TREEFROG THERAPEUTICS

At French biotech firm TreeFrog Therapeutics, researchers can grow 15 billion human induced pluripotent stem cells in a single batch.

Stem cells scaled up

Therapies based on induced pluripotent stem cells are hard enough to grow at a small scale. How will they be mass-produced for the clinic? **By Eric Bender**

In the laboratory, induced pluripotent stem (iPS) cells can seem like magic: derived from differentiated cells, they can then morph into surprisingly good replacements for pancreatic, brain, eye, heart and other cells. Some are being used in clinical trials to treat people with chronic conditions, including diabetes and Parkinson's disease, that are driven by damage to such cells (see page S8).

But it's magic done slowly, for one patient at a time. "Essentially, all the cells are made by hand, by highly trained scientists sitting in a clean room," says Nabiha Saklayen, a physicist and chief executive of Cellino Biotech in Cambridge, Massachusetts, a start-up developing a platform for manufacturing iPS cell therapies. "That's not scalable."

James Shapiro, a surgeon at the University of Alberta in Edmonton, Canada, concurs. Shapiro leads a team readying for a clinical study of pancreatic islet cells, created from iPS cells, that can take on the vital task of producing insulin in people with type 1 diabetes. Testing such transplants in a handful of patients "will be exciting and will move the needle quite a bit", he says.

"But it won't address the big challenge ahead for personalized medicine: how on Earth could we ever do this kind of work for thousands of patients?" Shapiro says. "Right now it takes a technician and a crew of other research associates working day and night to baby these cells along to grow them into islet-like cells."

To become practical therapies, stem-cell-based regenerative treatments must conquer two overlapping manufacturing challenges: achieving highly standardized automated production; and doing so in vastly greater volumes than at present.

To make an iPS-cell-based therapy, scientists first change the genes expressed by the starter cells to de-differentiate them into a pluripotent state. Gradual refinement of the techniques involved has made that relatively straightforward. But those pluripotent cells must then be differentiated at scale into the desired cell type — typically a much more formidable undertaking, says Jeffrey Millman, a bioengineer at Washington University in St. Louis, Missouri.

The cell whisperers

Success is currently highly dependent on the skill of the researcher. Some develop a gift for coaxing cells along. But the skills of a "cell whisperer", as Millman calls them, cannot be easily taught to another researcher or embedded in the protocols for automated manufacture.

The core of the problem is that the recipes for success are incomplete. That's partly because academic laboratories usually can't afford to measure exactly what's happening to the cells throughout the entire process. Moreover, identifying the best set of measurements to make is intimidating. Researchers don't always know which biomarkers will predict success for the final cells, although they often do know that the absence of certain markers guarantees failure, Millman explains.

Some cell types might be easier to evaluate than others. For instance, it's relatively

straightforward to perform functional tests for insulin-producing pancreatic islet cells *in vitro*. But it's much more difficult to assess the performance of iPS-cell-derived heart cells or dopamine-producing brain cells, because that depends on how well those cells integrate into the tissues around them, Millman says. In addition, many methods used to characterize cells kill them in the process. Some labs are trying to avoid this by analysing molecules secreted by the cells into the culture medium. This approach might eventually allow near-continuous measurements of progress throughout differentiation and scale-up, Millman says.

Given all these complications, measuring the crucial ingredients for efficacy is high priority, says Tom Bollenbach, a biochemist and chief technology officer at the Advanced Regenerative Manufacturing Institute's BioFabUSA initiative in Manchester, New Hampshire. "We're trying to tease out the needle in a haystack that helps us understand how this thing works, why it works, and how we can guarantee it'll work in every patient."

Most companies working on iPS-cell-based therapies do not aim to use a patient's own cells as starters — known as autologous cell therapy — but instead rely on lines of allogeneic iPS cells. These are developed from cells taken from one or more donors, and could be turned into off-the-shelf products to treat all patients who have a particular disease. This procedure requires scaling up iPS cell production and differentiation by orders of magnitude from the flasks used in the lab, which often contain only about 500 millilitres.

Cells are most commonly grown industrially in stirred-tank reactors, which can contain thousands of litres of tightly controlled media. These work well for the Chinese hamster ovary (CHO) cells that are commonly used to produce therapeutic proteins. CHO cells are robust and tend to float in the reactor as single cells, Millman says. But pluripotent stem cells are more fragile and need to grow in aggregates, which are likely to be sheared apart in the bioreactor. Any surviving single cells are unlikely to grow and differentiate correctly.

Different challenges arise for the autologous cell therapies that will be tailored to the individual patient. These treatments minimize threats from immune reactions to allogeneic cells, and the cells don't need to be churned out in high volumes. It might take a few billion pancreatic islet cells to treat one person's type 1 diabetes, but labs can currently create about half a billion of these cells in a flask.

Each individual cell line will, however, behave in its own idiosyncratic way as it is expanded and differentiated, which makes it daunting to find a manufacturing protocol that can ensure the safety and efficacy of the final product. "Different cell lines need different modifications to protocols," Millman says. For example, he explains, differentiation might fail if the density of the cells being grown is too high or too low.

"What makes cells so great is what makes them difficult to manufacture, which is that they're so variable," Bollenbach adds.

Biofactory builders

Biotech firms are responding to the challenge with an amazingly diverse range of technologies, says Bollenbach. Some companies are evolving production systems that were originally created to deliver other cell therapies, such as CAR-T cells used to treat blood cancers.

Other firms were launched to provide mass production and differentiation of iPS cells. In April, TreeFrog Therapeutics in Bordeaux, France, announced production of a single batch of 15 billion iPS cells in a week — an encouraging milestone. The company's technology allows cells in the bioreactors to self-organize into aggregates similar to those formed by natural stem cells and protects them from shear stresses. TreeFrog is now working with several partners to build towards clinical trials for Parkinson's disease and other conditions.

"What makes cells so great is what makes them difficult to manufacture, which is that they're so variable."

Other start-ups are developing platforms for automated, high-precision cell transfection, in which reagents are introduced into cells to modify their genomes or the genes expressed. Such platforms could speed up the differentiation of iPS cells into the required cell types.

Cellino Biotech, for example, offers a platform for fabricating autologous iPS cell therapies that incorporates stem-cell biology, laser physics and machine learning. The system features a nano-structured absorbent layer on the bottom of the culture vessel that generates bubbles when the layer is hit by laser light (N. Saklayen *et al. Biomed. Optics Express* **8**, 4756–4771; 2017). Large bubbles kill cells and smaller bubbles can deliver molecular cargoes into them, Saklayen says. The laser can focus on individual cells, and the system characterizes each one through machine learning. "You can individually target cells or clusters of cells to remove them or deliver cargo into them," she explains.

This approach could drastically cut today's unfeasibly high cost of autologous cell treatments. One dose of conventionally generated, clinical-grade cells derived from autologous iPS cells costs around US$1 million. "Our target manufacturing cost for 2025 is $30,000 per dose," Saklayen says.

Kytopen, another start-up firm in Cambridge, Massachusetts, has developed a microfluidic platform to create iPS cells and other forms of cell therapy. The system combines mechanical and electrical energy to deliver cargoes such as messenger RNAs across the cell membrane.

"We want to do minimally invasive surgery," says Kytopen co-founder Cullen Buie, a mechanical engineer at the Massachusetts Institute of Technology in Cambridge. The ability to adjust both mechanical and electrical processes means the system minimizes harm to the cells and maximizes their production — the company's demonstration system can transfect hundreds of millions of cells in a minute, at the push of a button.

Seeking approval

Even if manufacturing costs can be reduced, and standardized cells reliably created, there are still obstacles to obtaining regulatory approval, says Kapil Bharti, a molecular-cell biologist at the US National Institutes of Health in Bethesda, Maryland. "Part of the reason that there are so few iPS-cell-based therapies approved is that there is no regulatory framework," he says. "We are still figuring things out as we go along. Often, as scientists we're not trained in the regulatory aspects of things, so for us it's a very steep learning curve."

Bharti is leading the first cell-therapy trial using autologous iPS cells in the United States, for age-related macular degeneration. The US Food and Drug Administration approved the trial for the therapy of this eye disease in December 2019, after reviewing 12,000 pages of preclinical documentation. The first patient was enrolled in October 2020.

For iPS-cell-based therapies to fulfil their promise, "we must keep focusing on the most fundamental biology of the cells," says Bharti. "We also must figure out all the logistics of delivery, surgery, shipment and reimbursement. Only then can we make the right products."

But all these problems will be overcome, advocates say. "I want this to be commercialized and scaled up and completely normal for everyone," Bollenbach says. "Someday we're going to say, hey, remember way back when we couldn't make a heart for transplant? That was crazy."

Eric Bender is a science writer in Newton, Massachusetts.