Pig islets encapsulated in alginate.

Gordon Weir and Susan Bonner-Weir

ENCAPSULATE THIS

Insulin-producing islet cells could hold the secret to curing type 1 diabetes—if only scientists could figure out a way to encapsulate and transplant them into the body. But first, the right biocompatible material must be found to hold these precious cells. A team of bioengineers thinks it has discovered one. **Elie Dolgin** reports.

Arturo Vegas hits the play button on his tablet computer. A video pops up showing the inside of a monkey's abdomen. "You see this blistering?" asks Vegas, a chemist at the Massachusetts Institute of Technology (MIT) in Cambridge. He points to the lining of the abdominal cavity onto which hundreds of tiny balls resembling semi-translucent fish eggs are attached. "Those are all capsules, and what we're trying to do here is wash them out with a saline solution." A large needle comes into view and squirts the capsules with fluid in an effort to retrieve them for analysis. They don't budge.

The capsules have nothing to do with fish, but the shell is made of a material derived from the ocean. Measuring just half a millimeter in diameter, they are made of an ultrapure preparation of alginate, a gelatinous seaweed extract used widely in the food and medical industries. The idea is to embed inside each of these capsules a handful of islet cells, which in turn would secrete insulin into the body. For the estimated 30 million people worldwide living with type 1 diabetes (T1D), the immune system has unwittingly attacked and destroyed the islet cells in the pancreas that normally produce insulin. These individuals can receive insulin injections, but the jabs don't do such a great job of keeping glucose levels in check at all times, and remembering to take the shots while continuously monitoring blood sugar levels can be onerous.

For decades, researchers have sought a way to transplant insulin-producing islet cells into people without the need for immune-suppressing drugs. However, all attempts to cloak the cells in some sort of protective armor—an approach dubbed 'islet encapsulation'—have failed. The body often starts to go after the encased islets, covering implants in fibrous tissue like a scar. This type of immune reaction seems to have happened with the alginate capsules implanted into the monkey in Vegas's video. Even though these capsules don't contain any islets and even though they've only been inside the animal for two weeks, they're engulfed in a layer of scar tissue that has locked them into place.

The problem is that materials such as alginate just aren't biocompatible enough to be used in cell encapsulation. They might be suitable for hip replacements, say, where some collagenous build-up around the transplanted structure won't drastically affect its functionality. But islet cells are living entities. They need access to blood supplies and nutrient flows. They need ample oxygen and glucose to enter the capsule, and the insulin they produce needs to exit into the body. Simply put, the capsules can't be overgrown with fibrotic gunk.

Despite the pressing medical need, surprisingly little research has gone into finding a material that can achieve the stringent demands of islet encapsulation. "Basically, people have been blindly testing different materials without really knowing what contributes to this response," says Albert Hwa, the senior scientist in charge of beta cell therapies at JDRF, the New York–based nonprofit formerly known as the Juvenile Diabetes Research Foundation. "That's what's so frustrating about this field. There hasn't been a very systematic approach to it."

In an effort to change that, last year JDRF formed a consortium centered on encapsulation research. Vegas's team is one of the largest beneficiaries. Supported by a $9 million, seven-year grant from JDRF (first awarded in 2007), plus another $800,000 contribution from The Leona M. and Harry B. Helmsley Charitable Trust, a foundation headquartered in New York that also funds T1D research, his MIT group has completed the first-ever high-throughput

synthesis and biological screen of new modified alginate materials that might better escape the body's immune attack.

Their top hit looks promising. In the same type of monkey tests shown in Vegas's video, the MIT researchers could retrieve almost all the capsules when they were made from the new material; they came back looking clean and fibrosis-free. Moreover, in as-yet-unpublished rodent studies involving the team's new material, capsules packed with insulin-producing islet cells from rats could effectively cure a mouse model of diabetes. "We're excited by it," says Dan Anderson, a bioengineer at MIT who is leading the effort together with the renowned MIT scientist and entrepreneur Robert Langer. "We see some big differences."

Daniel Pipeleers, director of the Diabetes Research Center at Vrije Universiteit Brussel in Belgium, applauds the MIT effort. "By using other forms of alginate, one can achieve better biocompatibility and better outcomes," he says. "I'm a believer in that."

Breaking protocol

The ultimate goal of T1D research is to help people restore normal blood sugar control without the current need for daily injections of insulin. Clinical islet transplantation—in which islets are taken from the pancreases of deceased donors and transplanted into recipients with severe T1D who then receive immunosuppressive therapy to protect against cell rejection—have helped hundreds of people achieve this level of insulin independence since the procedure first began 25 years ago.

With the latest advances in islet preparation and antirejection drugs, the majority of islet transplant recipients now achieve good glycemic control for years on end without requiring insulin therapy, according to James Shapiro, director of the Clinical Islet Transplant Program at the University of Alberta in Edmonton, Canada, who helped develop and refine the most common method of islet transplantation known as the 'Edmonton protocol'. However, "it's still far from a cure because patients are needing heavy immunosuppression, and sometimes they need long-term top-ups if their transplants start to fail," Shapiro says. Additionally, cadaveric pancreases are hard to come by, and most recipients require cells from more than one donor organ to achieve insulin independence.

The cell source problem can be overcome in one of two ways: either with cells harvested from pig organs; or, as most researchers now hope, with islets derived from human embryonic or induced pluripotent stem cells. Whatever the cell source, porcine or human, the transplanted islets will still raise a red flag in the recipient's body. The only way to curb immune destruction without the blunt instrument of immunosuppressive drug therapy, experts say, is to hide the transplanted cells behind a protective, semipermeable barrier. Anderson likens the approach to "a teabag for islets."

Encapsulation technologies can be broken down into two main categories based on the size of the device. There are microcapsules, like those being tested by the MIT team. These have historically been made of alginate, with each capsule containing a few islets apiece, and hundreds of them are usually transplanted into the abdominal cavity of the recipient. (Alternatively, individual islets can be coated directly on the surface with a thin layer of protective hydrogel.) Then, there are macrodevices: typically flat, thin chambers containing large numbers of islets that can be implanted under the skin.

A big advantage of macrodevices, says Shapiro, comes down to their size and placement. Islets "need to be in a location that can be retrieved and can be biopsied and be looked at closely as we look at safety," he says. This is especially true of islets derived from human stem cells, which hold the potential to form benign tumors (although this has yet to be seen with any stem cell–derived products tested in a clinical setting).

Beta testing

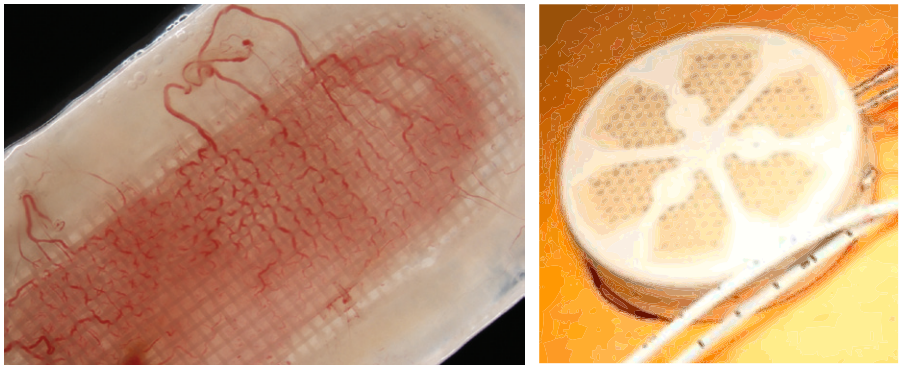
Shapiro is collaborating with the San Diego–based firm ViaCyte to advance an islet encapsulation device known as the Encaptra Drug Delivery System. Slightly larger than a stick of chewing gum, Encaptra contains a semipermeable membrane made of undisclosed polymers into which ViaCyte researchers load embryonic stem cell–derived precursors of insulin-producing beta cells. In preclinical studies, the cells then complete their differentiation over the course of a few months once implanted under the recipient animal's skin.

"There are certainly some unique aspects of this device," says Kevin D'Amour, chief scientific officer at ViaCyte. "In addition, our cell product is different from the types of cells people have used in the past in this kind of a macroencapsulation approach." ViaCyte developed Encaptra and the cells contained within—a combination product labeled VC-01—with tens of millions of dollars from JDRF, the California Institute for Regenerative Medicine and several private investors, including the venture capital arm of New Jersey's Johnson & Johnson (J&J). The company hopes to start human clinical trials later this year. BetaLogics, an internal division of J&J, is working on a similar system, too.

But even as islet cell–containing macrodevices advance toward the clinic, Gordon Weir, co-head of the Section on Islet Cell and Regenerative Biology at the Joslin Diabetes Center in Boston, doesn't hold out much hope for these platforms. "The packing densities just drive me crazy," he says. Weir crunched the numbers for how much islet tissue could be supported by sheet-shaped devices like those being developed by ViaCyte and BetaLogics. The answer: not much. According to Weir's calculations, each square centimeter of a planar macroencapsulation device could support only around 1,000 islets given the needs for oxygen supply from the surrounding vasculature[1].

The Encaptra device has a surface area totaling around 24 square centimeters, which translates to a capacity of around 24,000 islets. Considering that diabetics require around 400,000 islets to stop taking insulin shots, "if you go through the arithmetic, that turns into a fair amount of surface area," Weir says—and thus many Encaptras might be needed for any recipient to safely go off insulin therapy.

Beta-O_2 Technologies may have a solution for the packing density problem. Engineers at the Israeli company have designed a chamber, about the size of a hockey puck, made of alginate-impregnated Teflon that holds two layers of islets (each encapsulated again in alginate microspheres) separated by a central chamber of oxygen. Last year, the company published a case report involving a 63-year-old



ViaCyte, Beta-O_2

The big picture: Macroencapsulation devices from ViaCyte (left) and Beta-O_2 (right).

individual with T1D in Germany who received the Beta-O_2 device filled with cadaveric islets implanted in his abdomen; he did not take immunosuppressive drugs. Ten months after the procedure, the device was still producing some insulin (although not enough for the man to stop taking insulin shots), and the recipient's immune system did not show any signs of attacking or destroying the cells inside[2]. A follow-up eight-person trial is planned for later this year.

The device, dubbed βAir, still requires daily oxygen refills with a needle—which is more maintenance than needed for the ViaCyte device. But according to Beta-O_2's Avi Rotem, the increased oxygen supply means that around 200,000 islets can be supported in a circular device that measures just 68 millimeters across and 18 millimeters thick. "We proved that we could support a very high density of islets," says Rotem, chief technology officer at the company.

Micro management

Those scientists pursuing new takes on the microencapsulation approach have seen glimmers of hope in their approach, as well. David Scharp, president and chief executive of Prodo Laboratories, an Irvine, California–based company that provides human islets and services for diabetes researchers, was part of the first team to successfully transplant islets into a patient with T1D, in 1989. He has worked on encapsulation technologies for decades and serves as a senior advisor to the JDRF Encapsulation Consortium. Scharp is collaborating with polymer chemist Alexander Gorkovenko, president and founder of an Irvine-based startup called TRGel, to develop a novel glucose-based polymer of monomers for islet microencapsulation.

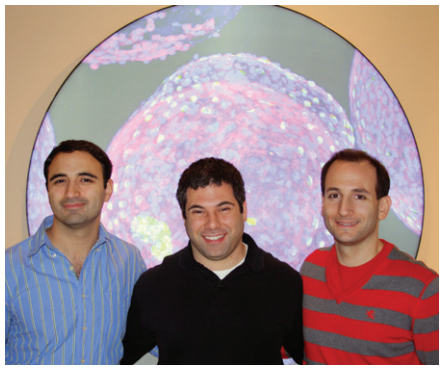
At the 2013 International Pancreas and Islet Transplant Association Congress in Monterey, California, Gorkovenko presented data showing that human islets encapsulated with the new material function well in response to glucose in cell culture. "*In vitro*, it does beautifully," says Scharp. "It has excellent glucose release kinetics; it's not harmful to the cells; it looks really good." Mouse studies with islet-filled capsules are next.

Igor Lacík, director of the Polymer Institute of the Slovak Academy of Sciences in Bratislava, is also advancing a new spin on microencapsulation. He and his colleagues started with alginate beads but then coated the capsules with a layer of another polymer called poly(methylene co-guanidine), or PMCG. José Oberholzer, chief of transplant surgery at the University of Illinois at Chicago, is now collaborating with the Slovakian group to test the capsules in primates.

But human trials, to date, have highlighted the challenges of microencapsulation. Alginate capsules containing human pancreatic islets have been transplanted into a handful of people with T1D. Recipients typically experience some metabolic relief from the treatment, but the effects are limited and transient. In one instance, doctors in Australia analyzed capsules recovered from one person with T1D 16 months after transplantation: they were surrounded by fibrous tissue and the islets in the middle were dead[3]. This, says scientists, drives home the need for the JDRF Encapsulation Consortium and the work it supports.

Integral derivative

In a sixth-floor laboratory at the MIT Koch Institute, a technician sits behind a droplet generator. A steady stream of tiny beads, each made from the MIT team's leading new material, falls into a metal bowl of water.



Elie Dolgin

Islet innovators: MIT's Vegas, Veiseh and Doloff.

The material, which, for now, has been given a placeholder name of 'E9', is one of 774 derivatives of alginate that Anderson, Langer and their colleagues synthesized in search of a more biocompatible building block for islet encapsulation. From this library, every material that could form a hydrogel, and thus was suitable for making capsules, went through a rigorous test for biocompatibility in what Anderson calls the "high-throughout mouse"—basically an animal in which the researchers place up to eight different materials just below the skin at different sites along a single mouse's back[4]. Using fluorescence imaging, they could then track inflammatory responses to the implanted biomaterials. "If it lights up, it's bad," Vegas explains.

With this approach, the MIT team whittled down the chemical library to E9 and two backup substances, all of which proved much less immunogenic than standard alginate. E9 has now gone into monkeys as empty capsules measuring either 0.5 or 1.5 mm across (there's some indication that larger capsules are less prone to fibrotic reactions). The researchers are also working with Dale Greiner at the University of Massachusetts Medical Center in Worcester to test the material in mice with humanized immune systems, and they've teamed up with Douglas Melton, co-director of the Harvard Stem Cell Institute in Cambridge, to fill the capsules with human embryonic stem cell–derived beta cell precursors. "Now that we have the right material, we still need a cell source to put in there," says Omid Veiseh, a postdoc working on the project who is already planning ahead for eventual human clinical trials.

In their longest experiment to date, the MIT scientists took rat islets, encapsulated them with E9, and implanted them into diabetic mice. The experiment had to be stopped after ten months because the chemical initially used to induce diabetes, a beta cell–killing substance called streptozotocin, appeared to have a delayed effect of causing facial tumors in the study animals. However, at the end of the experiment there was nothing wrong with the glucose metabolism in the mice. And, importantly, there were few signs of fibrosis. "The modified chemistry is actually changing the recognition of the material by the immune system," says postdoc Joshua Doloff. "You can still find islets that are perfectly alive and producing insulin just fine," adds Vegas.

Long-term studies involving islet-filled capsules in monkeys are planned for later this year. "We view that as a linchpin piece of data," says Anderson. "If it looks great that new capsules are clean and conventional capsules are dysfunctional in primates, then that, combined with the long-term cures in rodents, we see as an important step toward the clinic."

Oberholzer has teamed up with Anderson, Langer and their MIT colleagues to assist with the testing of their alginate derivative in monkeys. He is cautiously optimistic about the new material, but he notes there's a long way to go yet before an encapsulated islet therapy will be ready for use in patients with T1D. "This is step 1; you can put the material into a living animal or human and it's not going to react," Oberholzer says. "Step 2 is then to put in [islet] cells and show that the cells can actually survive in there and correct diabetes. And step 3 would be to show that they are going to function over a certain period of time without being rejected."

"We are still struggling with step 1," he says, "which is kind of amazing."

Elie Dolgin is senior news editor at* Nature Medicine *in Cambridge, Massachusetts.

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