these cells, researchers can now regulate protein expression directly, making it easier to develop new therapeutic strategies for such disorders.

Image: Kumar Varoon Agrawal



ENHANCED WATER EVAPORATION FROM NANOPOROUS GRAPHENE

With growing global water stress, there is a pressing need to develop potable water technologies with a lower carbon footprint. Enhancing the kinetics of liquid–vapour transition from nanoscale confinements is an attractive strategy for developing evaporation and separation applications. The ultimate confinement limit for evaporation is an atom-thick interface hosting Angstrom-scale nanopores.

Researchers led by Ganapathy Ayappa at the Department of Chemical Engineering and collaborators at EPFL, Switzerland have now developed a novel, low thermal input, evaporation-based process using single-layered graphene sheets synthesised with tunable-sized nanopores. They report, for the first time, a 35-fold enhancement in water evaporation rates from Angstrom-scale graphene nanopores relative to a bare liquid-vapour interface.

Using a vapour deposition process, the group at EPFL synthesised nanoporous graphene and studied for the first time the water evaporation rates as a function of the nanopore dimensions. This is in sharp contrast to earlier studies where evaporation had been studied through nanochannels, presenting a significant advance in the development of nanopore-based technologies. The group at IISc carried out extensive molecular dynamics simulations using the Institute's supercomputing facility to provide molecular insights to explain this enhancement.

Photo courtesy: Susmita Dash

BREAKING INTERFACIAL BARRIERS

SUSMITA DASH'S LAB STUDIES THE SCIENCE OF SURFACES, FLUIDS AND HEAT TO FIND SOLUTIONS TO PROBLEMS IN ENERGY, ENVIRONMENT, AND HEALTH

Growing up, Susmita Dash never thought that she would become a scientist. But that changed when she landed an internship at the Indian Institute of Technology, Kanpur, while pursuing her bachelor's degree. It was here that she was exposed to the world of experimentation. During the internship, she studied how ink flow patterns form when an oscillating circular cylinder is suspended in a liquid stream. This experience kindled a fascination in her for research. "I loved seeing what I saw … research looked fun," she says.

After her bachelor's degree, Susmita took a short diversion from academia to industry and joined Schlumberger, an oil and gas company, as a mechanical engineer. But she couldn't resist the lure of academia. "I liked research, I wanted to be a researcher," she says. She took up a PhD programme at Purdue University, USA. After graduating from Purdue, Susmita pursued her post-doctoral research at the Massachusetts Institute of Technology for two and half years. She then joined IISc as an Assistant Professor in the Department of Mechanical Engineering in January 2018.

Susmita's lab focuses on understanding how heat, fluids and material surfaces interact with one another at various scales, from the micro to the macro. They seek to develop solutions for global-scale challenges related to energy and transport.

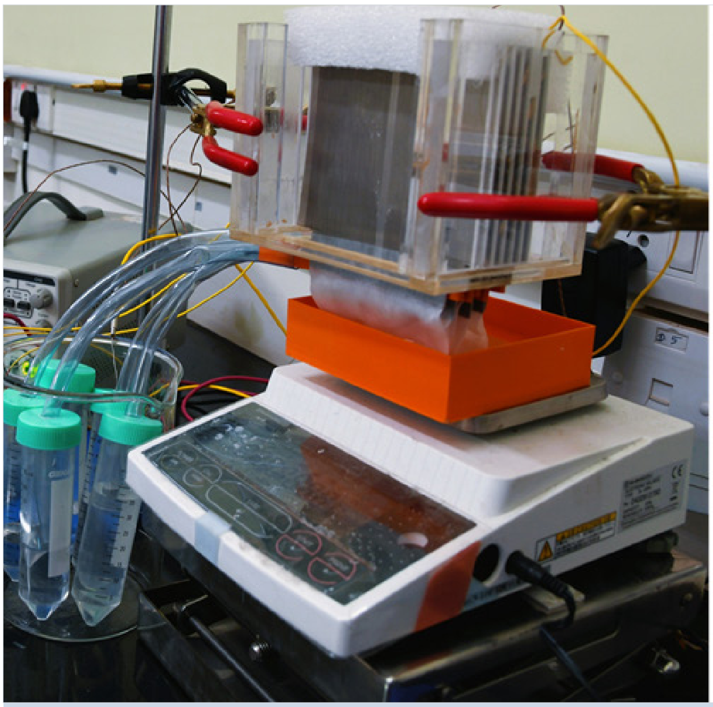
One area of interest is thermal management – the control and regulation of heat in a system or device. This is crucial in many areas such as electronics and automotive engineering, where excessive heat can cause damage to or reduce the efficiency of a system. "There is an ever-increasing demand for power, which creates a need for efficient thermal management techniques … often electronic devices are limited by their ability to dissipate heat, or how fast they can be cooled," Susmita says. Her team works on ways to maximise heat transport and increase device productivity.

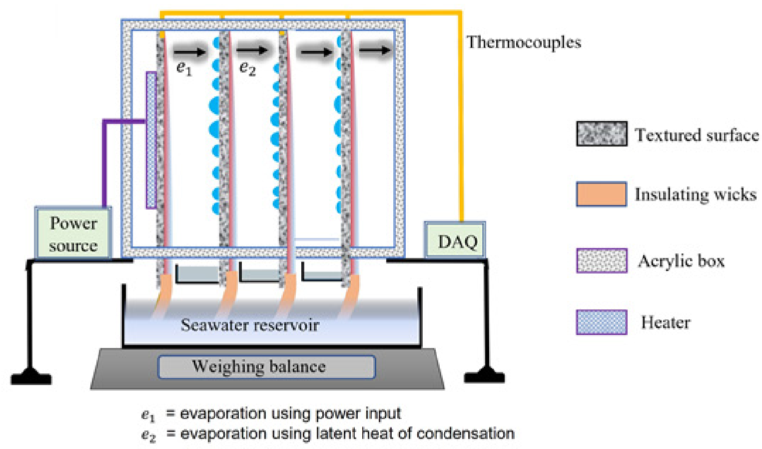
Along the same lines, her team is working towards designing and developing, and thrust characterisation of micro-thrusters that rely on localised evaporation of the propellant for propulsion of nanosatellites. Such nanosatellites, which are helpful for low-cost communication and surveillance, require compact and lightweight propulsion systems. The microthruster utilises a green propellant – water – as the working fluid. There are, however, several challenges in this area, which her group is currently working on. "The device needs to adapt to vacuum conditions and low temperatures," says Susmita.

Another challenge that the lab attempts to address is fouling – the build-up of undesirable particles on a surface. This can happen in a variety of situations, such as when salt accumulates and clogs pipes and heat exchangers. Fouling can have negative effects on the performance and efficiency of a system, and so it is important to understand and prevent it.

The lab also conducts research on desalination – the process of removing salts, minerals, and other contaminants from water, to make it fit for human consumption or irrigation – using solar energy, which is a low-cost, sustainable alternative compared to fossil fuels. Traditional desalination units convert saline water to vapour using the rays of sunlight. This vapour then cools down and gets collected as distilled water. But existing desalination systems are not very efficient at recovering large volumes of fresh water and also consume a lot of power. To overcome this problem, Susmita and her team are working on developing an efficient solar thermal desalination system by primarily focusing on improving the efficiency of the condenser. For this, they employ the concept of surface wettability – the tendency of a liquid or a gas to spread and interact with other liquids and solid surfaces – and wicking. "Think of it like a sponge. When placed over water, the water gets absorbed (wicked) because of

Interfacial thermal desalination system (Photo courtesy: Susmita Dash)





the sponge's tiny capillaries, which help collect the liquid," Susmita explains. The team uses the same strategy: a conducting substrate dipped in liquid on one side and exposed to sunlight on the other.

Food safety is yet another area that the lab works on. A recent study from the lab made headlines for offering a low-cost approach to detect adulterants in milk. In the study, Susmita and her team showed that evaporative deposition can help detect the presence of common adulterants in milk, such as water and urea, by observing changes in the patterns generated by non-volatile milk solids and the crystallisation of urea.

Like other young researchers, Susmita also found the early days of being a faculty member challenging: setting up an experimental lab, and teaching courses. The second part was particularly intimidating for her in the beginning. "When you are a new faculty member and are teaching the course for the first time, you are as nervous as the students … how to plan the course materials and how to put across the concepts so that the class understands … but when you want to learn something properly, the best way is to teach," Susmita explains.

In research, one has to "grease their own gears," she says, explaining that students have to constantly keep thinking of new approaches to their research problems, and think and plan their work independently.

Although mechanical engineering has historically been a male-dominated field, Susmita believes that the gender gap can be reduced by increasing awareness among school students about the exciting possibilities the field has to offer. Mechanical engineering is becoming more and more interdisciplinary, offering people from diverse backgrounds opportunities to pursue cutting-edge research. "People think that if you are a mechanical engineer, you have to hold a spanner, tighten a screw, or fix a machine, but that is not the real picture," she says.

- Seemadri Subhadarshini

Susmita Dash with her research group (Photo courtesy: Susmita Dash)

For placements and internships for IISc students, please contact the Office of Career Counselling and Placement (OCCaP) at placement.occap@iisc.ac.in | https://occap.iisc.ac.in

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