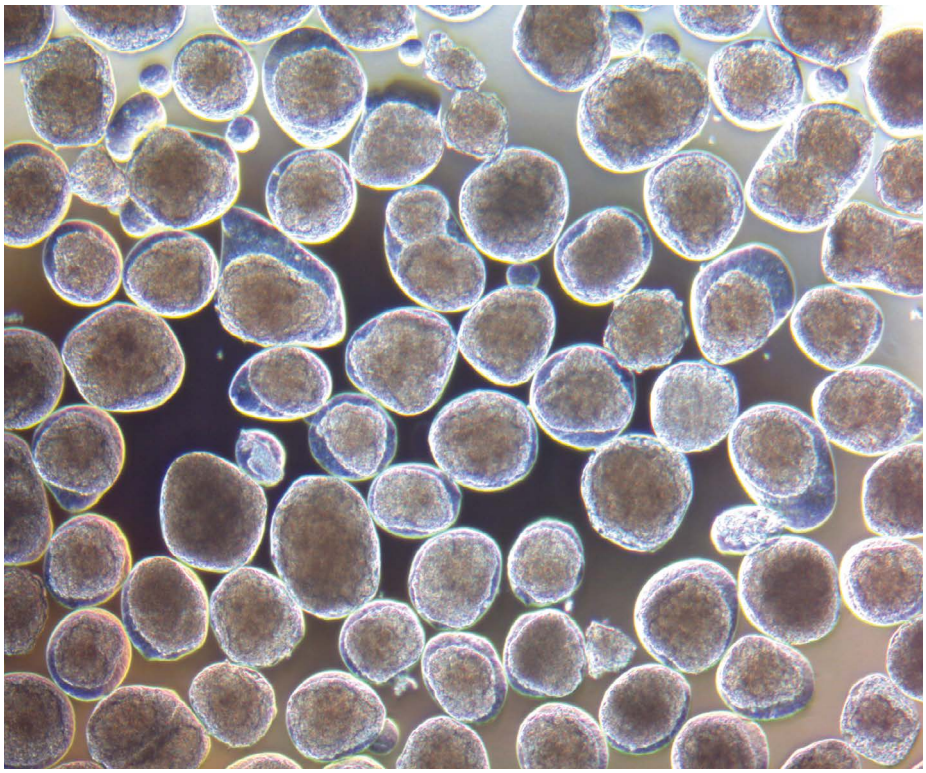


Encapsulated stem cell-derived islets could shield β cells from the immune system.

Beta testing

Trials to replace the pancreatic β cells that are destroyed by an errant immune system in type 1 diabetes are raising hopes of a cure. **By Liam Drew**

Insulin has been one of the most transformative discoveries in medicine. The isolation of this hormone in 1921 made type 1 diabetes (T1D) a treatable, rather than a terminal, illness. However, there is growing hope that 100 years later, insulin therapy for T1D might be on the brink of obsolescence.

Insulin is crucial to maintaining safe levels of glucose in the blood. It is produced in the pancreas by β cells, which continuously detect circulating glucose concentrations and secrete insulin accordingly – the higher sugar levels go, the more hormone is released to counteract the increase. In T1D, however, the β cells are destroyed by a person's own immune system.

The cause of this autoimmunity, which typically manifests in childhood, is incompletely understood, but the effect is clear: with neither β cells nor insulin, circulating sugar levels remain constantly, toxically elevated. This chronic hyperglycaemia damages blood vessels and nerves, leading to an accumulation of ill-health effects and, if untreated, death.

Today, the effects of T1D can be mitigated through a combination of careful blood glucose monitoring and insulin administration. However, despite advances in automated insulin delivery systems, for most people this means a life dominated by the need to conscientiously manage their own physiology. And even with people taking on this burden, the life expectancy for somebody with T1D is 12 years below average[1]. Frederick Banting, who won a share of the Nobel Prize in Physiology in 1923 for discovering insulin, knew his work offered no panacea – he concluded his Nobel lecture by saying "insulin is not a cure for diabetes".

Now, though, a growing number of scientists and physicians are talking about curing T1D. Their focus is not supplying the body with insulin, but rather replacing the β cells that make it.

At the turn of the 21st century, hard-won breakthroughs showed that transplants of β cells from deceased donors could successfully treat people with T1D[2]. Several complicating factors – not least a shortage of donors – limit this approach. But today, stem-cell biology allows the creation of glucose-sensing, insulin-dispensing cells in the laboratory – offering the possibility of an almost limitless supply of replacement cells.

In June, at the virtual annual meeting of the American Diabetes Association, regenerative medicine company ViaCyte, based in San Diego, California, reported that in a small clinical trial, cells derived from embryonic stem cells had helped people with T1D to gain better control of their blood sugars. "Seeing clinically relevant results that drastically change management for patients with T1D is incredibly exciting," says Manasi Sinha Jaiman, ViaCyte's vice-president of clinical development. "It is the culmination of 20 years of research."

ViaCyte's success and other scientific advances are drawing more companies into the field. But although the idea of making β cells and transferring them into people sounds simple, fundamental questions remain. Researchers still need to resolve which cells are best to use, where they should be implanted, and – crucially – how to ensure that they are protected from recipients' immune systems. REF. 8

Success from failure

T1D is considered one of the lowest-hanging fruits in regenerative medicine. It is a condition in which autoimmunity selectively kills a single cell type, so it is clear what needs replacing. Additionally, only a small amount of tissue is needed. In the pancreas, β cells are one of five endocrine cell types that reside in small pockets of hormone-producing cells known as islets of Langerhans. Although there are around one million islets dotted throughout the organ, they amount to only 1–2% of its overall mass.

Equally importantly, although the pancreas is the natural home, β cells could be placed elsewhere. Unlike in Parkinson's disease, where implanted neurons would have to integrate into existing brain networks to restore function, experiments in rats with diabetes in the early 1970s showed that the rats regained normal blood sugar control after isolated islets were infused into the portal veins of their livers[2]. All that β cells require to function, it seems, is good access to the bloodstream.

Attempts to replicate this result in people with T1D in the 1980s and 1990s – using islets taken from the pancreases of deceased donors – were rarely successful[2]. When surgeon James Shapiro arrived at the University of Alberta in Edmonton, Canada, in 1993, the university's

transplant programme had lapsed. "Nobody wanted to run this", he says, "because it was such a failure."

But reviewing the literature convinced Shapiro that if the cells actually engrafted and survived, they helped. He increased the dose of cells – using tissue from two to four donors per recipient – and cut the interval between harvesting and implantation to improve cell health. Crucially, he also realized that the drugs previously used to stop the immune system from attacking the transplanted tissue were probably also damaging the β cells. To avoid this, he designed a new drug regimen[3].

All seven of Shapiro's first patients were able to come off insulin for at least one year – one, remarkably, remains insulin free more than 20 years later. "We showed conceptually that islet cell transplantation can dramatically improve patients' lives," Shapiro says.

Shapiro's method, now known as the Edmonton protocol, is used today at a handful of centres to transplant β cells into people whose T1D is poorly controlled by insulin. The downside of the procedure is that recipients must commit to a long-term regimen of powerful immunosuppressive drugs, which leave them vulnerable to infections and other complications. And even if large numbers of people with T1D were willing to make this trade, too few donors exist to provide cells for more than a small fraction of recipients.

Unlimited supply

The key characteristic of stem cells is that they have the potential to differentiate into other cell types. Initial research involving human stem cells relied on cells taken from embryos of terminated pregnancies; these cells can grow into almost any type. Later methods created stem cells from adult cells, known as induced pluripotent stem cells (iPSCs). Scientists have worked to discover ways of directing stem cells along particular developmental trajectories by applying signalling molecules that switch the cells from one type to another.

Although guided by knowledge of normal development, uncovering the right signalling molecules – and their correct concentrations and exposure times – is a largely empirical process. Kevin D'Amour, who recently stepped down as chief scientific officer at ViaCyte but remains an adviser to the company, recalls "hundreds, if not thousands, of experiments" inching stem cells towards a β-cell phenotype.

A huge appeal of this technology is that stem cells can be turned into continuously dividing cell lines – cells from a single donor can give rise to trillions more. A self-renewing cell line that can be straightforwardly transformed into β cells (or hormone-making islet cells more generally) would therefore provide an essentially inexhaustible supply of implantable cells.

This cell product is regenerative medicine's equivalent of a drug. The field's primary challenge is developing a good one.

Doug Melton, a stem-cell biologist at Harvard University in Cambridge, Massachusetts, is something of a talisman for this field. He was a developmental neurobiologist until his six-month-old son was diagnosed with T1D in the early 1990s. He dropped everything to seek a cure for the condition, eventually entering the stem-cell field. He recalls telling his wife that he was going to work out a way to make β cells, and that it would take four or five years.

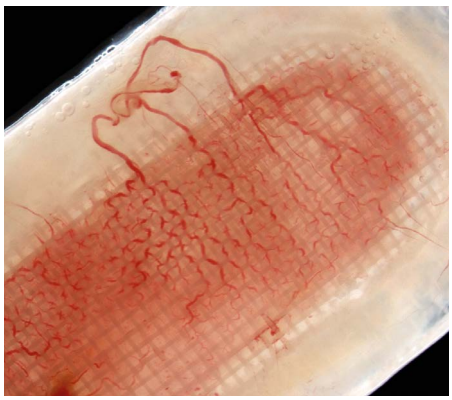
His estimate was a little off. Creating a β cell required coaxing stem cells through a series of intermediate stages, such as making cells that could become any cell of the pancreas. Working out all those steps and how to navigate them ultimately took Melton closer to 15 years. Melton's group – and, independently, that of Tim Kieffer at the University of British Columbia in Vancouver, Canada, – published their methods in 2014 (refs 4 & 5).

With patents secured on β-cell production protocols, in 2015 Melton co-founded a start-up company, which was acquired by Vertex Pharmaceuticals in Boston, Massachusetts, for US$950 million in September 2019. In March, Vertex announced that the US Food and Drug Administration had granted its proprietary mixture of insulin-making cells and other endocrine cells, now called VX-880, fast track designation – and that the company was commencing a 17-person phase I clinical trial that would be the first test in people of islet cells derived from stem cells.

"We showed conceptually that islet cell transplantation can dramatically improve patients' lives."

ViaCyte, meanwhile, has taken a slightly different approach. "We and everyone else in the field had the idea that we're going to take stem cells, we're going to make functional islets, then we're going to transplant," says D'Amour. But an experiment he did in the mid-2000s changed ViaCyte's strategy.

D'Amour's group was leading the race to make β cells, having pioneered methods for transforming human stem cells into pancreatic progenitor cells[6]. But rather than trying to further transform these into islet cells, they injected the pancreatic progenitors straight into diabetic mice. Over 2–3 months, the cells matured into islet cells and the immunosuppressed mice began to show controlled blood glucose levels[7].



VIACYTE, INC.

ViaCyte's pancreatic cells in a pouch.

Consequently, by the time Melton and others had determined how to make β cells, ViaCyte was commencing its first clinical trial implanting pancreas progenitor cells – and is now running its fourth and fifth trials.

Distinguishing which cells make the best therapies will take time. Melton thinks that implanting fully differentiated cells will provide better control of the cell dosage that people receive. Kieffer agrees that relying on progenitors to mature inside recipients introduces extra layers of variability and uncertainty, as well as months of waiting for functional cells to emerge. But he also notes that progenitor cells might be more resilient to implantation procedures, and that they're easier and cheaper to manufacture. Only clinical trials, he says, can determine which cell type is best.

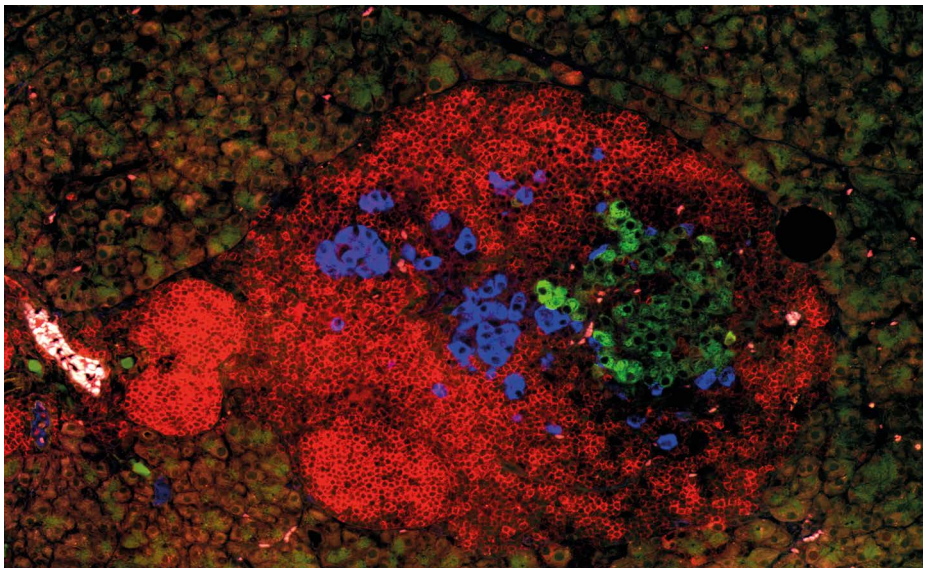
Immune protection

No matter which cells are implanted, however, recipients' bodies will not welcome them with open arms. Not only are these foreign cells, and therefore prime targets for any immune system, but they are also being administered to people whose bodies have a track record of specifically attacking β cells.

The simplest way to protect implanted cells from immune destruction, which could make the treatment pointless, is to administer them according to the Edmonton protocol, with a full regimen of immunosuppressive drugs. This is what Vertex is doing in its first trial. "There aren't a lot of cell therapies where you have such a strong clinical precedent for the type of therapy," says Vertex's Felicia Pagliuca, who was involved in the creation of β cells in Melton's laboratory. The trial will allow a comparison between VX-880 cells and islets transplanted from cadavers.

Ultimately, researchers want to spare recipients from lifelong immunosuppression. Current strategies focus on encapsulation, whereby

outlook



ALI ASADI (KIEFFER LABORATORY)

In type 1 diabetes, immune cells (red) attack β cells (green) in the pancreatic islets.

implanted cells are shielded from the immune system by a physical barrier. This encapsulation can be macro, in which a credit card-sized device is filled with cells and implanted, or micro, where islets are individually coated in a shielding polymer before delivery.

Encapsulation entails a delicate balance. Although the cells need to be isolated from the immune system, they must still have access to nutrients and oxygen to survive. And in the case of β cells, to respond to circulating glucose they need excellent access to the bloodstream, explains Alice Tomei, a biomedical engineer at the University of Miami in Florida. "If there is no vascularization, they can't really provide a good metabolic control," she says.

In 2014, ViaCyte's first clinical trial of a macro-encapsulation device failed: recipients' bodies recognized the device as foreign and deposited a layer of immune cells on it, killing the cells inside. The trial ViaCyte reported in June, showing that its cells could mature in humans and provide a degree of clinical benefit, used a stopgap encapsulation device punched with holes. The perforations allowed blood vessels to grow into the device, sustaining the cells, but recipients required chronic immunosuppression because immune cells could also enter.

ViaCyte and Vertex are now both developing new macro-encapsulation devices that use innovative materials designed to not provoke a foreign body response. Vertex's proprietary technology was developed in-house, whereas ViaCyte has partnered with materials company W. L. Gore in Newark, Delaware, best known for its Gore-Tex waterproof fabrics. The device they have worked on together is now entering phase II trials.

Tomei, meanwhile, is focused on micro-encapsulation and has developed a hydrogel-coating for cells that excludes immune cells while permitting β cell function[8]. She is collaborating with Sernova, a regenerative-medicine company in London, Canada, on an approach where a cell pouch is inserted below the skin two weeks before micro-encapsulated islets are introduced. In this two-week period, new blood vessels enter the pouch and the initial inflammation subsides – both of which should aid islet engraftment. Sernova is currently assessing a basic cell pouch in a phase I/II trial.

> **"Now I have a different goal. I don't want to make a normal cell – I want to make a super cell."**

A further crucial aspect of continuing trials is determining where cells can be optimally placed. "I don't think anybody knows where to put the devices," says Melton. The field has so far experimented with implanting them in various subcutaneous locations and is also, among other possibilities, considering the omentum – a large flap of fatty tissue that surrounds the intestines. Sites differ in their ability to generate vasculature in and around devices; their ability to hold devices and potentially tolerate multiple implantations, if and when cells need replacing; and also simply in how comfortable it is to have a device implanted there.

Super cells

An alternative to physically shielding islet cells from the immune system is to genetically modify them to evade immune detection. ViaCyte and Vertex are both exploring this, but are tight-lipped about their strategies.

Melton sees several possible solutions. One is to take cues from how cancer cells evade immune destruction. Oncological research has shown that expression of certain cell-surface proteins inhibits immune responses – a phenomenon that could be used to protect β cells.

Another option is to engineer the cells' antigen-presenting molecules. These could be removed or, as Melton is doing, replaced. He's studying what happens if islet cells express the fetal form of these molecules, which normally prevent the mother's immune system from attacking fetuses.

And the possibilities offered by gene editing do not end at cloaking the cells. The technology could also be used "to enhance engraftment, survival and performance", Kieffer says. Cells could be adapted to better tolerate the mild hypoxia associated with encapsulation devices or poorly vascularized implantation sites, for instance, or even to better tolerate being frozen for storage.

"When we started on this, our goal was to make a normal, natural β cell," Melton says. "Now I have a different goal. I don't want to make a normal cell – I want to make a super cell."

The concern with all these approaches is that the cells become too good at surviving. In the absence of functional immune surveillance, if implanted cells become infected with a virus or start to divide – potentially causing cancer or dangerously releasing excess hormones – there needs to be a way to eradicate them. Consequently, researchers are studying ways of spiking β cells with suicide genes, which would produce a cell-killing protein upon cell division or when people are given a specific drug.

Melton thinks that incorporating suicide genes is a straightforward challenge compared with evading immune destruction. He dreams of a future where cell therapy is routine and people can go years without thinking about their diabetes. "I think it's challenging," he says, "but I don't think that's crazy."

Liam Drew is a freelance writer near London.

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