SOPHIE CASSON FOR *NATURE*

Cell-based meat with a side of science

Growing a burger in a laboratory is one challenge, growing an industry to do it at scale is quite another. **By Elie Dolgin**

When Laura Domigan started her research group at the University of Auckland, New Zealand, in 2015, she hoped to continue her work developing protocols for growing cell-based meat in the laboratory. But with funding for cultivated-meat research practically non-existent in academia at the time, Domigan pivoted to working on biomedical materials for use in tissue engineering. A protein biochemist by training, she focused her efforts on creating artificial corneas for eye surgery — a far cry from anything resembling a lab-grown steak.

Still, she never gave up on her dream of studying *in vitro* meat. "I had to be super patient and keep trying," Domigan says. And although it took several years, Domigan's strategy eventually paid off.

Initially, she secured funding for a PhD student to begin developing formulations of nutrient media to grow cell-based meat. Then, in October 2020, a team led by Domigan won a multi-million dollar grant from the New Zealand and Singaporean governments to explore questions such as which cells are the best starting material for cultured meat, and is the nutritional profile of meat grown in a lab equivalent to the real thing. "There is so much research that needs to be done," Domigan says. And much of it is only beginning to happen, at least in any sort of transparent way.

Investors have poured hundreds of millions of dollars into cultured-meat research in the past few years, bringing hype and breathless news coverage about an agricultural revolution that could bypass the environmental and animal-welfare issues of conventional meat production. One estimate by the consulting firm Kearney in Chicago, Illinois, suggests that 35% of all meat consumed globally by 2040 will be cultured – a change that is projected to reduce greenhouse-gas emissions and antibiotic use. And thanks to the COVID-19 pandemic, which revealed crucial weaknesses in global food-supply chains, some people now expect the transition to cell-based meat to happen even faster.

Earlier this month, a US start-up called Eat Just announced that its chicken bites – which are 70% cultured chicken cells, with plant protein added for structure and flavour – had won regulatory approval for sale to consumers in Singapore, a global first for the cultivated-meat industry.

Scientists worry that the commercial push to bring palatable products to market means that fundamental studies are either not happening or remain cloaked in trade secrecy. Start-ups have made splashy demonstrations of their lab-grown chicken nuggets, pork sausages, steak strips and seafood dumplings. But these show only that companies "can do this on a small level", says Abhi Kumar, a venture partner at Lever VC, a New York-based venture capital fund that focuses on alternative-protein start-ups. The challenge now, he says, is making it work at scale.

Improvements in cell source material and the nutrient media required to fuel cell growth are needed, as are scaffolds to support 3D tissue structures. Next-generation bioreactor platforms that can grow huge numbers of cells at high densities are also a must. These are costly undertakings – so much so that many in the field are dubious that private financing can support them, and still yield an affordable product.

That's why leading thinkers in cellular agriculture, such as Erin Rees Clayton at the Good Food Institute (GFI), argue in favour of more open science and public investment. "There's a need for public-sector research in these areas," says Rees Clayton, who is associate director of science and technology at the GFI, a non-profit think tank in Washington DC. "There's a lot of room in the pre-competitive space for more work to be done in an open way, so we can all benefit from it and move forward more quickly."

To fill the funding gap, the GFI created a research-grant programme that has given out close to US$3 million over the past 2 years to 16 research teams working on cultivated-meat projects. Academic institutions are beginning to hire with the nascent discipline in mind – the Technical University of Munich in Germany, for example, is accepting applications for a professorship in cellular agriculture. And, as evidenced by Domigan's funding success, governments too are heeding the call for financial support.

The field of cellular agriculture is beginning to take on some of its biggest scientific and engineering challenges, and scientists from a range of backgrounds are entering the fray.

"This cellular-agriculture research is the stuff that gets me up in the morning," says Glenn Gaudette, a biomedical engineer at Worcester Polytechnic Institute in Massachusetts. For almost 20 years he has studied scaffold technologies for heart-regeneration therapies; now he is applying his expertise to the problem of how to grow meat. "Does it pay the bills? No, not yet – hopefully, one day – but it's really exciting."

The fight for funding

In the early 2000s, the US space agency NASA briefly supported efforts to grow goldfish muscle in the lab as a potential source of protein for astronauts on long missions. A few years later, the Dutch government sponsored a €2-million ($2.3-million) project to cultivate pork meat from stem cells – research that, with an extra €250,000 infusion from Google co-founder Sergey Brin, eventually led to the field's highest-profile moment so far, when vascular biologist Mark Post at Maastricht University in the Netherlands, unveiled the world's first cultured burger, in 2013.

> **"This cellular-agriculture research is the stuff that gets me up in the morning."**

But aside from intermittent funding opportunities to explore the social ramifications of producing meat from cell cultures, there have been few other public grants for cultivated-meat research. Government bodies stayed away from the field in large part, says Kate Krueger, former research director at the non-profit organization New Harvest in Cambridge, Massachusetts, because the science was unproven and crossed disciplines in ways that defied the conventional dividing lines of funding-agency bureaucracy.

"Cellular agriculture falls into this funding no-man's land between biomedical research and agricultural research," says Krueger, who now runs a consulting firm, also in Cambridge, centred around cellular agriculture.

Money from the GFI and New Harvest has plugged the funding gap to some extent. But the situation is changing. As scientific interest in the subject grows and grant applications increase, governments have begun to inject more cash into the field. Several large grants have been issued in the past few months alone. In November, for example, the government agency Flanders Innovation and Entrepreneurship began funding a €2.1-million, 4-year project called CUSTOMEAT, run by scientists at Ghent University and KU Leuven, both in Belgium. In the United States, the National Science Foundation (NSF) awarded around $3.5 million in September to back a cultivated-meat consortium at the University of California, Davis, for the next five years.

"Our hope is that we can provide basic knowledge and build a trained workforce," says chemical engineer David Block, who is leading the Davis effort. "Those are the kinds of things that you need to grow an industry."

Experts say that many cultivated-meat companies will probably over-promise and under-deliver. But academic science can help "keep credibility alive," says Johannes le Coutre, who led a research group at the Swiss food giant Nestlé before joining the University of New South Wales in Sydney, Australia, in 2019 to run a lab dedicated to cellular agriculture.

Amy Rowat, a biophysicist at the University of California, Los Angeles, notes that academia also offers the intellectual freedom for researchers to work on exploratory projects, using expertise in basic science to come up with innovative ways of thinking, or to tackle questions not directly related to product development but still significant to the overall domain. And according to David Kaplan, a bioengineer at Tufts University in Medford, Massachusetts, the next generation of scientists entering the field are "totally motivated to make a difference".

"I have never seen such driven, passionate students in all my decades of doing this," he says. Andrew Stout is a case in point. A PhD student in Kaplan's lab, Stout is rethinking the entire process of cellular agriculture, starting with the most basic ingredient: the muscle cells themselves.

Most companies growing lab meat either use cells taken directly from animal biopsies or cell lines that have spontaneously become immortal through natural mutations that allow indefinite proliferation in the lab. Few firms will consider genetically manipulating the cells for optimal performance because of a fear of consumer backlash. But Stout realized that genetic engineering offered a path to achieving the nutritional promise of cultivated meat.

Starting material

He inserted three genes into cow muscle cells[1]. Each encoded an enzyme involved in

Producing products such as the lab-grown meat burger at scale is still a long way from reality.

DAVID PARRY/PA IMAGES/ALAMY

the synthesis of an antioxidant that mitigates diseases associated with consuming red and processed meats, such as colon cancer. The enzymes might also help with the manufacture of cultured meat, because the unstable molecules that antioxidants attack reduce the proliferation of some lab-grown cells. If consumers are willing to accept these types of DNA enhancement, "genetic and metabolic engineering can offer a lot of impact and benefit to cultured meat", Stout says, "and could even allow us to create novel foods that we couldn't get any other way".

Other researchers are reconsidering whether the cells that go into cultivated-meat products need to come from species that are already commonly consumed in Western cultures. For example, Natalie Rubio, another Tufts graduate student, has explored growing meat from insect cells to create products that can be designed to taste like crab, prawns and other seafood. Using muscle cells from fruit flies (*Drosophila melanogaster*)[2] and the caterpillar of the moth *Manduca sexta*, Rubio showed that insect cells are easier and cheaper to grow than cells from conventional livestock species, and might also have nutritional advantages.

Meanwhile, other scientists hope to rally the research community around the idea of cell-based zebrafish fillets, at least as a vehicle for accelerating advances in the field. The zebrafish (*Danio rerio*) is an established model organism for studying the genetic, neuronal and behavioural basis of disease. Alain Rostain, executive director of Clean Research, a non-profit organization in New York, now wants to make it the go-to species for basic-research projects in cellular agriculture as well. "There's a lot of fundamental understanding that's not there yet," he says. "We need the participation of a lot of people to just think freely through the science together."

And, as Rostain and his colleagues have described[3], researchers can benefit from the extensive molecular toolkit already established for zebrafish. Plus, as a lean fish with little fat content in the muscle, zebrafish fillets should be easier to produce than comparable lab-grown cuts of fat-laced salmon, tuna, beef or pork. Cultivated zebrafish will probably taste similar to white fish, such as cod or haddock, Rostain says, and discoveries made with zebrafish should translate to any other edible species.

"I have never seen such driven, passionate students in all my decades of doing this."

Regardless of the starting material, all cells will require an optimized growth medium — the rich broth of chemicals and proteins needed to support proliferation and differentiation. Companies have already devised ways to eliminate the nutrient-rich fetal bovine blood that is the cornerstone of most *in vitro* culture media, creating the slaughter-free products that the industry demands. But this serum-free media is too expensive for cultivated meat to be affordable on the supermarket shelves. "It's difficult to find a cost-efficient option," says Ka Yi Ling, chief scientific officer at Shiok Meats, a cell-based seafood company in Singapore.

Media matters

According to an analysis by the GFI[4], growth media currently make up the bulk of total production costs for cultivated meat, and proteins known as growth factors are the most expensive ingredient. Costs are coming down, as start-ups dedicated to serving the cellular-agriculture industry devise ways to manufacture these products. But as Matt Anderson-Baron, co-founder and chief scientific officer at one such company, Future Fields in Edmonton, Canada, concedes, "There's still so much to be done on the optimization and discovery front."

Assuming researchers find the right cell line and growth medium combination, they then have to grow those cells on a scaffold — ideally one that is edible so that it doesn't have to be removed from the final product. For ground-meat products, such as burgers and sausages, small beads known as microcarriers can provide the surface properties needed by most muscle and fat cells for growth. But for anything with a more complex meat structure — a steak or an Iberian ham, for example — a more sophisticated tissue-engineering approach is required.

One option comes from a team at Harvard University in Cambridge, Massachusetts. Bioengineer Kevin Kit Parker and his colleagues have developed a spinning technique that works like a candy-floss machine to extrude long, thin fibres from gelatin[5]. The researchers put the gelatin, a protein product derived from collagen, into their machine and produced tiny threads — narrower than the width of a hair — that closely matched the architecture of fibres found in muscle tissue.

Last year, Parker and his colleagues showed that rabbit and cow muscle cells grown on the fibrous gelatin line up with the proper orientation[6]. The cells were still not as densely packed as real muscle, but Parker, together with three of his postdocs and students, has since created a company called Boston Meats to improve the technology further. "Now, with our scaffolds," he says, "you can move from hamburger to fillet."

Elsewhere, researchers have made scaffolds out of foods such as textured soya protein and various vegetables stripped of their cellular material so that only supporting structural sugar molecules and proteins remain. At the University of Ottawa in Canada, for example, biophysicist Andrew Pelling and his students have taken decellularized stalks of celery and shown that the grooves created by its natural structure help to promote the patterning and alignment of muscle cells[7]. And at Worcester Polytechnic, Gaudette's team has grown fat and muscle cells on cell-free spinach leaves – the plant's branching network of delicate veins provide ideal conduits for the nutrient medium to reach every cultivated meat cell.

Because muscle and fat cells require different growth media, however, researchers typically culture the two cell types separately, each on its own scaffold in a different nutrient bath. Some researchers, including Rowat, have devised strategies to then interlace the muscle and fat to achieve the flavour of a well-marbled steak. "The cells actually fuse together with the other partnering scaffold type on the time scale of hours to form these composite structures," Rowat explains. In unpublished work, she has created miniature marbled steaks out of mouse and rabbit cells, and has begun to work with cells from pigs and cows, as well.

But even with the latest scaffolding strategies, some muscle biologists worry that key aspects of tissue physiology are still being discounted. "This incredible focus that we have as an industry on cell division ignores fusion and maturation," says James Ryall, chief scientific officer at Vow, a start-up in Sydney working on cell-based meat from animals such as kangaroo and alpaca.

To form muscle tissue, thousands of individual precursor cells must first fuse together to become long myotubes. These cells require physical stimuli to mature into myofibres. Only then will muscle grown in the laboratory have the texture and nutritional properties of real meat, says Lieven Thorrez, a muscle-tissue engineer at the Kortrijk campus of KU Leuven. "And that is a process that takes time. You cannot just differentiate cells over a period of a few days and say the myofibres will be the same as those found in an adult animal," he says. "That is largely overlooked."

Scaling up

With so many scientific issues to resolve, cell-based meat research, whether in academic or private labs, remains at the experimental stage. For commercial viability, the industry will need to find ways to produce tissue at a massive, and unprecedented, scale.

Tissue engineer Che Connon at Newcastle University, UK, estimates that feeding the world's population with lab-grown meat would necessitate building systems for growing on the order of a septillion (10^{24}) cells

Ka Yi Ling and Sandhya Sriram are co-founders of Shiok Meats in Singapore.

annually, something that is not possible with the types of batch bioprocessing techniques currently used in mammalian-cell-based manufacturing. Global capacity could fulfil about one-billionth of that requirement. "It's a massive limiting factor," says Connon, who has developed a type of continuous cell-bioreactor platform that he plans to commercialize through a spin-off company called CellulaREvolution.

"This incredible focus that we have as an industry on cell division ignores fusion and maturation."

Meanwhile, computer scientist Simon Kahan, president of life-sciences software company Biocellion SPC in Seattle, is leading a team called the Cultivated Meat Modeling Consortium that formed in 2019 with the aim of optimizing bioprocessing techniques through modelling techniques. With funding from the German technology company Merck, the consortium has developed a proof-of-concept model of a stirred-tank bioreactor, involving little more than a spinning rotor and free-floating minute beads for growing muscle cells.

The bioreactor might be fairly rudimentary, but the consortium's computer modelling is anything but. The simulations of fluid dynamics and cellular biomechanics have revealed a central challenge to growing muscle or fat cells at scale. "You have these two competing interests," says Kahan. To support nutrient and gas exchange, "you're trying to keep things well mixed while at same time trying to subject the cells to very little mechanical fluid stress". With input from industry partners, the consortium plans to build more complexity into its models to inform the design of bioreactors in the real world.

Block's group, with its large NSF grant, isn't even attempting to work on bioreactor designs; there is plenty to keep his team busy tackling the issues of cell lines, media and scaffolds, as well as conducting feasibility assessments of the cell-based meat industry. "To me," says Block, "it's not clear yet that this is going to be a viable alternative" – whether from a technical, economic or sustainable standpoint. But with each new grant or research team entering the field, the goal of a perfectly grilled, medium-rare steak grown from cells gets a little closer to becoming a reality.

Elie Dolgin is a science journalist in Somerville, Massachusetts.

1. Stout, A. J., Mirliani, A. B., Soule-Albridge, E. L., Cohen, J. M. & Kaplan, D. L. *Metab. Eng.* **62**, 126–137 (2020).
2. Rubio, N. R., Fish, K. D., Trimmer, B. A. & Kaplan, D. L. *ACS Biomater. Sci. Eng.* **5**, 1071–1082 (2019).
3. Potter, G. *et al. One Earth* **3**, 54–64 (2020).
4. Specht, L. *An Analysis of Culture Medium Costs and Production Volumes for Cultivated Meat* (The Good Food Institute, 2020).
5. Reza Badrossamay, M., McIlwee, H. A., Goss, J. A. & Parker, K. K. *Nano Lett.* **10**, 2257–2261 (2010).
6. MacQueen, L. A. *et al. npj Sci. Food* **3**, 20 (2019).
7. Campuzano, S., Mogilever, N. B. & Pelling, A. E. Preprint at bioRxiv https://doi.org/10.1101/2020.02.23.958686 (2020).