Issue 8
July - September 2017

SINGAPORE

A*STAR Research

FAT ON THE INSIDE

Asia's hidden obesity epidemic

ORIGINS OF ZIKA
Unravelling the viral culprit

FACTORIES OF THE FUTURE
A revolution on the shop floor

FRESH BLOOD
A new purpose for skin cells

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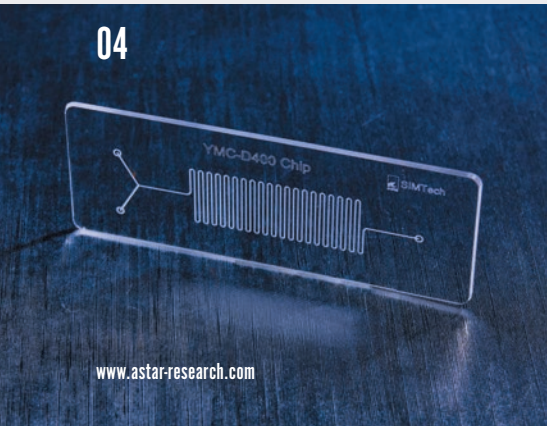

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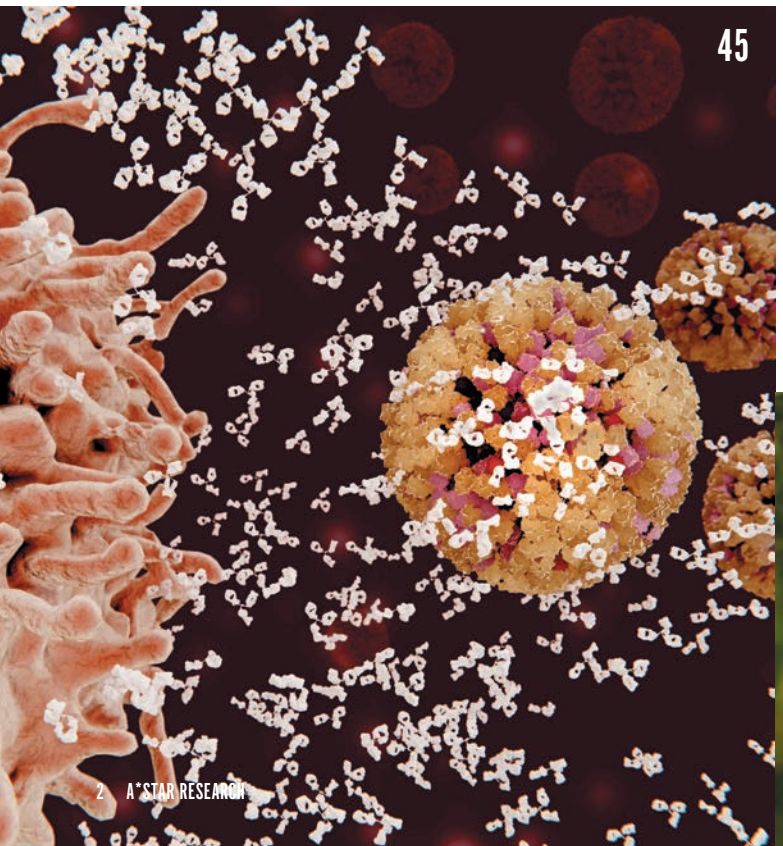
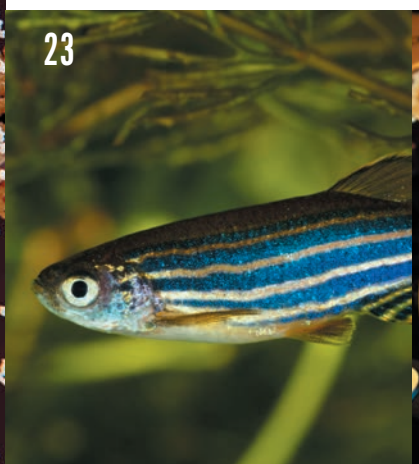
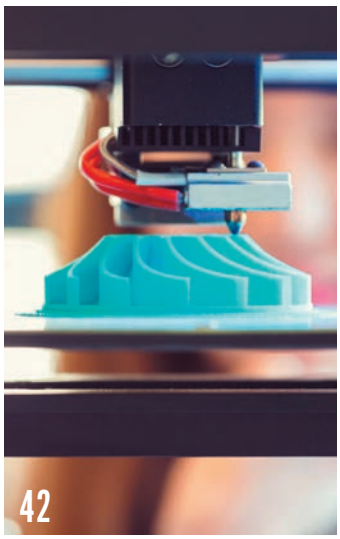






Notes from the editors

The Editorial Team introduces the latest issue of *A*STAR Research*

Welcome to the fourth edition of *A*STAR Research* for 2017, where we showcase some of the most exciting and ground-breaking research from A*STAR, as highlighted on our website from July to September 2017.

Obesity continues to be a major global health problem, and roughly a third of the world's current population is overweight or obese. In Asia, obesity often takes on a more insidious form — that of slender people with abundant visceral fat, with grave health consequences, as explained in our cover story on page 32. Another article on fats demonstrates a surprising use for lipids — where fatty acids in excess lung fluid are used to diagnose cancer (page 8). Progress has also been made in diagnosing stomach cancer, by mapping regions of the genome known as 'super-enhancers' (page 48).

We also present an update on Zika research, a year after the August 2016 outbreak in Singapore, in which researchers from all across Singapore worked together to trace the Asian origins of Zika's global spread (page 12). Another story on a mosquito-transmitted disease — the chikungunya virus — discusses a promising new treatment that utilizes a drug normally used for treating multiple sclerosis (page 49).

Another of our features explore the factories of the future, that look poised to usher in a new era of efficiency, precision and speed, thanks to additive manufacturing (page 42). Many other advances in manufacturing technology are also showcased in this issue, such as the development of a rapid and accurate electromechanical actuator (page 7), and a simple and versatile method for fabricating hybrid lasers (page 51).

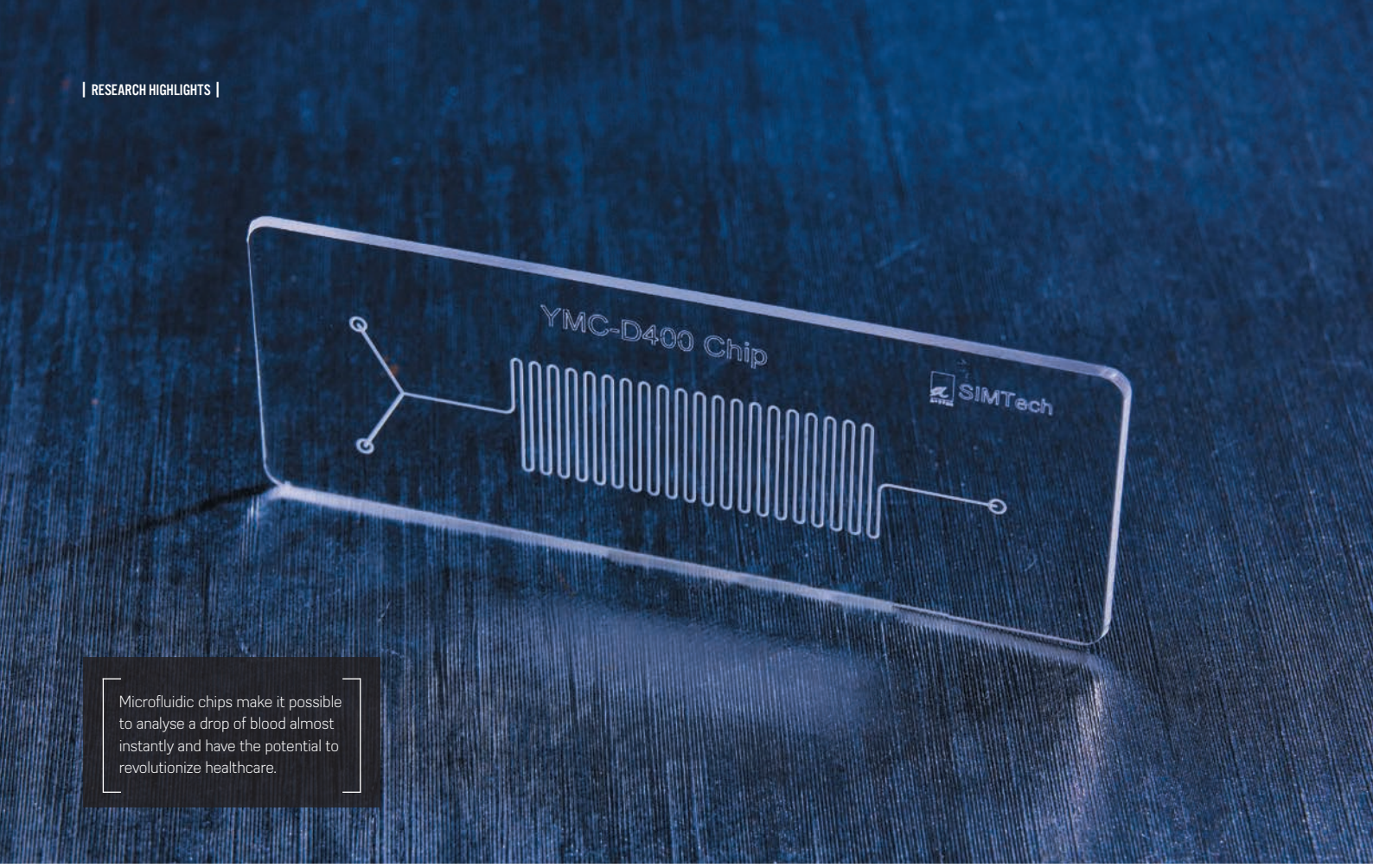
Interesting developments on the chemistry side of things are also highlighted — including fluorescent polymers that can be used to detect miniscule amounts of explosives (page 50), and new near-infrared dyes that may have valuable applications in medical imaging (page 31).

As usual, this is just a glimpse into the offerings of this issue, and we hope you will enjoy the rest of the magazine.

COVER IMAGE

The obesity epidemic might not look like you think it does ... [p.32]

Texture: © Rita Petersone-Lazdina/EyeEm/Getty



Microfluidic chips make it possible to analyse a drop of blood almost instantly and have the potential to revolutionize healthcare.

Microfluidics

BETTER BONDS, LESS BUBBLES

A COMPOSITE FILM IMPROVES THE BONDING OF PLASTICS IN HIGH-PRECISION MICROFLUIDIC CHIPS AS A CRITICAL STEP TOWARD MASS PRODUCTION.

Point-of-care medicine could be revolutionized by cheap, disposable card-sized plastic 'microfluidic chips' capable of diagnosing a range of conditions on the spot from a drop of blood. A simple bonding technique could help to make this a reality.

This technology has been under development for many years, but has been hampered by the difficult and expensive task of bonding the plastic parts of the chips in a way that preserves the integrity of the microchannels needed to achieve diagnostic functions.

Now, a bonding technique that overcomes many of the shortcomings of existing bonding methods has been developed by researchers from A*STAR's Singapore Institute of Manufacturing Technology (SIMTech) in collaboration with coworkers from Nanyang Technological University and Seoul National University of Science and Technology.

> "A KEY CHALLENGE IN THE COMMERCIALIZATION OF MICROFLUIDIC DEVICES IS LOWERING THE MANUFACTURING COST."

"A key challenge in the commercialization of microfluidic devices is lowering the manufacturing cost," explains Gary Sum Huan Ng from the SIMTech team. "The bonding process to seal the microchannels is often the bottleneck in microfluidics manufacturing."

Microfluidic devices are essentially comprised of microchannels etched into plastic, which route a sample of biofluid to reservoirs of fluids or substrates that are active toward an analyte that may be present in the sample. Many methods are used to bond the various parts of a microfluidic device together, but most are slow and painstaking, hindering mass production. Ultrasonic welding, which applies high-frequency vibrations to create a solid-state weld, is promising for scaling up production because it's fast, produces a strong bond, and uses compact automated equipment. However, the quality of bonds produced by ultrasonic welding is difficult to control due to excess melting and the entrapment of air bubbles.

The researchers have shown that sandwiching a composite film between the two

plastic parts to be bonded is very effective in preventing air bubbles and melt flow during ultrasonic welding. The key was to incorporate thermoplastic microspheres in the composite film to help confine and control melting.

"The thermoplastic microspheres, which are scattered within an elastomer matrix to form the composite film, act as micro energy directors that melt and collapse when the ultrasonic energy is applied to create the weld," says Ng. "The ultrasonic directors are restrained by the elastomeric matrix, which effectively prevents uncontrolled melt flow as well as the generation of trapped air bubbles. This method may overcome the critical bonding issue in the mass manufacturing of microfluidic devices." ■

1. Chana, W. X., Ng, S. H., Li, K. H. H., Park, W.-T., & Yoon, Y.-J. Micro-ultrasonic welding using thermoplastic-elastomeric composite film. *Journal of Materials Processing Technology* **236**, 183–188 (2016).

Health

ATTENDING TO DIABETES DURING PREGNANCY

CHILDREN BORN TO MOTHERS WITH DIABETES DURING PREGNANCY ARE AT RISK OF DEVELOPING ATTENTION PROBLEMS

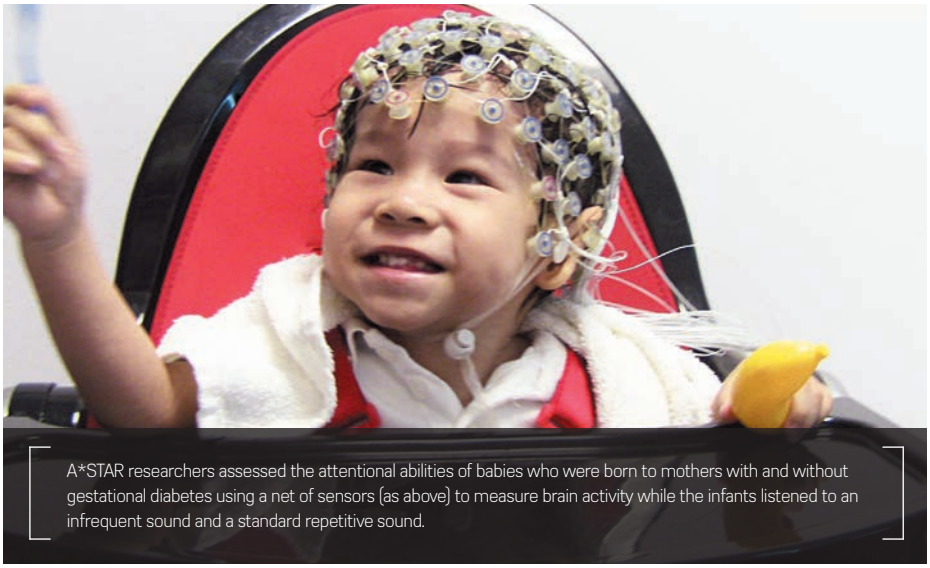
Babies born to mothers who develop diabetes during pregnancy — known as gestational diabetes (GDM) — are shown to have attentional deficiencies as early as 6–18 months of age, suggesting the need to reduce insulin resistance prior to pregnancy.

The study, conducted by Anne Rifkin-Graboi from the A*STAR Singapore Institute for Clinical Sciences (SICS) and Shirong Cai from the National University of Singapore, is part of a large-scale, ongoing collaborative study of mothers and children before and after birth, called 'Growing Up in Singapore Towards healthy Outcomes (GUSTO)'[1].

The researchers measured brain activity in infants listening to a 'standard' repetitive sound, which was expected to become familiar and therefore elicit less brain activity, interrupted by an infrequent sound. Responses differed between children born to mothers with and without GDM at 6 and 18 months of age. Notably, at 18 months, children born to mothers with GDM responded more to the 'standard' sound compared to those born to mothers without GDM.

Such responses have been linked to adverse developmental outcomes such as attention deficit hyperactivity disorder (ADHD). However, Rifkin-Graboi notes that "early presentation of such differences may enable higher-risk children to be identified earlier, to allow interventions to prevent or alleviate the development of attention-related problems."

The researchers also examined memory function using behavioral tasks and found no differences between children born to mothers with and without GDM. Their subtle findings



A*STAR researchers assessed the attentional abilities of babies who were born to mothers with and without gestational diabetes using a net of sensors (as above) to measure brain activity while the infants listened to an infrequent sound and a standard repetitive sound.

may be due to the sensitivity of techniques used to measure brain activity.

This research examined 473 children from the GUSTO study, the mothers of whom were screened and treated for GDM. "GDM was fairly well-controlled, as demonstrated by the comparable birth weights between GDM and control: GDM babies tend to be larger," says Cai.

"Our results suggest that the effect of well-controlled GDM on child neurodevelopment is subtle," says Cai. "This may encourage women with GDM to manage their condition to ensure better offspring outcomes."

The researchers are planing a follow-up study to determine whether children born to mothers with GDM develop attention-related problems later in life.

"If the association between GDM and attention deficiency persists, then pre-conception and early pregnancy prevention programs should be considered for women at risk for gestational diabetes, as should interventions for their offspring," says Rifkin-Graboi.

GUSTO is a collaboration between Singapore's National University Health System (NUHS), KK Women's and Children's Hospital (KKH), and SICS. This research is supported by the Singapore National Research Foundation (NRF) and administered by the Singapore Ministry of Health's National Medical Research Council (NMRC). ■

1. Cai, S., Qiu A., Broekman B. F. P., Wong E. Q., Gluckman P. D. *et al*. The influence of gestational diabetes on neurodevelopment of children in the first two years of life: a prospective study. *PLoS ONE* **11**, e0162113 (2016).

Photonics

TEXTURED LED GIVES GREEN LIGHT TO LI-FI

USING A GREEN LED AS A DETECTOR CAN HELP TO BOOST DATA TRANSMISSION RATES.

Standard light-emitting diodes (LEDs) used for home lighting can now transmit data more rapidly between electronic devices, thanks to new research from A*STAR.

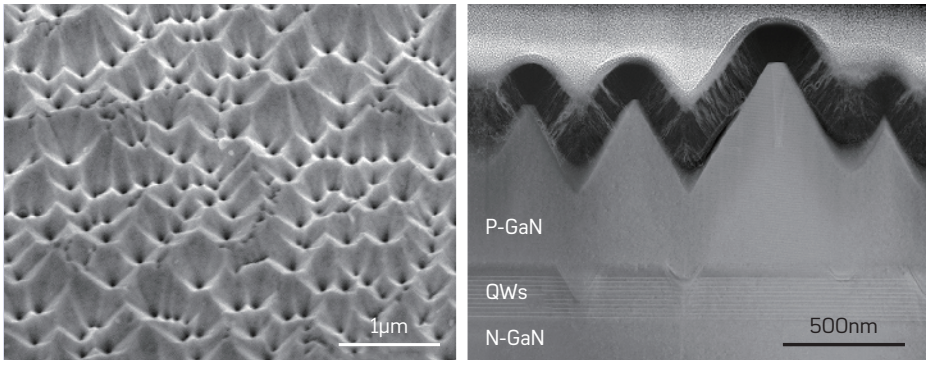
Wireless visible light communication — also known as Li-Fi — relies on data signals encoded in incredibly brief pulses of light, far too quick for the eye to see. By supplementing congested Wi-Fi networks, Li-Fi could increase the capacity and speed of data transmission in offices, homes and public spaces. However, white LEDs typically use a phosphor coating to create a natural-looking white light, and the time it takes for the phosphor's glow to fade away limits how quickly the LED can transmit data.

Previous solutions typically required installing new types of white LEDs. Instead, Ee Jin Teo of the A*STAR Institute of Materials Research and Engineering, and colleagues, have developed a Li-Fi receiver that overcomes these problems. Rather than using a conventional silicon photodiode to detect transmissions, they found that an indium gallium nitride (InGaN) LED is an effective data receiver[1].

Crucially, the team's InGaN LEDs can detect only the 'fast' blue component of the phosphor's white light, which fades in only one nanosecond, and not the 'slow' yellow component which takes more than 50 nanoseconds to fade away.

The researchers also gave their InGaN LED a textured surface, so that every square centimeter was covered with one billion V-shaped pits, roughly 150 nanometers deep. These V-pits scatter incoming light, allowing the LED's active layers to absorb more than twice as much blue light as an LED with a smooth surface.

Tests with a white LED showed that the InGaN LED with V-pits was a much better receiver than a standard silicon photodetector. "Using a silicon photodetector, the white LED can reach a switching speed of five megahertz — this typically means a data transmission rate of up to 100 megabits per second," says Teo. "With our InGaN LED as a detector, this switching speed can be increased by four times, enabling faster data transmission rates from white LEDs."

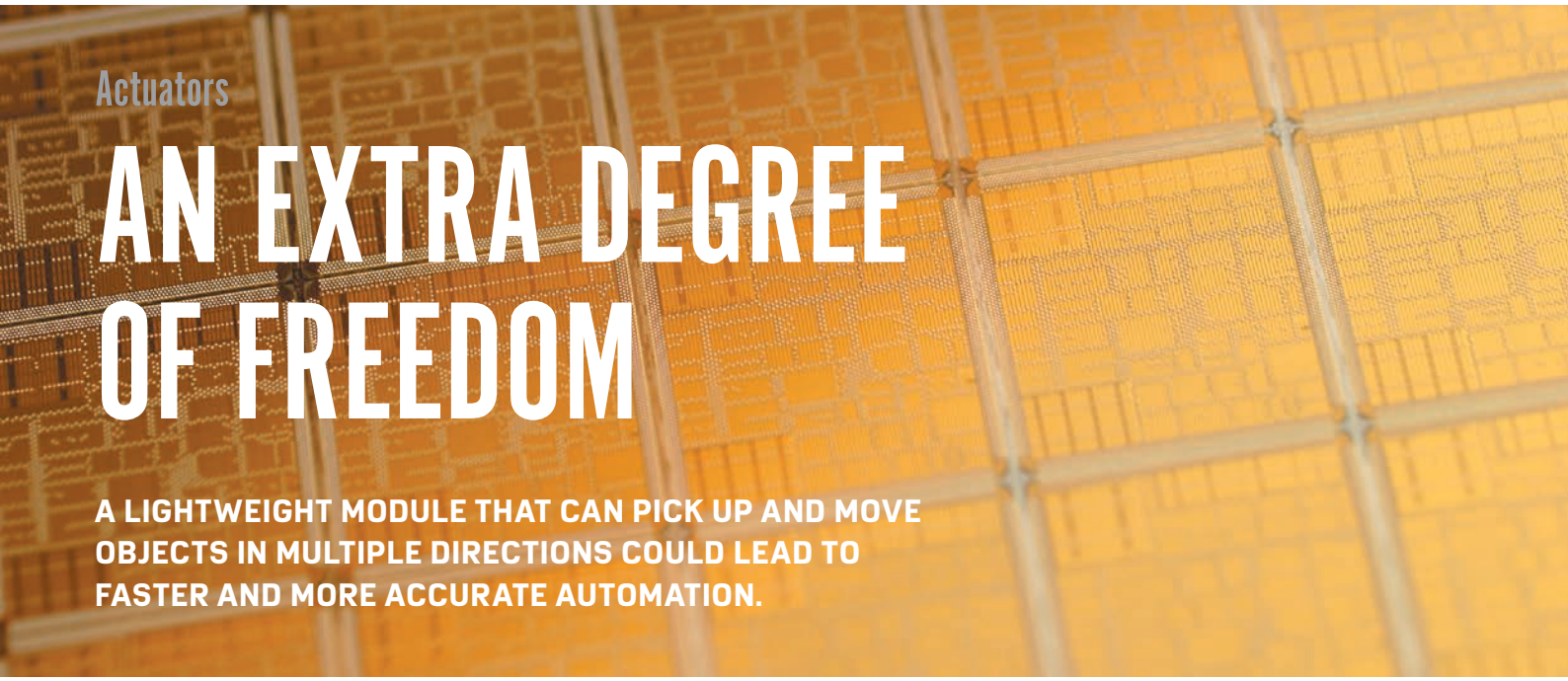


V-pits on the surface of an InGaN LED (left) scatter light into the device's active layers, known as quantum wells (QWs – right).

She notes, however, that since the receiver is only picking up part of the white LED's light, it may reduce the range over which data can be transmitted.

"The next stage of our research," she adds, "is to implement this concept into a dongle where the same LED can be used for transmission as well as detection of data." ■

1. Yang, C., Turaga, S. P., Bettiol, A. A., Balamuniappan, P., Bosman, M. *et al.* Textured V-pit green light emitting diode as a wavelength-selective photodetector for fast phosphor-based white light modulation. *ACS Photonics* **4,** 443–448 (2017).



Actuators

AN EXTRA DEGREE OF FREEDOM

A LIGHTWEIGHT MODULE THAT CAN PICK UP AND MOVE OBJECTS IN MULTIPLE DIRECTIONS COULD LEAD TO FASTER AND MORE ACCURATE AUTOMATION.

A module for rapid, accurate and versatile positioning of semiconductor chips has been developed by Singaporean researchers. It features a novel electromechanical actuator that can move objects both linearly and rotationally.

Actuators are transducers: they convert electrical energy into physical energy; mechanical motion or force. Electromagnetic motors can perform this task with a high-force output and highly accurate positioning — which makes them ideal for automated manufacturing systems in which objects must be picked up and moved quickly. But most actuators tend to move linearly in only one direction, or they offer a rotational motion.

"For surface-mount technology (SMT) assembly, it is important that an actuator can provide both linear and rotary motion concurrently so that assembly line can achieve high throughput and high accuracy ," says Daniel Tat Joo Teo and colleagues from the A*STAR Singapore Institute of Manufacturing Technology (A*STAR SIMTech) and National University of Singapore (SIMTech-NUS) Joint Lab.

> "HOW FAST AND HOW ACCURATE THE ACTUATOR CAN PERFORM THIS TASK WILL DETERMINE THE OVERALL THROUGHPUT AND ACCURACY OF THE AUTOMATED SYSTEM"

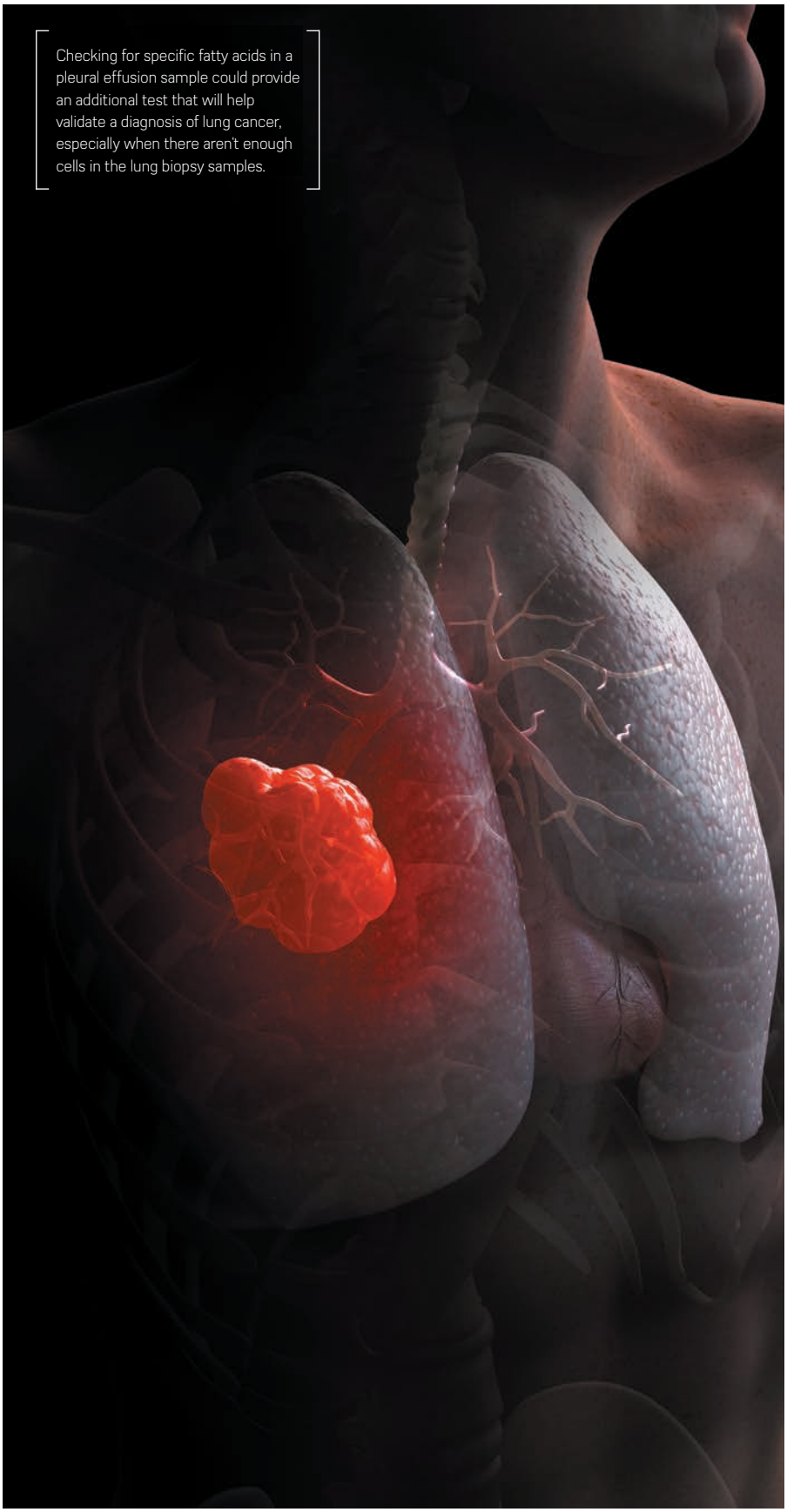
"For example, when picking up a chip, the actuator can rotate it to compensate the angular misalignment based on the feedback from a camera before placing it on to the lead frame," says Teo. "How fast and how accurate the actuator can perform this task will determine the overall throughput and accuracy of the automated system."

Teo and co-workers designed, modeled and developed a novel type of actuator that delivers decoupled linear and rotary motions, which is both light and accurate. Their device comprised separate translational and rotary modules and included a cylindrical Halbach magnet array. This formed a closed-loop magnetic circuit that concentrated the magnetic field within an air-core coil rotator and reduced magnetic field leakage. These features made the actuator lighter and permitted a high-speed and dynamic response. Similarly, the translational module was made of two permanent magnets facing each other, which also focused the magnetic field on the active moving coil region.

The team built a prototype of their actuator design and demonstrated a linear movement range of ten millimeters and a rotational displacement of up to 90 degrees. The device could achieve a high throughput of 9000 unit-per-hour pick-and-place tasks with a linear and rotational accuracy of 20 micrometers and 0.66 degrees respectively.

The researchers propose that their actuator could be used in the semiconductor industry for SMT assembly and sorting silicon wafers. "We hope to make the design even more compact by reducing the number of components and the size the electromagnetic modules," says Teo. ■

1. Teo, T. J., Zhu, H., Chen, S.-L., Yang, G. & Pang, C. K. Principle and modeling of a novel moving coil linear-rotary electromagnetic actuator. *IEEE Transactions on Industrial Electronics* **63,** 6930–6940 (2016).



Checking for specific fatty acids in a pleural effusion sample could provide an additional test that will help validate a diagnosis of lung cancer, especially when there aren't enough cells in the lung biopsy samples.

Lung cancer

THE FATS THAT IDENTIFY CANCER

EXCESS FLUID SURROUNDING THE LUNGS IN SOME LUNG CANCER PATIENTS HAS A UNIQUE LIPID PROFILE, WHICH COULD HELP DIAGNOSE AND TREAT THE DISEASE.

The lipid contents of a fluid that surrounds the lungs in some diseases contains specific fats that could be used as a biomarker to distinguish people with and without lung cancer. It can also identify a subtype of the cancer that needs to be treated with drugs that are different from those used in other types of the disease.

Lung cancer is the leading cause of cancer-related deaths worldwide. Its predominant form, called non-small cell lung cancer (NSCLC), represents more than 85 per cent of cases and is usually diagnosed by examining cells from a lung biopsy. But sometimes biopsies don't contain enough cells for diagnosis. "In the event of insufficient cancer specimens, a false-negative finding might indicate the absence of cancer which would be incorrect," explains Ying Swan Ho of A*STAR's Bioprocessing Technology Institute. "This could result in a lung cancer patient walking out of the hospital without receiving the appropriate treatment."

Ho and a team of colleagues in Singapore examined the contents of 'pleural effusion' removed from 30 people who did not have lung cancer and 41 people who did. Pleural effusion accumulates around the lungs in up to 30 per cent of people with lung cancer. It also can be found in people with tuberculosis, pneumonia and other lung conditions.

The team found two polyunsaturated fatty acids in pleural effusion that were each highly sensitive and specific for NSCLC. Looking for either fatty acids in a pleural effusion sample distinguished between people with and without the disease.

There were also differences in the levels of seven lipids found in the pleural effusions of NSCLC cases who had a mutation in a gene called *EGFR* compared to cases without the mutation. Lung cancer patients with *EGFR* mutations respond better to a specific targeted anti-cancer treatment than they do to standard chemotherapies. Identifying them early could help provide them with more effective treatment.

"Upon further validation, we think that quantifying the levels of lipids can potentially complement traditional approaches in cancer diagnosis, particularly when cancer cells and tissues are limited or unavailable," says Ho.

Next the team plans to examine a larger number of people to validate their study's results and also to determine the levels of lipids, when found, that can accurately detect the disease and its subtype. They are also currently investigating the roles of lipids in the development of lung cancer. ■

1. Ho, Y. S., Yip, L. Y., Basri, N., Chong, V. S. H., Teo, C. C. *et al.* Lipidomic profiling of lung pleural effusion identifies unique metabotype for EGFR mutants in non-small cell lung cancer. *Scientific Reports* **6**, 35110 (2016).

Materials

HARNESSING THE PROPERTIES OF A REMARKABLE 2D MATERIAL

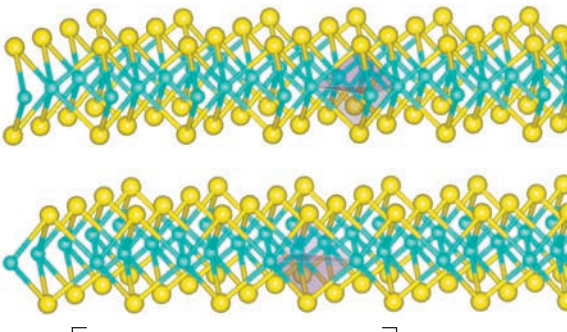
DETERMINING THE THERMAL PROPERTIES OF A VERSATILE MATERIAL COULD LEAD TO NEW APPLICATIONS IN ENERGY STORAGE, OPTOELECTRONIC AND FLEXIBLE ELECTRONIC DEVICES.

Characterizing the thermal properties of crystalline molybdenum disulfide, an important two-dimensional (2D) material, has proven challenging. Now researchers from A*STAR have developed a simple technique that could pave the way for its use in a wide range of new applications in energy storage, optoelectronic and flexible electronic devices.[1]

Hexagonal molybdenum disulfide (MoS_2), one of the dichalcogenides — a family of semiconducting transitional metals — has attracted considerable attention as a two-dimensional (2D) material thanks to its remarkable electronic and optoelectronic properties. It is also notable for its impressive strength and flexibility, which arise from the hexagonal lattice of molybdenum atoms sandwiched between layers of sulfur atoms.

Determining the thermal characteristics of MoS_2 is key to unlocking its astonishing properties, but its complex geometry and the many required calculations for phonons — the different vibrational modes of atoms in a crystal lattice — are a costly and time-consuming computational process.

Chee Kwan Gan and Yu Yang Fredrik Liu from the A*STAR Institute of High Performance Computing have now developed a numerical technique that dramatically reduces the number of calculations, allowing the thermal expansion coefficient — which determine how their shape and size change in response to changes in temperature — of MoS_2 crystals to be accurately and efficiently calculated, and could also be applied to other important 2D materials.



The structure of the MoS_2 crystal.

"Think of a phonon as a particle tied to a spring, where it vibrates with a fixed pattern at a fixed frequency," explains Gan. "There are many phonon modes in a crystal like molybdenum disulfide, and the challenge is to calculate all of them."

By deforming a crystal of MoS_2, the researchers determined the change in frequency for each phonon in the lattice structure, and by applying a numerical method, based on perturbation theory, to these altered frequencies; they were able to estimate the crystal's thermal characteristics, known as the Grüneisen parameters. These parameters were then used to calculate the thermal expansion coefficients for hexagonal MoS_2.

"Our method uses the full symmetry of the hexagonal structure to reduce the amount of computation to only four sets of phonon calculations compared with quasi-harmonic approximation — the traditional approach — that requires many more," says Gan.

The work presents, for the first time, an accurate and simple method for determining the thermal properties of MoS_2, and provides a deeper understanding of thermal conduction in 2D materials.

"Our long-term aim is to extend the approach to other technologically important semiconducting, two-dimensional materials, such as bismuth selenide," says Gan. ■

1. Gan, C. K. & Liu, Y. Y. F. Direct calculation of the linear thermal expansion coefficients of MoS_2 via symmetry-preserving deformations. *Physical Review B* **94**, 134303 (2016).

Biomaterials

DISSOLVEABLE PINS AND SCREWS FOR BROKEN BONES

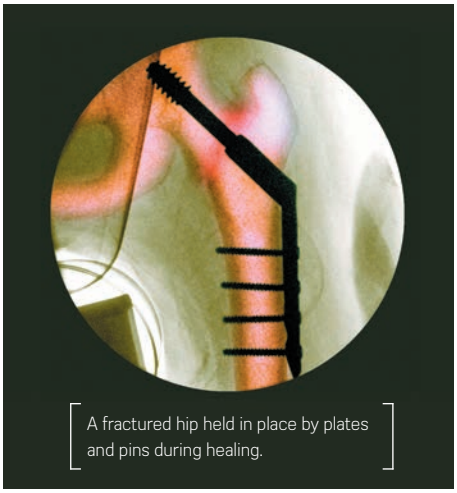
A VERSATILE COATING FOR MAGNESIUM COULD EVENTUALLY LEAD TO BETTER IMPLANTS FOR MENDING BROKEN BONES.

Broken bones may be repaired more effectively by using a biodegradable coating for magnesium-based metal implants.

Some bone breaks require a temporary metal implant for support while they heal. "However current implants, such as screws and braces made of titanium, steel or cobalt-chromium alloys, have serious drawbacks," explains Sandor Nemeth of the A*STAR team involved in developing the innovative coating. Firstly, the difference in mechanical properties between the metals and the healing bone can loosen the implants and damage the bone. In addition, the temporary implants must eventually be surgically removed, resulting in further risk and inconvenience.

The A*STAR team is developing a biodegradable implant that is a better mechanical match with bone, and might also can directly contribute to the healing process.

Magnesium is a strong and flexible metal, but its reactivity means it cannot be used alone because it would corrode too quickly inside the body. The tendency to corrode, however, could become an advantage as the rate of degradation could be controlled. The trick is to find a coating that degrades slowly enough to support the bones during healing, then safely disperses when the job is done.



A fractured hip held in place by plates and pins during healing.

Nemeth and his PhD student, Sara Kaabi, at the A*STAR Singapore Institute of Manufacturing Technology, collaborated with Ming Jen Tan of Nanyang Technological University to explore potential coatings. "We turned to calcium phosphate because in nature this inorganic material is a key component of bone and other strong materials such as sea shells," says Nemeth.

Incorporating carbon-based organic polymers produced a composite coating with several key advantages: varying the mix of components allows the mechanical and biodegradable properties to be adjusted for different requirements and avoids the brittleness of purely inorganic coatings. Studies with cultured cells suggest the calcium phosphate might also actively assist the healing process by providing some raw materials to sustain bone growth. The magnesium is coated from a solution of pressurized hot water

in a one-step process, offering a simplicity that may be crucial for commercial-scale production.

"Animal trials are an obvious next step, but would require an industry partner," says Nemeth.

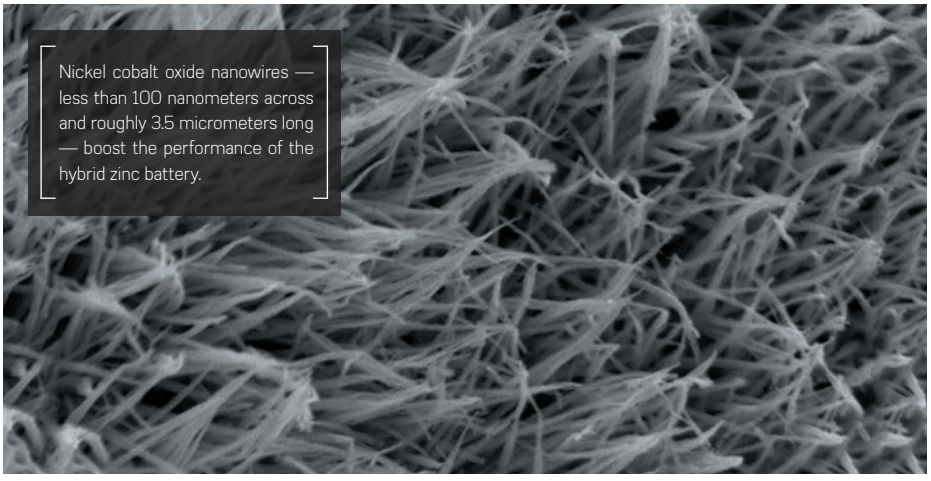
The researchers are also exploring options to make the coating even more useful by incorporating antibiotics and other drugs that could permeate the surrounding tissues and bone and contribute to healing. ■

1. Asl, S. K. F., Nemeth, S. & Tan, M. J. Novel biodegradable calcium phosphate/polymer composite coating with adjustable mechanical properties formed by hydrothermal process for corrosion protection of magnesium substrate. *Journal of Biomedical Materials Research B: Applied Biomaterials* **104**, 1643–1657 (2016).

Energy

HYBRID ZINC BATTERY BEATS ITS RIVALS

A NEW LOW-COST BATTERY OFFERS A HEFTY VOLTAGE AND SUSTAINED ENERGY CAPACITY.



Nickel cobalt oxide nanowires — less than 100 nanometers across and roughly 3.5 micrometers long — boost the performance of the hybrid zinc battery.

A zinc-based battery that delivers a high voltage and substantial energy capacity could be set to rival conventional lithium-ion batteries, A*STAR researchers have found[1].

The proliferation of electric vehicles and renewable energy sources is driving demand for rechargeable batteries that store and deliver large amounts of energy safely, efficiently and inexpensively. Zinc-based batteries offer some key advantages over lithium-ion, including low-cost and non-flammability. Kilo for kilo, zinc-air batteries can potentially store five times more energy than lithium-ion. Zinc-nickel batteries produce relatively high voltages which is potentially useful because fewer batteries would be needed to power a device. Yet zinc batteries also tend to lose their energy storage capacity after just a few hundred recharging cycles, and no zinc battery has yet combined both a decent voltage of more than 1.5 volts and a high energy storage capacity.

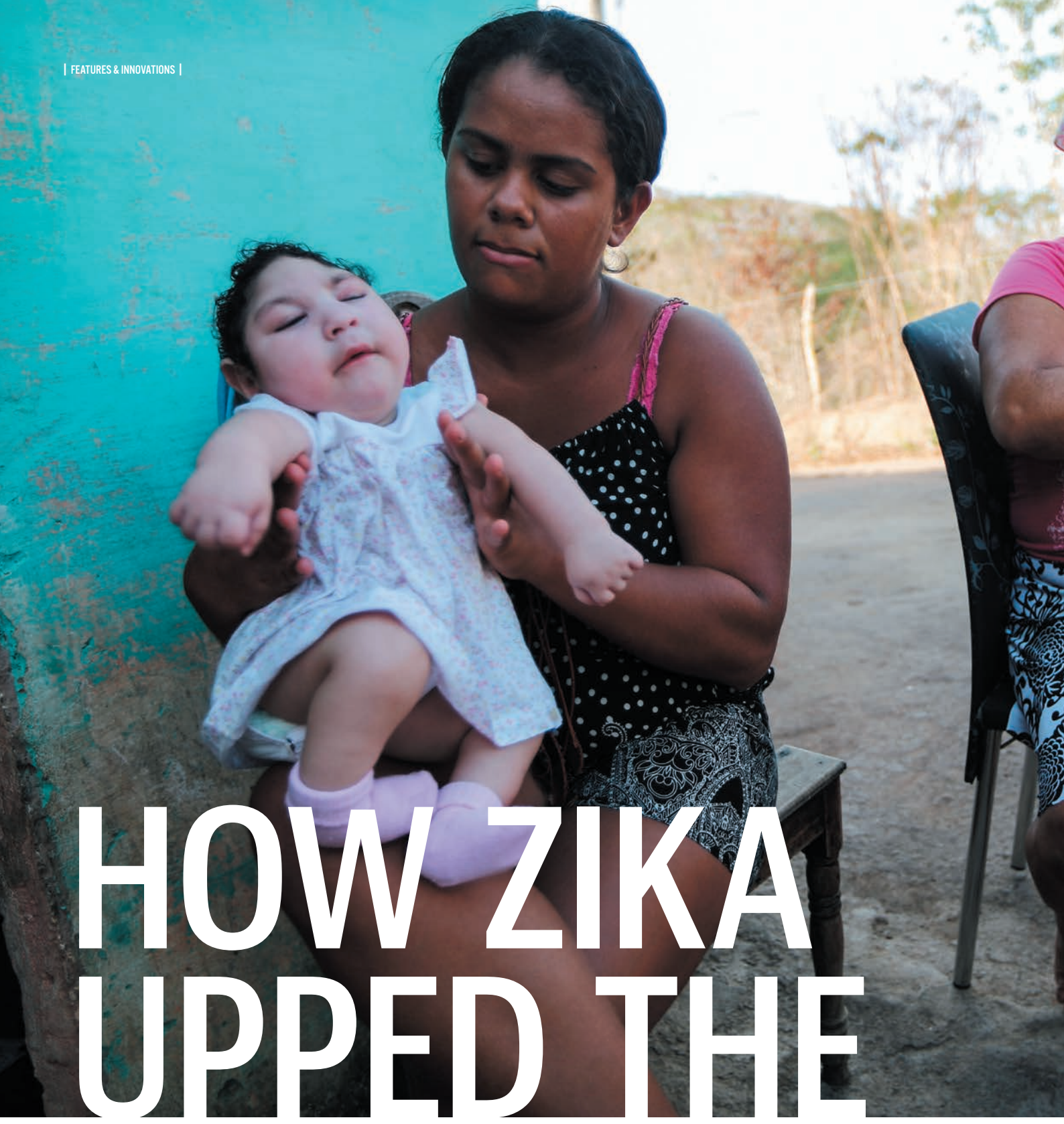
Yun Zong and Zhaolin Liu of the A*STAR Institute of Materials Research and Engineering and colleagues have now developed a hybrid zinc battery that combines the best of zinc-air and zinc-nickel technologies, completing over 5,000 charging cycles with no loss of performance. The battery has a zinc anode, while its cathode is based on a carbon-coated nickel foam covered with nickel cobalt oxide nanowires. The liquid electrolyte between the electrodes contains hydroxide anions dissolved in water.

A key reason for the battery's excellent performance is that the cathode works in two distinct ways during charging and discharging. When the battery charges, hydroxide ions from the electrolyte react with metal oxides in the cathode to produce oxyhydroxide compounds, freeing electrons. But the metals in the cathode also act as a catalyst, combining hydroxide anions to produce oxygen, water, and more electrons. These electrons flow around the circuit to the anode, where they combine with zinc ions in the electrolyte to produce zinc metal. During discharge, these electrochemical processes are reversed.

The battery has a stable two-step discharge voltage between 1.75 and 1.0 volts, and maintained its performance over three months of continuous testing, vastly outstripping previous zinc batteries. Zong estimates that the battery can store about 270 Watt-hours per kilogram, with potential for improvement. "This is already on a par with lithium-ion batteries available on the market," he says.

The two chemical processes at the cathode produce different voltages, which could be an advantage for applications that initially require a higher voltage, such as unmanned aerial vehicles that need an energy boost to get airborne and then a lower voltage to sustain their flight. The team now hopes to improve the battery's cycle life, perhaps by using a porous zinc anode, and to increase the capacity of the zinc-nickel portion of the battery. ■

1. Li, B., Quan, J., Loh, A., Chai, J., Chen, Y. *et al.* A robust hybrid Zn-battery with ultralong cycle life. *Nano Letters* **17**, 156–163 (2017).



HOW ZIKA UPPED THE ANTE

Singaporean researchers are tracing the Asian origins of Zika's global spread.

Singapore's Zika strain may be a clue to why more than 4,000 Brazilian babies were born with microcephaly, while there have been less than a handful in Asia.

Genomic detectives have traced the most recent outbreak of Zika — a mosquito-borne virus that became a pandemic linked to neurological defects — back to a strain in South-east Asia.

A Zika strain was first identified in South-east Asia in the 1960s, and it is thought to have maintained a low-key presence across the region since. Two mosquito species, *Aedes aegypti* and *Aedes albopictus*, are the virus' principal carriers and can be found across a wide band straddling the equator, extending patchily beyond the subtropics. A member of the Flavivirus genus, which also contains the dengue and West Nile viruses, Zika usually causes no or mild symptoms — a fever, rash, and aching joints.

Given its localized history, scientists are still baffled as to why a Zika strain suddenly headed east to French Polynesia in 2013, causing a suspected 32,000 cases within a year. From there, it moved on to the Caribbean and Brazil, where it exploded. Most worryingly, between late 2015 and early 2016, more than 4,000 Brazilian infants were born with abnormally small heads; a characteristic of microcephaly, stunted growth of sections of the fetal brain. Brazil is previously thought to have had roughly 1,000 cases of microcephaly annually. In February 2016, the World Health Organization (WHO) declared neurological complications linked to Zika a public health emergency. To date there are thought to have been between three and four million cases of Zika across 84 countries.

Part of the global scientific response was the consolidation of a cross-disciplinary group of A*STAR researchers, already connected after working together on SARS and later swine flu. They were uniquely positioned to study the first known Zika outbreak in Singapore in August 2016, identifying it as separate from the strain that had reached South America — which some consider an important clue about the evolution of the virus in Asia. Some of these insights might help explain why the pandemic suddenly spread east from Asia.

THE SINGAPORE OUTBREAK

The city-state's researchers, agencies and clinicians were already well-prepared when the Singaporean outbreak started in August 2016. Identified first in the Kallang-Aljunied neighbourhood, a total of 455 cases were reported across three months and were centered around 15 major clusters. Through enhanced surveillance coupled with intensive vector control, Singapore's Ministry of Health (MOH) and

National Environment Agency (NEA) quickly identified and managed infected people, and eradicated mosquitoes and removed breeding sites. New cases were reduced by 48 per cent within a month.

Zika patients were initially isolated in the Communicable Disease Centre at the multidisciplinary Tan Tock Seng Hospital, one of the city's largest. Staff there had a chance to trial a new diagnostic test they had been developing with Masafumi Inoue, a molecular pathologist at the A*STAR Experimental Therapeutics Centre (ETC), along with colleagues at the Environmental Health Institute (EHI) and the Bioinformatics Institute (BII) also at A*STAR. Inoue reports that it worked exceedingly well on real Zika infections. "Clinical results showed the test is between six and 32 times more sensitive to Zika and dengue than the hospital's old dengue antibody assay test," he says.

The test itself uses a blood sample, from which ribonucleic acid (RNA) is extracted and copied through a technique called polymerase chain reaction (PCR), improving analysis. It harnesses common hospital machinery, yielding results in three to four hours, and tests for all four key strains of dengue and all lineages of Zika — important, as approximately 12,000 cases of dengue were also reported to have been circulating for the first nine months of 2016. Inoue now hopes to submit the test for use internationally.

Crucially, within a week of Singapore's first localized case of Zika, the National Public Health Laboratory (NPHL) and A*STAR's Bioinformatics Institute (BII) had looked at the whole genome of the virus and confirmed that this wasn't the Brazilian Zika doubling back to Asia via international travel. "To our surprise, the Zika strain causing the local outbreak was derived from a local version that has been circulating in South-east Asia since the 1960s," says BII virus bioinformatician, Sebastian Maurer-Stroh.

Zika strains identified prior to 2013 have not been linked to severe neurological complications.

WHICH STRAIN OF ASIAN ZIKA CAUSED THE ZIKA OUTBREAK?

There have only been a few confirmed cases of microcephaly in Asia — in Thailand and Vietnam. The initial BII analysis suggested that Singapore's new Zika strain diverged in early 2010 from the strain that spread to Brazil. Comparing emergent South-east Asian or Pacific strains like this one, says Maurer-Stroh, could be key to understanding why the microcephaly pathology was a huge problem in Brazil and not in Asia.

Researchers from A*STAR's Singapore Immunology Network (SIgN), Lisa F.P. Ng and Florent Ginhoux, have recently helped link the Polynesian strain, which sits between the Asian and the Brazilian in strain lineage, to brain inflammation in fetal brain cells. But, they question why microcephaly hasn't been observed in other parts of Asia. "We don't know why there's microcephaly in Brazil and not in other places in the world where the virus is still circulating like parts of the Pacific or even Singapore," Ng says. The link between Zika and microcephaly is still unclear, she adds, but studies at SIgN have observed a link to immunodeficiency in mice. One theory is that parts of the Americas population could have been immunodeficient or genetically predisposed to microcephaly. To Ng, two of the key questions that researchers should be asking are whether host genetics are at play, and whether the population in Brazil might have been pre-exposed to other risk factors.

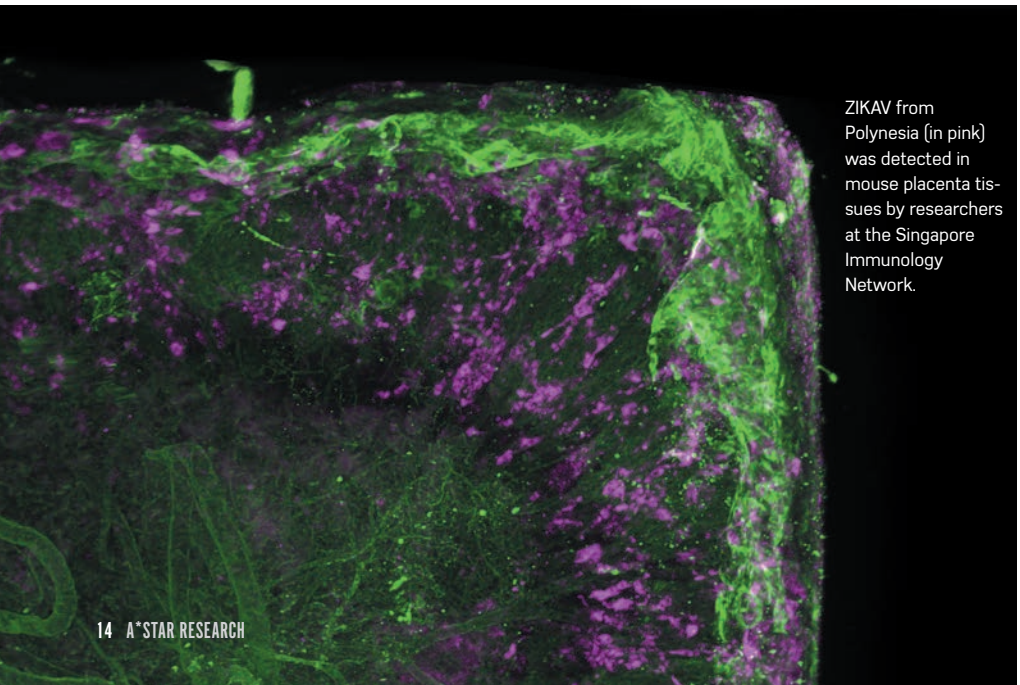
Maurer-Stroh has another hypothesis. Microcephaly, he says, could be the result of an unidentified genetic mutation in the virus that developed somewhere between South-East Asia and Brazil. "That it's been under the radar in Asia for so long would indicate that the change happened after the first big outbreak," says Maurer-Stroh.

He's working with Bruno Reversade, a molecular biologist at the A*STAR Institute of Medical Biology and the NEA, comparing Asian strains and the Brazilian strain in search of important changes in toxicity and pathogenicity. Maurer-Stroh says it's a work in progress, but they are starting to see differences.

One of the probable evolutions could have been in virus concentration levels. Indeed, in April, an international group of scientists including Singaporean researchers Julien Pompon, Menchie Manuel, Jun Hao Tan and October Sessions, at the Duke-NUS Signature Research Program noted a key difference between the South American and the Asian strains. By feeding mosquitoes Zika-infected blood, the consortium found that the Americas strain of Zika is more effectively transmitted than the Polynesian strain by *Aedes aegypti*— showing in the mosquitoes' saliva faster and at significantly higher concentrations.

DEVELOPING A SEARCHABLE 'ZIKASURVER'

As this strain information becomes clearer Maurer-Stroh hopes to amass it in a computer-based server through which users could



ZIKAV from Polynesia (in pink) was detected in mouse placenta tissues by researchers at the Singapore Immunology Network.

An apartment block being fumigated during a outbreak of Zika in Singapore in 2016.

"Clinical results showed the test is between 6 and 32 times more sensitive to Zika and dengue"

search for different Zika strains — similar to the BII's FluSurver, which is a functioning part of WHO's virus surveillance network. The 'ZikaSurver' would help governments determine the risk associated with the strain, and perhaps eventually help inform individual prognoses and treatments.

Releasing genomic information particularly in Asia-Pacific will be the key, says Maurer-Stroh. "The fact that we found this intermediate strain in Singapore … fast filled a gap in what we know about the global spread and evolution of the virus. But, globally I haven't seen the same release of information, especially in other south-east Asian countries that have the same virus," he says, and suggests that trepidation from tourism and business bodies might play a role.

A*STAR is trying to lead by example. BII released an early analysis of Zika genomes to WHO in March 2016, and after the recent outbreak, Singapore was again quick to share. "Because no genomes from this recent regional strain were known," says Maurer-Stroh, "NPHL immediately shared the genome sequence with WHO and published the initial results within three weeks of the first detected case while the outbreak was ongoing". A*STAR's Genome Institute of Singapore (GIS) also sequenced another 100 Zika genomes from the Singapore outbreak using a novel enrichment technology developed by Duke-NUS and GIS affiliate, October Sessions. Ng agrees that the limited sampling in Asia has hindered efforts to identify the source of the recent outbreak, but she's hopeful.

THE BIG PROBLEM WITH FLAVIVIRUSES

Another obstacle is that there is still no specific and licensed treatment for Zika. Ng's theory is that there are still multiple strains of Zika in circulation with "wide antigenic diversity and immunity". She says there is no generic therapy, so many approaches of "vaccines, antiviral medicines, antibody treatment" should be made available, but must be complementary.

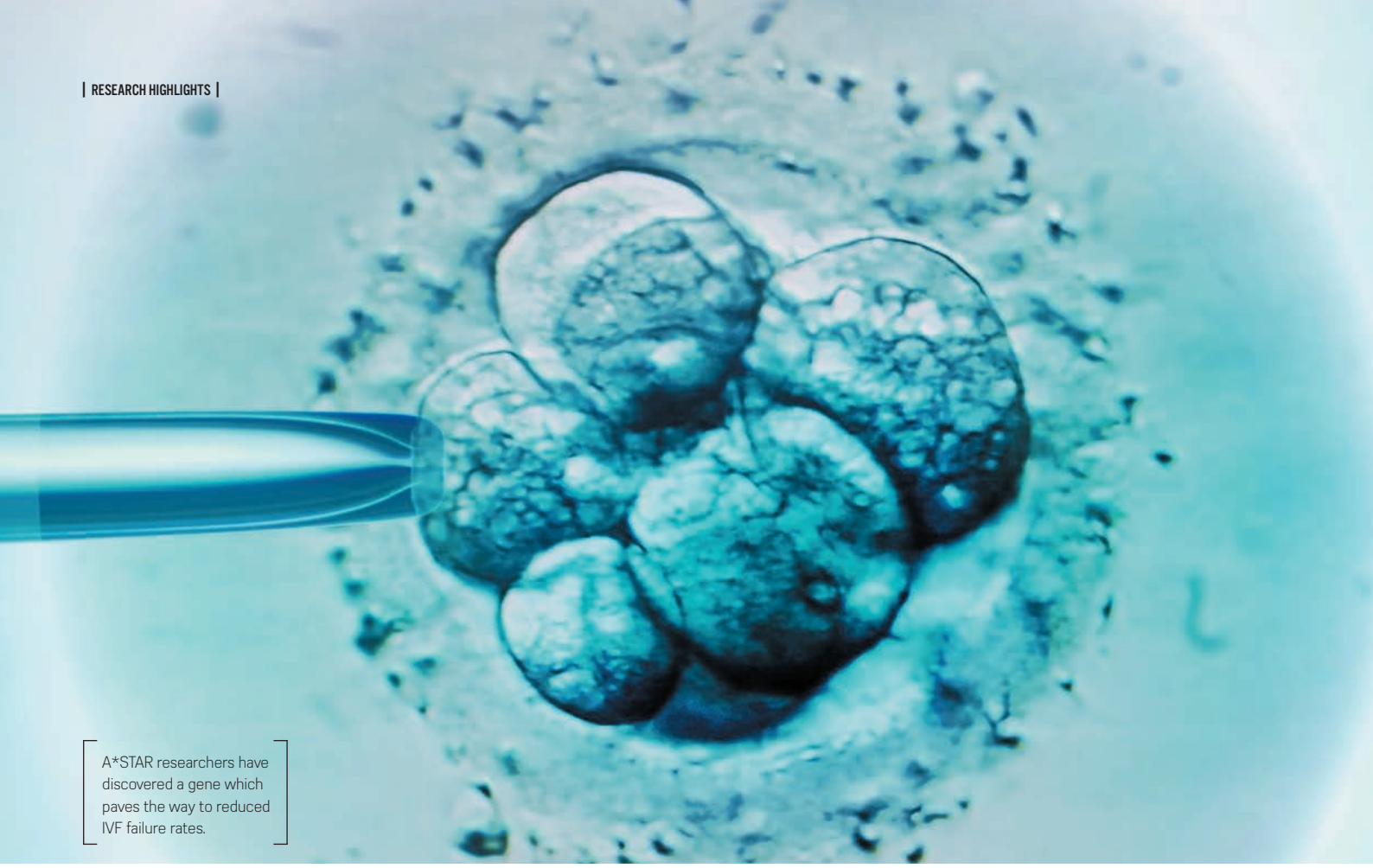
Recently, a team led by Laurent Rénia, the executive director of SIgN, has been working on using the antibodies of fellow flavivirus dengue to treat Zika. Flaviviruses are all largely mosquito-borne — and include tropical diseases like Ross River Fever and Yellow Fever. Zika is so similar to dengue that this is yielding results says Rénia.

The team at SIgN has identified a particularly promising Zika-neutralizing antibody and shown that it has an effect even on mice with immune deficiencies. This is especially significant in light of the link SIgN has observed between immune deficiency and microcephaly. Two mutations of this dengue antibody he notes also lowered Zika viral loads in fetal organs and placentas in pregnant mice, and were able to stop related fetal growth problems. "Of course, these findings provide the basis for any partner with pre-clinical vaccine candidates to screen for drugs," says Rénia. Once pre-clinical trials are done, these antibodies could be developed into treatments.

The similarity of Zika to dengue is also a concern. Dengue has been particularly resistant to vaccine development for many decades because of a unique phenomenon called 'antibody dependant enhancement' or ADE. Because dengue strains are similar but not identical, antibodies against one strain can bind to other strains, but imperfectly. As a result, when a person contracts two different strains of dengue over the years, the antibodies he has against first strain may enhance the new infection instead of fighting it, leading to more severe illness. The worry is that Zika will do the same. Ng says this underlines the need to develop a wide a range of treatment and prevention options.

For now, the global impact of Zika appears to be waning. Maurer-Stroh notes this is probably due in part to the protective effect of prior infections, but also because of the effectiveness of targeted responses such as that in Singapore. Nonetheless, Zika is still being found in countries with the vector mosquito species, and research on treatments, detection and genomic tracing will continue. ■

For references, visit the online version of this article at:
www.research.a-star.edu.sg/feature-and-innovation/7740/how-zika-upped-the-ante



A*STAR researchers have discovered a gene which paves the way to reduced IVF failure rates.

Epigenetics

LOCKDOWN GENES TO HELP REDUCE IVF FAILURE RATES

A HUNT FOR THE SPECIAL GENES THAT DEFY THE TREND IN EMBRYOS COULD HELP BOOST FERTILITY TREATMENT SUCCESS.

Embryos kickstart a vibrant genetic program to thrive, but if the wrong genes are active the cells can self-destruct. A*STAR scientists have discovered one of the genes that needs to be tightly locked down for an embryo to develop; a finding that could improve IVF success rates.

Human egg and sperm cells have their genes trained on a single purpose — to fertilize. Once their mission is complete, the developing embryo begins the complicated genetic program that turns a single cell into a healthy fetus.

This program is possible thanks in part to epigenetic changes to the DNA, such as the removal of methyl group 'locks' by enzymes, which allows many more genes to be read.

Some specialized genes however need to be locked down during development, as their genetic messages cause problems for the embryo.

"Everything that goes wrong in embryos has the potential to cause infertility or early pregnancy abortions," explains Daniel Messerschmidt from the A*STAR Institute of Molecular and Cell Biology. "We are keen to discover the genomic locations which impact on that development."

Messerschmidt's team previously discovered that a protein called Trim28 locks methyl groups to certain regions in the genome. Now, the researchers looked for the targets of Trim28 to find what genes lies within these regions.

The scientists sequenced the RNA of more than 30 embryos lacking Trim28 and discovered that a gene called *Rbmy1a1* was unusually active.

"It's an interesting gene which is not expressed anywhere in the body during development except for spermatogonia in the testes – it has no place to be expressed in the embryo," says Messerschmidt. He proposes that the enzyme encoded by *Rbmy1a1* produces mRNA transcripts which are harmful to the developing embryo.

Messerschmidt's team is now looking for more of these 'special attention' genes. If the activity of detrimental genes such as *Rbmy1a1* can be detected before an embryo is implanted, then it could improve rates of IVF success, says Messerschmidt.

"We want to find out whether we can do epigenetic diagnostics in the same way as when we screen for a suspected genetic disease," he says. "Ultimately, having an overall understanding of these processes will give us a basis for what to look at."

Messerschmidt adds that an epigenetic diagnostic tool for embryos may allow doctors to compare IVF methods which differ between labs. "If we can compare different methods, perhaps we can point doctors to techniques that improve efficiency," he says. ■

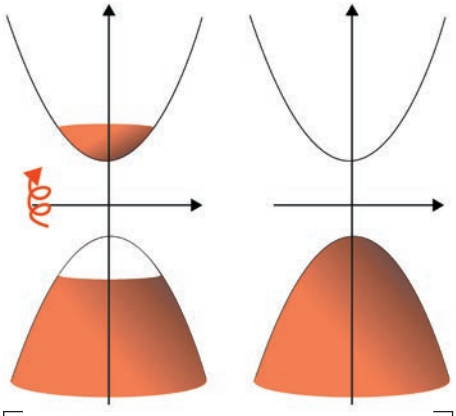
1. Kumar, A. S., Seah, M. K. Y., Ling, K. Y., Wang, Y., Tan, J. H. L. *et al.* Loss of maternal Trim28 causes male-predominantly early embryonic lethality. *Genes & Development* **31**, 12-17 (2017).

Two-dimensional materials

LIGHT FORCES ELECTRONS TO FOLLOW THE CURVE

AN EFFECT IN WHICH AN ELECTRON FOLLOWS A SKEWED TRAJECTORY CAN BE REALIZED IN TWO-DIMENSIONAL STRUCTURES CONSISTING OF DIFFERENT MATERIALS.

An exotic phenomenon usually associated with high magnetic fields can be achieved without a magnetic field, according to theoretical predictions by researchers from A*STAR and the United States. Their analysis could open the path to a novel type of optoelectronic device operating at long wavelengths.



Circularly polarized light creates electrons in one band structure 'valley' and not the other, and this can lead to a Hall effect without a magnetic field.

A charged particle in an electric field experiences a force that drives it along the direction of the field, creating a current. The moving particle can also experience a force perpendicular to its motion. This can happen in the presence of a magnetic field for example, and can lead to a range of unusual properties, particularly when the perpendicular component dominates and the electron starts to follow a skewed trajectory. But this so-called Hall regime often requires large magnetic fields which are impractical for real devices.

Justin Song from the A*STAR Institute of High Performance Computing, working with his colleague Mikhail Kats from the University of Wisconsin-Madison, have theoretically predicted that an unusual Hall-type motion can be harnessed at room temperature and without a magnetic field in a new class of materials known as gapped Dirac materials[1].

"Dirac materials are semi-metals because of their material symmetries," explains Song. "Narrow-gapped Dirac materials gently break these symmetries, opening up small bandgaps."

The alternative route to a Hall effect investigated by Song and Kats is based on so-called 'valleys' in these gapped Dirac materials. A valley, in the context of the electronic band structure of a material, is a minimum into which electrons can settle. If there are two valleys with identical energy, the electrons in each of the valleys of gapped Dirac materials feature contrasting trajectories.

Song and Kats exploited this contrast by inducing an imbalance of electrons in one valley over the other via circularly polarized light illumination. They revealed a photo-induced Hall effect (Hall photoconductivity) with strength determined heavily by the wavelength of the light, increasing by a factor of up to one million when switching from visible light to the far infrared.

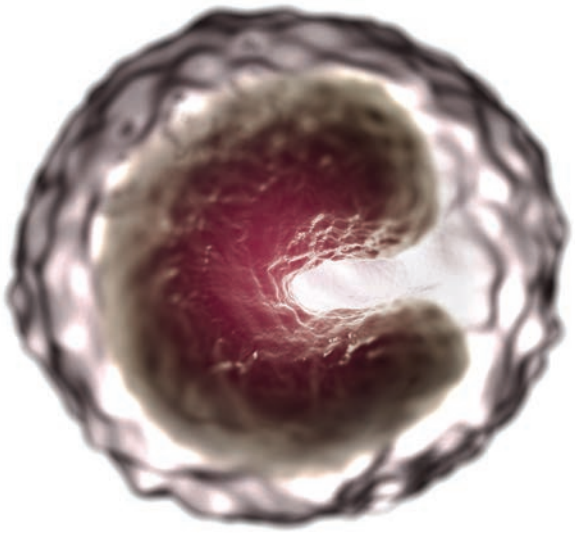
This means that gapped Dirac materials with a smaller electronic bandgap, such as graphene-boron-nitride heterostructures, are more effective than those with a larger bandgap including molybdenum disulfide.

This phenomenon could be useful for the development of novel far infrared and terahertz optoelectronics. "A particularly tantalizing prospect is a new type of photodetector concept that measures the Hall current in these gapped Dirac materials," says Song. "Such a photodetector could potentially possess zero net dark current even with a large bias voltage." ■

1. Song, J. C. W. & Kats, M. A. Giant Hall photoconductivity in narrow-gapped Dirac materials. *Nano Letters* **16**, 7346–7351 (2016).

Immunology

MONOCYTES NOT ALL CREATED EQUAL



DISCOVERY OF WHICH MONOCYTES SECRETE A PRO-INFLAMMATORY PROTEIN COULD LEAD TO NEW THERAPIES FOR AUTOIMMUNE DISEASE.

Different populations of white blood cells secrete different levels of IL-1β, a pro-inflammatory protein that normally helps the body fight off infection and injury, but may also trigger autoimmune disease and inflammatory diseases. An investigation by A*STAR researchers and collaborators shows that a regulatory protein called Hsp27 is responsible for some of these differences in subsets of monocytes[1].

The results help explain some of the diversity and sub-specialization of the immune system — and could yield new treatment strategies for patients with rheumatoid arthritis, colitis, diabetes and other ailments.

"These findings represent the first comprehensive and unifying data on IL-1β production by monocytes, and identify the Hsp27 pathway as a main player and potential novel therapeutic target for inflammatory disease," says Wong Siew Cheng from the A*STAR Singapore Immunology Network, who led the research project.

Wong teamed up with Heather Wilson, an immunologist at the University of Sheffield in the United Kingdom, to study IL-1β production in different subsets of monocytes. The two researchers jointly supervised a PhD student, Éva Hadadi, who used surface markers to sort monocytes isolated from human blood into three subpopulations: classical, intermediate and non-classical monocytes.

Hadadi then induced IL-1β production and maturation through a two-step stimulation protocol. In this way, the researchers observed that classical and intermediate monocytes produced and released more than twice as much IL-1β as non-classical monocytes.

They then searched exhaustively and systematically for reasons to explain this differential production of IL-1β. Non-classical monocytes were no less receptive to the stimulation protocol, they found, so that didn't account for their low IL-1β output. Nor were these white blood cells generally deficient in their ability to produce pro-inflammatory signaling proteins or process their maturation.

"Instead," says Wong, "these cells expressed abundant Hsp27," an essential subunit of a protein complex involved in breaking down the RNA transcripts of IL-1β before they can be turned into proteins. As a result of this differential Hsp27 activity, overall IL-1β levels remained low in non-classical monocytes but high in classical monocytes.

The findings indicate that IL-1β-spewing classical monocytes are the true culprits behind inflammatory disease and that therapies designed to boost Hsp27 levels in these cells might help tamp down unwanted inflammation. However, Wong notes that Hsp27 is also known to promote other pro-inflammatory molecules. "Hence," she says, "the type of treatment option to utilize will be based on the type of disease." ■

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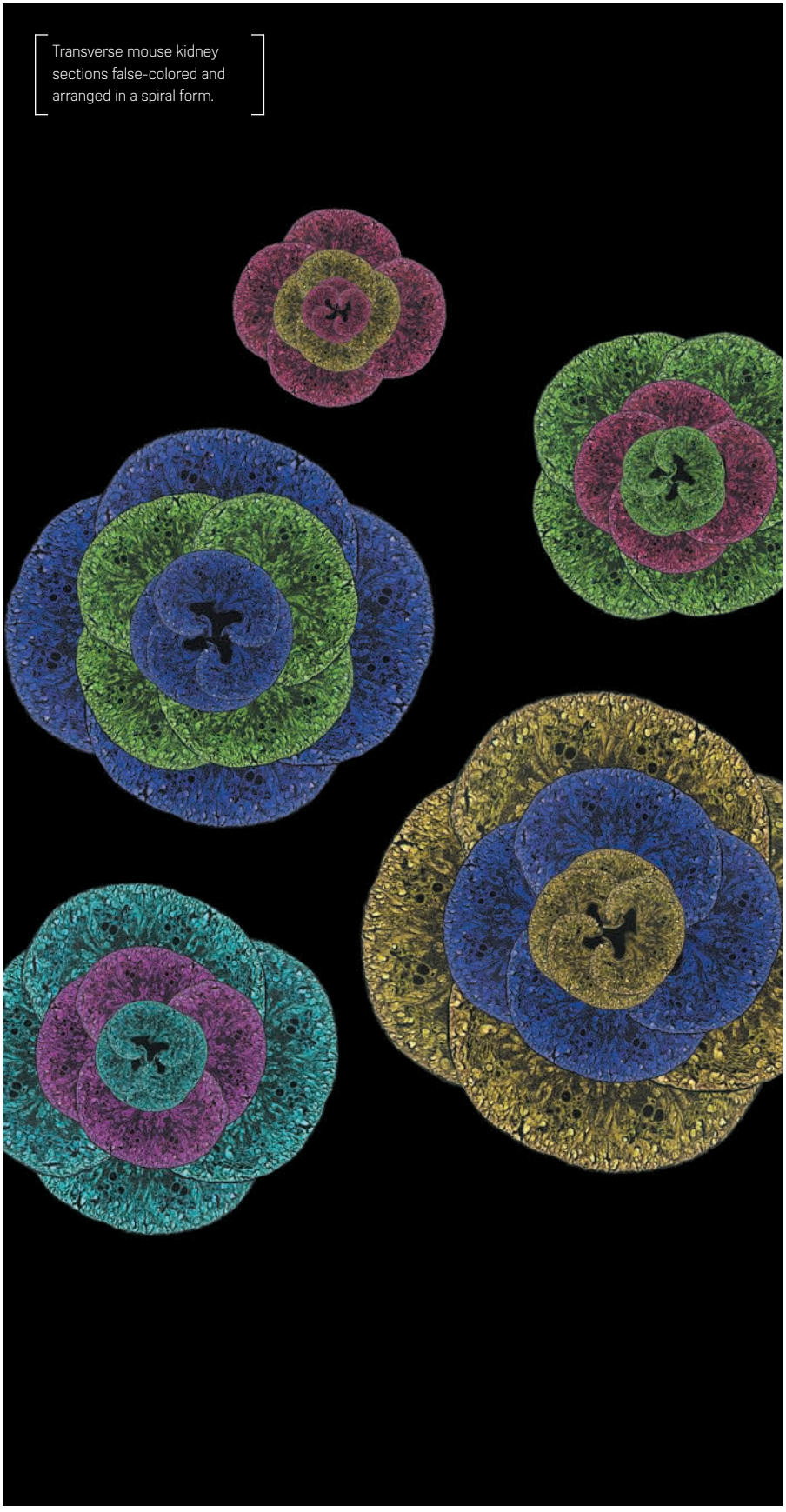


Transverse mouse kidney sections false-colored and arranged in a spiral form.

Kidney disease

ENEMY AT THE GATE

A SECOND GENE ASSOCIATED WITH A HERITABLE FORM OF KIDNEY DISEASE IS SHOWN TO ACT AS A GATEKEEPER IN 'MOLECULAR ANTENNA'.

Mutations in a single gene have long taken sole responsibility for a rare type of kidney disease. Now A*STAR researchers find a second culprit by demonstrating that mutations in another gene also cause the disease.

Polycystic kidney disease (PKD) is a genetic disorder that produces fluid-filled cysts, reducing kidney function and leading to organ failure. Autosomal dominant PKD is more prevalent and typically affects adults, whereas the rarer autosomal recessive PKD (ARPKD) is more aggressive and affects infants and children. The mortality rate of infants with ARPKD can be as high as 50 per cent and most sufferers need a transplant before their tenth birthday.

Mutations in the gene, polycystic kidney and hepatic disease 1 (*PKHD1*) were thought to be responsible for ARPKD. Now Sudipto Roy at the A*STAR Institute of Molecular and Cell Biology in Singapore and colleagues demonstrate that ARPKD is also caused by mutations in DAZ interacting protein 1-like (*DZIP1L*). "Finding that the disease is genetically heterogeneous is surprising," said Roy.

The authors found that seven patients from four different families carry mutations in *DZIP1L*. Furthermore, they show that kidney function is compromised in both mice and zebrafish bearing *DZIP1L* mutations, suggesting that the role of DZIP1L is conserved across the vertebrates.

DZIP1L encodes a protein that localizes to cilia, hair-like structures on cell surfaces, which

are vital for kidney cell function. The primary cilium functions as a molecular antenna conveying important messages to the cell about the local environment. Experiments in cells show that DZIP1L localizes to the base of the primary cilium at what is known as the transition zone. This region is important for regulating the transport of proteins in and out of the cilium.

Although the number of cilia is unaffected in *DZIP1L* mutant tissue, loss of this protein stops two proteins that are important for preventing cyst formation, polycystin-1 and -2, from reaching the primary cilium. As Roy explains, "the ineffective access of polycystin-1 and -2 to cilia could be the cause of cystic kidney disease in patients with mutations in *DZIP1L*".

Roy and colleagues also show that DZIP1L interacts with septin2 (SEPT2) to create a diffusion barrier at the transition zone that helps maintain ciliary subcompartments.

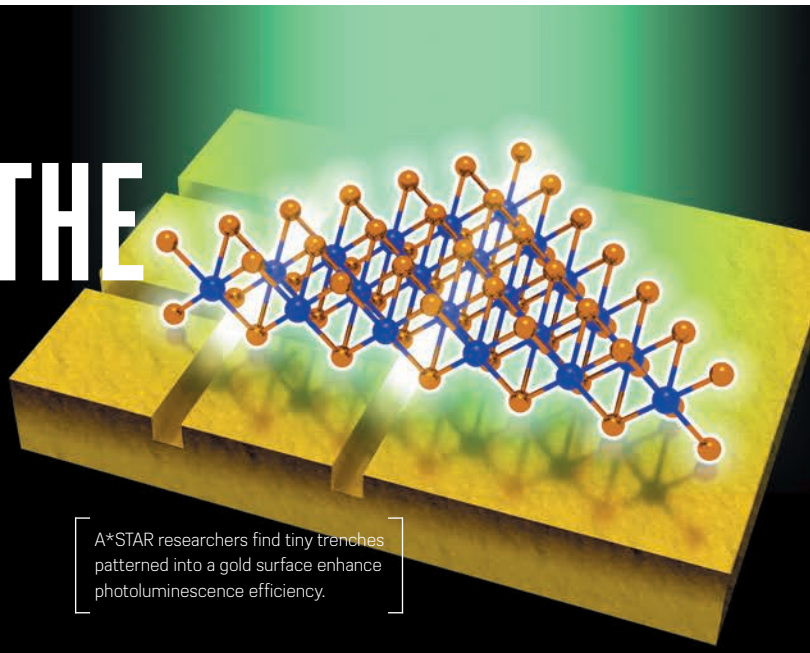
The team are now focusing on understanding the mechanism by which DZIP1L functions at the transition zone and determining the extent to which mutations in DZIP1L mutations cause ARPKD. Future research will also investigate therapeutic strategies that facilitate the proper localization of ciliary proteins. ■

1. Lu, H., Galeano, M. C. R., Ott, E., Kaeslin, G., Kausalya, P. J. *et al.* Mutations in DZIP1L, which encodes a ciliary-transition-zone protein, cause autosomal recessive polycystic kidney disease. *Nature Genetics* **49,** 1025–1034 (2017).

Optoelectronics

EFFICIENCY IN THE TRENCHES

THE KEY TO ULTRATHIN HIGH-EFFICIENCY SENSORS AND SOLAR CELLS COULD BE MATERIALS COVERED WITH TINY TRENCHES.



A*STAR researchers find tiny trenches patterned into a gold surface enhance photoluminescence efficiency.

Future ultrathin solar cells and light sources could have their surfaces covered by tiny trenches, after A*STAR researchers found such structures enhance efficiency by four orders of magnitude.

Joel Yang from the A*STAR Institute of Materials Research and Engineering was part of an international collaboration that achieved a 20,000-fold increase in the photoluminescence of a one atom-thick layer of tungsten diselenide, by mounting it on a gold surface patterned with narrow trenches[1].

Tungsten diselenide is promising for ultra-sensitive, ultra-thin light sensors, solar cells and light-emitting diodes, because of its ability to absorb light and re-emit at a different frequency. However this effect only occurs for a single atom layer, so its efficiency is very low — most of the light passes straight through.

> "IT WAS VERY SURPRISING THAT SUCH A LARGE ENHANCEMENT COULD BE POSSIBLE"

Yang's inspiration was to mount the layer on a gold surface and trap the light energy at the interface of the two layers in the form of surface plasmons. To enhance the absorption of light, they added trenches to the gold layer under the tungsten diselenide.

"It was very surprising that such a large enhancement could be possible," says Yang.

The key was matching the trench size to the energy so that the plasmons were trapped in the trenches through a resonant process known as the Purcell effect.

The team shone 633-nanometer light onto the sample and measured the output at 750 nanometers. They found 12 nanometer wide trenches in a grid pattern with spacing 200 nanometers gave the highest photoluminescence — 20,000 times more than a bare layer of tungsten diselenide.

To create the structure, the team etched a very flat silicon crystal to create a grid of ridges. Next they deposited a layer of gold onto the silicon and then peeled it off to reveal trenches where the ridges had been.

"The narrowness of the trenches and the flatness of the metal film is important," Yang says. "Any roughness will interact detrimentally with the two-dimensional material."

The gold was immersed in water and a film of tungsten diselenide floated on the water's surface. The gold was then slowly raised out of the solution, emerging with the thin layer on top.

The simple structure has many advantages, says Yang. "The entire surface is exposed to the user, which makes it easy for further research, such as functionalizing the surface with chemicals or adding electrodes".

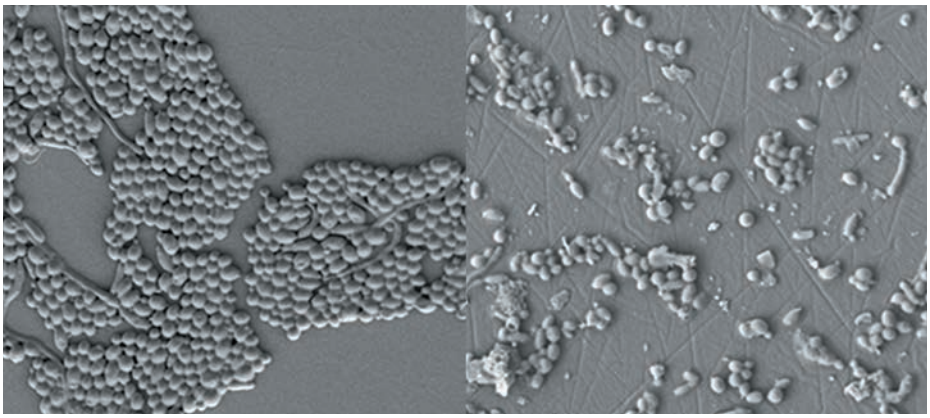
It is also easier to manufacture than other plasmonic devices, which require a second layer above the thin layer, creating a sandwich. ■

1. Wang, Z., Dong, Z., Gu, Y., Chang, Y. H., Zhang, L., Li, L. J., *et al.* Giant photoluminescence enhancement in tungsten-diselenide–gold plasmonic hybrid structures. *Nature Communications* **7,** 11283 (2016).

Drug development

EYE CAN SEE CLEARLY NOW

SYNTHETIC MOLECULES PROMISE TO RELIEVE DEBILITATING EYE DISEASE.



A *Candida albicans* biofilm before (left) and after (right) treatment with one of the synthetic peptides.

A*STAR researchers have created synthetic molecules to treat fungal keratitis – an infection of the cornea that causes visual disability[1]. The new molecules are a first step toward developing effective drugs to combat this widespread disease.

According to the World Health Organization, corneal ulcers resulting from keratitis cause 1.5–2 million people to lose vision in one eye each year, with fungal infections accounting for nearly half of keratitis cases caused by microbes. "There are no safe and effective antifungal agents for clinical treatment of fungal keratitis," says Jackie Ying, the executive director of the A*STAR Institute of Bioengineering and Nanotechnology (IBN), Singapore.

Ying and IBN group leader Yi Yan Yang led a team of researchers who developed a series of short, synthetic protein fragments designed to mimic antimicrobial peptides produced by the immune system. The synthetic peptides were broadly effective against bacteria and *Candida albicans*, one of the pathogens that can cause fungal keratitis. However, they had not been tested against fungal keratitis, where *C. albicans* aggregate to form a biofilm that protects the fungus.

The team evaluated three synthetic peptides to determine which structural characteristics were effective against fungal keratitis. All three peptides were designed around a core spiral structure known as an alpha helix, but each was slightly different. The first peptide was a short, simple helix, the second was a helix capped with the amino acid cysteine, and the third was a longer helix.

While all three peptides slowed the growth of *C. albicans*, the simple helix had little effect when tested against fungal biofilms. Both the other peptides were able to disrupt the biofilm and eradicate 90 per cent of the fungus within 24 hours, with the longer peptide being effective at lower doses. Both peptides also relieved keratitis symptoms in mice infected with *C. albicans*.

"We were excited when we saw that, since we may have discovered a solution to treat severe fungal keratitis," says Yang. The peptides are a first step toward developing antifungal agents to treat the disease. Overall, the two peptides performed as well as the antifungal drug amphotericin but with promise to overcome some of its drawbacks, such as its high cost and limited clinical applicability due to low solubility and instability.

Next, the team will move to trials in rabbits, testing the best-performing candidates from this and other studies to confirm their activity and evaluate their safety. ■

1. Wu, H. Liu, S., Wiradharma, N., Ong, Z. Y., Li, Y. *et al.* Short synthetic α-helical-forming peptide amphiphiles for fungal keratitis treatment *in vivo*. *Advanced Healthcare Materials* **6,** 1600777 (2017).

INNOVATION BEYOND BORDERS

The Leaders in Science Forum, organized by A*STAR and JTC and supported by Science Centre Singapore and Mediacorp, was held on 16 August 2017 at Biopolis as part of the one-north Festival. This year's theme was 'Innovation Beyond Borders', and the forum brought together over 400 science and technology leaders, policy makers, captains of industry and academics to exchange insights on research strategies and examine how the government, industries, and the arts and social sciences communities are collaborating to shape Singapore's economy and society, through research, innovation and enterprise.

Genetically modified zebrafish can offer quick, useful insights into whether a drug may be harmful to humans.

Drug toxicity

ZEBRAFISH LIGHT THE WAY TO SAFER MEDICATION

GENETICALLY MODIFIED ZEBRAFISH ARE PROMISING IN THE DETECTION OF HARMFUL COMPOUNDS FOR SCREENING DRUG CANDIDATES.

The common zebrafish is a useful proxy for testing whether drug candidates cause organ damage. Now, researchers in Singapore have created two modified types of the fish, one that glows when experiencing toxicity, and another that metabolizes drugs in a similar way to humans. Combined, these may help pharmaceutical companies develop better drugs.

"Roche, our partner in the study, want a way to quickly identify which of their drugs may be damaging to the liver," says Tom Carney from the A*STAR Institute of Molecular and Cell Biology.

Zebrafish despite being very different from humans, experience toxicity from similar drugs, making them a great model. Carney's team monitored the fish to identify what genes are 'switched on' when they were dosed with a range of drugs known to be damaging to liver cells, or 'hepatotoxic', and identified four common genetic sequences that produced enzymes to neutralize the drugs. "The fish are trying to clear the drugs out, and are doing so by using a detoxification process of which these genes are parts," explains Carney.

With this knowledge, the team produced a breed of modified zebrafish that expressed a fluorescent protein when these genes were switched on, creating fish that glow in response to liver-damaging drugs[1].

In practice, the fish could offer an easy way for pharmaceutical companies to remove harmful drugs from their pipeline reducing R&D expenditure.

Despite zebrafish and humans sharing similar detoxification pathways, the detoxifying enzymes differ between the species and therefore drugs can be metabolized differently. Addressing this, the scientists produced a second line of zebrafish in which the liver was supplemented with a key human liver enzyme. The result was the first demonstration of a 'humanized' zebrafish model that detoxified drugs in a much more similar way to humans[2]. This is especially important considering that drug metabolites can be more damaging than the originally ingested medication.

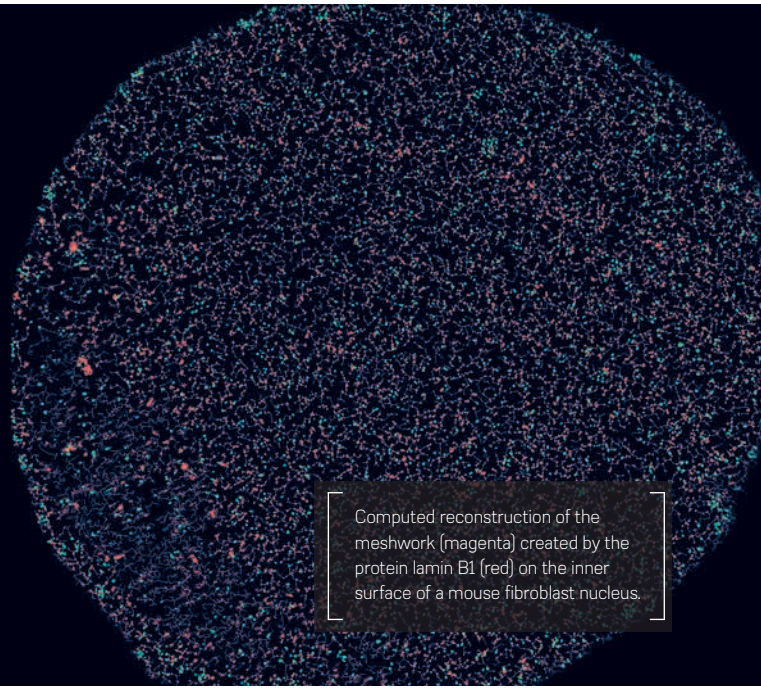
These models offer a promising line of inquiry into a drug testing technique that is scalable, affordable, and allows for high-throughput screening. In the future, Carney's team may combine their two models in order to create a line of zebrafish that accurately metabolizes toxic medications and provides an immediate, visible signal for drugs that may harm patients. ■

1. Poon, K. L., Wang, X., Lee, S. G. P., Ng, A. S., Goh, W. H. *et al.* Transgenic zebrafish reporter lines as alternative in vivo organ toxicity models. *Toxicological Sciences* 1**56,** 133–148 (2017).
2. Poon, K. L., Wang, X., Ng, A. S., Goh, W. H., McGinnis, C. *et al.* Humanizing the zebrafish liver shifts drug metabolic profiles and improves pharmacokinetics of CYP3A4 substrates. *Archives of Toxicology* **91,** 1187–1197 (2017).

Cell Biology

MODELING MEMBRANES

SUPER-RESOLUTION MICROSCOPY REVEALS DETAILS OF THE MESH AT THE INNER SIDE OF THE NUCLEAR MEMBRANE.



Computed reconstruction of the meshwork (magenta) created by the protein lamin B1 (red) on the inner surface of a mouse fibroblast nucleus.

All creatures of the animal kingdom share one thing: the nuclear membrane. Wrapping the genetic core of the cell, this membrane, together with all the attached proteins, plays a vital role in biological functions. Despite its importance, details of its architecture are still missing.

Scientists at the A*STAR Institute of Medical Biology, led by Brian Burke, have constructed a nanoscale model of the inner side of the mammalian nuclear membrane, where threadlike proteins called lamins form a mesh. Lamins, as well as providing support for the nuclear membrane, are involved in cell division, chromatin organization and DNA repair and more. Mutations in lamins have been connected to more than a dozen human diseases, including muscular dystrophy, heart disease and progeria, a premature aging syndrome.

> "SIGNIFICANTLY, A-TYPE AND B-TYPE LAMINS ASSOCIATE ONLY WITH OTHER LAMINS OF THE SAME TYPE, FORMING INDEPENDENT NETWORKS"

Because the nuclear periphery is so crowded with protein and DNA molecules, it has proven extremely difficult to determine the lamin arrangement using either light or electron microscopy. Now Burke's team has used super-resolution microscopy to go beyond the capabilities of conventional microscopy and to record the locations of single lamin molecules.

Burke explains the process: "Fusing 10,000 images, each fluorescently-labeled lamin appeared as a bright spot. Then, we applied a mathematical technique to link these spots. As a result we saw that irregular filament networks made of different types of lamins cover the entire nuclear inner surface," he says. "Significantly, A-type and B-type lamins associate only with other lamins of the same type, forming independent networks."

Although the different lamins are almost identical, they interact with distinct proteins and therefore seem to have different functions. For example, the A-type lamin, LaC, binds to a protein that is part of the channels surrounding the holes on the nuclear membrane. The nuclear membrane has pores that allow the transfer of material between the nucleus and the rest of the cell. These channels are known as nuclear pore complexes (NPCs) and look like hoops with a

basketball net. The interaction between LaC and a protein in the basket could mean that LaC filaments regulate the distribution of the NPCs in the nuclear membrane, a mechanism which may be linked to the development of progeria.

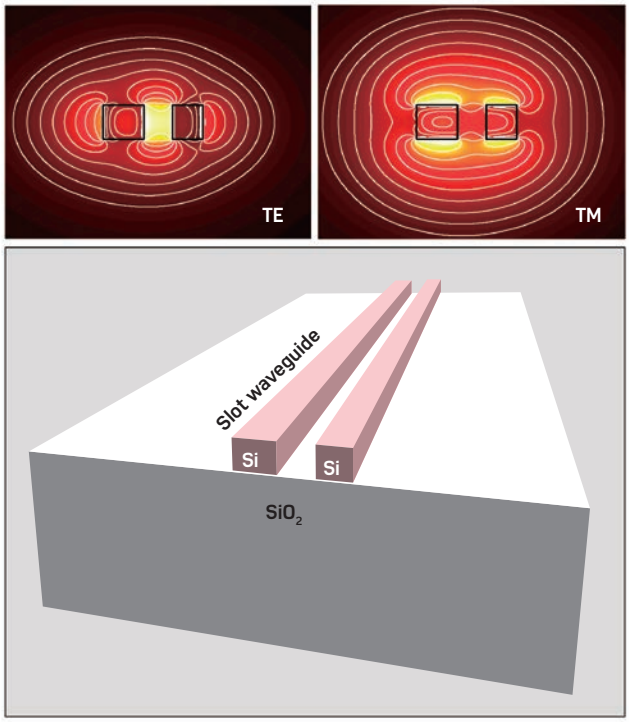
Burke says the next challenges for the team are to better understand the different functions of the lamin networks and to explore their interactions with other nuclear components. Their findings may explain how even subtle changes in nuclear lamin organization can give rise to a bewildering array of human diseases. ■

1. Xie, W., Chojnowski, A., Boudier, T., Lim, J. S., Ahmed, S. *et al.* A-type lamins form distinct filamentous networks with differential nuclear pore complex associations. *Current Biology,* **26,** 2651-2658 (2016).

Photonics

ON-CHIP OPTICS FIND THEIR GROOVE

CLEVER DESIGN OF RAIL-LIKE WAVEGUIDES MAKES IT SIMPLER TO MINIATURIZE PHOTONIC COMPONENTS ON TO SILICON WAFERS.



Optimizing the layout of slot waveguides can eliminate differences in transverse electronic (TE) and magnetic (TM) light refraction, creating a single beam that is significantly easier to incorporate into photonic circuits.

High-speed optical circuits and sensors generally require strict control over light polarization to minimize loss and cross-talk in photonic devices such as waveguides. An A*STAR team now predicts that noise resulting from imperfect polarizations can be eliminated using micro-structures known as 'slot' waveguides[1].

Discovered just over a decade ago, slot waveguides trap electromagnetic fields into a narrow region between two microfabricated strips of materials, such as silicon. Differences in the refractive indices between the slots and rails help focus the light into the slot with optical intensity and power not seen in typical waveguides. These properties impart improved sensitivity to sensors and generate useful amplification effects.

One difficulty with photonic waveguides, however, is splitting incoming radiation into electric and magnetic polarization components within nanometer-scale spaces. "Inevitably, there will be contamination from the light source or defects along the waveguides," says Jun Rong Ong from A*STAR's Institute of High Performance Computing. "Unwanted polarization acts as noise, and this deteriorates device performance."

Ong, along with colleagues Valerian Chen and Ching Eng Png, hypothesized that a special state known as 'zero birefringence' might negate the need for specialized splitter devices currently used in photonic waveguides. Birefringence describes how light with a mix of polarizations can refract into two directions when passing through crystals with specific shapes. The team undertook a systematic theoretical analysis to determine if changes to the waveguide's height, angle, and slot size could remove birefringence from the waveguide, leaving just a single ray.

"By having zero birefringence, we can process the inevitable mix of both polarizations simultaneously," explains Ong. "This means the device footprint could be effectively halved."

The trio's simulations showed that many structural parameters could produce zero birefringence in the waveguide, but some were more effective than others. Surprisingly, they discovered the two rails need not be symmetric — having unequal widths enabled one side to confine greater amount of light, and give better control over the waveguide's refractive index. Conversely, when the team

tested waveguides with bent orientations to go around corners, symmetrical rails proved most effective.

Currently, the tolerances needed to produce the researchers' zero birefringence waveguides could only be realized through electron beam lithography, a relatively slow process. However, they are confident that practical demonstrations of this technology are within reach.

"It would be useful to explore if short devices, less than a few hundred micrometers, can be polarization-independent on a wafer scale," says Ong. "This could lead to applications with real impact." ■

1. Chen, V. H., Ong, J. R. & Png, C. E. Polarisation independent silicon-on-insulator waveguides. *Scientific Reports* **6**, 37760 (2017).

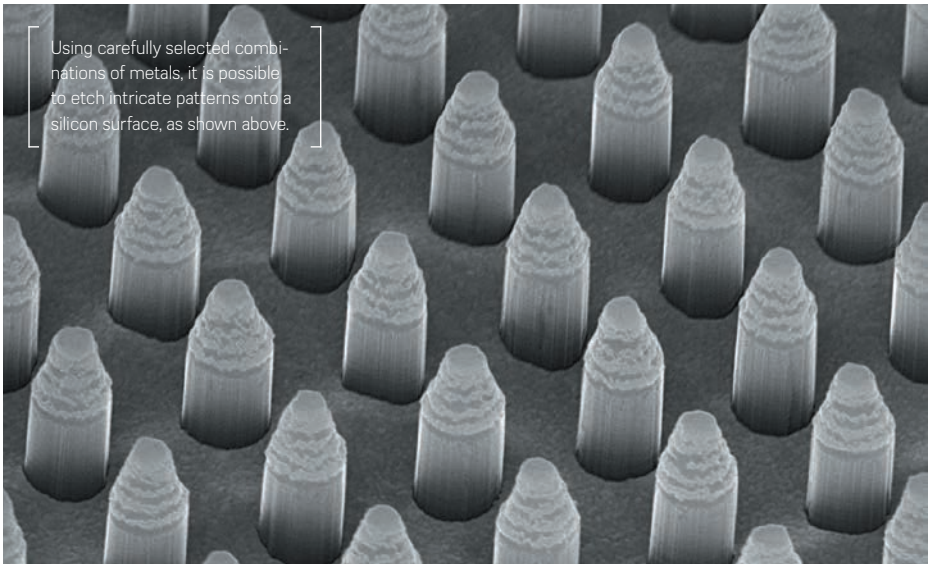
Nanostructured silicon

BRISTLING WITH POTENTIAL

BETTER UNDERSTANDING THE PRINCIPLES OF SILICON ETCHING LEADS TO IMPROVED SURFACE PATTERNING.

From solar cells that capture more light, to medical devices that resist colonization by bacteria; there are many applications for materials given a bristly coating of silicon nanowires. Creating these nanostructured silicon surfaces can be challenging — but A*STAR researchers have now discovered how to control at least one route.

Metal-assisted chemical etching (MacEtch) is one of the most scalable and cost-effective ways to form these surfaces, but researchers frequently encounter discrepancies between existing MacEtch models and the process in reality.



Using carefully selected combinations of metals, it is possible to etch intricate patterns onto a silicon surface, as shown above.

Sing Yang Chiam at the A*STAR Institute of Materials Research and Engineering and his colleagues have now discovered the key governing mechanism by which MacEtch works[1]. "We were very surprised by our discoveries," says Chiam. "Only after many repeated tests, and studying it from many angles, did we become convinced by our model."

> "ONLY AFTER MANY REPEATED TESTS, AND STUDYING IT FROM MANY ANGLES, DID WE BECOME CONVINCED BY OUR MODEL."

MacEtch is based on silicon's interaction with a catalyst (such as gold) in a hydrogen peroxide 'etching solution'. When coated on silicon, the catalyst accelerates hydrogen peroxide's attack on its surface. The process can be controlled, however, by putting certain blocking metals between the catalyst and the silicon. If this intermediate layer is placed in a dot pattern across the silicon, when the hydrogen peroxide is added, the silicon beneath the dots is protected from etching. These protected points become silicon nanowires as the silicon around them is dissolved.

Chiam and his team recently showed chromium metal is a good blocking layer. However, why chromium worked well, and what other metals might also perform well, were not known. "We set out to find the fundamental governing mechanism," Chiam says. "Then we could more easily determine whether one material should or shouldn't work."

After systematically studying different blocking metals, the researchers soon overturned the prevailing idea the catalyst controls etching by helping to inject positive charges at the catalyst/silicon interface.

Instead, they showed etching is controlled by a chemical 'redox' reaction between the catalyst and the silicon. Only metals with a high enough redox potential can react with and remove silicon atoms. This discovery helps reconcile previous experimental discrepancies like the chromium result and means MacEtch catalysts or blocking materials can be chosen simply by looking up their redox potential.

The team is already using its new understanding to produce even more finely detailed, more deeply etched silicon nanostructures, Chiam says. Applications range from filtration to microelectronics, he adds. "We look forward to finding the right partner in taking our discovery and technology forward." ■

1. Kong, L., Dasgupta, B., Ren, Y., Mohseni, P. K., Hong, M. *et al.* Evidences for redox reaction driven charge transfer and mass transport in metal-assisted chemical etching of silicon. *Scientific Reports* **6,** 36582 (2016).

Encapsulation

MAKING THE UNPALATABLE PALATABLE

MASKING THE BITTER TASTE OF ANTIOXIDANT QUERCETIN COULD ENABLE ITS USE IN A WIDE RANGE OF FOOD PRODUCTS.

Encapsulating the antioxidant quercetin in wax could mask its bitterness and enable its use in a wide range of food products, according to new research from Singapore.

Quercetin is a type of plant pigment called a flavonoid, and is one of the most abundant antioxidants in our diet. Laboratory research suggests it could have a range of beneficial effects, including anti-cancer, anti-obesity, anti-inflammatory and antimicrobial properties, in addition to being a potent antioxidant.

However, the opportunities to incorporate quercetin into fortified food products have been limited by its bitter taste. Encasing quercetin particles in a bland or even pleasant-tasting coating could offer one way to mask the taste.

"We see masking taste as a challenge for us to ensure that this doesn't get released at the wrong time, wrong place, wrong amount, and microencapsulation comes naturally to us as a solution to the problem," says Wai Kiong Ng from the Division of Crystallisation & Formulation Science at the A*STAR Institute of Chemical and Engineering Sciences.

The group identified three substances that could work as the outer coating: carnauba wax from the Brazilian palm tree, shellac — a natural resin secreted by a species of insect called the lac bug, and zein, a water-insoluble protein derived from corn gluten. These products are already widely used in the food and pharmaceutical industry, but the team needed to find out which option would remain intact in the mouth, masking the taste of quercetin, but dissolve inside the gastrointestinal tract.

The microencapsulation process involved mixing quercetin powder with powdered carnauba wax, shellac or zein, then feeding the powders into a heated device called a hot-melt extruder.

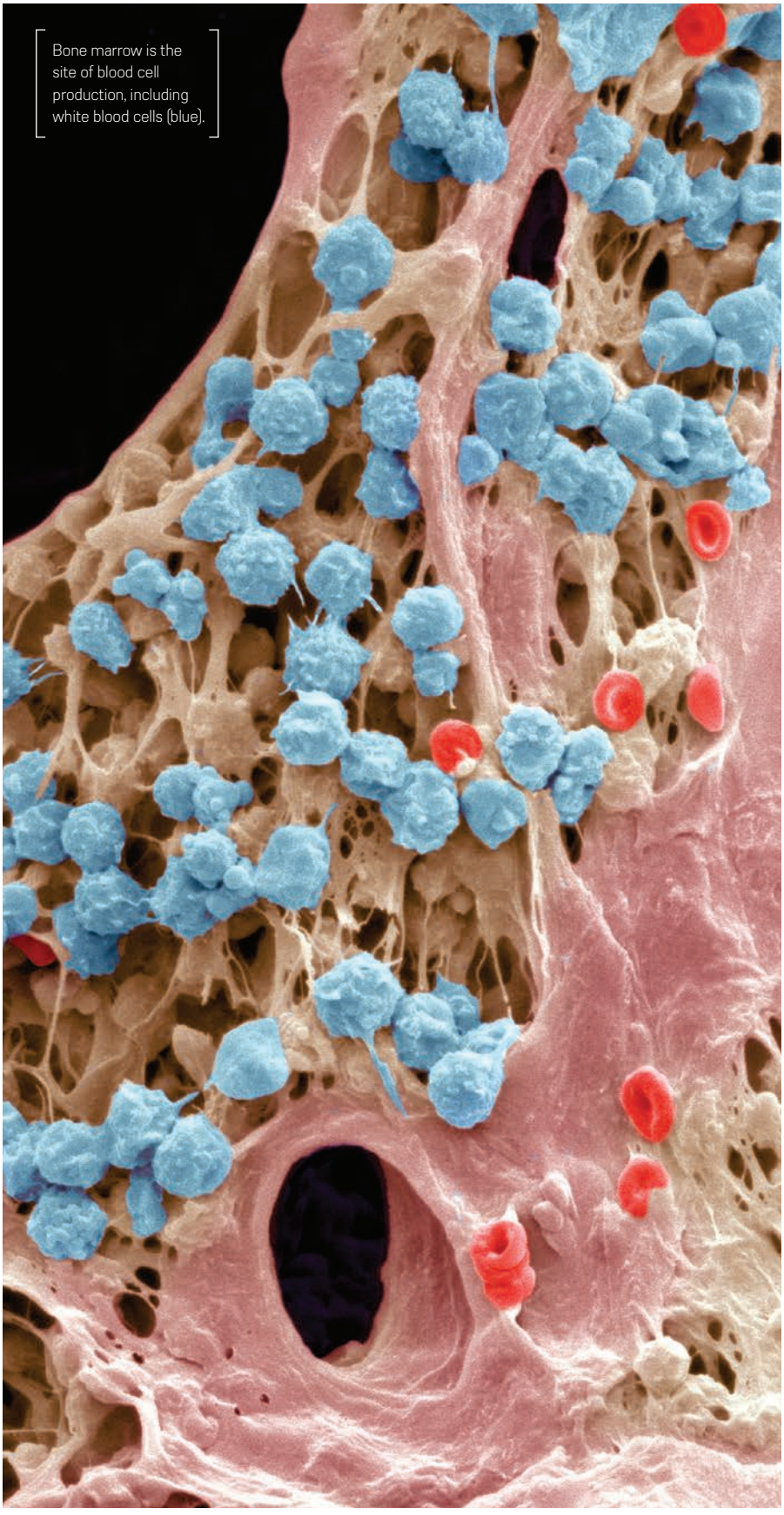
The temperature of the hot-melt extruder had to be controlled to melt the products sufficiently to coat the quercetin powder, without altering the chemistry of the quercetin. The resulting mix was then cooled and milled.

The team then tested how the coated materials behaved in solutions designed to mimic conditions throughout the gastrointestinal tract. They found the carnauba wax-microencapsulated quercetin powder stayed intact in the mouth but still dissolved well in the acid environment of the stomach.

Testing with an electronic taste sensor showed that the carnauba wax-coated product possessed an almost neutral taste, which was verified by human tasting of quercetin-fortified bread by Zhou Weibiao's group at the Division of Food Science & Technology, National University of Singapore. This paves the way for the microencapsulated quercetin to be used in products such as bread and powdered beverages. ■

The milled carnauba wax-encapsulated quercetin powder.

1. Khor, C. M., Ng, W. K., Kanaujia, P., Chan, K. P. & Yuancai Dong. Hot-melt extrusion microencapsulation of quercetin for taste-masking. *Journal of Microencapsulation* **34,** 29-37 (2017).



Bone marrow is the site of blood cell production, including white blood cells (blue).

Immunology

A TEAM EFFORT IN THE BONE MARROW

A NEWLY DISCOVERED POPULATION OF IMMUNE CELLS HELPS REPLENISH THE BONE MARROW'S SUPPLY OF INFECTION-FIGHTING MONOCYTES.

Infection-fighting immune cells known as monocytes consist of two distinct subpopulations in the bone marrow, an A*STAR investigation has found. One of these acts as a reservoir for the other in order to maintain a stable pool of monocytes circulating through the bloodstream, a discovery that could inform future drug development.

"We discovered a new population of bone marrow monocytes, debunking the popular view that monocytes constitute a homogenous population," says Lai Guan Ng of the A*STAR Singapore Immunology Network (SIgN), who led the research. "Since monocytes are increasingly being recognized as attractive therapeutic targets, our findings provide critical biological knowledge that could form the basis for improved therapeutic treatments of disease."

Ng and his colleagues used a cell sorting technique called flow cytometry to categorize monocytes in the bone marrow on the basis of six surface receptor proteins. One of these

proteins, CXCR4, stood out because its expression levels on the cells clearly demarcated two subsets of monocytes — one with high CXCR4 activity that serves as a kind of transitional pre-monocyte, and a more mature monocyte with low CXCR4 levels.

Gene activity analyses and experiments in mice showed that the CXCR4-expressing cells were actively proliferating and slowed their division as they matured to replenish supplies of monocytes that were ready to move from the bone marrow to the bloodstream. As Ng explains, "This newly defined transitional phase is believed to act as a regulatory checkpoint to maintain a stable pool of circulating monocytes throughout the body."

The SIgN team, in collaboration with scientists from the A*STAR Institute of Molecular and Cell Biology and around the world, went on to define a number of other novel functions of CXCR4 in monocyte biology. Aside from CXCR4's role in retaining transitional pre-monocytes in the bone marrow, the researchers found that CXCR4 activity also continues to affect migration and localization of mature monocytes after they have left the bone marrow and entered the rest of the body.

In fact, by inhibiting CXCR4, Ng and his colleagues showed that they could reduce the number of monocytes that congregate in the blood vessel walls of the lungs of endotoxin-exposed mice, thereby limiting lung injury and the risk of sepsis-induced death. "These findings may pave the way for future CXCR4-based therapies," says Ng, noting that monocytes are increasingly being recognized as critical mediators of inflammation in conditions such as heart disease, multiple sclerosis, and liver fibrosis. ■

1. Chong, S. Z., Evrard, M., Devi, S., Chen, J., Lim, J. Y. *et al.* CXCR4 identifies transitional bone marrow premonocytes that replenish the mature monocyte pool for peripheral responses. *Journal of Experimental Medicine* **213**, 2293-2314 (2016).

Medical imaging

THE NEXT SEGMENT

A DATA-DRIVEN COMPUTATIONAL APPROACH THAT RECOGNIZES FILAMENTARY SECTIONS OF NEURONS AND BLOOD VESSELS MAY ENHANCE MEDICAL DIAGNOSIS.

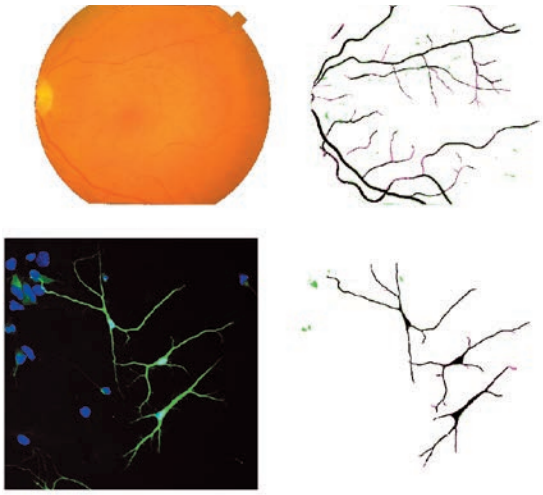
A computational tool is now available for faster and more reliable screening and diagnosis of serious vascular and neurological conditions, including glaucoma, diabetic retinopathy, and Alzheimer's disease, in their early stages. Developed by A*STAR researchers, the software automatically segments filamentary structures, such as retinal blood vessels and neurons, in various biomedical images.[1]

Clinicians and biologists often need to evaluate the shape of filaments in biomedical images to determine the presence and severity of vascular and neurological diseases. Filaments however can present a problem: as well as crossing and overlapping, they show variable diameters and degrees of twisting. This makes filamentary sections difficult to distinguish from backgrounds and, consequently, can skew visual assessments.

Several computer programs have been created to automate the segmentation process. These time-saving programs reduce human error, but their performance is typically optimized for specific tasks, which limits their use.

> "WE WERE VERY HAPPY TO FIND OUT THAT OUR ALGORITHM CAN WORK ACROSS DIFFERENT TASKS AND PERFORMS WELL."

To widen the scope of these programs, Li Cheng and colleagues from the A*STAR Bioinformatics Institute, in collaboration with the Beijing Institute of Technology, have developed an algorithm that tackles segmentation as a general problem. "We have come up with a data-driven approach based on learning structured and contextual features," says Cheng.



Retinal and neuronal segmentation images (right column) can be produced from empirical two-dimensional datasets (left column) thanks to a new computational approach.

The researchers designed a two-step algorithm that sequentially extracts structured and contextual information from experimental data sets. During structured feature learning, the program captures informative image patches consisting of unique foreground textures that collectively help the machine distinguish them from backgrounds. This first step produces features that serve as input for the subsequent learning stage.

Next, contextual characteristics among patches are encoded into the algorithm using pre-existing machine learning tools, called

boosted tree classifiers, to acquire more of the global information.

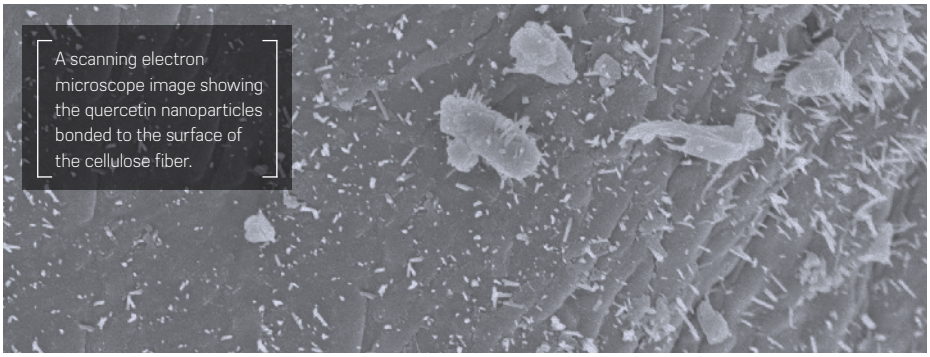
"We were very happy to find out that our algorithm can work across different tasks and performs well in general," says Cheng. Compared to existing state-of-the-art methods, the algorithm delivered competitive results over many different datasets. According to Cheng, the new algorithm represents a stepping stone in his team's long-term efforts to cure eye-related problems and decipher the structure and inner working of neuronal diagrams. His team is developing a series of tools to reconstruct, segment, and separate these elementary objects in imaging data. "We expect these tools to provide a solid platform for clinicians and biologists," he adds. ■

1. Gu, L., Zhang, X., Zhao, H., Li, H. & Cheng, L. Segment 2D and 3D filaments by learning structured and contextual features. *IEEE Transaction on Medical Imaging* **36,** 596–606 (2017).

Antioxidants

DISSOLVING OBSTACLES TO A USEFUL ANTIOXIDANT

INSOLUBLE DIETARY FIBER COULD HELP MAKE ANTIOXIDANT QUERCETIN MORE SOLUBLE.



A scanning electron microscope image showing the quercetin nanoparticles bonded to the surface of the cellulose fiber.

A common plant pigment that is also a potent antioxidant could soon be a mainstream health supplement, as A*STAR researchers get closer to making it soluble.

Laboratory and animal studies of quercetin — one of the most abundant dietary plant-based antioxidants — suggest it could offer antioxidant, anti-cancer, anti-obesity, anti-inflammatory and anti-microbial benefits if incorporated in food supplements. However its use has been limited by the fact that it does not dissolve in water, which reduces its absorbability.

The research team, led by Yuancai Dong from the A*STAR Institute of Chemical and Engineering Sciences faced two key challenges; the first was to find a way to break the quercetin down into nano-sized particles that would enable it to be better absorbed in the gastrointestinal tract, and the second was to stabilize the nanoparticles so they did not clump together into a less soluble form.

"There are some publications in the literature about the preparation of quercetin nanoparticles, but these nanoparticles become highly agglomerated immediately after formation and/or during the drying process, so their benefits are severely diminished," says Dong

To solve these problems, the team first formed nanoparticles from quercetin in an ethanolic solution by adding water, to which they added insoluble dietary fibers in the form of cellulose or resistant starch. "We used dietary fiber as the matrix former, so the quercetin nanoparticles can be individually deposited on to the fibers' surface, which guarantees the full benefits of a faster dissolution rate," says Dong.

Normally when quercetin nanoparticles are formed, they agglomerate and grow into large particles within minutes. But with the addition of the insoluble dietary fiber, the quercetin nanoparticles bind to the surfaces of the fiber particles instead. The solution was then spray-dried to a powdered form, yielding nanoparticles that were stable and non-clumping.

This technique avoided the clumping problems that had previously thwarted efforts to create quercetin nanoparticles, and the resulting nanoformulation was found to be significantly more soluble than raw quercetin.

As an added benefit, the cellulose and resistant starch brought their own health benefits that further boosted the antioxidant effects of the quercetin.

"We think the quercetin nanoparticle/dietary fiber formulation could be used as a novel food ingredient or as a supplement," Dong says. However given quercetin has a less-than-pleasant taste, further processing is necessary to make it a feasible food additive (see page 27). ■

1. Khor, C. M., Ng, W. K., Chan, K. P. & Dong, Y. Preparation and characterization of quercetin/dietary fiber nanoformulations. *Carbohydrate Polymers* **161,** 109-117 (2017).

Organic chemistry

TEACHING AN OLD DYE NEW TRICKS

FLUORESCENT COMPOUNDS USED FOR BIOLOGICALLY SAFE IMAGING AND LABELING CAN BE MODIFIED ON-DEMAND WITH A SPECIAL MOLECULAR SCAFFOLD.

A library of dye molecules synthesized with a 'modular' chemical approach may lead to improved imaging of live cells.

Radiation in the near-infrared region is invisible, but can deeply penetrate living tissue without damaging it. Dye molecules that produce near-infrared light consequently have valuable applications in medical diagnostics, and A*STAR researchers have developed a synthetic approach that can quickly identify ways to fine-tune their emission properties[1].

One dye known as dihydroxanthene (DHX), although discovered nearly 20 years ago, has attracted a flurry of renewed interest after chemists discovered that small tweaks to a central 'scaffold' — an interlinked framework of three aromatic rings — could switch on bright, near-infrared fluorescence. Current synthetic methods, however, are ill-equipped to access a variety of analogs from a single DHX scaffold. This makes it difficult to comprehend how certain structures can maximize fluorescence.

Jean-Alexandre Richard from A*STAR's Institute of Chemical and Engineering Sciences and co-workers aimed to explore DHX's potential by taking a lead from medicinal chemists, who often generate libraries of potential drug candidates by reacting a common intermediate with a set of reagents. This technique, also known as divergent synthesis, significantly simplifies efforts to screen compounds with desirable properties.

> "OUR APPROACH GIVES ACCESS TO A NUMBER OF MOLECULES WHICH WOULD HAVE BEEN TOO TIME-CONSUMING TO OBTAIN THROUGH PURELY *DE NOVO* SYNTHESIS."

"I saw potential for developing new chemistry to make these dyes because the reported routes were not flexible enough," says Richard. "Our approach gives access to a number of molecules which would have been too time-consuming to obtain through purely *de novo* synthesis."

To build their library of dyes, the team devised a divergent synthesis where two 'chemical handles' were attached to either end of the DHX scaffold. By giving the handles opposing electron-donating and -accepting capabilities, the team envisioned they could create conditions for a wide range of fluorescence levels. They identified that, by using aldehyde and aryl bromide handles, they could produce the initial scaffold in just one step and on a gram scale.

The researchers first systematically replaced the bromine handle with more than 20 amino-based donors, each with slightly different linear, cyclic, and aromatic structures. Then, they directly swapped the aldehyde handle with a charged aromatic ring group to boost DHX's electron-pulling properties. Optical tests of the dye library enabled the team to rank the analogs in terms of their fluorescence intensity — data that may prove critical for tracking different components in complex biosystems.

The team is excited about the dye's new potential. "The DHX dyes will complement the rather small number of near-infrared dyes now available, and encourage people to consider them a viable option for microscopy, diagnostics and imaging," says Richard. ■

1. Ong, M. J. H., Srinivasan, R., Romieu, A. & Richard, J.-A. Divergent synthesis of dihydroxanthene-hemicyanine fused near-infrared fluorophores through the late-stage amination of a bifunctional precursor. *Organic Letters* **18**, 5122–5125 (2016).

OBESITY IN ASIA

Genetics mean that Asian populations are more likely to store fat as dangerous internal fat wrapped around their organs. People with problematic amounts of this internal fat may still look quite slender, but develop serious obesity-related health issues.

Singaporean researchers tackle one of the world's most pressing obesity problems. It's invisible, insidious and very often fatal.

"The obesity you see in Asia is internal, it's visceral," explains Christiani Henry, director of Clinical Nutritional Sciences at A*STAR's Singapore Institute of Clinical Sciences (SICS). "You may look skinny and have a pretty impressive BMI, but also suffer from an insidious metabolic condition." For a variety of genetic reasons, obesity in Asia isn't always as obvious as in the West, but it is a major problem — complicated by the fact that Asian studies come back with very different metabolic responses.

As a result, Asian people who don't fit the typical expectations of what obesity looks like, may still be described by its formal definition — fat accumulation that has an effect on health, such as causing diabetes.

ASIAN METABOLISMS

"The diabetes rate in Asians is very high for example, even among those who have the same body weight as Caucasians," says Shigeki Sugii from A*STAR's Singapore Bioimaging Consortium (SBIC). A greater percentage of Asians with fat wrapped around internal organs, known as visceral fat, is probably why they have a higher prevalence of metabolic and obesity-induced diseases.

The most recent data from the International Diabetes Federation indicates that in 2015, 415 million people had diabetes, more than half of whom lived in Asia. "If you have an HbA1C — glycated haemoglobin, which identifies average plasma glucose concentration — of say seven in Europe and you talk to a doctor," says Henry, "they say, 'Well that's pretty good, well done'." But he says in Asia, people with seven and eight, "are ending up with [diabetes related] renal failure".

About six years ago Henry moved from Oxford in the United Kingdom to set up a center studying metabolism in Singapore, the perfect place to launch studies specific to Asian ethnicities. Singapore is the world's most densely populated city and home to large cohorts of Malay, Chinese and Indian residents — which means that Henry's scientists at the SICS Centre for Clinical Nutritional Sciences are leaders in the study of Asian metabolic responses.

Asian groups across the board — whether Malay, Indian or Chinese — says Henry, all have a significantly higher glycaemic response to carbohydrates than western groups. Glycaemic responses are the effect a meal has on your blood sugar and insulin levels, which can increase your fat accumulation from carbohydrates and heighten the risk of developing diabetes, one of the most serious consequences of obesity. "So you can't use the normal BMI cut-off to articulate [an Asian person's] risk of getting Type II diabetes," says Henry.

Diabetes dramatically increases the risk of cardiovascular problems, nerve damage (neuropathy) and kidney damage (nephropathy), among other things — all expensive burdens on healthcare systems. Henry says increasing knowledge

"You may look skinny and have a pretty impressive BMI, but also suffer from an insidious metabolic condition."

about Asian metabolic responses will change what foods governments advocate for Asian populations. High carbohydrate diets send Asian insulin levels "through the roof" says Henry. Rice, in particular is a problem. "In Asia people are eating up to 700 grams of cooked rice in a day, sometimes at breakfast, lunch and supper... We need to spend much more resources and money to look at food competence as we have done with pharma, because how on earth are we going to manage up to 50 million people who have Type II diabetes in China alone?"

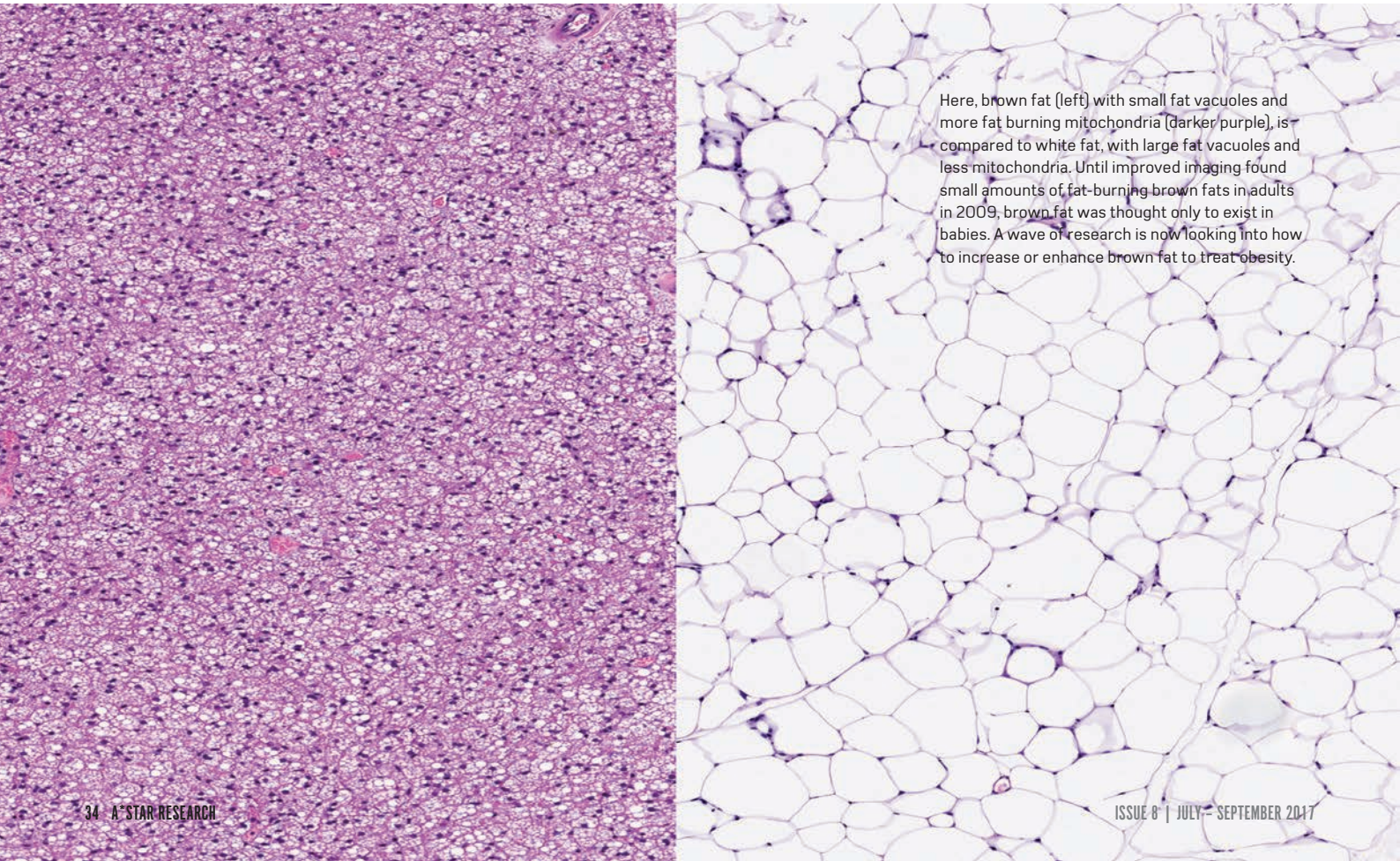
The classical nutrition paradigms say we should eat less fat, therefore more carbohydrate, Henry says. "That may be the worst thing for us to advise Asians because high carbohydrate, high glycaemic index foods may be the reason why people in Asia, who are on a very low fat diet are highly susceptible to Type II diabetes and obesity."

GOOD FATS – WHITE, BROWN AND BEIGE

But, tackling obesity could become even easier. Feng Xu, who now works for the A*STAR Institute of Molecular and Cell Biology (IMCB), is another researcher who has moved across the world, in this case from California, to work at A*STAR's biomedical research hub. He's focused on one of obesity's hottest topics, brown and beige fat.

Unlike white fat, whose primary role is to store energy, brown fat is a rarer type of fat that burns energy for heat when a person is even slightly cold — a process known as thermogenesis. Once thought to be present only in babies, brown fat was re-discovered in adult humans in 2009 by improved PET-CT scan sensitivity. In the flurry of research since, it has been found that white fats can also be browned by an increase in their internal fat burning engines, mitochondria — making them more like brown fats. These have been dubbed beige fats.

Weight-loss medications have had a bad name since the detrimental effects of amphetamine — notably heart attacks — forced the US Food and Drug Administration to remove them from the US market in the 1960s. However, treatments that enhance



Here, brown fat (left) with small fat vacuoles and more fat burning mitochondria (darker purple), is compared to white fat, with large fat vacuoles and less mitochondria. Until improved imaging found small amounts of fat-burning brown fats in adults in 2009, brown fat was thought only to exist in babies. A wave of research is now looking into how to increase or enhance brown fat to treat obesity.

THE SUBTLETIES OF SMELL, TEXTURE AND TASTE

Tackling obesity often starts with looking at the foods you eat and how you eat them. Ciarán Forde is a principal investigator at the Clinical Nutrition Research Center (CNRC) where he and his team have been studying how Singaporean children eat, and how this relates to energy intake and body composition.

Their research, which was published earlier this year, highlights that children who eat with larger bites that are chewed less tend to consume more energy within meals, and have higher fat mass and body weight. The team describe this 'obesogenic eating' style as an opportunity to intervene and change eating behaviours using what they call a 'food-based' approach.

"The sensory properties of foods can stimulate food-related behaviors and directly influence energy intake over time" says Forde. Food texture, for example, has a direct effect on bite size and chew rate and can influence the calories consumed during a meal through its effect on eating rate. Odours can stimulate sensory-specific appetites and people receive signals on nutrient density from the taste quality and intensity of their food.

His team looked into ways of using sensory experiences to influence calorie selection and intake, and have developed novel approaches to reducing calorie density and maintaining satisfaction using approaches like slowing eating rates with harder, more textured foods.

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brown fat could be akin to the magic bullets these once claimed to be, as increased brown fat activity may improve our bodies' capacity to burn fat. Recently, Xu used epigenomic profiling and bioinformatic analysis to search for novel regulators that promote brown fat's energy expenditure. In addition to multiple protein factors identified in the study, he also discovered a micro-RNA known as miR-32, which enhances brown fat activity. In the study published in *Cell Reports* in May, Xu and co-workers found that miR-32 is significantly induced in brown fat when exposed to the cold. And up-regulation of miR-32 leads to increased production and secretion of a circulating factor called FGF21 from brown fat, which further promotes white fat browning.

This study provides the first example of a brown fat microRNA that enhances subcutaneous white fat browning through a long-range effect (mediated by FGF21). In short, with a better understanding of this process Xu thinks "there is a potential for miRNA-32 mimics to be explored for their function in promoting thermogenic activity in the human brown fat to enhance the fat burning effect".

Shigeki Sugii, group leader of the Fat Metabolism and Stem Cell Group at SBIC also wants to promote brown fat's effects and uses stem cells to study and target visceral fat – the 'bad fat' that heightens metabolic problems. Earlier in his career he worked on a small molecule drugs. "At that time I felt the limitations of small molecule in drugs particularly for metabolic diseases, because diabetes is a chronic disease and you take the drugs for a long time," says Sugii. Most drugs are made from small molecules, as their size lets them diffuse across cell membranes to effect bodily functions, but, cautions Sugii, these molecules often have side-effects.

Sugii instead wants to improve visceral fat function by inserting brown/beige fats made from stem cells into it — making it more like the fat just under the skin (subcutaneous fat), which is known to have more beige fats. To do this he's currently working on improving the method to make stem cells into beige fat (and to image this process). The long-term goal is definitely to have people use stem cells to treat obesity. "It's more effective and it can be more targeted," he says.

SHORT- AND LONG-TERM SOLUTIONS

In another study completed recently, Xu and co-workers found narciclasine, a natural compound purified from wild daffodils, has the potential to be developed into a novel anti-obesity drug. "Plant extracts containing this compound were used 2,000 years ago by Greek people treating tumors. From literature we knew that many cancer drugs changed the metabolism in cancer patients, so we thought that maybe narciclasine could also make a change in the metabolism," says Xu. In this study published in February, his team fed the drug to mice being fed excessive calories and found that the compound prevents the mice from getting obese by increasing energy expenditure. Moreover, narciclasine promotes fat clearance from peripheral metaboloic tissues, improves blood metabolic parameters, and protects these mice from the loss of voluntary physical activity. Further investigation suggested that narciclasine achieves these beneficial effects by promoting fatty acid consumption in the skeletal muscle.

Henry points out that one of the most obvious issues with obesity research in Asia is that most of the studies to date come from the West, and do not focus on the Asian physique. This is coupled with the fact that increased living standards and consumption across the region have accelerated the occurrence of obesity.

However, there is plenty of work in the pipeline — Asia is a huge market, says Henry, and many of the world's top companies are already investing a lot of money in understanding and solving the region's unique issues.

For references, visit the online version of this article at:
https://www.research.a-star.edu.sg/feature-and-innovation/7757/clever-fats-weighing-in-on-asia-s-obesity-issue

Microwaves could boost the speed and energy efficiency of hard drive memory.

Materials

HARD DRIVE BOOST COMES IN LAYERS OF IRON AND COBALT

THIN LAYERS OF IRON AND COBALT SHOW PROMISE AS MATERIAL FOR FAST, LOW-ENERGY HARD DRIVES.

A*STAR researchers have created a promising new material from thin layers of iron and cobalt that could enable magnetic recording technologies such as hard drives to be boosted with microwaves[1].

Zhou Tiejun, Chung Hong Jing and colleagues at the A*STAR Data Storage Institute fine-tuned both the magnetic properties and the microwave response in the iron and cobalt thin layers, creating an ideal material to drive a tiny quantum-powered microwave generator called a spin torque oscillator.

The team had previously studied layers of cobalt and iridium and found a surprising magnetic irregularity — the material strongly preferred having its magnetic field aligned in one particular direction, a property known as magnetic anisotropy[2]. With careful alignment of the material, its anisotropy would make it easier to magnetize and demagnetize.

In this new work, the team found that sandwiching cobalt with iron, instead of iridium, produced stronger magnetic anisotropy and had superior microwave performance.

Microwaves generated by a spin torque oscillator embedded in the read-write head of a hard drive would make writing the data more energy efficient, Chung said.

"The microwaves effectively lower the energy barrier for flipping the direction of the magnetic domains," says Chung.

The microwave signal would aid the switching of magnetization required to write data to a hard drive by setting the magnetic fields of the atoms in the hard drive weaving in circles, in the same way that a spinning

top wobbles in circles, an effect known as precession. The cobalt-iridium stack lost the microwave energy quickly, like a top spinning on a thick carpet, an effect known as damping. However, in the cobalt-iron stack, the damping was much lower, like a top spinning on a hard polished floor.

The breakthrough came from the team's work in separately engineering the magnetic and microwave properties of the stack, said Chung.

"We take a lot of care to achieve the desired interfacial quality of the layers. Control at the nanometer level is utterly important," he said.

The team tested more than 30 combinations of materials, first exploring the effect of layer thickness, annealing temperature and sputtering rate and temperature. Finally, they tested them in a full stack configuration, concluding cobalt and iron in equal layers of 0.625 nanometers thickness was optimal.

Chung says there is much work still to be done to bring this technology to fruition.

"It's difficult, because of the complexity of the material design and the challenges of integrating the spin torque oscillator into the magnetic read-write head." ■

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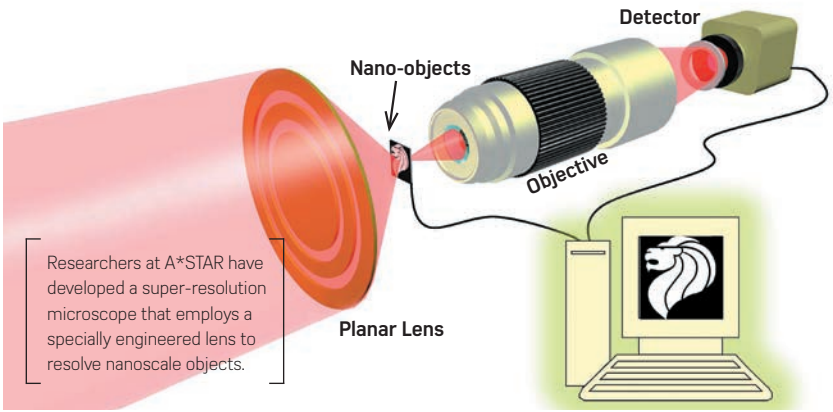
Microscopy

PEERING INTO THE NANOSCALE

A MICROSCOPE WITH A SPECIALLY ENGINEERED LENS OVERCOMES A FUNDAMENTAL LIMITATION.

A*STAR researchers and collaborators at the National University of Singapore have developed a non-invasive optical microscope that can image smaller objects than conventional microscopes, and does not require samples to be dyed[1].

It is hard to overestimate the incredible contribution the humble optical microscope has made to science and industry. But microscopes suffer from a fundamental limitation that prevents them from distinguishing between two objects that are closer than about 200 nanometers. In recent years, various ingenious ways to overcome this limit have been demonstrated, but most of them require either placing the lens extremely close to the sample — both of which could be damaged during focusing — or staining the sample with fluorescent dyes, reducing the usefulness of these so-called super-resolution microscopes.



Researchers at A*STAR have developed a super-resolution microscope that employs a specially engineered lens to resolve nanoscale objects.

Now, Jinghua Teng of the A*STAR Institute of Materials Research and Engineering and colleagues have developed a super-resolution microscope that can distinguish objects separated by just 65 nanometers and does not suffer from either disadvantage.

The microscope has a specially engineered lens, known as a supercritical lens. This flat lens has transparent concentric rings at certain radii and focuses down to a much narrower spot than a conventional lens. By using this lens to focus a laser beam and then scanning the focused beam across a sample, it is possible to build up a high-resolution image of the sample. While other research groups have fabricated supercritical lenses in the past five years, they have various drawbacks. Teng and his team have overcome these disadvantages through improved lens design based on computer simulations.

Besides enabling super-resolution imaging, the lens has several other important advantages. It is easy and inexpensive to make because, unlike previous supercritical lenses, it has micrometer-scale features rather than nanometer-scale features. It also has a long, needle-like focal region, meaning that samples will remain in focus even if they move slightly up or down relative to the lenses. Furthermore, the distance between the lens and the sample is about ten times greater than that for previous supercritical

lenses. Finally, since the imaging process is completely physical and captured in real time, there is no need for special sample preparation or mathematical post-processing of images, making the microscope quick and easy to use.

The team compared the performance of their microscope with those of a conventional optical microscope and a confocal laser scanning microscope, and found that theirs had superior resolution to both.

"This technique is highly attractive for developing the next generation of confocal laser scanning microscopes. There are huge potentials for planar-lens technology in general," notes Teng. "We hope to commercialize the planar-lens technology within three to five years. We're already having discussions with optic companies," he adds. The researchers are working on optimizing the specifications of their microscope in preparation for commercialization. ■

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Nanofabrication

A SLICK ROUTE TO SMART SURFACES

CONTROLLED RUPTURING OF THIN FILMS CAN MAKE LOW-COST NANOPATTERNED ARRAYS FOR SOLAR CELLS AND BIOMOLECULAR DETECTION.

The phenomenon of 'dewetting' — usually considered a nuisance as it causes solids to bead up into islands, much like raindrops on glass — has been harnessed for a useful application. An A*STAR-led team has clarified how dewetting can assemble arrays of 3D nanostructures for applications including single molecule sensing[1].

Solid state films freshly applied to microelectronic devices sometimes split apart at temperatures much lower than typical melting points, due to the high energy at the interface between the film and substrate. This dewetting effect is increasingly problematic at nanoscale film dimensions; however it has also inspired researchers looking for an easy way to produce patterned substrates.

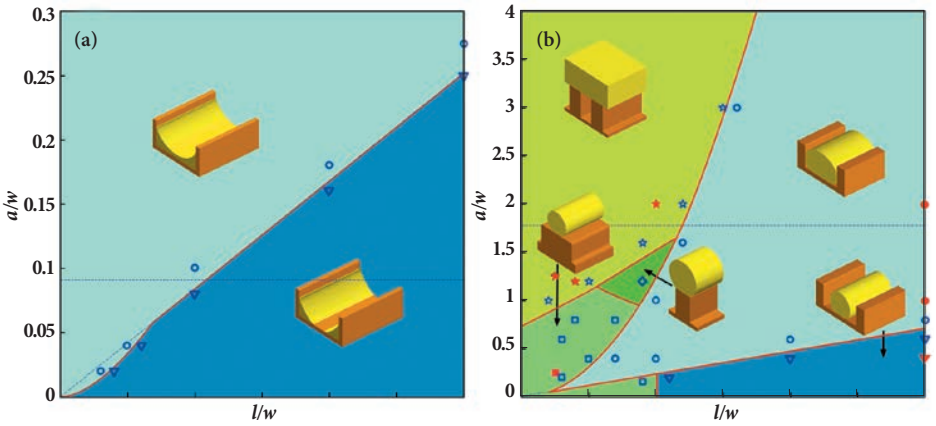
Liangxing Lu from the A*STAR Institute of High Performance Computing and co-workers recently demonstrated that metal films can be transformed into 'nano-aperture' arrays — tiny pores with dimensions controllable down to 10 nanometers — by performing dewetting on surface templates containing 3D ridges and ripples. However, the team found that the templates only produced nano-apertures from metal films of a certain thickness; otherwise, random nanodot features appeared.

"Many factors influence the dewetting process, and also there are many types of equilibrium structures," says Lu. "Finding the conditions for select morphologies is complex and difficult."

To use dewetting for other nanostructure shapes, Lu and colleagues developed a custom algorithm to simulate solid state dewetting. Their technique calculates all possible nanopatterns for a dewetting film on a template and spots the lowest energy configuration. Then, diffusion calculations expose how movements between adjacent nano-islands lower the system's total free energy.

"This model ignores the detailed kinetics, and instead analyzes the diffusion paths of equilibrium morphologies on a given substrate," explains Lu. "The only driving forces are the surface and interface energies, which simplifies the problem."

Through their calculations, the researchers produced detailed descriptions of droplet



Researchers have developed a recipe book of different nanopatterned surfaces possible by combining dewetting techniques with 3D surface templates (as above).

coalescence inside pit-shaped templates, and beading on top of table-like 'mesa' templates. Then, they generated phase diagrams that identified possible dewetting behavior on differently shaped templates — guidelines that proved useful for fabrication trials.

Collaborators at A*STAR's Institute of Materials and Research Engineering verified this analytical approach by coating 100-nanometer high mesa templates with gold films, and then induced dewetting by heating the substrate. With electron microscopy images, they captured gold nanopatterns that matched their phase simulations, with only one exception: defects, such as grain boundaries, disrupted the natural dewetting patterns.

Lu believes that such fundamental fabrication insights could help optimize dewetting techniques for metallic interconnects and gratings, as well as the growth of special morphologies, such as nanowires. ■

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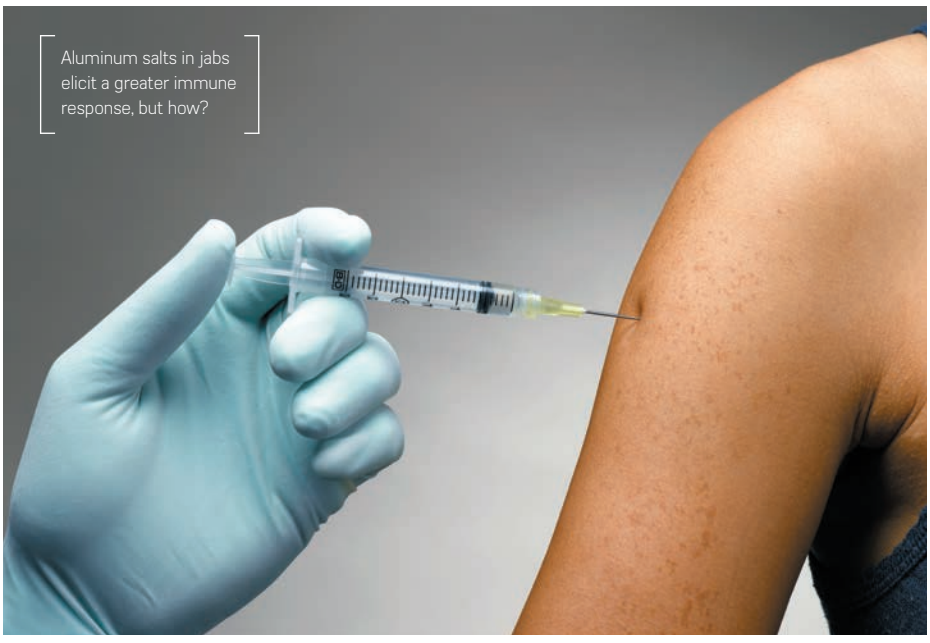
Immunology

THE "IMMUNOLOGIST'S DIRTY LITTLE SECRET"

STUDYING IMMUNE RESPONSE TO ALUMINUM SALTS CAN EXPLAIN HOW THESE CHEMICALS BOOST VACCINE'S EFFICACY.

Adjuvants are often included in vaccines to stimulate the immune system and make a vaccine more effective. Now an A*STAR team, led by Alessandra Mortellaro from the Singapore Immunology Network, has explained a new immune pathway of a commonly used vaccine adjuvant, aluminum salts or 'alum'.

Components of disease-causing microorganisms contained in vaccines are not always sufficient to elicit a strong immune response. In some cases, unrelated chemicals, called adjuvants, are needed to further stimulate the immune system. The A*STAR team has taken up the challenge of explaining this enigma, known as the "immunologist's dirty little secret".



Aluminum salts in jabs elicit a greater immune response, but how?

The immunity-boosting effect of alum was discovered in the 1920s: scientists in London found that aluminum potassium sulfate enhanced the efficacy of diphtheria vaccines considerably. Nowadays, alum is included in inoculations against various diseases, including common ones such as seasonal flu, tetanus and human papillomavirus infection. Paradoxically, despite the fact that millions of doses of aluminum-containing jabs have helped prevent and eradicate several pathologies, the details of alum's mechanism of action are not fully confirmed.

Mortellaro's team discovered that alum triggers immune cells called dendritic cells (DCs), to release IL-2 proteins. These act as a bridge between innate immunity and immunological memory. The former defends the organism against any foreign substances entering the body, while the latter is specific for a certain infectious agent and can quickly detect and attack it upon subsequent encounters.

By injecting an alum-adjuvated vaccine to mice which are either able or unable to produce DC-derived IL2, the team found that this protein is required to spark immune protection and memory against the vaccination's target.

"We found that the release of DC-derived IL-2, promoted by alum, produces the typical signs of an efficient long-term immunization, where white blood T cells help other immune cells (B cells) to differentiate into antibody-producing cells," explains Mortellaro. Specifically, the researchers found an increase in both in the number of $CD4^+$ T cells and of antibodies specific for the antigen present in the vaccine.

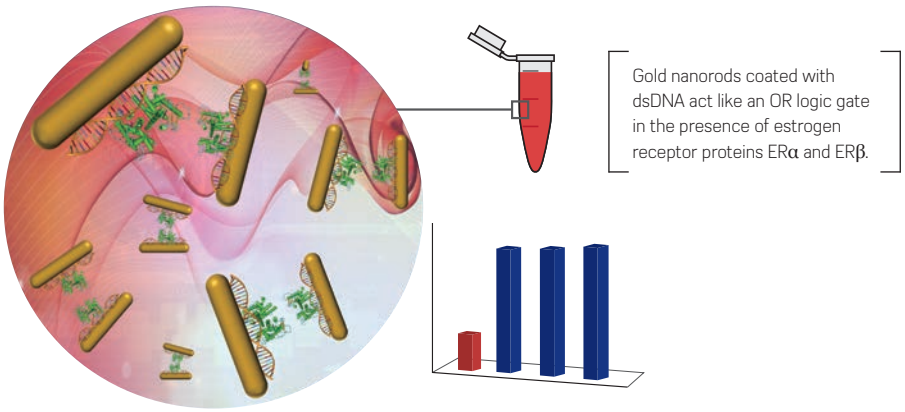
The release of DC-specific IL-2 is the last step of a molecular pathway, of which A*STAR scientists clarified the specifics. "It is an immune pathway shared by mouse and man, so these findings on alum and mouse immunity could be translated into the clinic," Mortellaro points out. "Moreover, we can leverage the knowledge about this pathway to improve vaccine formulation and development, and to test whether new adjuvants and alum alternatives have the same effect on DCs." ■

1. Khameneh, H. J., Ho, A. W., Spreafico, R., Derks, H., Quek, H. Q. & Mortellaro, A. The Syk–NFAT–IL-2 pathway in dendritic cells is required for optimal sterile immunity elicited by alum adjuvants. *The Journal of Immunology* **198**, 196–204 (2017).

Biocomputing

DNA GIVES A LOGICAL SOLUTION

A SOLUTION OF DNA AND GOLD NANORODS CAPABLE OF SIX FUNDAMENTAL LOGIC OPERATIONS DEMONSTRATES THE POTENTIAL OF MOLECULAR BIOCOMPUTING



Gold nanorods coated with dsDNA act like an OR logic gate in the presence of estrogen receptor proteins ERα and ERβ.

By adding strands of DNA to a solution containing gold nanorods, A*STAR researchers have created a remarkably simple system that can 'compute' basic logic operations like OR and NOT in response to specific molecular inputs[1]. This has potential applications in rapid and complex diagnostic systems.

Clinical diagnostics often rely on the detection of pathogens and disease biomarkers from samples of blood or other biological fluids from patients. Most such tests produce a simple 'true' or 'false' result for the presence of a single biomarker. The ability to perform logic operations such as AND and OR for two or more biomarkers could greatly increase the diagnostic power of such tests. Progress in building biomolecular logic gates is hampered by the complex and chemically demanding modifications required to produce practical logic systems.

Xiao Di Su and colleagues from the A*STAR Institute of Materials Research and Engineering and University College London have devised a highly versatile and reliable diagnostic system using gold nanorods, DNA and proteins, that is both easy to create and offers the potential for sophisticated logic-based computing operations.

"We have taken human gene regulation, one of the most precise mechanisms in nature, and applied it to develop the basis of a new technology in the field of biocomputing," says Su.

Gold nanorods absorb light at specific wavelengths determined by the rods' dimensions, but the degree of absorption is controlled by the aggregation of the nanorods in solution. Su and her colleagues found that when double-stranded DNA (dsDNA) was added to the nanorod solution, the aggregation of the nanorods could be reliably controlled by the dsDNA concentration.

To demonstrate the system, the researchers created the solution using DNA segments containing the DNA sequence for estrogen receptor (ER) elements, which would allow the system to respond to the addition of ER proteins. They found that the system could be configured to give an OR result — a change from a 'low' to 'high' absorbance level when one or both of the two different ER variants (ERα and ERβ) were added to

the solution — as well as a NOT result in response to dsDNA addition. The team then demonstrated other logic functions (IMPLY, FALSE, TRUE and BUFFER), which when arranged in series formed the basis for more complex logic operations.

"This is a simple, yet highly versatile platform that does not rely on nanomaterial functionalization or other complicated fabrication methods, and demonstrates the huge potential of nature-inspired applications using biological binding events to manipulate the optical properties of nanomaterials," says Su. ■

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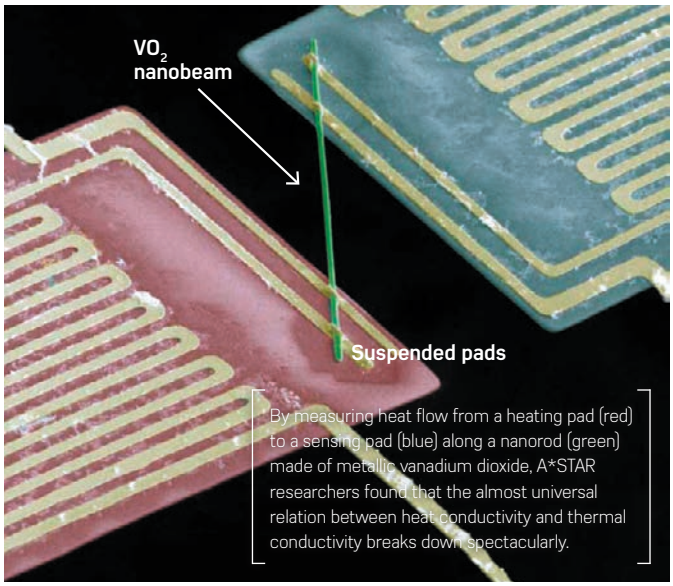
Solid-state physics

METAL BUCKS THE TREND

THE VIRTUALLY UNIVERSAL RELATIONSHIP IN METALS BETWEEN HEAT AND ELECTRICAL CONDUCTIVITY BREAKS DOWN SPECTACULARLY IN VANADIUM DIOXIDE.



By measuring heat flow from a heating pad (red) to a sensing pad (blue) along a nanorod (green) made of metallic vanadium dioxide, A*STAR researchers found that the almost universal relation between heat conductivity and thermal conductivity breaks down spectacularly.

An A*STAR researcher has, together with an international team, uncovered an exception to the longstanding rule that effective heat-conducting metals are also good conduits for electricity[1]. This anomaly could eventually be harnessed in thermoelectric devices that convert waste heat from appliances and engines into useful electric power.

Metals conduct both heat and electricity, which is why they are used to make frying pans as well as electrical wires. This is because electrons can flow freely in metals, carrying thermal energy and electrical charge with them as they move.

This phenomenon is so universal that it has been enshrined in an empirical rule known as the Wiedemann–Franz law, which was discovered more than 150 years ago. While some metals that break this law have been found in recent years, these exceptions usually occur at very low temperatures close to absolute zero, making them impractical for use in applications such as thermoelectric devices and thermal switches.

Now, Kedar Hippalgaonkar at the A*STAR Institute of Materials Research and Engineering and collaborators in an international team have found a large deviation from this law at temperatures above room temperature. Specifically, by measuring heat flow along a vanadium dioxide (VO_2) nanorod, they discovered that the thermal conductivity attributable to the electrons in the material is ten times lower than that predicted by the Wiedemann–Franz law.

The effect was observed in the temperature range –33 to 67 degrees Celsius. Above 67 degrees Celsius, vanadium dioxide suddenly switches from being an electrical insulator to a conductor, accompanied by a small change in its crystal structure.

"This deviation is only possible because of new physics that govern the motion of collective electrons in this unique class of materials, which exhibit a similar metal-to-insulator transition," says Hippalgaonkar. "In particular, in metallic vanadium dioxide, electrons flow like a liquid, which manifests as them carrying charge effectively, but not heat." This electron movement contrasts with the more random movement of electrons in a regular metal, which resembles that of molecules in a gas.

It is also possible to tune the heat conduction of vanadium dioxide by introducing tungsten as an impurity. This ability could be exploited in thermal switches.

"Our study puts the spotlight on the class of materials that exhibit a metal-to-insulator transition as well as those harboring correlated electron systems," says Hippalgaonkar. "It's just the beginning. As well as exploring other material systems that provide improved performance compared to this proof-of-concept study, we intend to delve deeper into the physics underlying this phenomenon." ■

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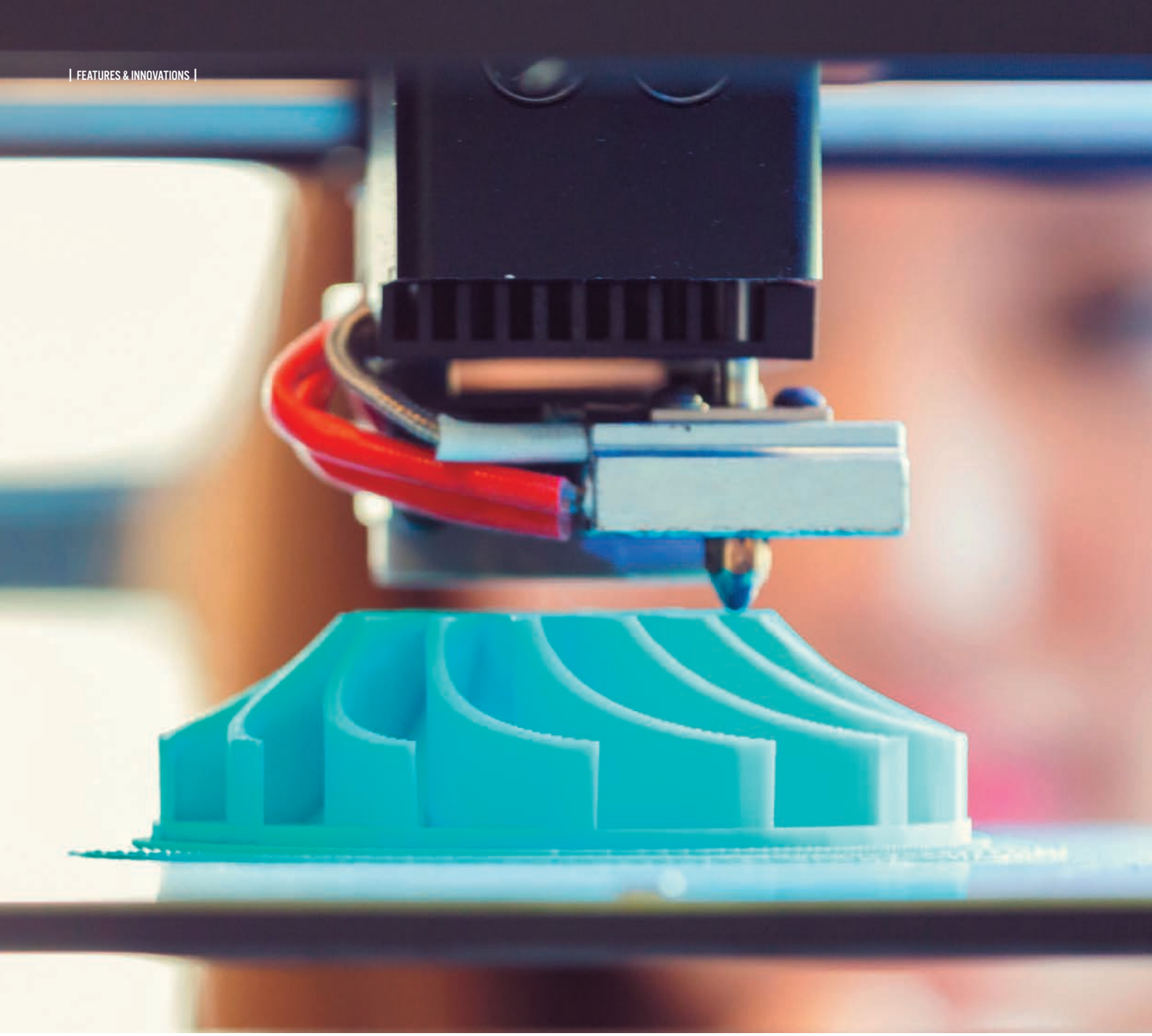


TOWARD THE FACTORIES OF THE FUTURE

The plants of tomorrow will usher in a new era of efficiency, precision and speed, thanks to additive manufacturing.

Automation, robotics, advanced computer-aided design, sensing and diagnostic technologies have revolutionized the modern factory, allowing the building of complex products, from microchips to cars and even airplanes, with unprecedented cost-efficiency, scale and reliability. The modern factory represents the pinnacle of mass production technology, refined over a hundred years or more, to produce identical items for mass consumption at the lowest cost.

Every manufacturing line is uniquely designed and configured for one specific product or component. Setting up an assembly line to produce a new item is a costly and painstaking process, involving iterative design and prototyping within the constraints of available mass production technologies. This includes the creation and configuration of molds, installation and configuration of equipment, designing, testing and troubleshooting processes, and quality testing of the final product. The lengthy process results in long lead times and presents significant obstacles to item customization and the production of small batches or very complex pieces.

Additive manufacturing, known as 3D printing, looks set to turn this traditional model on its head. In contrast to using a dedicated assembly line for a single item, additive manufacturing uses a single high-technology production line to create many different items without the design constraints and startup costs of conventional mass production.

"Additive manufacturing is today mainly used for high-value, high-complexity and low-volume production," says A*STAR's Advanced Remanufacturing and Technology Centre (ARTC) Senior Group Manager, Stuart Wong Sow Long. "With the advancement of technologies such as high-performance computing and sensor technology, however, the industrialization of additive manufacturing is accelerating."

The envisaged 'smart factories' seamlessly bring technologies such as the 'internet of things', cloud computing, advanced robotics, real-time analytics and machine learning together around a versatile additive manufacturing hub, and enable the production of customized products at mass scales, cheaply and quickly.

Stuart notes that "future factories will be able to create products that were not possible before, producing design geometry impossible to manufacture by traditional machining processes." Accordingly, A*STAR has established the Factory of the Future program at the Advanced Remanufacturing and Technology Centre to ensure that Singapore will become one of the key global players in this emerging sector.

THE RISE OF ADDITIVE MANUFACTURING

Although additive manufacturing has been around since the 1980s, the technology has advanced rapidly over the past few years. Many companies in the aerospace, automotive and machine tool production industries have introduced additive manufacturing for rapid prototyping as a cost-effective part of the design process, and it is increasingly being used for custom fabrication in medical applications, as well as dental products such as crowns.

"The product is built up layer by layer," explains Tan Teck Leong from A*STAR's Institute of High Performance Computing (IHPC). "This is in contrast to traditional 'subtractive' manufacturing, where material is removed to form the final product shape."

While thermoplastics are the most widely used materials for additive manufacturing, industrial objects typically need to be produced from metallic alloys, a more advanced manufacturing approach. In metal additive manufacturing, a volume of metal powder is precision heated using a laser or electron beam to fuse the metal into a contiguous whole with almost no material waste. "Future factories will use additive manufacturing because it allows practically any design to be printed, including intricate shapes such as gears, engines and blades," says Leong.

Persistent drawbacks of printing-based methods need to be addressed before it can compete. These drawbacks center around the poorer mechanical properties of printed parts, compared with cast or machined parts.

"Particularly for metallic materials, improving mechanical properties is one of the biggest challenges in additive manufacturing," says Leong. "Many processing factors, including powder morphology, laser power and speed, need to be perfected in order to minimize the formation of defects such as pores and brittle impurities, which can compromise the mechanical properties of the printed product."

GAME-CHANGING SIMULATIONS

One of the most significant ways in which A*STAR researchers are contributing is through simulation of the manufacturing process using fundamental principles of materials physics.

"Physics-based simulations support additive manufacturing in multiple ways," says Guglielmo Vastola from the IHPC. "For large end users of 3D printed parts, simulations show if and where the part could fail during the manufacturing process, and can therefore suggest the optimal way to print the product before sending the part to the printer. The equipment and raw materials are very expensive, so this means direct savings. The manufacturers of 3D printers can also use our simulations to understand the exact functioning of their machines, and to get clues on how to further improve them to increase product quality and reliability."

The IHPC is leading a cross-disciplinary effort to develop a comprehensive simulation toolkit for additive manufacturing, involving fluid dynamics to simulate the flow and melting process of the powder precursors during laser melting, photonics to simulate the interaction between laser and processing material, and materials science to simulate the microstructure, chemical composition and mechanical properties of the final product.

> "The time is right for [additive manufacturing] to enter the shopfloor as the foundation for future factories."

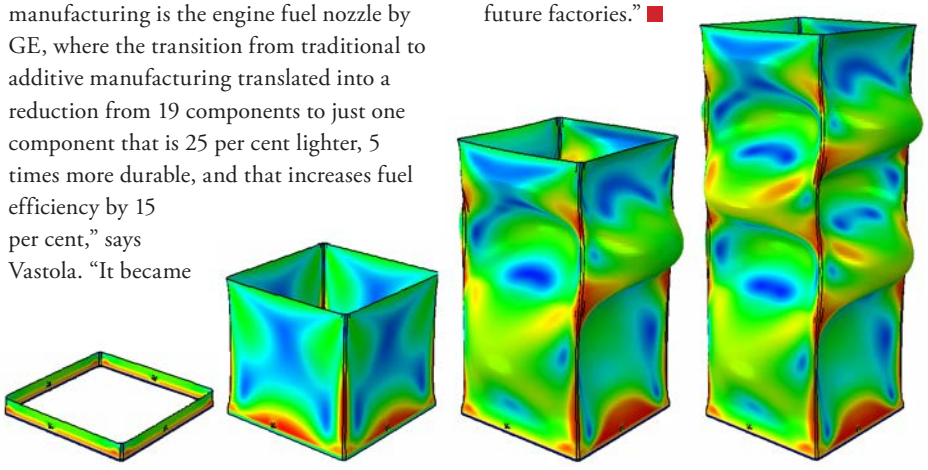
"Modeling and simulation will be the 'killer app' for additive manufacturing," says Vastola. "Because additive manufacturing is such a complex and difficult process, and at the same time so economically important, developing simulation software to support the technology will be a tremendous contribution."

OPPORTUNITIES AND CHALLENGES

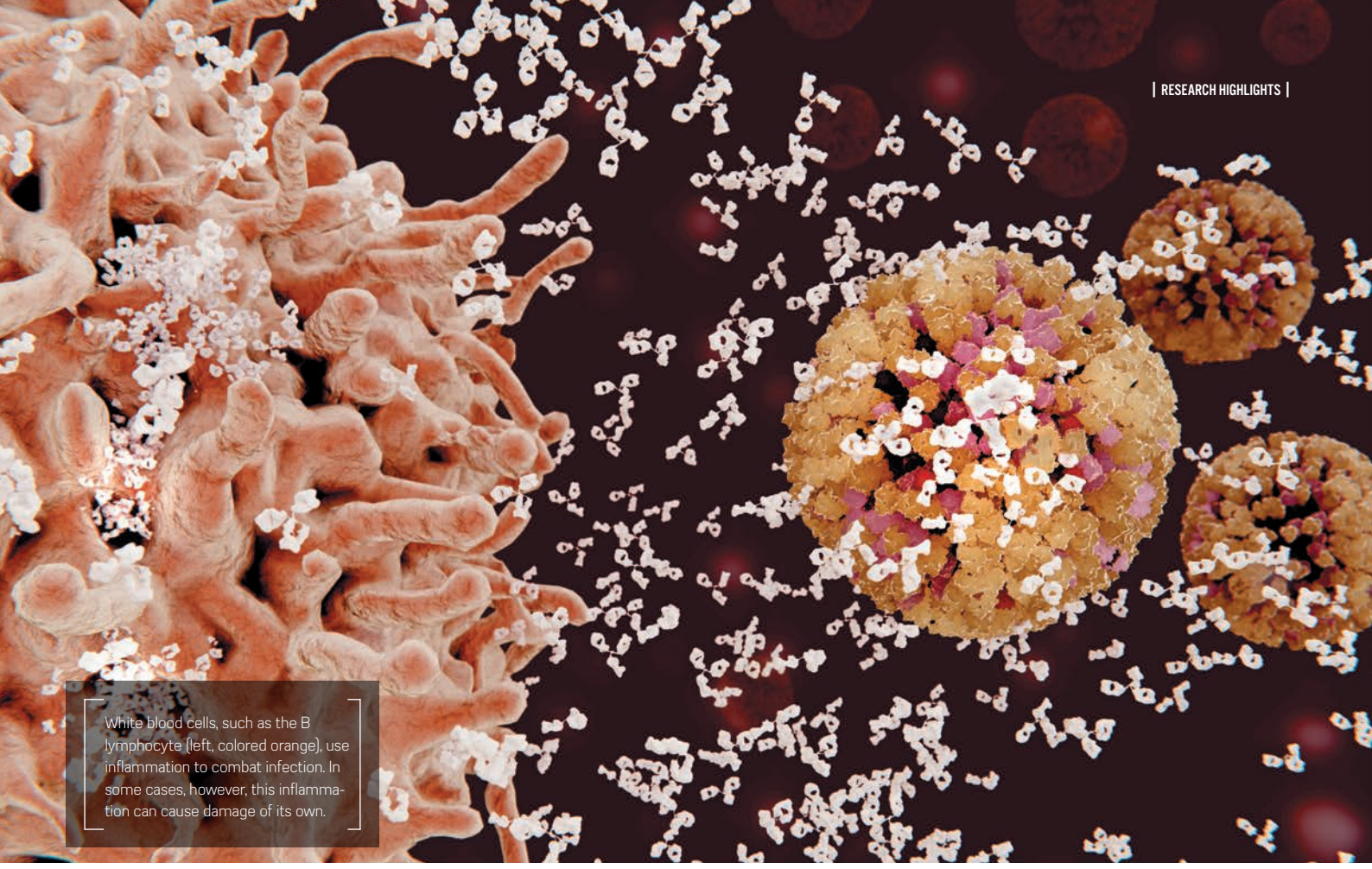
"The classic example of the benefits of additive manufacturing is the engine fuel nozzle by GE, where the transition from traditional to additive manufacturing translated into a reduction from 19 components to just one component that is 25 per cent lighter, 5 times more durable, and that increases fuel efficiency by 15 per cent," says Vastola. "It became possible to produce a complex part like a fuel nozzle easier and faster than before, in a single component run. And the design can be made even more complex if need be, because manufacturing is not a constraint anymore. That is the essence of the opportunity."

To realize these benefits, A*STAR researchers are working hard on the two main obstacles to a commercially-ready future factory concept: repeatability, and standardization. The repeatability of the process is critical. Currently, fabricating the same part using the same material, but on different machines will result in different final product quality. Even starting the part from a different position on the same machine can result in differences in mechanical properties. Standards are under development, but the additive process is very complex and therefore standardization is remains a challenging task.

"In five to ten years, we will see more parts being moved from traditional to additive manufacturing on a purely economic basis," says Vastola. "The GE fuel nozzle can be regarded as the pioneer of many more parts to come. And as the technology becomes more widely adopted, it should move upstream all the way to parts designers. Then, once the freedom of complexity is embraced at the design level, we will start to see truly 'new' parts that simply would have never been thought of before. Additive manufacturing is truly an enabling technology, and the time is right for this technology to enter the shopfloor as the foundation for future factories." ■



A simulation of the additive manufacturing process of a thin-wall structure, showing the distortion experienced by the part as it is being built. Color scale: Red = high residual stress — blue = low residual stress.



White blood cells, such as the B lymphocyte (left, colored orange), use inflammation to combat infection. In some cases, however, this inflammation can cause damage of its own.

Inflammatory disease

A CAUTIONARY TALE FOR IMMUNOTHERAPY

A FAILED TREATMENT FOR CHIKUNGUNYA HIGHLIGHTS THE NEED FOR EXTREME CAUTION WHEN MANIPULATING THE IMMUNE SYSTEM.

A*STAR researchers found that a treatment they developed for chikungunya, a disease causing painful joint inflammation, was successful when administered closely preceding an infection, but made things much worse if subjects — in this case, mice — were already in the grip of the virus.

Lisa F. P. Ng, who has studied chikungunya virus immunity and infection for the past decade, and her team from A*STAR's Singapore Immunology Network (SIgN) had previously shown a specific antibody complex, IL-2/JES6-1, could prevent chikungunya virus-mediated inflammation when administered before infection[1].

As it is not always feasible to predict an infection and administer prophylaxis, Ng and the team went on to test the treatment on mice already infected with the chikungunya virus[2].

"We hoped to see a similar improvement, but unfortunately the contrary happened. It didn't protect the mice — it made them worse," says Ng.

The antibody complex stimulated the production of regulatory white blood cells called Tregs. Ng and her team, in collaboration with Olaf Rotzschke, a Tregs expert, and Laurent Rénia, an expert in $CD4^+$ T cells, found that, in healthy animals, these cells protected against virus-induced inflammation. If the host was already infected, however, the body had already generated high levels of activated immune cells and adding more Tregs to the mix initiated a cascade that led to hyper-inflammation.

Ng cites another example of immunotherapy gone awry — the 2006 TeGenero/PAREXEL clinical trial disaster which ravaged the organs

of its six healthy volunteers, caused loss of limb and left some participants with lasting damage to their immune systems. "It was terrifying," Ng recalls. "By the time they got to injecting the final participant, the first one had already collapsed."

The ramifications of the SIgN study are clear. "The impact of this research — the take-home message? It's a word of caution," warns Ng. "We've shown here, down to the mechanism of action, why this line of research is dangerous."

She adds, "Modulating the immune system can yield treatment options, but scientists need to take heed: these approaches can have serious adverse effects."

Chikungunya emerged in the 1950s; however, despite decades of reports of sporadic infection in Africa and South-East Asia, it was only thrust into the global research spotlight after a 2005 outbreak in the French territory of Reunion Island. "It's a rarely fatal disease, so priorities were elsewhere for a long time," says Ng. ■

1. Lee, W. W. L., Teo, T-H., Her, Z., Lum, F-M., Kam, Y-W. *et al.* Expanding regulatory T cells alleviates chikungunya virus-induced pathology in mice. *Journal of Virology* **89**, 7893-7904 (2015).
2. Lee, W. W. L., Teo, T-H., Lum, F-M., Andiappan, A. K., Amrun, S. N. *et al.* Virus infection drives IL-2 antibody complexes into pro-inflammatory agonists in mice. *Scientific Reports* **6**, 37603 (2016).

Stem cells

MAKING BLOOD FROM SKIN

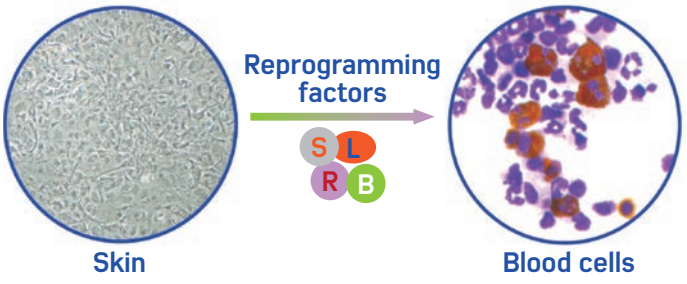
DESPITE BEING VERY DIFFERENT KINDS OF CELLS, SKIN CELLS HAVE BEEN USED TO MAKE BLOOD CELLS IN MICE.

Researchers have long dreamed of producing blood, thereby making donations a thing of the past. Now, thanks to a recent A*STAR study that has successfully made mouse blood and immune cells from skin cells, they are closer to achieving this goal.

"This represents a first step toward the engineering of new human blood cells from skin cells or artificial sources," notes Bing Lim of the A*STAR Genome Institute of Singapore, who led the study.

Blood is in short supply and the demand is likely to increase as populations age and fewer people donate. Medical advances which require large volumes of blood, such as chemotherapy, organ transplants and heart surgery, also draw on dwindling stocks.

Previous studies generated new mouse blood cells from skin cells, but the blood cells produced lasted only two weeks after being injected back into mice. Now, Lim and his co-workers have succeeded in producing artificially skin-derived blood cells that can last up to four months in mice. They identified a cocktail of four transcription factors — proteins involved in the translation of DNA into RNA and that turn nearby genes on and off by binding to DNA — that 'reprogram' mouse skin, causing it to develop into different types of blood and immune cells.



A*STAR researchers have identified four transcription factors that can 'reprogram' mouse fibroblast cells, causing them to become different types of blood and immune cells.

> "THIS REPRESENTS A FIRST STEP TOWARD THE ENGINEERING OF NEW HUMAN BLOOD CELLS FROM SKIN CELLS OR ARTIFICIAL SOURCES."

"We've been interested in finding out whether it might be possible to rewrite the identity of cells, specifically to turn skin into blood," says Lim. "Skin cells and blood cells couldn't be more different. It's amazing that we have artificially generated blood cells from mouse skin cells."

The demonstration has implications beyond just the artificial production of blood. "This is not only of practical importance for regenerative medicine in terms of potentially yielding a source of new blood or immune cells," says Lim. "It is also interesting from a fundamental biological perspective that two very different cells like skin and blood can be interconverted."

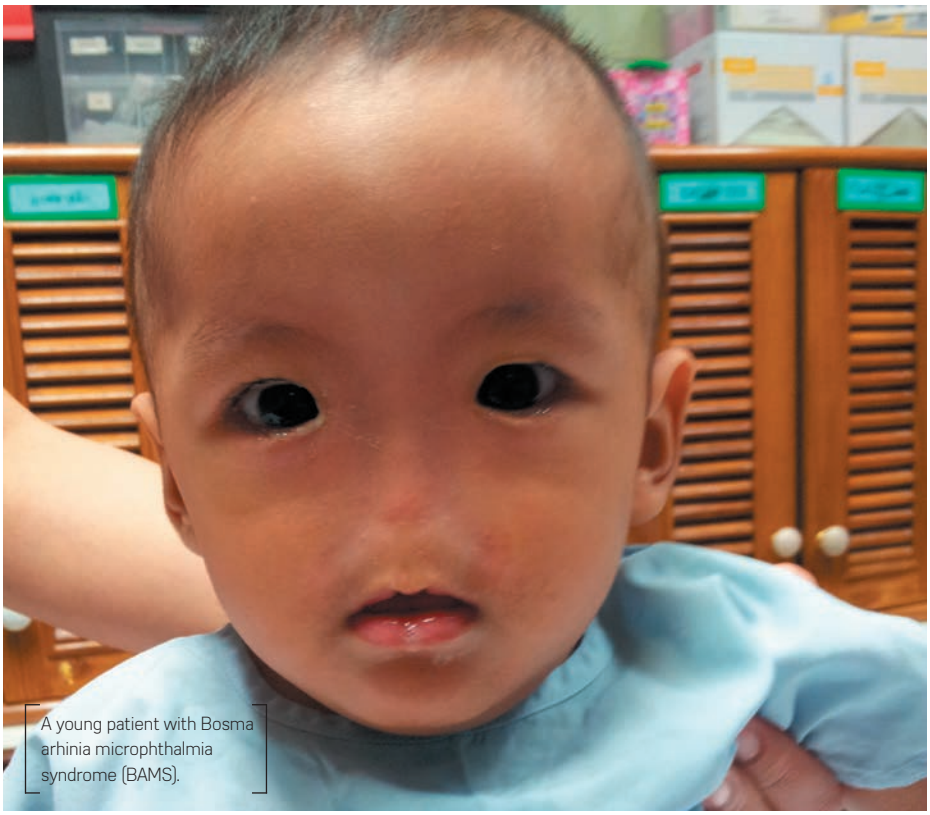
The researchers intend to continue to improve their protocol in mice, but they have their sights fixed on extending the technique to human cells so that it can provide a platform to produce new human blood and immune cells. ■

1. Cheng, H., Ang, H., Y.-K., EL Farran, C. A., Li, P., Fang, H. T. *et al.* Reprogramming mouse fibroblasts into engraftable myeloerythroid and lymphoid progenitors. *Nature Communications* **7**, 13396 (2016).

Human genetics

MUTATION FOUND IN PATIENTS WITHOUT A NOSE

RESEARCHERS DISCOVER A MASTER REGULATOR OF NOSE DEVELOPMENT.



A young patient with Bosma arhinia microphthalmia syndrome (BAMS).

A mutated gene in patients lacking a nose has been identified by an international team, a first step toward understanding nose development and possible therapies for another condition.

The team, led by researchers from Bruno Reversade's lab at the A*STAR Institute of Medical Biology and the Institute of Molecular and Cell Biology, sequenced DNA from 14 people with Bosma arhinia microphthalmia syndrome (BAMS). BAMS is a congenital condition in which patients are born without a nose, and often with eye defects and stunted release of sexual hormones.

All 14 people had mutations in the gene *SMCHD1*, none of which were present in the parental DNA samples, indicating that they were spontaneous mutations. *SMCHD1* is known to regulate chemical modifications to DNA which silence or repress genes, and this study showed that it is expressed in the developing nasal and optical tissues of mouse embryos.

SMCHD1 is already implicated in a more common disease, facioscapulohumeral muscular dystrophy (FSHD). "FSHD is a very different disease," says Shifeng Xue, a research fellow in the Reversade lab who led the project. "It's an adult onset degenerative disease, whereas BAMS is an embryological developmental disease. The only similarity is that they have mutations in the same gene."

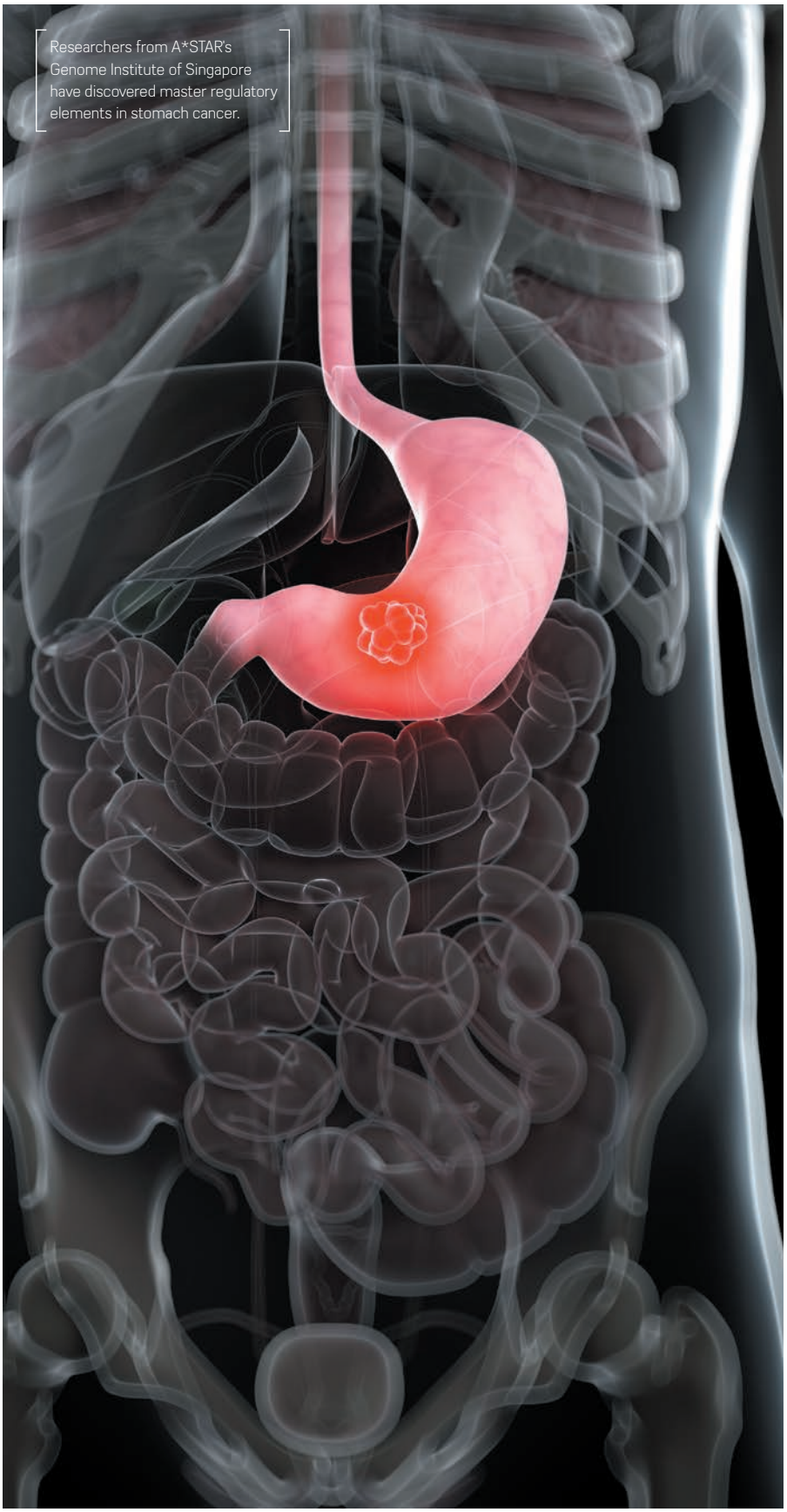
The diseases are also driven by entirely different types of mutations. While FSHD2 results from mutations that knock out *SMCHD1*, the team believes that BAMS is caused by increased activity of the gene. Introducing the mutant gene into frog embryos led to craniofacial defects similar to those of BAMS patients, and the team observed the same defects when they increased expression of the unmutated form of the gene.

While *SMCHD1* is clearly a key gene regulating nose development, its precise function remains a mystery. "*SMCHD1* is a repressor," explains Xue. "We think the gain-of-function mutations cause it to silence more genes than it's supposed to, including genes involved in nose development."

"Finding SMCHD1 gives us a handle to tackle this question," she adds. Developmental genetics research often uses mice as models, but there are no mutant mice lacking a nose; mice cannot mouth breathe, so they die if they are born without a nose. "Now that we know this gene is a master regulator, we can explore its downstream targets and signaling pathways," says Xue.

Reversade calls the discovery "a fascinating example of how rare conditions can provide insights into common diseases," adding that the team hopes to translate their findings into potential FSHD therapies. ■

1. Gordon, C.T., Xue, S. Yigit, G., Filali, H., Chen, K. *et al.* *De novo* mutations in *SMCHD1* cause Bosmia arhinia microphthalmia syndrome and abrogate nasal development. *Nature Genetics* **49**, 249-255 (2017).



Researchers from A*STAR's Genome Institute of Singapore have discovered master regulatory elements in stomach cancer.

Stomach cancer

CANCER ENHANCER SWITCHES

FIRST ROADMAP OF STOMACH CANCER SUPER-ENHANCERS PAVES THE WAY FOR NEW TREATMENTS.

A*STAR researchers have homed in on a potential new way to diagnose and treat stomach cancer, through the mapping of an unprecedented catalog of almost 3,800 super-enhancers from stomach cancer tumor cells.

Gastric or stomach cancer is the world's fifth most common cancer and the third leading cause of cancer death globally, according to Patrick Tan, the Deputy Executive Director of A*STAR's Biomedical Research Council (BMRC).

Tan led the A*STAR Genome Institute of Singapore research team that mapped more than 100 epigenomic profiles from stomach tumors, surgically removed from patients in Singapore, and compared them to normal stomach cells. They validated these profiles using gastric cancer data from scientific institutions in Japan, the United States and South Korea.

Tan says the study is yielding new insights into gastric cancer that have been overlooked by previous approaches.

The epigenome consists of chemical tags that attach to the DNA and instruct it. Tan says, "what makes each cell type different — one cancerous and one not, is really which genes are turned on or off. This is a process we refer to as gene expression."

Tan explains that enhancers are "control circuits in the genome which determine which elements are switched on or off".

Recently, researchers have discovered super-enhancers: clusters of enhancers localized to specific regions of the genome. They have broad and powerful effects on gene expression, and are pivotal to cancer and other disease processes.

Tan says: "When one looks at the gene expression patterns controlled by the super-enhancers, the function of these genes are all related to different traits of cancer, including resistance to cell death and uncontrolled proliferation."

Analyzing stomach cancer and matched normal samples, the research team investigated almost 37,000 enhancers and found 3,759 predicted super-enhancers. The active super-enhancers are recognized by a special DNA tag, known as H3K27ac.

They also studied 848 gastric cancer patients and showed that those with a high level of super-enhancer associated genes had a worse survival rate than other patients.

The research team will now focus on cancer therapies that target factors that allow the super-enhancers to promote gastric cancer growth. They want to develop a method of early diagnosis for stomach cancer by testing for these gastric cancer-specific super-enhancers.

The team plans to open a laboratory that will offer the epigenomic profiling platform they developed, to collaborators investigating other cancers and diseases. ■

1. Ooi, W. F., Xing, M., Xu, C., Yao, X., Ramlee, M. K., Lim, M. C. *et al.* Epigenomic profiling of primary gastric adenocarcinoma reveals super-enhancer heterogeneity, *Nature Communications* **7,** 12983 (2016).

Immunology

EXISTING DRUGS COULD TREAT CHIKUNGUNYA

INSIGHT INTO HOW CHIKUNGUNYA SYMPTOMS ARE TRIGGERED SUGGESTS EXISTING TREATMENTS COULD BE REPURPOSED TO HELP PATIENTS.

Chikungunya virus infection could be treated with autoimmune therapies currently used for other conditions, according to research led by A*STAR scientists.

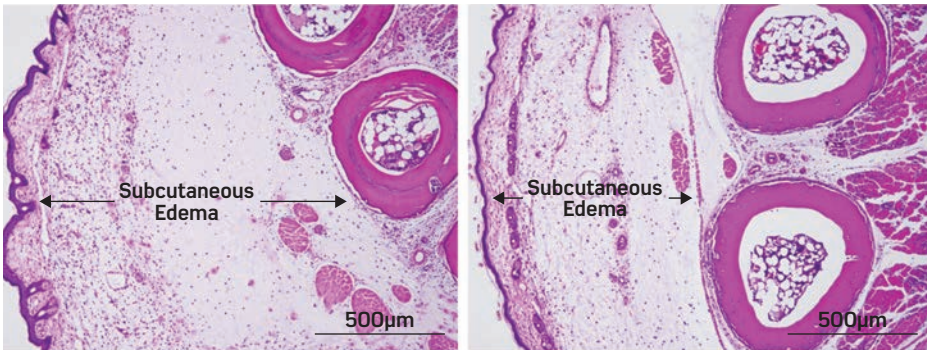
When transmitted to humans by mosquitoes, chikungunya causes high fever, headaches, joint inflammation and debilitating joint pain. Since 2004 there have been major outbreaks in Africa, Asia, the Caribbean, and South and Central America. There are currently no approved treatments.

A group led by Lisa F. P. Ng at the A*STAR Singapore Immunology Network (SIgN) demonstrated in 2013 that $CD4^+$ T cells, which mediate immune system responses, play a central role in triggering chikungunya-induced joint swelling.

To investigate, Ng and Laurent Renia, her colleague at SIgN led the team to transfer $CD4^+$ T cells from both healthy and chikungunya-infected mice into T cell receptor-deficient mice. Only those that received cells from infected donors suffered joint swelling, inflammation and skeletal muscle damage — confirming the key role of these T cells in triggering chikungunya symptoms.

They then carried out proteome wide screening assays in which $CD4^+$ T cells taken from chikungunya-infected mice were tested against all proteins generated by the chikungunya virus.

This identified specific segments in two viral proteins, nsP1 and E2, as those that stimulate $CD4^+$ T cell responses to chikungunya. The discovery could be used to help design vaccines against chikungunya and related viruses.



The degree of subcutaneous edema during Chikungunya infection (left) and the fingolimod treated mice (right) during the peak of joint swelling.

Applying their findings, the researchers treated groups of chikungunya-infected mice with three clinically-approved T cell suppressive drugs. Tissue samples were then visually assessed by histopathologists.

Fingolomid, usually used to prevent multiple sclerosis relapses, successfully reduced joint swelling, inflammation and muscle damage in the rodents. It did so both when given as a prophylactic, and following infection as a standard therapeutic treatment. The other two drugs — cyclosporin A and rapamycin — failed to control the disease symptoms in mice.

Ng and her colleagues stress that the use of fingolomid to treat chikungunya patients with chronic joint pain has yet to be evaluated, but hope their study will lead to further research on whether it and other immunosuppressive drugs could help those with the condition.

"We were pleased with these findings," says Ng. "They demonstrate our previous theory about the pathogenic role of $CD4^+$ T cells was correct, and suggest existing T cell suppressive drugs could provide viable treatment options for patients."

The group believes such drugs could also be used to treat inflammation caused by other insect-spread viruses that is mediated by virus-specific $CD4^+$ T cells. ■

1. Teo, T.-H., Chan, Y.-H., Lee, W. W. L., Lum, F.-M., Amrun, S.N. *et al.* Fingolimod treatment abrogates chikungunya virus-induced arthralgia. *Science Translational Medicine* **9**, eaal1333 (2017)

Polymer chemistry

AN EYE FOR HIGH EXPLOSIVES

FLUORESCENT POLYMER POINTS THE FINGER AT TRACES OF EXPLOSIVE DEVICES.

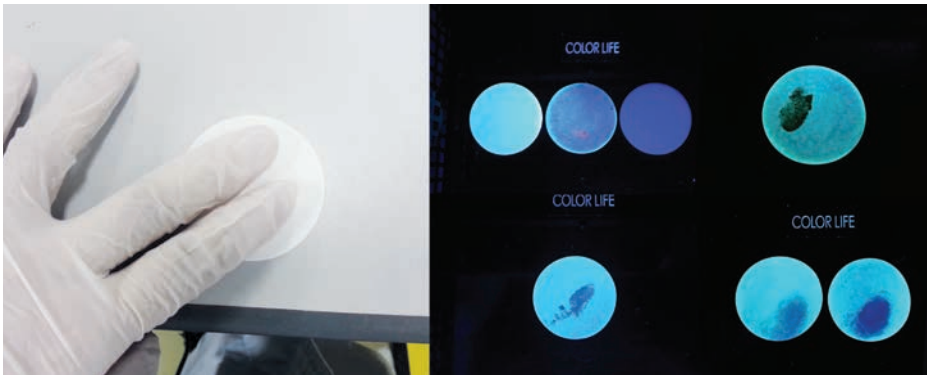
Bomb plots could be thwarted with the help of a portable system for detecting traces of high explosives using fluorescent polymer nanoparticles[1], developed by A*STAR. Coated on to paper, these polymers display an explosive-detection performance far more robust than previous materials with similar properties.

Many methods have been developed to detect traces of high explosives such as trinitrotoluene (TNT), but most require large, lab-based analytical instruments such as UV absorption detectors or mass spectrometers, says Jianwei Xu from the A*STAR Institute of Materials Research and Engineering, who led the work. "We aimed to develop a portable, quick, inexpensive and highly sensitive alternative."

Xu focused on fluorescent polymers whose light emission dims when exposed to vapors of molecules such as TNT that contain 'nitro-groups' — the nitrogen-rich structure that give high explosives their energetic properties.

Although explosive-detecting fluorescent polymers have been developed before, there have been obstacles to their practical use, Xu explains. Some lost part of their fluorescence in solid form through a process called aggregation-caused quenching. Others lacked the necessary porosity for explosives vapor penetration, only retaining an effective response when formed in layers of around 2.5 nanometers — so thin, the sensor would be very difficult to fabricate cheaply.

Xu and his colleagues have been developing polymers that, rather than losing their light emission in the solid state, become brighter, an effect known as aggregation-induced emission (AIE). "Our previous research demonstrated that porous films obtained from AIE active copolymers show a strong response to the vapor of nitro-compounds," says Xu. The team has now developed two new polymers, poly(triphenyl ethene) (PTriPE) and poly (tetraphenyl ethene) (PTPE), designed to maximize AIE, porosity and nitro group sensitivity. The polymers' multiple bulky phenyl groups help keep them rigid and maximize light emission following aggregation.



The polymer-coated polymer readily could detect fingerprints contaminated with traces of four common high explosives, including TNT.

But in the presence of nitro-groups, electron transfer between polymer and analyte rapidly quenches the light.

"Paper sensors fabricated by absorbing the polymer nanoparticles on to filter paper showed high sensitivity for nitro-compound particles," says Xu. PTriPE proved to be the pick of the pair, detecting TNT at a concentration of five parts per billion, even in polymer layers 1,000 nanometers thick.

"This technology could be used at airports, ports and for border control for detecting explosives in any explosive-contaminated containers, clothing, boxes or bags," says Xu.

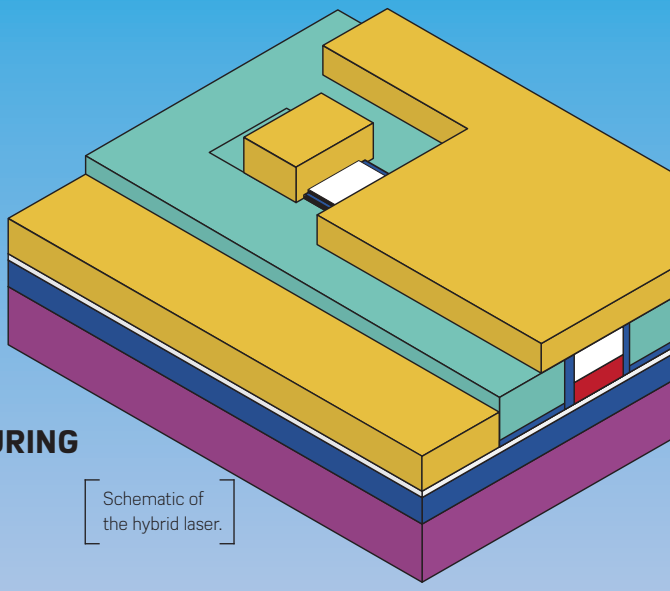
"We are seeking co-operation with industry to commercialize this technology." ■

1. Zhou, H., Wang, X., Lin, T. T., Song, J., Tang, B. Z. & Xu, J. Poly(triphenyl ethene) and poly(tetraphenyl ethene): synthesis, aggregation-induced emission property and application as paper sensors for effective nitro-compounds detection. *Polymer Chemistry* **7**, 6309 (2016).

Materials

BEDDING IN NEW LASERS

A SIMPLE AND VERSATILE TECHNIQUE FOR MANUFACTURING HYBRID LASERS ON DIFFERENT MATERIALS OPENS THE DOOR TO NEW APPLICATIONS FOR PHOTONIC DEVICES.



Schematic of the hybrid laser.

Fabricating hybrid semiconductor lasers on materials other than the commonly used silicon-on-insulator (SOI) substrates has proved challenging. Now, A*STAR researchers have developed an innovative technique that can integrate the lasers on to a range of different materials.[1]

Hybrid lasers combine the light-emitting properties of group III-V semiconductors like gallium arsenide and indium phosphide, with conventional silicon technologies, offering inexpensive photonic and microelectronic devices for application in optical telecommunication systems.

Their range of applications, however, is limited by the poor light-emitting characteristics of the silicon-on-insulator (SOI) wafers mostly used as substrates in the fabrication process. This spurred Doris Keh-Ting Ng and colleagues from the A*STAR Data Storage Institute to develop an innovative technique for bonding III-V lasers on to other substrates, be it silicon, quartz, or metal alloys.

By using an ultrathin layer of silicon oxide to bond the lasers to a silicon substrate, the researchers developed a simpler, safer and more flexible technique than direct bonding, which relies on chemical bonding between the surfaces.

"The challenge is to produce a smooth, extremely thin layer of silicon oxide on the surface of the substrate," explains Ng. "By growing the film on the silicon substrate, but not on the III-V substrate, we greatly reduced the complexity of the process and improved the strength of the bond between the two materials."

After first cleaning the surfaces with an organic solvent, the researchers exposed the surface to an oxygen plasma to increase its adhesive properties. They then initiated the bonding process at ambient temperature by bringing the two substrates slowly together, to reduce the air trapped between them, ensuring a much stronger bond.

The bonding was then completed at relatively low temperatures of around 220 degrees Celsius, allowing the ultrathin layer of silicon oxide to conduct heat between the layers, reducing potential damage to the materials, strengthening the bond and avoiding the need for hazardous chemicals, such as Piranha solution and hydrofluoric acid, used in direct bonding.

The work demonstrates a versatile on-chip laser that can be integrated on to any material platform and could lead to new applications for photonic devices, such as detector-on-chip and modulator-on-chip technologies.

"The low temperature interlayer approach is simpler and much safer than direct bonding, and means that laser manufacturers are not restricted by the choice of substrate," says Ng. ■

1. Lee, C. W., Ng, D. K. T., Ren, M., Fu, Y. H., Kay, A. Y .S. *et al.* Generic heterogeneously integrated III–V lasers-on-chip with metal-coated etched-mirror. *IEEE Journal of Selected Topics in Quantum Electronics* **22**, 1500409 (2016).

VOICES FROM A*STAR

www.research.a-star.edu.sg/blog

*Voices from A*STAR* is a monthly blog published on the *A*STAR Research* website. It features a personal account of the challenges and rewards of a life in science by A*STAR researchers from a range of disciplines. Staff interested in contributing to the *Voices from A*STAR* blog are encouraged to contact the Managing Editor.

Anand Andiappan

Senior Research Scientist, SIgN

"We all know that the genetic code of DNA is made up of the bases A (adenine), T(thymine), G(guanine) and C(cytosine). Just like any language, meaning comes from reading letters in context. To understand DNA, the language of genetics, we need the context of epigenetics."

Keri McCrickerd

Research Fellow, SICS

"If a young child is showing healthy weight gain as they grow, the portion sizes they are consuming day-to-day are probably appropriate for their needs. Here, urging children to finish everything on their plate may be unnecessary and could prevent children attending to their own feelings of hunger and fullness."

NEXT ISSUE

Here's a sneak peek of the material covered in the next issue of *A*STAR Research*

Photonics

INVISIBILITY IS WITHIN SIGHT

The theoretical discovery of transparent particles that break the previously accepted limit of visibility opens a new door in the search for perfect transparency

Immunology

THE ROLE OF CRYSTALS IN THE 'DISEASE OF KINGS'

Gout is associated with unhealthy living, but its underlying mechanism has been a mystery

Glaucoma

EYES OPEN TO A PROTECTIVE MUTATION

A genetic mutation that protects against glaucoma has been uncovered during an international study into exfoliation syndrome

Vaccines

A DENGUE TREATMENT THAT IS ONE 'FOUR' ALL

An antibody that targets all four strains of the virus renews hope for effectively treating and preventing dengue

Agency for
Science, Technology
and Research

The Agency for Science, Technology and Research (A*STAR) is Singapore's lead government agency dedicated to fostering world-class scientific research and talent for a vibrant knowledge-based economy.

A*STAR actively nurtures public-sector research and development in biomedical sciences, physical sciences and engineering, and spurs growth in Singapore's key economic clusters by providing human, intellectual and industrial capital to our partners in industry and the healthcare sector.

A*STAR currently oversees the following research institutes, consortia and centers and supports extramural research with universities, hospital research centers, and other local and international partners.

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Bioinformatics Institute (BII)
Bioprocessing Technology Institute (BTI)
Clinical Imaging Research Centre (CIRC)
Data Storage Institute (DSI)
Experimental Power Grid Centre (EPGC)
Experimental Therapeutics Centre (ETC)
Genome Institute of Singapore (GIS)
Institute of Bioengineering and Nanotechnology (IBN)
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