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Editorial

From blood spots to hearts, transporting biological samples is critical for saving lives. Researchers at IISc are working on innovative designs that can help preserve the integrity of such samples while they are being rushed to the diagnostic centre or hospital. Read more about their efforts in this issue of *Kernel*.

We also feature a civil engineer who uses imaging and modelling tools to probe the complex interior of granular materials. Other stories focus on space bricks for construction on Mars, paper strips that can sense hydrogen peroxide, and microplastics that are polluting the Cauvery river.

GOING FOR A RIDE

Prototype of LifeBox, an organ transportation device developed by IISc researchers (Photo courtesy: Deval Karia)

RESEARCHERS ARE DEVELOPING INNOVATIVE TOOLS TO TRANSPORT A VARIETY OF BIOLOGICAL SAMPLES

The COVID-19 pandemic has underscored the need for robust mechanisms to ensure rapid diagnostics and testing, especially for infectious diseases. Biological samples, such as swab samples collected for RT-PCR tests, need to be stored, maintained and transported to diagnostic centres quickly and safely. When it comes to the transportation of organs, which is crucial for saving the lives of patients with organ failure, the situation becomes more complicated. A major issue is that the infrastructure and transportation systems in Indian cities are not well equipped to handle the rapid and efficient movement of such organs. In this context, some researchers at IISc are working on different ways to improve these modes of transportation.

Biological samples are usually transported in fluid form via a cold chain in refrigerators or freezer boxes – a challenging prospect in remote locations. Blood samples can be transported in the dry state using blood spot cards, but these can carry only small volumes. The advantage of transporting samples in the dry state is that it doesn't need a cold chain. This makes transportation of biological samples for diagnosing diseases like tuberculosis (TB) easier.

Caused by *Mycobacterium tuberculosis (Mtb),* TB is the number one cause of death by a single infectious agent worldwide, with a majority of deaths occurring in developing nations. Accurate diagnosis is

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L: Priyanka Agarwal & Shruti Soni, PhD students in Bhushan Toley's lab, who worked on developing the SPECTRA-tube. R: Azooka team that has developed RNAWRPR
(Photos courtesy: Bhushan Toley and Fathima Benazir)

key to controlling TB. An important step in this process is storing and transporting sputum – a type of mucus in the respiratory tract, coughed up during an infection – taken from patients. There have been only a few studies to transport sputum in dry form in the past in low volumes. It is necessary to collect a large amount of sputum from each patient to diagnose TB effectively, as the concentration of Mtb in the infected sputum can be very low.

To enable such transport, a team of researchers at IISc led by Bhushan Toley, Assistant Professor at the Department of Chemical Engineering, has recently designed a prototype device called specimen transportation tube (SPECTRA-tube). SPECTRA-tube consists of a cassette, comprising multiple layers of glass fibre membranes, in which the sputum sample is collected. Compared to conventionally used paper-based membranes – to which samples end up sticking – glass fibre membranes allow more efficient recovery of the sample in the end. The collected sample is dehydrated using desiccant packs that reside within the SPECTRA-tube. The SPECTRA-tube containing the sputum sample can then be transported to the testing facility, where it is reconstituted using a rehydration buffer. This technique has enabled almost complete recovery of the sample, an immense improvement over previous attempts, according to Bhushan.

"Because SPECTRA-tube allows a large volume of sample collection and transportation in the absence of a cold chain, this prototype can be distributed to rural areas for better diagnosis in low resource countries," he explains.

He and his team also plan on extending this approach to urinary tract infections (UTIs), as the SPECTRA-tube can allow at-home collection of urine samples in large volumes, which can then be transported without refrigeration to diagnostic centres.

"The design of our SPECTRA-tube is quite intricate. So, manually assembling each component of this system is fine for the lab. However, for larger applications … we plan on 3D printing the design for large scale manufacturing. We [also] plan on further improving the current design of the SPECTRA-tube to increase its holding capacity, improving the shelf life and [for] better stabilisation of the sample in the future," he adds.

During the COVID-19 pandemic, there was an unprecedented increase in the transportation of biological samples – particularly RNA (ribonucleic acid), which is the genetic material of SARS-CoV-2. The RNA is usually collected and placed in a refrigerator for transport to the testing centre, but poor refrigeration or handling can damage the sample. Fathima J Benazir, co-founder and Chief Scientific Officer of IISc-incubated startup, Azooka Labs Pvt Ltd, and her team have created a solution called RNAWRAPR. Their device preserves the integrity of RNA and inactivates all substances that could affect its quality – such as enzymes or other biological molecules that can act on it – allowing its transportation without the requirement of a cold chain. "Samples remain stable at room temperature upto seven days after collection," explains Fathima.

"Being in the field of molecular biology, we wanted to contribute towards the COVID-19 crisis. This led to the idea of creating a solution that can preserve the integrity of RNA samples and can be transported without any hassle and zero contamination," she adds. "One of its greatest values is that it inactivates the virus in the media. Therefore, in cases of accidental spillage or exposure, it remains safe for the frontline health workers."

Azooka is also working on extending the applications of RNAWRAPR to the transportation of other biological samples. "We have a series of products addressing sample collection for nucleic acid integrity across various sample specimens right from blood to biological fluids to tissues and so on, and also for specified applications like cell-free DNA. We are also working on targeting infectious disease sample collection, including TB," Fathima adds.

While samples like RNA need smaller devices, moving entire organs is a much bigger challenge. They are currently transported via ice boxes and depend on civic authorities establishing green corridors (traffic-free routes) along the way. This has several drawbacks; it requires massive coordination between hospitals and several regulatory authorities and often, the time window is too narrow for the organ to reach the recipient. Deval Karia, a former MDes student at IISc, was inspired after attending a course by faculty members B Gurumoorthy and Ashitava Ghosal at the Centre for Product Design and Manufacturing (CPDM), IISc, to develop LifeBox – a drone-based portable system that can extend the out-of-body time for the organ being transported. The project is currently led by B Gurumoorthy, Ashitava Ghosal and Manish Arora at CPDM, along with Dr K Balakrishnan at MGM Hospital.

The team decided to develop a system that would allow transportation of hearts for transplant surgeries. LifeBox uses a perfusion fluid and a patent-pending, novel refrigeration system. It is also equipped with multiple sensors that monitor the health of the heart, making it easier for the surgeon to make critical decisions. "A peristaltic pump drives the perfusion fluid from a reservoir to the heart which is housed within a chamber. Waste fluid from the heart is then collected. The heart in this setup is only intermittently perfused and does not beat," explains Deval.

Currently, patients in need of a heart transplant spend long periods on waitlists for organs. For such patients, inventions like LifeBox could make a significant difference. Deval explains, "Factors such as debilitating illness, loss of employment, anxiety, isolation, and clinical costs take a severe toll on family members as well. Any method that reduces this waiting time will provide significant relief to all stakeholders within the system. Compared to aerial transport in chartered/commercial airplanes, this would significantly reduce costs for the recipient family too."

Deval adds that they also plan on extending their novel refrigeration system to storing and transporting other organs, which may help in saving more lives.

- Monmita Bhar

Photo: Nitin Gupta

USING BACTERIA TO BUILD SETTLEMENTS ON MARS

In collaboration with the Indian Space Research Organisation (ISRO), a team of researchers from IISc has developed a sustainable method for making bricks out of Martian soil, using bacteria and urea. These "space bricks" can be used to build structures on Mars that could facilitate human settlement on the red planet.

The method for making these space bricks has been outlined in a study published in *PLOS One.* A slurry is first created by mixing Martian soil simulant with guar gum, a bacterium called *Sporosarcina pasteurii,* urea and nickel chloride. This slurry can be poured into moulds of any desired shape, and over a few days the bacteria convert the urea into crystals of calcium carbonate. These crystals, along with biopolymers secreted by the microbes, act as a cement, holding the soil particles together.

An advantage of this method is the reduced porosity of the bricks, which has been a problem with other methods used to consolidate Martian soil into bricks. "The bacteria seep deep into the pore spaces, using their own proteins to bind the particles together, decreasing porosity and leading to stronger bricks," says Aloke Kumar, Associate Professor in the Department of Mechanical Engineering at IISc, one of the senior authors of the paper.

The research group had previously worked on making bricks out of lunar soil simulant, using a similar method. However, the previous method could only produce cylindrical bricks, while the current slurry-casting method can also produce bricks of complex shapes. The slurry-casting method was developed with the help of Koushik Viswanathan, Assistant Professor in the Department of Mechanical Engineering, IISc, whose lab works on advanced manufacturing processes. In addition, extending the method to Martian soil proved challenging. "Martian soil contains a lot of iron, which causes toxicity to organisms. In the beginning, our bacteria did not grow at all. Adding nickel chloride was the key step in making the soil hospitable to the bacteria," explains Kumar.

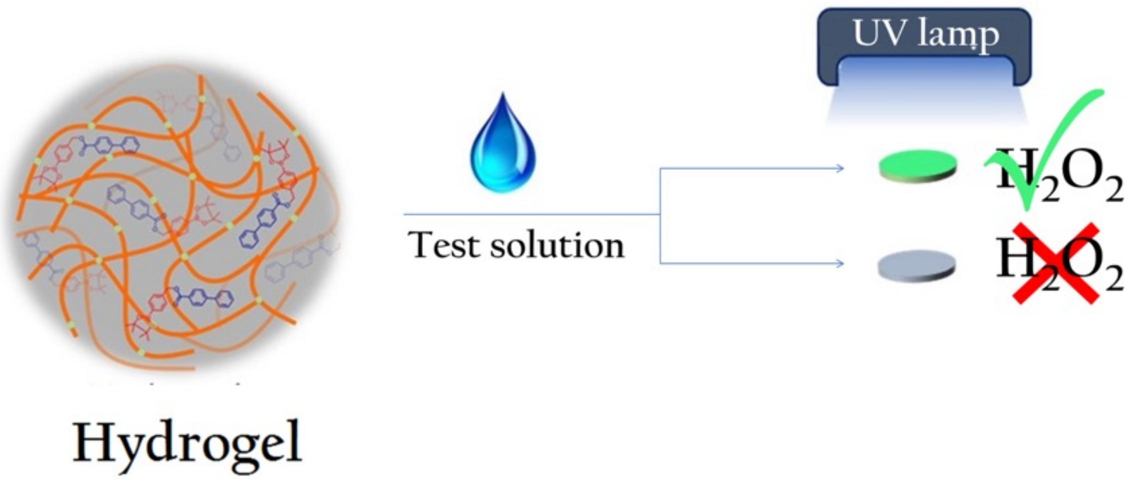
The group plans to investigate the effect of Mars' atmosphere and low gravity on the strength of the space bricks. The Martian atmosphere is 100 times thinner than Earth's atmosphere, and contains over 95% carbon dioxide, which may significantly affect bacterial growth. The researchers have constructed a device called MARS (Martian AtmospheRe Simulator), which consists of a chamber that reproduces the atmospheric conditions found on Mars in the lab.

The team has also developed a lab-on-a-chip device that aims to measure bacterial activity in microgravity conditions. "The device is being developed keeping in mind our intention to perform experiments in microgravity conditions in the near future," explains Rashmi Dikshit, a DBT-BioCARe Fellow at IISc and first author of the study, who had also previously worked on the lunar bricks. With ISRO's help, the team plans to send such devices into space, so that they can study the effect of low gravity on the bacterial growth.

"I'm so excited that many researchers across the world are thinking about colonising other planets," says Kumar. "It may not happen quickly, but people are actively working on it."

- Rohith KMS

Image: Arnab Dutta



PAPER DISCS THAT CAN PICK UP HYDROGEN PEROXIDE

A paper-based sensor developed by IISc researchers can help detect even tiny volumes of hydrogen peroxide. This chemical is used widely in household and healthcare products like hand sanitiser as a disinfectant, in rocket fuel as a propellant, and is also found in biological cells.

The technique they used involves preparing a gel from a solution containing a specially designed molecule, treated with a liquid that has hydrogen peroxide, and air-drying them on a thin paper disc about 0.45 cm in diameter. The paper disc emits green light when placed under a UV lamp, only in the presence of hydrogen peroxide. The intensity of the light was found to be directly proportional to the concentration of hydrogen peroxide.

"You can actually visualise this green emission (photoluminescence) with the naked eye. You don't need any sophisticated instruments. All you need is a simple UV light source," explains Arnab Dutta, PhD student in the Department of Organic Chemistry and first author of the study published in *ACS Sensors.*

Because the paper disc is low-cost, biodegradable and easy to use, it could serve as a powerful tool in low-resource settings, even for testing biological fluids like blood. Detecting hydrogen peroxide efficiently is also crucial in other fields; peroxide-based explosives, for example, can be traced using hydrogen peroxide which is sometimes used as a starting material.

When the researchers used their technique to randomly test five different hand sanitiser brands, they found that only three of them contained the level of hydrogen peroxide mandated by the World Health Organisation – 0.125%. A fourth appeared to have much lower than 0.125% and one had almost zero hydrogen peroxide.

"Hydrogen peroxide can be detected on a larger scale using titration and other experiments, but those are cumbersome and require training. This method is easy because of its simplicity," says Uday Maitra, Professor in the Department of Organic Chemistry and senior author of the study.

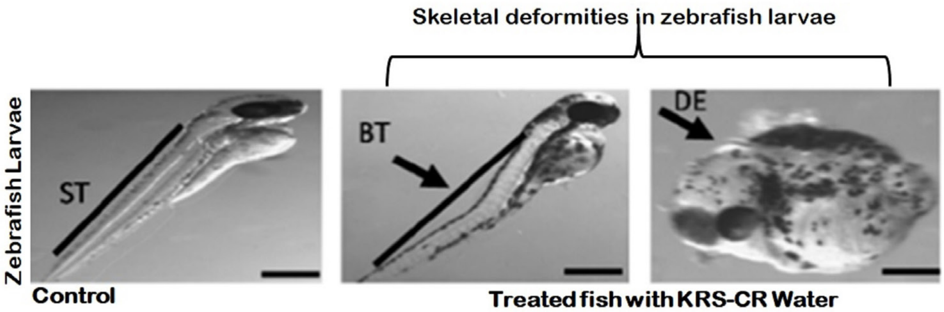
Maitra's lab has been working on developing several 'sensitiser' molecules that turn on the photoluminescence of elements called lanthanides in the presence of specific chemicals or compounds. They have previously developed paper-based sensors for detecting specific antioxidants in green tea – and thereby testing its quality – as well as sensors for various enzymes.

The sensitiser molecule they designed in this study enables a metal called terbium to emit green light under a UV lamp. When the sensitiser is combined with a masking agent, the green light vanishes. When hydrogen peroxide is added to this combination, it unmasks the sensitiser molecule, making it glow green once again. "The way we designed the mask … it is very specifically unmasked by hydrogen peroxide," says Maitra.

Currently, the team is working on cutting down the reaction time; it takes a bit longer if the concentration of hydrogen peroxide is lower. Maitra adds that they are also working on developing a small portable device where the detection can be done in a more automated manner. "We are in touch with a start-up company in Chennai. We have a few prototypes made with UV LEDs and a camera, to generate the emission, take a photograph, and use an image processing app to quantify the amount of hydrogen peroxide."

- Ranjini Raghunath

Images: Abass Toba Anifowoshe and Upendra Nongthomba



MICROPLASTICS IN THE CAUVERY RIVER MAY BE HARMING FISH

Pollutants like microplastics may be causing growth defects in fish in the Cauvery River, a new study in *Ecotoxicology and Environmental Safety* reveals. The study was led by Upendra Nongthomba, Professor at the Department of Molecular Reproduction, Development and Genetics (MRDG).

Nongthomba likes his fish. "Over the years, I have cherished going to the backwaters of the Krishna Raja Sagara [KRS] Dam and having fried fish on the Cauvery River bank," he says. But in recent times, he has been noticing physical deformities in some of them. He began to wonder whether the quality of water may have something to do with it.

"Water is essential for everyone, including animals and plants. When it is polluted, it is capable of causing diseases, including cancer," adds Abass Toba Anifowoshe, a PhD student in Nongthomba's lab, and the first author of the study. Nongthomba's lab, therefore, conducted a comprehensive study of pollution at the KRS Dam and its potential effects on fish. They collected water samples from three different locations with varying speeds of water flow – fast-flowing, slow-flowing, and stagnant – since water speed is known to affect the concentration of pollutants.

In the first part of the study, Nongthomba's team analysed the physical and chemical parameters of the water samples. All but one of them fell within the prescribed limits – dissolved oxygen (DO) levels were much lower than they needed to be in samples collected from the slow-flowing and stagnant sites. Water from these sites also had microbes such as *Cyclops, Daphnia, Spirogyra, Spirochaeta and E. coli,* well-known bio-indicators of water contamination.

Using a technique called Raman spectroscopy, the researchers detected microplastics – minute pieces of plastic often invisible to the naked eye – and toxic chemicals containing the cyclohexyl functional group. Microplastics are found in several household and industrial products, and chemicals containing the cyclohexyl group are commonly used in agriculture and the pharmaceutical industries.

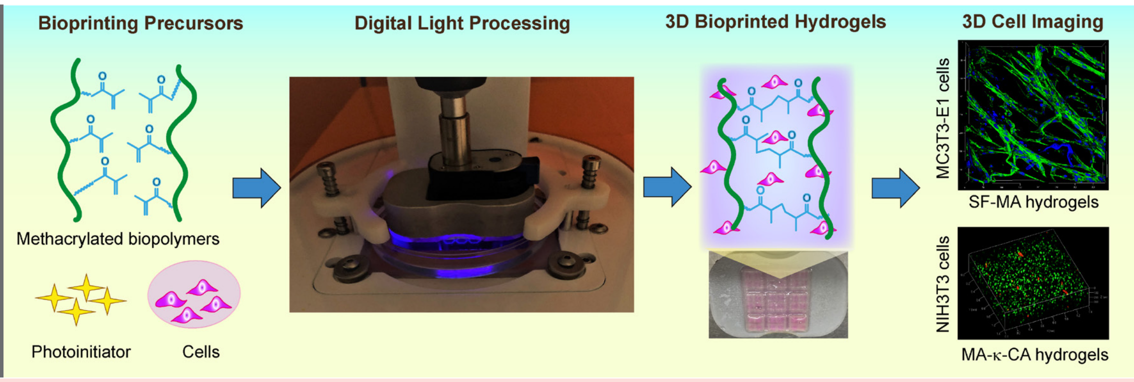
Next, Nongthomba's team investigated whether pollutants in water could account for the developmental abnormalities seen in wild fish. They treated embryos of the well-known model organism, zebrafish, with water samples collected from the three sites, and found that those exposed to water from the slow-flowing and stagnant sites experienced skeletal deformities, DNA damage, early cell death, heart damage, and increased mortality. These defects were seen even after microbes were filtered out, suggesting that microplastics and the cyclohexyl functional groups are responsible for the ailments in the fish.

The researchers also found unstable molecules called ROS (Reactive Oxygen Species) in the cells of the fish that developed abnormally. ROS build-up is known to damage DNA and affect animals in ways similar to what Abass and Nongthomba saw in fish treated with water from the slow-flowing and stagnant sites. Other studies have shown that microplastics and chemicals with the cyclohexyl group lead to decreased DO, which in turn triggers ROS accumulation in animals like fish.

A recent study from the Netherlands has shown that microplastics can enter the bloodstream of humans. So, what do the results from Nongthomba's lab mean for the millions of people who use Cauvery water? "The concentrations we have reported may not be alarming yet for humans, but long-term effects can't be ruled out," he says. But he also admits that before they answer the question conclusively, they need to understand how exactly microplastics enter and affect the host. "This is something which we are trying to address now," Nongthomba says.

- Praveen Jayakumar

Image courtesy: Kaushik Chatterjee



3D BIOPRINTING OF TISSUES USING NATURAL BIOMOLECULES

Tissue engineering focuses on tissue and organ regeneration by creating scaffolds using biomaterials called bioinks. Bioinks comprise of cultured cells and biocompatible materials like a hydrogel, and are specific to the tissue being transplanted. The scaffolds are 3D printed, maintaining structural and functional compatibility with human tissues ranging from soft tissues to bone.

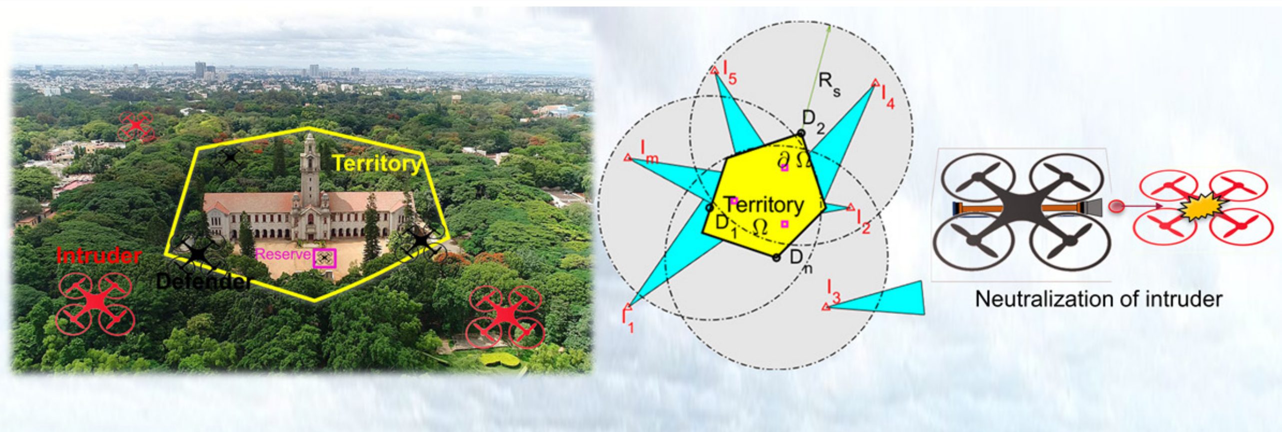
In the past, digital light processing (DLP)-based 3D bioprinting has been attempted where UV light selectively crosslinks the macromolecules to generate the scaffold. However, UV light is known to cause DNA damage in the cells during printing. Kaushik Chatterjee, Associate Professor at the Department of Materials Engineering, and his team addressed this issue by using visible blue light radiation (405 nm) instead of UV.

This new technique shows promise in building tissue scaffolds that mimic the tissue microenvironment structurally and functionally.

In one study, they successfully prepared bioprinted silk fibroin scaffolds for bone tissue regeneration. In another, they carried out experiments with k-carrageenan, a naturally occurring polysaccharide resembling essential components of connective tissues, and demonstrated that it could be used as a component in bioinks for soft tissues.

- Monmita Bhar (with input from authors)

Image: Shridhar Velhal



DREAM FOR PROTECTION OF AIRSPACE

Drones are now commonly used in defence, surveillance, and other activities. A foreign or unidentified drone could potentially breach the security of a protected place or facility. To safeguard such critical airspace and infrastructure, researchers resort to game theory – mathematical modelling of strategic interactions among a set of players, which includes intruders and defenders. A defender can only neutralise a single homogeneous intruder moving with constant speed in a convex territorial space. But in real life, intruders are more likely to be moving at varying speeds in more than one direction.

Suresh Sundaram and his group in the Department of Aerospace Engineering have now developed a novel approach called DREAM (Dynamic REsource Allocation with decentralised Multi-task assignment).

DREAM can tackle heterogeneous intruders, including sudden intruder pop-ups, and ensures that fewer defenders are utilised against a larger number of intruders.

DREAM is more efficient, simpler, scalable, has a superior success rate, and is better at using resources, compared to existing models. The authors plan to extend DREAM to 3D problems with constrained navigational space in hard-to-observe regions, and apply these ideas to an ongoing anti-drone project for protecting restricted airspace.

- Mohammed Asheruddin

Photo courtesy: S Balaji

TO SEE THE WORLD IN A GRAIN OF SAND

TEJAS G MURTHY'S LAB STUDIES THE MECHANICAL BEHAVIOUR OF GRANULAR MATERIALS USING COMPUTED TOMOGRAPHY

Matter is often classified into solids, liquids and gases. But if you think about sand, these lines begin to blur. Each particle of sand is a solid, but a collection of sand particles flows and takes the shape of the container it is poured into, like a liquid. Thus, the properties of a collection of particles can be very different from those of each constituent particle. Such properties that emerge only when the parts interact as a whole are called emergent phenomena. Granular materials such as sand, snow, or cement, which are a collection of solid particles (or grains) in close contact with each other, show a wide variety of such emergent phenomena.

"Granular materials are endlessly fascinating," says Tejas G Murthy, Associate Professor at the Department of Civil Engineering at IISc. Using computed tomography (CT), his team investigates the microscopic structure of granular materials and how the interactions between the individual grains lead to their overall complex behaviour. Widely used in chemical, metallurgical, pharmaceutical and food processing industries, granular materials have generated significant interest among researchers across disciplines. "After perhaps fluids, granular materials are the most commonly handled system in industries. For example, food grains, cement, and pharmaceuticals are all granular materials. So, there is an interest in this field from several different disciplines," explains Tejas.

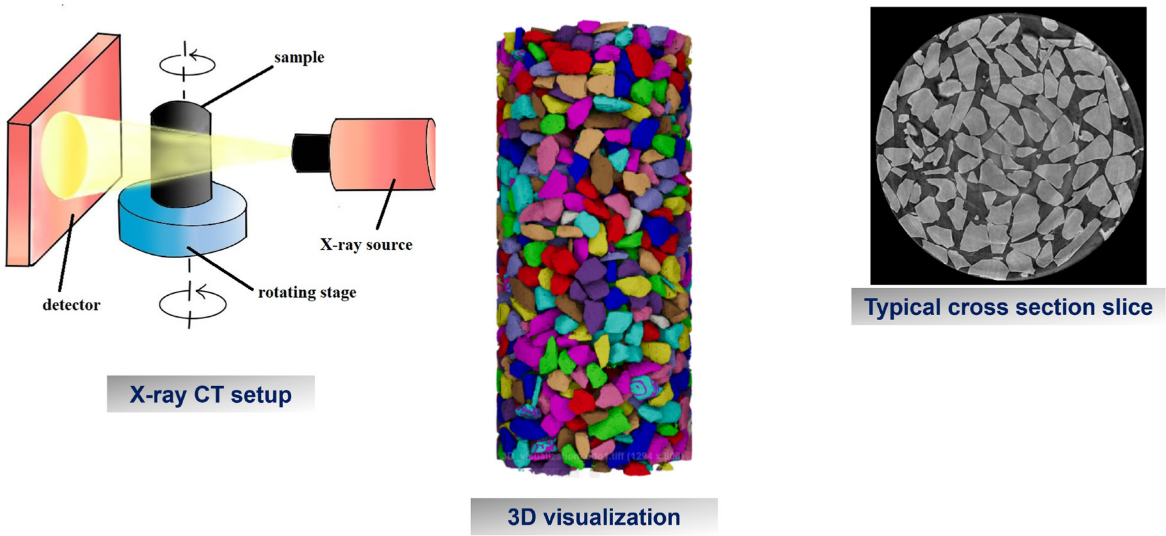
Tejas' interest in granular materials was kindled when he was a teaching assistant for a course taught by his eventual PhD advisor, Rodrigo Salgado, Professor at the School of Civil Engineering at Purdue University. In his early work, Tejas developed theoretical models to predict how the behaviour of a granular material emerges from the motion of the individual grains that constitute it. However, he needed experimental evidence to corroborate his theories. After getting tenured at IISc, he went on a sabbatical in 2017 as a Fulbright-Nehru fellow to the Georgia Institute of Technology. He spent a year there learning about CT techniques, which would later help his team gain valuable insights into the microstructural changes that take place in these materials under experimental conditions. Tejas also learnt about various image analysis techniques. "The most important part of tomography is the image analysis. It's very easy to collect a bunch of CT images. Analysing the images is the hard part," says Tejas.

In a recent collaboration with Vijay Natarajan, Professor at the Department of Computer Science and Automation, Tejas has developed a new segmentation algorithm to single out individual particles in the CT image of a granular material. When compared with conventional techniques, this algorithm has the ability to segment images of more complex geometries in a much shorter time, and provide better insight into the connectivity of the particles with each other.

The team now conducts experiments at the Cornell High Energy Synchrotron Source (CHESS) in Ithaca, New York, a high energy X-ray source used to obtain high-quality CT images. "These are massive datasets," he says, referring to the CT images from CHESS. This has allowed the team to move from studying simple systems – such as a collection of spherical steel balls of similar shape and size – to probing highly complex systems with particles of different sizes, shapes and connectivity, which are more representative of real-world granular materials. The CHESS data also includes diffraction data, which allows the team to map out the forces at the contact points between grains. In a recent paper, they have shown that a small amount of such inter-particle forces can drastically change how granular materials respond when a load or force acts on a collection of them.

Tejas' expertise in image analysis has led to an interesting offshoot – his

Microstructure characterisation of geomaterials using X-ray Computed Tomography (Image: Manasa Bhat)



collaboration with Hema Somanathan, Professor at IISER Thiruvananthapuram, on studying the web architecture of a species of social spider, *Stegodyphus sarasinorum*. Social spider webs support and catch prey for the entire colony, and it was previously thought that in larger groups of spiders, each spider would have to produce less silk compared to a smaller group. This was thought to be one of the advantages of social living among spiders. However, when Hema's team tracked the growth of *S. sarasinorum* webs of different populations using the image analysis techniques suggested by Tejas, they discovered that the per capita silk investment did not decrease in larger groups as expected. This suggests that other benefits of group living such as the ability to capture larger or more prey, protection from predators, better survival rates and access to mates, play a more important role in the spiders' tendency to socialise.

Spiders aren't the only "creepy-crawly" creatures Tejas has studied. He has also collaborated with Renee Borges, Professor at the Center for Ecological Sciences, to study how termites build their mounds. They found that termites use moisture to strengthen their mounds, and secrete special proteins to make them weather-resistant.

When asked if he ever envisioned himself investigating creatures like termites and spiders, he immediately responds, "No, absolutely not! I am afraid of anything creepy-crawly, but I have to admit that they are really fascinating." It is this fascination – the rise of emergent behaviour in both the living and non-living – that keeps Tejas going forward in his quest to visualise the world, one grain at a time.

- Rohith KMS

Tejas G Murthy and his lab members (Photo courtesy: Manasa Bhat)

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