Biopharmaceutical firm CureVac's RNA printer can rapidly supply mRNA vaccine candidates.

CUREVAC

SEVEN TECHNOLOGIES TO WATCH IN 2021

COVID considerations unsurprisingly dominate the tech developments that could have a big impact in the coming year. **By Esther Landhuis**

This year looks to be a promising one for technology development. From advances in vaccines to olfaction, neuroscience to mass spectrometry, researchers describe the tools and techniques generating excitement in their disciplines.

NICK JACKSON
THERMALLY STABLE VACCINES

At the Coalition for Epidemic Preparedness Innovations (CEPI), a global coalition launched in 2017 to develop vaccinesimp against emerging infectious diseases, we're interested in vaccine technologies that drive speed, scale and access. That includes the speed at which vaccines are proven safe and effective, and how they can be manufactured at scale and delivered to vulnerable populations so that everyone has access.

At no other time has that proven more urgent than in the continuing COVID-19 pandemic, during which messenger RNA vaccines inside lipid nanoparticles have gone from sequence to clinical proof-of-concept to interim analysis in record time. It took less than four months for the biotechnology company Moderna and the drug firm Pfizer to go from sequence to phase I trial, which is incredible when development typically takes years or decades. These vaccines are already being rolled out to the public for emergency use.

But they could be made to work even better. One powerful recent innovation has been the use of ionizable lipids in the nanoparticles that deliver mRNA into cells[1]. The particles remain neutral at physiological pH but when they enter a cell's endosome, they build up charge in the organelle's acidic environment, which then aids the release of its mRNA payload. The next generation of ionizable lipid nanoparticles under development would use a receptor-binding process to target the particles to specific tissues or cell types.

Other innovations are improving access. Some technologies, for example, use sugar molecules to allow efficient freeze-drying without damaging the vaccines' refined structure or formulation, making them easier to store and transport.

Another avenue for increasing vaccine access is developing portable RNA-printing technology. Few countries have the capital and expertise to produce high-quality vaccines at scale, but in February 2019, CEPI invested US$34 million in the biopharmaceutical company CureVac's efforts to develop a fully transportable unit that could enable low-resource areas to produce their own mRNA vaccines. This sort of innovation is going to make vaccines even more accessible. And it provides a glimpse of the future: it means more countries will be better prepared for the inevitable next outbreak.

Nick Jackson is head of programmes and technology for research and development at CEPI in London.

OFER YIZHAR
HOLOGRAMS IN THE BRAIN

Optogenetics — techniques for controlling the activity of defined brain cells and circuits — has generated excitement in the neuroscience field since it emerged in 2005. In 2021, I anticipate these tools will have an even bigger impact.

With optogenetics, researchers can shine light into a tissue, and all neurons that express the tool will respond. Yet in reality, brain activity is much more nuanced. Neurons respond only to particular stimuli. The timing matters; the sequence matters; the neurons rarely fire all together. From 2005, optogenetics allowed us to manipulate specific types of neurons, but still couldn't recapitulate the language cells use to communicate with each other.

To address this shortcoming, some neuroscientists developed new light-responsive proteins — for example, by changing the colour of light that activates the channel, or making the channel stay open for longer. Some of these modified proteins allowed us to stimulate neurons more precisely using two-photon excitation, a technique for high-resolution imaging of living tissue. However, there are limits to how fast a laser beam can activate individual neurons, and this constrained how reliably we could design stimulation patterns to mimic natural activity.

At the same time, others made advances in optics. Over the past few years, holography and other optical approaches for

single-neuron manipulation have matured enough to be adopted by non-specialist labs. By splitting laser light into many beamlets that form the shape of neurons, it's possible to generate holograms to stimulate neurons precisely, in complex temporal patterns, in three dimensions[2].

Whereas a single laser beam might take 10–20 milliseconds to stimulate a neuron, holography lets you stimulate that cell in less than a millisecond — considerably faster than the 4–5 milliseconds it generally takes to transmit a signal from one neuron to another. You can also generate multiple holograms at the same time, or in a particular sequence.

This type of experiment used to be limited to specialized labs with the know-how to build custom microscopes. Now, microscope companies such as Bruker and 3i have incorporated holography in their two-photon imaging systems. Neuroscientists can take a picture through the microscope, mark the neurons they want to activate, and the software generates holograms to match these activation patterns. With converging developments in optogenetic tools and optical techniques, we can begin to explore the neural code with single-neuron precision.

Ofer Yizhar is a systems neuroscientist at the Weizmann Institute of Science in Rehovot, Israel.

ALICIA CHENOWETH
BUILDING BETTER ANTIBODIES

Antibodies have been used as therapies since the mid-1990s. However, it's only in the past couple of years, as scientists have worked out how the structure of antibodies influences their function, that we've really started to uncover their potential. Amid the continuing pandemic, antibody therapeutics have taken on new urgency.

Most antibody therapies are just regular, unmodified antibodies that bind to a particular target — for instance, a protein on the surface of a virus or tumour cell. However, many of these antibodies are ineffective at engaging immune cells to get rid of the targeted material. With advances in molecular biology, we can quickly modify antibodies to make them better at harnessing the immune system to fight disease[3].

My laboratory has been using two different strategies for doing that. Using the PIPE (polymerase incomplete primer extension) platform, a rapid and efficient molecular-cloning method developed at the Genomics Institute of the Novartis Research Foundation in San Diego, California, we have introduced point mutations into antibodies to make it easier for them to interact with natural killer cells, which increases the death of cancer cells in a breast cancer model in mice.

Separately, we have started investigating antibodies based on immunoglobulin E (IgE). Most therapeutic antibodies are based on an immunoglobulin G backbone. People typically think of IgE as this really awful antibody associated with allergic reactions. Yet in fact, if you use IgE antibodies to unleash that powerful inflammation, it could be a great way for targeting cancer cells for killing[4].

The beauty of engineered antibodies is that, because of their versatile nature, they can be applied to almost any disease as long as you have something to target. So, while we're looking into cancer, other scientists are engineering antibodies to shut down the immune system to treat autoimmunity and allergies, or to aid the immune response against infectious diseases, including COVID-19. The possibilities really are endless.

Alicia Chenoweth is a cancer immunologist at King's College London and co-chair of the 2022 Antibody Biology and Engineering Gordon Research Conference.

CORAL ZHOU
THE SINGLE-CELL POWER OF THREE

The body's cells have many different functions. Yet they all derive from a single cell and a single genome. How does one cell give rise to all these different types?

I'm excited about three new single-cell sequencing technologies that can help to address this question at the earliest stages of embryonic development. One uses Hi-C — a method for studying the 3D architecture of the genome — to examine maternal and paternal chromosomes in single cells of mouse embryos at distinct stages of early development. Using this approach, researchers reported last March that parental genomes do not mix immediately after fertilization — there is a moment between the 1- and 64-cell stage when the structure of the maternal genome looks different to that of the paternal genome[5]. Although we don't know exactly why this brief asymmetry exists, the authors speculate that it has a role in establishing sex-specific gene expression programs later in development. Until then, I don't think we had the technology to discover something like this.

Another technique called CUT&Tag tracks specific biochemical 'marks' on the genome to help scientists study how these chemical modifications switch genes on and off in individual live cells[6], while SHARE-seq combines two sequencing methods to identify regions of the genome that are accessible to transcription-activating molecules[7].

By applying these tools to the developing embryo, we can create a roadmap for how specific features of genomic architecture determine cell fate as an embryo develops.

Coral Zhou is a cell and developmental biologist at the University of California, Berkeley and co-chair of the 2021 Chromosome Dynamics Gordon Research Conference.

TAKANARI INOUE
FEELING THE FORCE

Besides growth factors and other molecules, cells also sense physical force. Force sensation can regulate gene expression, proliferation, development and possibly cancer.

Force is hard to study because you only see its effects — when you push something, there's deformation or movement. But now, using two cutting-edge tools to visualize and manipulate force in living cells, scientists can probe causal relationships between physical force and cellular functions as never before.

GenEPi, developed at the Swiss Federal Institute of Technology (ETH) in Zurich, fuses two molecules. One, called Piezo1, is an ion channel that conducts calcium ions through its pores when it senses tension on the cell membrane. These ions are detected by the second molecule — which fluoresces more brightly when it binds to calcium.

Previous studies used physical probes or other invasive devices to study the impact of force on cells. With GenEPi you can study intact cells in physiologically relevant conditions. And unlike previous sensors that broadly monitor cytoplasmic calcium, GenEPi measures only calcium activity linked to force sensation through Piezo1. As proof of principle, the researchers altered GenEPi fluorescence by stimulating heart muscle cells with the tip of an atomic force microscope cantilever[8].

The second tool, ActuAtor, is one that we generated using ActA, a protein from the pathogenic bacterium *Listeria monocytogenes*. When the bacterium infects a mammalian host cell, ActA hijacks the host's machinery to trigger actin polymerization on the microbe's surface. This generates force that pushes the bacterium through the cytoplasm.

We repurpose this hijacking by engineering ActA to polymerize actin at specific sites inside cells when given a light or chemical stimulus[9]. With ActuAtor we can exert force deep inside cells. For example, we liberated ActuAtor on the surface of mitochondria, causing the organelles to get chopped up in minutes. We found that these damaged mitochondria are more susceptible to degradation by mitophagy but

that key mitochondrial functions such as ATP synthesis were unaffected.

Previously it was hard to address such processes because we lacked tools to deform organelles specifically and non-invasively in living cells. ActuAtor is one of the first tools to be able to do that, to our knowledge.

Takanari Inoue is a synthetic cell biologist at the Johns Hopkins School of Medicine, Baltimore, Maryland, and was a co-organizer of the Tools and Devices for Cell Biology subgroup for the American Society for Cell Biology 2019 conference.

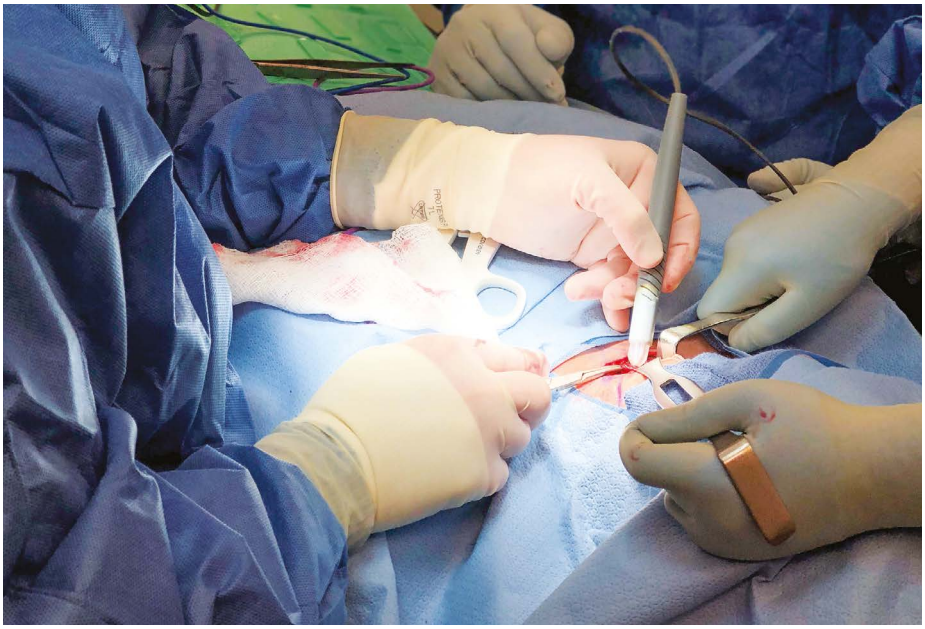
LIVIA SCHIAVINATO EBERLIN
MASS SPEC IN THE CLINIC

Mass spectrometry can rapidly analyse hundreds to thousands of molecules from complex samples with high sensitivity and chemical specificity. Biomedical research on these methods proceeds largely at two extremes. Some scientists are developing high-performance technologies to probe biological tissues more deeply. Researchers in our lab are simplifying mass spectrometry tools so physicians can use them for clinical decisions.

MALDI (matrix-assisted laser desorption/ionization) is a mass spectrometry imaging technology that has been used to analyse biological tissues. But releasing molecules from tissues and ionizing them under vacuum conditions can be cumbersome. In 2017, researchers developed a MALDI system that allowed them to manipulate ions in the open air, rather than under vacuum conditions[10]. That development simplified the MALDI process and allowed it to be combined with other technologies, including fluorescence *in situ* hybridization microscopy, bioluminescent imaging, blockface imaging and magnetic resonance imaging. These multimodal capabilities have enabled researchers to, for instance, probe host–microbe interactions and metabolic changes with greater molecular and histological precision than previously possible[11,12].

On the clinical side, our lab has created MasSpec Pen, a handheld mass spectrometry system that helps surgeons to identify tumour tissues and their boundaries[13]. Our device focuses on metabolites — end products of enzymatic reactions in the body that can distinguish normal tissue from tumour tissue. The process delivers a water droplet onto the tissue, dissolving the metabolites and then sending the molecular contents to the mass spectrometer for analysis. We already know the molecular profiles that characterize metabolism in normal tissue versus tumour tissue in the lab. Now we are testing the tool in the operating room[14].

This year, we plan to continue evaluating the MasSpec Pen in people undergoing surgery for breast, ovarian and pancreatic cancer, or robotic prostate-cancer surgery. We've licensed the technology to Genio Technologies.



JAMES SULIBURK/BAYLOR COLLEGE OF MEDICINE

Genio Technologies' MasSpec Pen is designed to detect tumour tissues and their boundaries.

Livia Schiavinato Eberlin is an analytical chemist at the University of Texas at Austin, co-founder and chief scientific officer at Genio Technologies, Tulsa, Oklahoma, and vice co-chair of the 2021 Chemical Imaging Gordon Research Conference.

JONG-HEUN LEE
SNIFFING OUT SICKNESS

To detect mixtures of gases that could indicate environmental risks or diseases, including COVID-19, researchers would like to mimic human olfaction, to know what we are smelling. However, unlike sight, hearing and touch, chemical sensors for smell are complex. They involve detecting mixtures of several hundred or even thousands of chemicals, often at trace concentrations.

In my lab we are taking several approaches to developing the next generation of artificial olfaction[15]. One involves increasing the diversity of gas-sensing materials using a bilayer design. For example, we could coat each of 10 different sensing materials with 10 catalytic layers that fine-tune each material's gas-sensing characteristics, to make a total of 10×10 or 100 different sensors. This is much easier than separately coating 100 different sensing materials.

We also need to make the sensors respond more rapidly. One strategy is to make the sensing material porous by mimicking nature's hierarchical assemblies, such as trees, which maximize surface area to absorb sunlight for photosynthesis, or lungs, which have a large surface area within a small volume that maximizes transport from main airway vessels to smaller branches.

Artificial olfaction technology can be used for medical diagnosis, for example, to detect higher concentrations of nitric oxide in the breath of people with asthma. Other applications include monitoring air pollution, evaluating food quality and smart farming based on signals from plant hormones.

Jong-Heun Lee is a materials scientist at Korea University, Seoul, and a steering committee member for the 2021 International Meeting on Chemical Sensors.

Interviews by Esther Landhuis.
These interviews have been edited for length and clarity.

1. Zeng, C., Zhang, C., Walker, P. G. & Dong, Y. 'Formulation and Delivery Technologies for mRNA Vaccines' in *Current Topics in Microbiology and Immunology*. https://doi.org/10.1007/82_2020_217 (Springer, 2020).
2. Papagiakoumou, E., Ronzitti, E. & Emiliani, V. *Nature Methods* **17**, 571–581 (2020).
3. Chenoweth, A. M., Wines, B. D., Anania, J. C. & Hogarth, P. M. *Immunol. Cell Biol.* **98**, 287–304 (2020).
4. Chauhan, J. *et al. Antibodies* **9**, 55 (2020).
5. Collombet, S. *et al. Nature* **580**, 142–146 (2020).
6. Kaya-Okur, H. S. *et al. Nature Commun.* **10**, 1930 (2019).
7. Ma, S. *et al. Cell* **183**, 1103–1116 (2020).
8. Yaganoglu, S. *et al.* Preprint at bioRxiv https://doi.org/10.1101/702423 (2019).
9. Nakamura, H. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2020.03.30.016360 (2020).
10. Kompauer, M., Heiles, S. & Spengler, B. *Nature Methods* **14**, 90–96 (2017).
11. Cassat, J. E. *et al. Sci. Transl. Med.* **10**, eaan6361 (2018).
12. Geier, B. *et al. Nature Microbiol.* **5**, 498–510 (2020).
13. Zhang, J. *et al. Sci. Transl. Med.* **9**, eaan3968 (2017).
14. Zhang, J. *et al.* Preprint at medRxiv https://doi.org/10.1101/2020.12.14.20248101 (2020).
15. Jeong, S.-Y., Kim, J.-S. & Lee, J.-H. *Adv. Mater.* **32**, 2002075 (2020).

Correction

This Technology feature erroneously stated that GenEPi was developed at Imperial College London. In fact, it was developed at the Swiss Federal Institute of Technology (ETH) in Zurich.