

Designing a retrievable and scalable cell encapsulation device for potential treatment of type 1 diabetes

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Cell encapsulation has been shown to hold promise for effective, long-term treatment of type 1 diabetes (T1D). However, challenges remain for its clinical applications. For example, there is an unmet need for an encapsulation system that is capable of delivering sufficient cell mass while still allowing convenient retrieval or replacement. Here, we report a simple cell encapsulation design that is readily scalable and conveniently retrievable. The key to this design was to engineer a highly wettable, Ca²⁺-releasing nanoporous polymer thread that promoted uniform in situ cross-linking and strong adhesion of a thin layer of alginate hydrogel around the thread. The device provided immunoprotection of rat islets in immunocompetent C57BL/6 mice in a short-term (1-mo) study, similar to neat alginate fibers. However, the mechanical property of the device, critical for handling and retrieval, was much more robust than the neat alginate fibers due to the reinforcement of the central thread. It also had facile mass transfer due to the short diffusion distance. We demonstrated the therapeutic potential of the device through the correction of chemically induced diabetes in C57BL/6 mice using rat islets for 3 mo as well as in immunodeficient SCID-Beige mice using human islets for 4 mo. We further showed, as a proof of concept, the scalability and retrievability in dogs. After 1 mo of implantation in dogs, the device could be rapidly retrieved through a minimally invasive laparoscopic procedure. This encapsulation device may contribute to a cellular therapy for T1D because of its retrievability and scale-up potential.

cell encapsulation | diabetes | medical device | cell transplantation | retrievable

Type 1 diabetes (T1D) is an autoimmune disease where the patients' insulin-producing pancreatic islet cells are mistakenly destroyed by their own immune system (1). Although current treatment by daily injections or infusion of exogenous insulin provides blood glucose (BG) control, the approach requires constant attention and strict compliance. It does not cure the disease or prevent the many devastating effects associated with diabetes, such as blindness, hypertension, kidney disease, and vasculitis (2). Islet transplantation provides a potential alternative to treat T1D and has been shown to restore normoglycemia (3). However, to avoid immune rejection of transplanted islets, long-term immunosuppressive drug administration is necessary, which is known to cause deleterious side effects (4).

Cell encapsulation allows transplantation of islets or stem cell-derived beta-like cells without immunosuppression and has therefore become a very promising approach for T1D treatment (5–12). The encapsulating material or device protects the cells from the host immune rejection while simultaneously allowing mass transfer to maintain cell survival and function. Despite tremendous research efforts worldwide and much significant progress that has been made, clinical application of cell encapsulation has remained elusive (5, 8, 12). Currently there are two major types of

islet encapsulation systems: macroscopic devices and hydrogel microcapsules. Both systems have been shown to be functional in numerous preclinical studies but unfortunately have had serious limitations for clinical applications. For example, macroscopic devices such as diffusion chambers have a small surface area for mass transfer and consequently low encapsulation capacity (8–10, 13, 14). Even though the capacity problem may be addressed by increasing the cell packing density, dense packing will also inevitably lead to a hypoxic environment and associated impairment in insulin secretion (15, 16). Thus, the scale-up to a capacity sufficient to cure a human patient has been challenging (5, 8–10, 14, 17, 18). Alginate hydrogel capsules, on the other hand, have a large surface area for mass transfer and can, in principle, deliver a sufficient number of islets (19–24). In particular, the biocompatibility of alginate materials has been significantly improved in recent years through advanced purifications, formulations, and chemical modifications (7, 11, 25–31). However, one major problem with capsules is that it is almost impossible to completely retrieve or replace them after implantation in the peritoneal cavity due to the complicated organ structures and the large capsule number required (i.e., ~100,000 capsules needed for a human patient) (4, 8, 18, 32). This raises significant risks and concerns in

Significance

Cell encapsulation holds great potential as a better treatment for type 1 diabetes. An encapsulation system that is scalable to a clinically relevant capacity and can be retrieved or replaced whenever needed is highly desirable for clinical applications. Here we report a cell encapsulation device that is readily scalable and conveniently retrievable through a minimally invasive laparoscopic procedure. We demonstrated its mechanical robustness and facile mass transfer as well as its durable function in diabetic mice. We further showed, as a proof of concept, its scalability and retrievability in dogs. We believe this encapsulation device may contribute to a cellular therapy for type 1 diabetes and potentially other endocrine disorders and hormone-deficient diseases.

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the event of transplant failure or medical complications (5, 8, 12, 33, 34). Retrieval is also an important issue associated with the regulatory approval process (35).

To address these challenges, here we report a simple and translatable cell encapsulation design that is both scalable and retrievable. The design involves a one-step in situ cross-linking of an alginate hydrogel around a nanoporous, wettable, Ca^{2+} -releasing polymer thread. We term the design as TRAFFIC (thread-reinforced alginate fiber for islets encapsulation). In TRAFFIC, the hydrogel encapsulating cells provide the necessary mass transfer and biocompatibility similar to conventional hydrogel capsules, while the polymer thread imparts mechanical strength and enables easy handling, implantation, and retrieval. The device may be extended to meters long and still be entirely retrievable through a minimally invasive laparoscopic procedure. To demonstrate the therapeutic potential of the device, we encapsulated and transplanted rat islets into diabetic C57BL/6 mice or human islets into SCID-Beige mice and obtained diabetes correction for up to several months. As a proof of concept, we scaled up the device and showed its retrievability in dogs. Given its simplicity and translatability, this device may contribute significantly to a cell encapsulation therapy for T1D and potentially many other diseases.

Design and Fabrication of the Device

The TRAFFIC device consists of a tough polymer thread and a uniform, strongly adhered alginate hydrogel layer with controllable thickness (Fig. 1A). To obtain strong adhesion between the thread and the hydrogel, we designed the thread by mimicking the highly adhesive, nanoporous silks that certain spiders use for capillary-enabled water collection and retention (36). To achieve uniform, controllable hydrogel formation, we incorporated a Ca^{2+} -releasing mechanism into the thread design. The fabrication process (Fig. 1B) is simple but involves several material design principles. First, to create the spider silk-like, nanoporous (and mechanically tough) thread, we chose to coat a nylon suture with 7% (wt/vol) poly(methyl methacrylate)/N,N-dimethylformamide (PMMA/DMF) solution. Under humid conditions, the drying of the polymer solution resulted in evaporative cooling and phase separation, leading to nanoporous structures (37, 38). However, due to the surface tension-driven Rayleigh Instability (39, 40), the coating solution dewetted on the suture and formed patchy beads instead of continuous coatings (Fig. 1C and *SI Appendix, SI-1*), leading to a “beads-on-a-string” morphology after drying. To overcome this problem, we twisted two sutures (typically 5-0) together to form a double helix and folded the helix in the middle. Upon folding, the torsion generated from the twisting spontaneously led to a stable

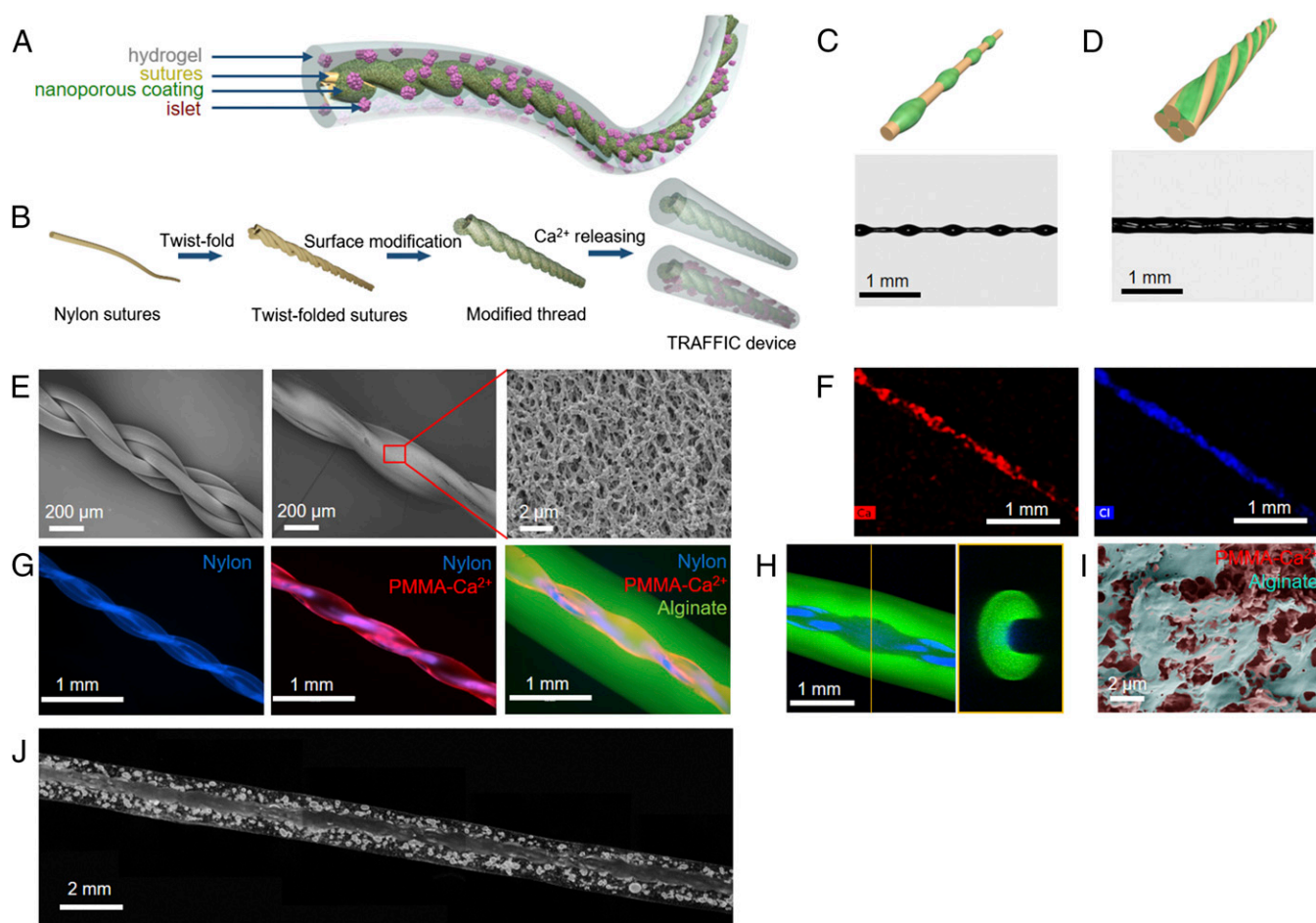


Fig. 1. Schematics and fabrication of the TRAFFIC device. (A) Schematic illustration of the design of TRAFFIC. (B) Schematic illustration of the fabrication process. (C and D) Schematic illustrations and optical images of the PMMA/ CaCl_2 /DMF solution coating on a monofilament nylon suture (C) and a thread made of twist-folded sutures (D). (E) SEM images of the thread and the uniform nanoporous surface modifications. (F) EDS element mapping of Ca and Cl on a modified thread. (G) Fluorescent microscopic images of the thread, modified thread, and a TRAFFIC device (without cells). (H) Confocal images of a TRAFFIC device. (I) An SEM image of the interface between alginate hydrogel and the modified thread after lyophilization. (J) A composite microscopic image (stitched from three individual microscopic images of 2x magnification) of a TRAFFIC device encapsulating rat islets.

four-strand thread with a “ridges-in-grooves” structure (Fig. 1 *D* and *E* and *SI Appendix, SI-2*). In contrast to the coating on the smooth suture, the solution formed liquid wedges along the grooves of the twist-folded, four-strand thread. This simple method prevented the dewetting and resulted in a continuous nanoporous coating (Fig. 1 *D* and *E* and *SI Appendix, SI-2*) on the thread. Lastly, within the nanoporous coating, we incorporated the cross-linking agent CaCl_2 by taking advantage of an unusual and unique property of CaCl_2 —its solubility in DMF. By dissolving 2.5% (wt/vol) CaCl_2 in the PMMA/DMF coating solution, we obtained a nanoporous thread with a uniform distribution of Ca and Cl as evidenced by energy dispersive spectrometer (EDS) X-ray element mapping (Fig. 1*F*).

After the thread was made, the formation of an alginate hydrogel layer or the fabrication of a TRAFFIC device was achieved through a simple, one-step, in situ cross-linking process (Fig. 1*B*). In a typical process, a 2% (wt/vol) alginate solution [e.g., ultrapure sterile SLG100 alginate dissolved in a 0.9% (wt) NaCl solution] was placed in a tubular mold or a channel reservoir, and a modified thread was then inserted into the alginate solution. After a 4-min cross-linking, the entire device was taken out and placed in a Ca/Ba solution (typically 95 mM CaCl_2 and 5 mM BaCl_2) for 5 min for further cross-linking. The hydrogel layer that formed around the thread was uniform (Fig. 1 *G* and *H*), and the thickness could be controlled by adjusting the amount of incorporated CaCl_2 and the cross-linking time (see *SI Appendix, SI-3* for details). In addition, examination of the interface between the nanoporous thread and the hydrogel in a lyophilized sample (Fig. 1*I*) seemed to suggest that the hydrogel infiltrated into the porous surface, which was believed to contribute to the strong thread–hydrogel adhesion. For cell encapsulation, the process was similar

and an alginate solution containing cells or cell aggregates such as rat islets (Fig. 1*J*) was used for cross-linking. It is noted that the continuous nanoporous modification of the thread, resulted from the twisted helical structure and the evaporative cooling, and the internal release of Ca^{2+} are both of great significance to this encapsulation design. Simple sequential dipping of a conventional thread (i.e., bare suture) into a CaCl_2 solution and an alginate solution resulted in much less well-controlled hydrogel formation (*SI Appendix, SI-4*) (41). Our biomimetic thread may be used as an off-the-shelf, ready-to-use product for cell encapsulation conveniently at the research or transplantation place.

Characterizations of the Mechanical Robustness, Mass Transfer Property, and Biocompatibility

To show the mechanical robustness of the TRAFFIC device, we measured both the tensile strength of the whole device and the adhesion between the thread and the hydrogel. Compared with neat alginate hydrogel fiber, TRAFFIC exhibited drastically higher strength (Fig. 2*A*). More importantly, the hydrogel–thread adhesion in TRAFFIC was also remarkably high, much higher than the device made from the nonuniform beads-on-a-string thread or the one made by sequential dipping of a bare suture (Fig. 2*B* and *SI Appendix, SI-5*). We attributed the high adhesion to both the nanoporous surface structure (similar to the adhesion of water droplets to spider silks) and the macroscopic helices from the twisting of sutures (see *SI Appendix, SI-5* for detailed analysis). To further demonstrate the necessity of the thread reinforcement for easy handling, we compared the TRAFFIC with a neat alginate fiber. As shown in Fig. 2*C*, the TRAFFIC was much more mechanically robust and easier to handle (also see *Movies S1* and *S2*). In addition, the neat alginate fiber can clump or entangle with

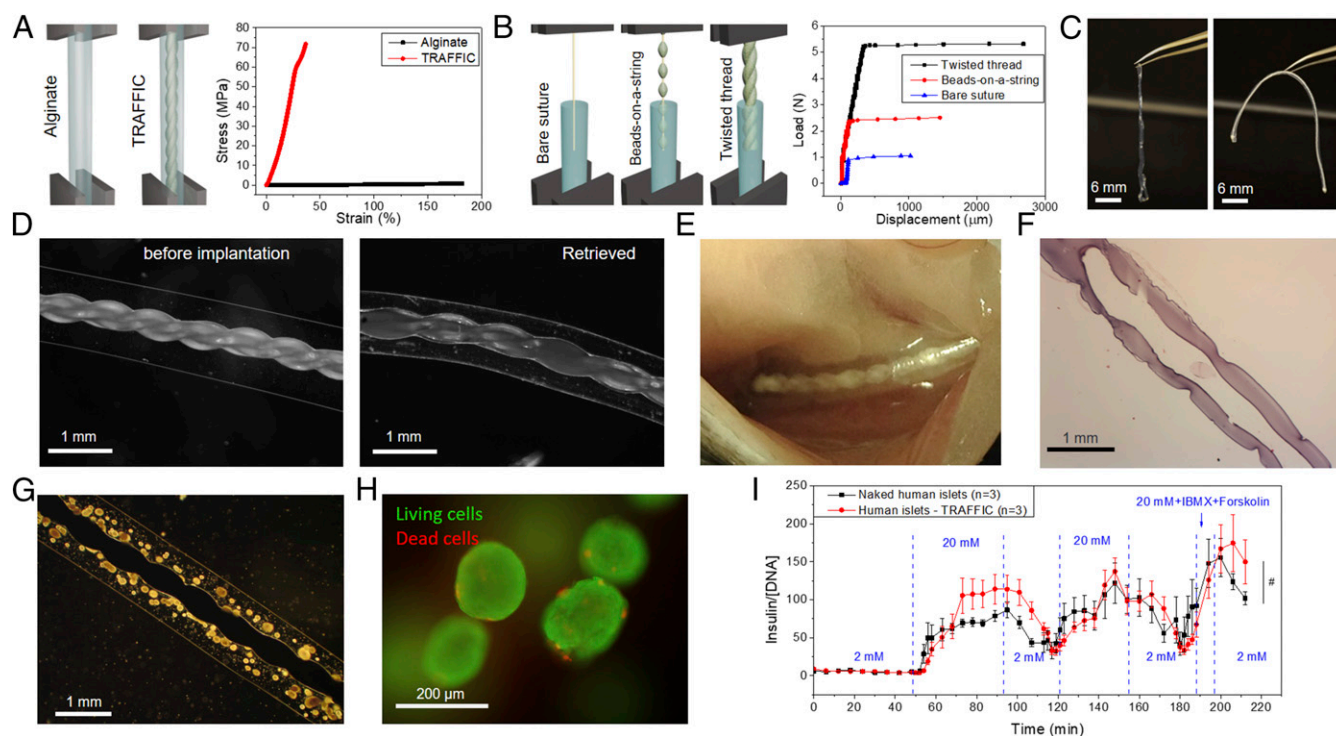


Fig. 2. Characterizations of the mechanical robustness, biocompatibility, and mass transfer property of TRAFFIC. (A–C) Mechanical robustness test: (A) strain-stress measurement, (B) load-displacement measurement, and (C) comparison between a neat alginate fiber and a TRAFFIC device in handling. (D–F) Biocompatibility characterization: (D) Microscopic images of TRAFFIC before implantation and after 7-mo implantation in mice. (Note that the devices shrank slightly after being transferred into a 4% paraformaldehyde for fixation.) (E) Digital photo of the device in the i.p. space of a mouse during the retrieval. (F) An H&E-stained image of a retrieved device ($n = 11$; see *SI Appendix, SI-7* for complete data). (G–I) Mass transfer property: (G) microscopic image of the TRAFFIC device encapsulating human islets, (H) live (green)/dead (red) staining of encapsulated human islets, and (I) the glucose-stimulated human insulin secretion in a dynamic perfusion test, $n = 3$, mean \pm SEM, $^{\#}P > 0.05$.

itself within the peritoneal cavity, jeopardizing the mass transfer or causing fibrosis (SI Appendix, SI-6). The mechanical robustness and easier handling are important advantages during implantation and retrieval.

Next, we investigated the biocompatibility of TRAFFIC. In cell encapsulation, the biocompatibility of the encapsulating material is one of the most important factors; foreign body reaction-induced fibrosis can negatively affect the mass transfer and the viability of encapsulated cells (42, 43). In recent years, much progress has been made on the biocompatibility of alginate materials, including both purity and formulation (25, 30). Particularly, it has been shown that increasing the size of intraperitoneally implanted alginate capsules from $\sim 500\ \mu\text{m}$ to $\sim 1.5\ \text{mm}$ reduced fibrosis (29). Inspired by these studies, we tested TRAFFIC with different thicknesses of the alginate layer in immunocompetent C57BL/6 mice and found that the thick device (overall diameter $\sim 1.3\ \text{mm}$) had less cellular overgrowth than the thinner ones ($\sim 500\ \mu\text{m}$) after 2 wk of i.p. implantation (SI Appendix, SI-7). We believe this difference could be caused by many factors such as stiffness and surface area (SI Appendix, SI-7), and future work and more quantitative immunological studies are needed to elucidate the exact mechanisms. Nevertheless, encouraged by the short-term results, we further conducted longer term studies (3 mo, $n = 6$; 7 mo, $n = 11$). In the 3-mo study (SI Appendix, SI-7), while the thin devices induced variable cellular overgrowth, the thick ones had significantly less, consistent with the 2-wk results. Interestingly, even after 7 mo of implantation, 10 out of the 11 thick devices remained almost free of fibrosis (Fig. 2 D–F and SI Appendix, SI-7). In the following tests of function and demonstration of scalability and retrievability, the thick device design was used.

Another factor that is critical to the function of an encapsulation device is the mass transfer property (44). In the TRAFFIC design, since the cells are encapsulated in alginate hydrogel that has been proven to have facile mass transfer and since all of the cells are near the surface, we hypothesized that the mass transfer would be sufficient to support the cell survival and function. Indeed, experiments with human islets (Fig. 2 G and H) and several other types of cells (SI Appendix, Fig. S18) confirmed the cell viability in *in vitro* cultures. In addition, we performed a dynamic glucose-stimulated insulin secretion (GSIS) experiment using an islet perfusion system. The islets were subjected to Krebs–Ringer bicarbonate Hepes (KRBH) buffers with low (2 mM) or high (20 mM) glucose concentrations continuously in an alternating pattern, and the secreted insulin was measured. The results showed that both the nonencapsulated and encapsulated human islets could sense the glucose concentration change and secrete insulin (Fig. 2I). Application of 3-isobutylmethylxanthine (IBMX) and forskolin was included as a positive control and increased the insulin secretion in both cases. Although there appeared to be a delay in response for encapsulated islets, statistical analysis (see details in *Methods*) showed no significant difference in both total insulin secretion and the kinetics in this experiment. The facile mass transfer was probably due to the short diffusion distance (i.e., proximity of encapsulated islets to the surface). Previous studies have shown that the short diffusion distance was beneficial for not only cell survival but also glucose responsiveness (14, 45). We also measured the mass transfer of TRAFFIC more directly using different molecular weight, FITC-labeled dextran standards. The results indicated that the device would prevent the diffusion of molecules larger than $\sim 250\ \text{kDa}$ (SI Appendix, SI-8).

Diabetes Correction in Mice

After confirming the mechanical robustness, biocompatibility, and mass transfer property of TRAFFIC, we explored its therapeutic potential. We transplanted encapsulated rat islets into the peritoneal cavity of streptozotocin (STZ)-induced C57BL/6 diabetic mice ($n = 10$ pooled from two independent experiments). This “rat-to-mouse” model has been used extensively in the field, and

alginate hydrogel materials have been shown to be effective to protect xenografts without immunosuppression (27). Each diabetic mouse received a device of $\sim 1\text{-inch}$ length containing $\sim 475 \pm 25$ islet equivalents (IEQs) (Fig. 3A) (46). (Note that the cell survival posttransplantation was not studied, and therefore, the actual, functional IEQs inside a mouse was unknown.) The BG level of the mice decreased to the normal glycemic range ($\text{BG} < 200\ \text{mg/dL}$) 2 d after the transplantation, and the mice remained cured for 4 wk before the devices were retrieved. After retrieval, the mice returned to a diabetic state, indicating the effectiveness of the device in regulating the BG (Fig. 3B). An i.p. glucose tolerance test (IPGTT) was conducted on day 28 after transplantation, before retrieval. The BG increased to more than $400\ \text{mg/dL}$ in both healthy and diabetic mice after an i.p. injection of glucose solution (2 g of glucose per 1 kg of body mass). The BG of the transplanted mice gradually dropped to normal range within 120 min, further confirming the function of transplanted islets (Fig. 3C). The retrieved devices showed no tissue adhesion or significant fibrosis (Fig. 3D). An *in vitro* static GSIS (Fig. 3E) test of the retrieved devices suggested that the islets were responsive to glucose increase and secreted insulin, indicating the viability and normal function of the retrieved islets. Moreover, histological studies (Fig. 3F) showed minimal cellular overgrowth around the devices and normal morphology of islets with positive staining of insulin (Fig. 3G).

In another set of 1-mo transplantation experiments, we also included a control group of rat islets encapsulated in neat alginate fibers and a control group of BALB/c mouse islets encapsulated in TRAFFIC (Fig. 3H and SI Appendix, SI-9). With a similar number of rat islets ($\sim 475 \pm 25$ IEQ), TRAFFIC performed similarly to the neat alginate fiber, confirming that the modified thread in TRAFFIC had no negative effect on the therapeutic potential. The results from rat islets and BALB/c mouse islets were also similar, although we used a lower IEQ ($\sim 380 \pm 20$) for mouse islets. These control experiments confirmed the immunoprotective function of TRAFFIC for at least 1 mo, similar to neat alginate fibers. However, compared with the neat alginate fibers, TRAFFIC had much more robust mechanical properties that were critical for handling, retrieval, and scale-up. To examine whether TRAFFIC could have a longer term function, we conducted two independent 3-mo transplantation experiments using the rat-to-mouse model with a control group of unencapsulated rat islets transplanted in kidney capsules (in total, $n = 14$ for TRAFFIC and $n = 8$ for kidney capsules). For the mice with kidney capsule transplantation of unencapsulated islets, the BG level was decreased briefly after transplantation, suggesting that the primary graft function was achieved. However, the transplants were rapidly rejected, all within 2 wk. In contrast, TRAFFIC protected the islets from rejection and cured 10 out of 14 mice for at least 3 mo (Fig. 3I and SI Appendix, Fig. S21). The normal morphology of islets retrieved from cured mice as shown by the H&E staining and the positive immunohistochemical staining of insulin and glucagon confirmed the islet function in the device (Fig. 3J).

To further demonstrate the function of TRAFFIC, we performed more experiments in a different transplantation model using human islets. Given the large species difference between human and mouse, we chose immunodeficient SCID-Beige mice as recipients to minimize the complication of immune responses that occur in xenogeneic transplantation (47). We first tested the viability of encapsulated human islets (Fig. 4A) in nondiabetic SCID-Beige mice. The devices were retrieved after 1 mo and had minimal cell attachment and fibrosis (Fig. 4 B and C). Live/dead staining (Fig. 4D) and *in vitro* GSIS (Fig. 4E) of the retrieved islets indicated high viability and expected function. Next, we transplanted encapsulated human islets into STZ-induced diabetic SCID-Beige mice. We considered the mice to be diabetic when we had two consecutive measurements over $300\ \text{mg/dL}$, which we acknowledge was a relatively low standard for diabetes. However,

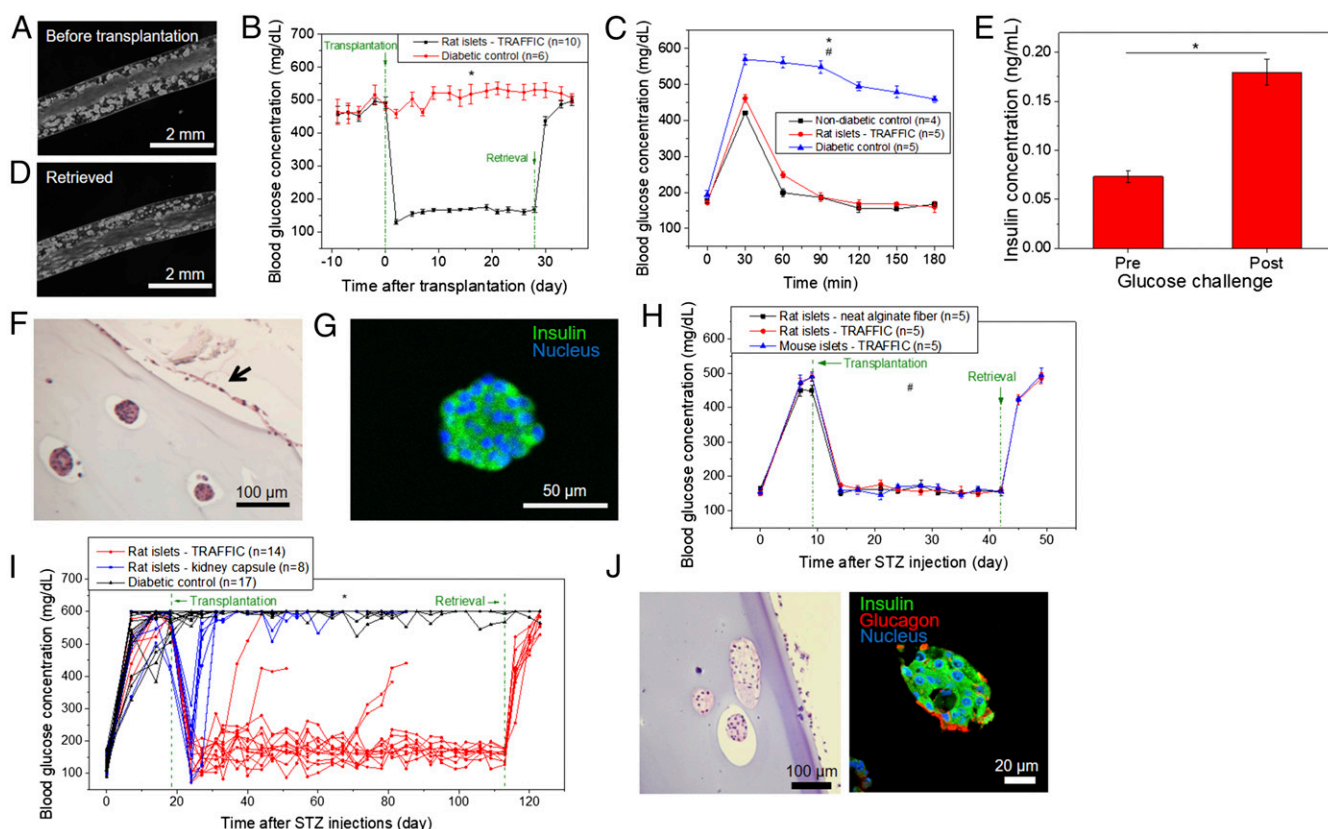


Fig. 3. Demonstration of therapeutic potential of TRAFFIC using rat islets. (A) Microscopic image of a TRAFFIC device encapsulating rat islets before transplantation. (B) BG concentration of diabetic C57BL/6 mice after transplantation of encapsulated rat islets, $n = 6-10$, mean \pm SEM, $*P < 0.05$. (C) IPGTT before retrieval on day 28, $n = 4-5$, mean \pm SEM, $*P < 0.05$ (diabetic control versus rat islets—TRAFFIC), $\#P > 0.05$ (nondiabetic control versus rat islets—TRAFFIC). (D) A microscopic image of the retrieved device. (E) In vitro GSIS test of the retrieved rat islets, $n = 3$, mean \pm SEM, $*P < 0.05$. (F) H&E staining of cross-sections of retrieved islets (the arrow points to the cellular overgrowth on the device). (G) Immunohistochemical staining of the islets in a retrieved device (green, insulin; blue, nuclei). (H) BG concentrations of diabetic C57BL/6 mice after transplantation of rat islets encapsulated in neat alginate fiber or TRAFFIC and allogenic mouse islets encapsulated in TRAFFIC, $n = 5$, mean \pm SEM, $\#P > 0.05$ (among all three groups). (I) BG concentrations of mice from 3-mo transplantation studies, $n = 8-17$, mean \pm SEM, $*P < 0.05$ (diabetic control versus rat islets—TRAFFIC). (J) H&E staining and immunohistochemical staining of retrieved islets in TRAFFIC.

when we measured mouse c-peptide for the nontransplanted control groups, we found that the average was 42.7 pM for the STZ-treated group (94 d after STZ treatment) and 123.2 pM for the nontreated group. The c-peptide comparison in combination with the BG levels indicated that the mice had lost a substantial part of the beta cell mass due to the STZ treatment. Each STZ-treated mouse received two devices ~ 1 inch long containing $\sim 1,900 \pm 100$ IEOs in total (48, 49). Nonencapsulated human islets of a similar number were transplanted in kidney capsules as a control. The BG and body weight were monitored over time. It took over 2 wk after transplantation to reverse the hyperglycemia. This slow response could be due to the species difference and/or the islet quality; similarly, slow BG reduction was observed previously following transplantation of human islets into kidney capsules of immunodeficient diabetic mice (49). However, after the initial diabetes correction, the BG levels of the mice in both the device and kidney capsule groups were maintained within the normal range for more than 4 mo until the experiment ended (Fig. 4F). In addition, the mice in both groups gained weight after transplantation, compared with the diabetic control group (Fig. 4G). Immunohistochemical staining showed positive staining of human insulin, Nkx-6.1, and glucagon in the islets retrieved from both kidney capsule and TRAFFIC, similar to the islets before transplantation (Fig. 4H). Assembled together, these data provide an important proof of concept for the use of TRAFFIC for T1D treatment.

Scale-Up and Test of Retrievability in Dogs

Two important advantages of the TRAFFIC design are scalability and retrievability. It is estimated that 500,000 IEOs may be needed to cure a human T1D patient (50). To deliver such a large number of islets, scalability is critical to any successful cell encapsulation system. Due to the thin cylindrical geometry, TRAFFIC can be scaled up in the longitudinal direction to a large capacity and can still be retrieved through minimally invasive laparoscopic procedures. To prove this concept, we fabricated ~ 10 -inch TRAFFIC devices using a custom-made thread holder and a channel reservoir (SI Appendix, Fig. S22A and B). The device can be further scaled up by using a zigzag channel reservoir or tying multiple TRAFFIC devices together (see SI Appendix, SI-10 for details). Next, we performed some large animal experiments using dogs. In an initial pilot experiment, a ~ 1.5 mm-diameter, 10 inch-long device (without cells) made from the beads-on-a-string thread (SI Appendix, Fig. S22B) was laparoscopically implanted into each of two dogs. The procedure was relatively simple and fast. Briefly, the device was placed in a pipette, which was then inserted through a laparoscopic trocar (SI Appendix, Fig. S22C and D and SI Appendix, SI-11). Under the laparoscopic visualization, the device was pulled into the peritoneal cavity using laparoscopic forceps (SI Appendix, Fig. S22C, Left) and placed in the cranial abdomen near the liver. Two weeks later, the device was retrieved by grasping and pulling the device using a similar laparoscopic procedure (SI Appendix, Fig. S22C, Right). In one dog,

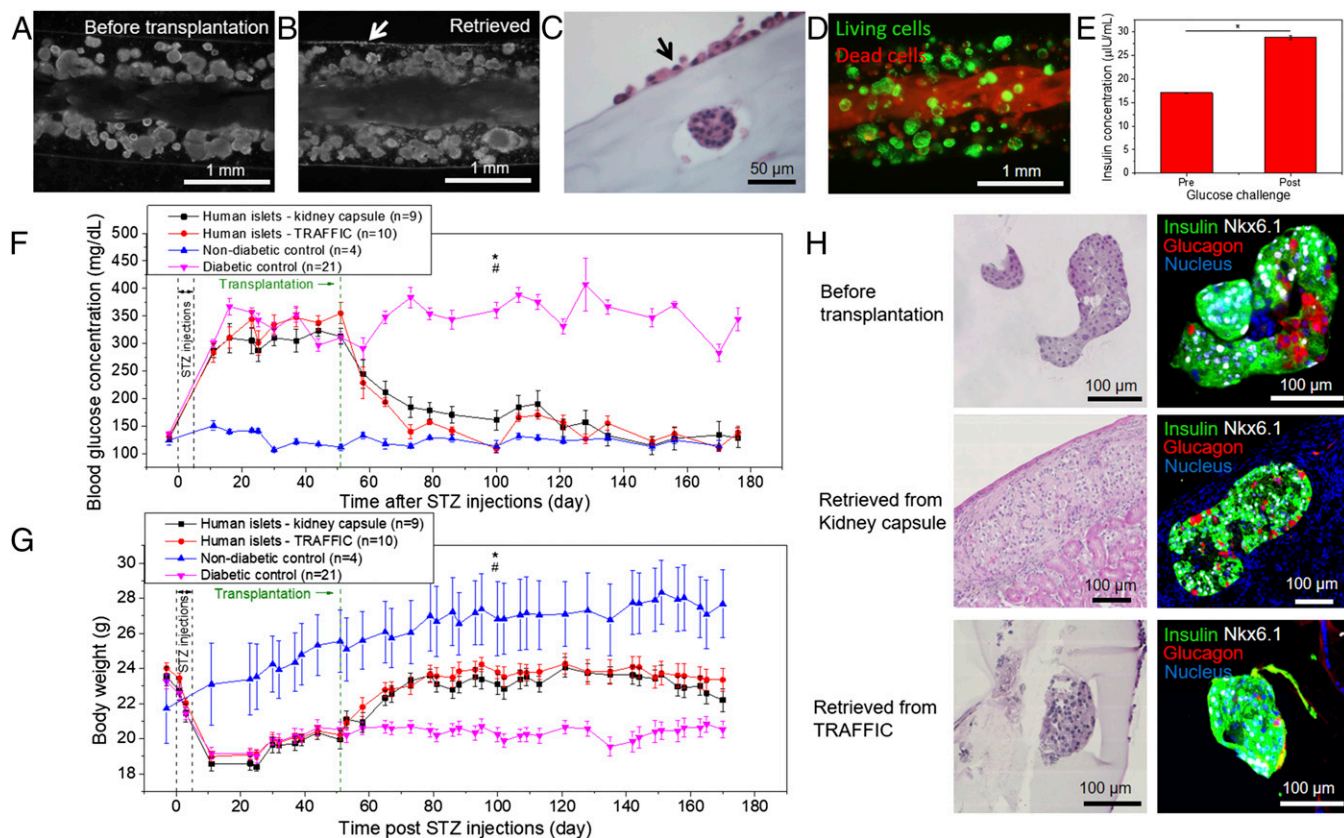


Fig. 4. Demonstration of the therapeutic potential of TRAFFIC using human islets. (A) Microscopic image of a TRAFFIC device with encapsulated human islets before transplantation. (B) Microscopic image of a TRAFFIC device retrieved after 1 mo. (C) H&E staining of a cross-section of the device (the arrow points to the minimal cellular overgrowth on the device). (D) Live (green)/dead (red) staining of human islets in retrieved device. (E) In vitro GSIS test of human islets in a retrieved device, $n = 3$, mean \pm SEM, $*P < 0.05$. (F) BG concentrations of diabetic SCID-Beige mice after transplantation of human islets, $n = 4-21$, mean \pm SEM, $*P < 0.05$ (human islets—TRAFFIC versus diabetic control), $^{\#}P > 0.05$ (human islets—kidney capsule versus human islets—TRAFFIC). (G) Body weights of the mice after transplantation. $n = 4-21$, mean \pm SEM, $*P < 0.05$ (human islets—TRAFFIC versus diabetic control), $^{\#}P > 0.05$ (human islets—kidney capsule versus human islets—TRAFFIC). (H) H&E staining and immunohistochemical staining of human islets before transplantation and retrieved from kidney capsules or TRAFFIC.

minor tissue adhesion occurred between the omentum and a segment of the device in which the hydrogel had detached and the thread was exposed (SI Appendix, Fig. S25). However, the device was still retrievable in its entirety after excising the adhered tissue. In the other dog, there was no adhesion or any gross fibrosis on the device (SI Appendix, Fig. S22 E and F). Remarkably, even though the device left an indentation on the liver (SI Appendix, Fig. S22E), there was no fibrosis or histologic indication of inflammation in the tissue (SI Appendix, Fig. S22G). In both dogs, there were no significant changes in bloodwork (i.e., fibrinogen concentration, white blood cell count, liver enzyme concentrations), suggesting that no inflammation was induced.

Although this first pilot experiment was promising, comparison of the results from the two dogs highlighted the importance of the thread design to ensure strong hydrogel–thread adhesion. To overcome the hydrogel detaching problem that occurred in one of the dogs, we repeated the implantation experiment with three more dogs using a device (diameter of ~ 2 mm) made from the continuously modified, twist-folded thread. One dog was implanted with an empty device while the other two were implanted with devices encapsulating subtherapeutic doses of rat islets ($\sim 1,000$ IEQs per device). The devices were retrieved after 1 mo. All three devices remained intact, and no tissue adhesion occurred (SI Appendix, Fig. S26 and Movie S3). To illustrate the convenient retrieval, we took videos both inside and outside the dog (Fig. 5 A and B). The retrieval was simple and rapid; it took merely 10 s from

grasping the device to removal (Movies S4 and S5). The retrieved devices had only thin layers of attached cells (Fig. 5 C and D). The xenogeneic rat islets induced some local inflammation (SI Appendix, Fig. S26), however there was no tissue adhesion and the devices were still easily retrievable. More importantly, the islets in retrieved devices were still viable and functional, as evidenced by live/dead staining (Fig. 5E), insulin staining of cross-sectioned islets (Fig. 5F), and in vitro GSIS (Fig. 5G).

Discussion

Recent development of stem cell biology has brought tremendous hope that one day human stem cells may be differentiated into functional beta-like cells and transplanted into T1D patients (11, 51, 52). However, to prevent immune and autoimmune attack, the exogenous cells need to be encapsulated or immunoprotected. Developing an encapsulation system that can be clinically used has unfortunately been a great challenge (5, 7, 11, 12). Planar diffusion chambers such as the Viacyte device have shown great promise in preclinical small animal studies (53). However, there are considerable challenges to scale up the device to human patients due to the intrinsically low surface area for mass transfer; high cell packing density may lead to deficiency of oxygen and nutrients (7, 13, 14). The other major type of encapsulation system is the hydrogel capsule. Hydrogel capsules have a larger surface area and can potentially deliver a higher number of islets (5, 7, 29, 54). Importantly much progress has been made recently on the biocompatibility of

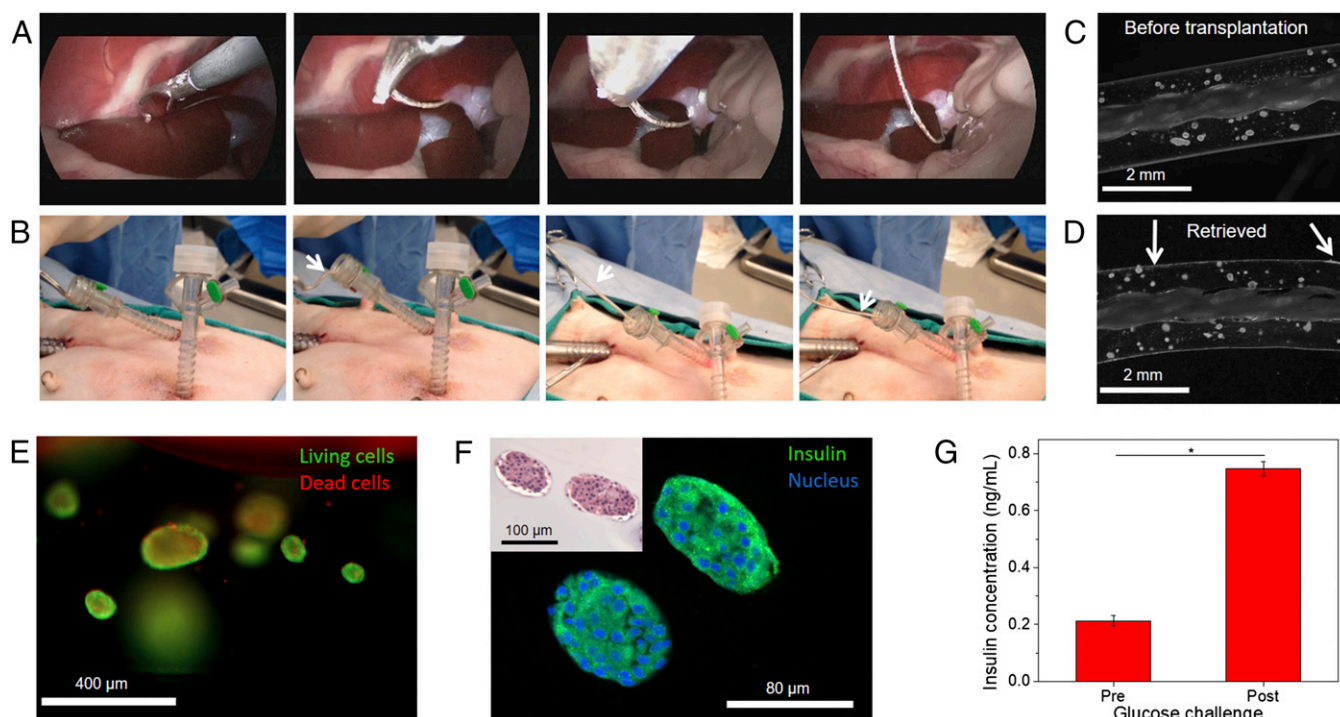


Fig. 5. Demonstration of rapid device retrieval and cell survival in a dog model. (A) A series of laparoscopic images showing the retrieval process. (B) A series of digital images showing the device being pulled out from a trocar. (C and D) Microscopic images of the device with encapsulated rat islets before (C) and after (D) transplantation in dogs (the arrow points to the minimal cellular overgrowth on the device). (E) Live/dead staining of retrieved rat islets. (F) Insulin staining of retrieved islets. (Inset) H&E staining. (G) In vitro GSIS, $n = 3$, mean \pm SEM, $*P < 0.05$.

alginate hydrogels (30, 43), which makes the capsules more functionally durable and easier to retrieve. However, common retrieval techniques such as aspiration and lavage are time-consuming, and it is a great challenge to ensure complete retrieval given the large capsule number and the complicated organ structures inside the peritoneal space (8, 18, 32). Incomplete retrieval can cause serious safety concerns when stem cell-derived cells are used due to the potential of teratoma formation (5, 55).

In this work, we report an encapsulation design that takes advantages of alginate hydrogel and is both scalable and retrievable. The key to the design is a tough central thread with continuous nanoporous modification and uniform Ca^{2+} release. The device is in contrast to conventional neat hydrogel fibers, which although previously proposed for cell encapsulation (56–61) were soft and easy to break and therefore not suited for clinical applications. The uniform and controllable hydrogel thickness as well as the strong thread–hydrogel adhesion are important for both function and translation. Convenient retrieval with a minimally invasive surgery would be particularly attractive for clinical applications. For any transplantation, straightforward removal after completion of therapy or failure of transplant would address patients' concerns of having foreign materials and cells permanently implanted in the body (5, 12, 33). As therapeutic cells derived from stem cells or adult cells become a potential alternative to primary cells, retrievability is more highly desired to mitigate the concerns of teratoma formation (5, 55). We believe this encapsulation design will minimize the risks and discomfort associated with transplantation, make repeated transplantation a more acceptable option, and therefore likely accelerate and contribute to the translation of cell encapsulation for T1D and potentially many other diseases. Furthermore, our modified thread device could be available to researchers or clinicians as an off-the-shelf, ready-to-use product, and cell encapsulation may be performed at site via a one-step in situ cross-linking.

Methods

Chemicals. Calcium chloride (CaCl_2), barium chloride (BaCl_2), sodium chloride (NaCl), PMMA, DMF, glucose stock solution, human serum albumin, and IBMX were purchased from Sigma-Aldrich Co. Forskolin was purchased from Tocris Bioscience. Glutamax was purchased from Life Technologies. KRBH was purchased from VWR. Glucose was purchased from Mallinckrodt Pharmaceuticals. Ultrapure, sterile sodium alginate (SLG100) was purchased from FMC BioPolymer Co. [Note that alginates used in this study had no significant amount of endotoxin according to a previous publication (30).] Water was deionized to 18.2 M Ω -cm with a Millipore purification system.

Animals. C57BL/6 and BALB/c mice were purchased from The Jackson Laboratory. SCID-Beige mice for transplantation experiments were obtained from Taconic Farms. Sprague–Dawley rats for isolation of pancreatic islet cells were obtained from Charles River Laboratories. Beagle dogs for implantation were obtained from Marshall Bioresources. All animal procedures were approved by the Cornell Institutional Animal Care and Use Committee. Transplantation of encapsulated human islets in diabetic SCID-Beige mice was performed at Novo Nordisk A/S, and the protocols were approved by the Danish Animal Experimentation Inspectorate and carried out by trained and licensed personnel.

Characterizations. The samples were characterized by different analytical techniques. Scanning electron microscopy (SEM) and EDS element mapping were performed by using a field emission scanning electron microanalyzer (LEO 1550). Optical and fluorescent microscopic images were observed using a digital inverted microscope (EVOS fl). Conventional macrotensile measurements were performed using a dynamic mechanical analysis (DMA Q800). All samples were mounted between holders at a distance of ~ 1.5 cm. Tensile testing was conducted at a rate of 0.5 N/min at room temperature (23 $^{\circ}\text{C}$). Stress (MPa) and strain (%) were automatically calculated by the software. Confocal images were taken by using a Laser Scanning Confocal Microscope (LSM 710).

Fabrication of Modified Threads. Typically, two sterile sutures (Ethilon nylon suture, 5–0, monofilament, Ethicon, Inc.; KRUUSE nylon suture, 5–0, Jørgen Kruuse A/S) were twisted together to accumulate some torsion. Then, the twisted sutures were folded in the middle, which causes the sutures to self-twist again to release the torsion. Knots were made at the end of the

twisted sutures to prevent the sutures from untwisting. Then, the sutures were submerged into 7% (wt/vol) PMMA/DMF solution, containing 2.5% (wt/vol) CaCl_2 , for ~ 3 s. The sutures were taken out from the polymer solution and air dried. After the modification was finished, all of the sutures were sterilized by ethylene oxide or by autoclave in dry mode before use.

Rat Islet Isolation and Purification. Sprague–Dawley rats from Charles River Laboratories weighing ~ 300 g were used for harvesting islets. All rats were anesthetized using 3% isoflurane in oxygen and maintained at the same rate throughout the procedure. Isolation surgeries were performed as described by Lacy and Kostianovsky (62). Briefly, the bile duct was cannulated, and the pancreas was distended by an *in vivo* injection of 0.15% Liberase (Research Grade, Roche) in RPMI 1640 media solution. The pancreas was digested in a 37 °C water bath for 30 min. The digestion was stopped by adding 10–15 mL of cold M199 media with 10% heat-inactivated FBS and a slight shaking. Digested pancreases were washed twice in the same aforementioned M199 media, filtered through a 450 μm sieve, and then suspended in a Histopaque 1077 (Sigma)/M199 media gradient and centrifuged at $1,700 \times g$ at 4 °C. This gradient centrifugation step was repeated for higher purity islets. Finally, the islets were collected from the gradient and further isolated by a series of gravity sedimentations, in which each supernatant was discarded after 4 min of settling. Purified islets were hand-counted by aliquot under a light microscope and then washed three times in sterile PBS. Islets were then washed once in RPMI 1640 media with 10% heat-inactivated FBS and 1% penicillin/streptomycin and cultured in this medium overnight for further use.

Cell Encapsulation. For a typical mouse device, the modified thread was inserted into a polyethylene tube (~ 1.5 mm inner diameter). A predetermined amount of cell-loaded alginate solution [e.g., ~ 500 IEQs dispersed in ~ 60 μL 2% (wt/vol) alginate solution] was filled into the tube using a micropipette and left for 4 min. Then, the TRAFFIC device was carefully pulled out of the tube using tweezers. Most alginate and cells were incorporated into the device, and the leftover alginate in the tube was usually unnoticeable with a small number of islets adhered to the tube wall. It was estimated from multiple observations that the encapsulation efficiency was at least 90% (data analyzed and estimated from five isolations with ~ 30 independent trials). Given the ~ 500 IEQs with which we started, we determined the actual IEQ for transplantation was 475 ± 25 . The device was further cross-linked by a cross-linking buffer containing 95 mM CaCl_2 and 5 mM BaCl_2 . Next, the device was washed three times with 0.9% (wt/vol) saline and transferred into corresponding cell culture medium. Islets were typically treated as following for encapsulation. Immediately before encapsulation, the cultured islets were centrifuged at $392 \times g$ for 1 min and washed with Ca-free Krebs–Henseleit (KH) buffer (4.7 mM KCl, 25 mM Hepes, 1.2 mM KH_2PO_4 , 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 135 mM NaCl, pH ~ 7.4 , osmotic pressure ~ 290 mOsm). After the wash, the islets were centrifuged again, and all supernatants were aspirated. Then, the collected islets were encapsulated following the procedure described above. The TRAFFIC devices encapsulating cells were checked under microscope to ensure that no cells were exposed on the hydrogel surface. Since the islets had variable sizes (50–400 μm), the total number of encapsulated islets were converted into islet equivalences (IEQs, normalized to 150 μm size) based on a previously published method (46).

Static GSIS Assay. Krebs Ringer Bicarbonate (KRB) buffer [2.6 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2 mM KH_2PO_4 , 4.9 mM KCl, 98.5 mM NaCl, and 25.9 mM NaHCO_3 (all from Sigma-Aldrich) supplemented with 20 mM Hepes/Na-Hepes (Roche) and 0.1% BSA (Serological)] was prepared beforehand. Encapsulated islets were incubated for 2 h in KRB buffer at 37 °C, 5% CO_2 , and then incubated for 75 min with 2.8 mM or 16.7 mM glucose under the same condition. Insulin concentrations in the buffer solutions were determined by using ultrasensitive mouse/rat insulin ELISA kit (Crystal Chem) or human insulin ELISA kit (ALPCO) per suppliers' protocols. For devices retrieved from mice, three out of five retrieved TRAFFIC devices were tested with the GSIS assay (the remaining two devices were fixed right after retrieval and submitted for histology); for devices retrieved from dogs, three independent regions of the retrieved devices were cut and tested with the GSIS assay. All of the ELISA results were normalized to the IEQs.

Perfusion Test. Human islets were purchased from Prodolabs. Upon receipt, viability was assessed with the LIVE/DEAD Viability/Cytotoxicity kit (calcein-AM staining indicating living cells and ethidium homodimer-1 staining indicating dead cells) according to the manufacturer's instructions (Invitrogen). Dynamic insulin release was examined with a perfusion system from Biorep according to the manufacturer's guidelines. In short, 250 hand-picked human islets or 1/3 device (expected 500 IEQ) were loaded on columns surrounded by Bio-Gel P-4 beads (Biorad) and allowed to acclimate for 60 min

in KRBH containing 2 mM glucose at 100 $\mu\text{L}/\text{min}$ flow (kept constant during the experiment). Islets were then exposed to different glucose concentrations and/or factors, and secreted insulin was collected in defined time frames. After the experiment, islets were collected and lysed [tissue extraction reagent I (Invitrogen) + tissue lyzer (Qiagen)]. The insulin concentration was measured and normalized to DNA concentration (PicoGreen dsDNA kit; Thermofisher).

Implantation and Retrieval in Mice. Immune-competent male C57BL/6 mice and immunodeficient SCID-Beige mice were utilized for transplantation. To create insulin-dependent diabetic mice, healthy mice were treated (50 mg/kg mouse) with freshly prepared STZ (Sigma-Aldrich) solution (7.5 mg/mL in sodium citrate buffer solution) for 5 consecutive days. The BG levels of all mice were retested before transplantation. Only mice whose nonfasted BG levels were above 300 mg/dL for two consecutive measurements were considered diabetic and underwent transplantation. The nondiabetic or STZ-induced diabetic mice were anesthetized with 3% isoflurane in oxygen, and their abdomens were shaved and sterilized using betadine and 70% ethanol. Preoperatively, all mice received 0.3 mL of 0.9% saline s.c. to prevent dehydration. A ~ 1 -mm incision was made along the midline of the abdomen, and the peritoneal lining was exposed using blunt dissection. The peritoneal wall was then grasped with forceps, and a ~ 1 -mm incision was made along the linea alba. The device was then inserted into the peritoneal cavity through the incision. The incision was closed using 5-0 taper polydioxanone (PDS II) absorbable sutures. The skin was then closed over the incision using a wound clip. The devices were retrieved from mice at desired time points postimplantation or -transplantation (with encapsulated cells) using similar procedures except that larger (~ 5 mm) incisions were made in the skin and the peritoneum. The device was located, grasped, and pulled out by using blunt tweezers. After retrieval, the TRAFFIC devices were first submerged into a cross-linking solution containing 95 mM CaCl_2 and 5 mM BaCl_2 for ~ 5 min and then transferred into 4% paraformaldehyde for fixation or a cell culture medium for postcharacterization.

For the SCID-Beige mice and human islet study, the mice were rendered diabetic by five consecutive doses of STZ (70 mg/kg). During the first week after the STZ treatment, the mice were not intervened. After the first week, if the body weight of a mouse dropped more than 10%, the mouse was treated with insulin (NPH 100 nmol/kg $1 \times$ daily). The insulin treatment was independent of whether the mouse was from the experimental or control groups. Normally, the diabetic mice were used 3–4 wk after STZ treatment, but for this batch of experiments, mice were used about 45 d after treatment due to the logistics associated with human islet isolation and delivery. The TRAFFIC device transplantations were performed as described above, but anesthesia and analgesia procedures were performed as for the kidney capsule transplantation.

For the kidney capsule transplantation, mice were anesthetized using 5% isoflurane with O_2 flow set at 1 L for anesthetic induction (approx. 1–2 min). The anesthesia was maintained using 1–1.5% isoflurane. Rimadyl, 5 mg/kg s.c., was administered 30 min presurgery and 24 and 48 h postsurgery. Temgesic, 0.05 mg/kg, was dosed s.c. 30 min presurgery and 3–6 h postsurgery.

The sedated mice were shaved in the surgical area, more specifically the left lateral side of the mouse $\sim 3 \times 3$ cm and about 2 cm from the last rib down. The skin was washed with iodine and ethanol. The shaved area was covered with barrier drape. A small incision through the skin and muscle of the left flank of the animal was made, and the kidney was exposed outside the body using two saline-moistened cotton-tipped applicators. The kidney capsule was sliced using a small needle leaving a small hole. A pouch was made using a rounded glass capillary, and a transfer pipette was used to transplant clusters of islets into the pouch. The kidney was returned to the peritoneal cavity, and the muscle layer was closed with a 4-0 absorbable suture and the skin closed with two to three staples.

Laparoscopic Implantation and Retrieval in Dogs. Dogs were premedicated with glycopyrolate and butorphanol, induced with propofol, and anesthetized with isoflurane and oxygen. The abdomen was clipped and prepared for sterile surgery. A 10-mm laparoscopic camera port and two 5-mm instrument ports were percutaneously inserted into the abdomen. The abdomen was insufflated to 12 mm Hg pressure with CO_2 . The device was inserted into the abdomen through the left-side instrument port. A laparoscopic probe was introduced through the right-sided 5-mm port and was used to manipulate the device so that it was placed between the liver and the diaphragm. The remaining ports were then removed, and the port sites were closed with 3-0 polydioxanone suture material. For retrieval of the devices, the procedure was similar using one 10-mm camera port and one or two 5-mm instrument ports. The previously implanted device was located and photographed. The device was grasped with laparoscopic Kelly forceps

inserted through a 5-mm port and dissected from the attached omentum if necessary using laparoscopic scissors. The device was then removed from the abdomen through the 5-mm port. The remaining laparoscopic ports were removed, and the dogs were humanely euthanatized.

BG Monitoring. BG levels were monitored three times a week in mice following transplant surgery. A small drop of blood was collected from the tail vein using a lancet and tested using a commercial glucometer (Clarity One, Clarity Diagnostic Test Group). Mice with unfasted BG levels below 200 mg/dL were considered normoglycemic. For the BG test in SCID-Beige mice, blood samples were obtained from the tip of the tail by puncturing the tail capillaries with a lancet in conscious mice. The blood (5 μ L) was collected into Na-heparinized capillary tubes and transferred to 250 μ L eBioscience hemolysing buffer. The glucose concentration was measured on Biosen 5040 S line glucose analyzer (Eppendorf).

Histological Analysis and Immunostaining. The retrieved devices were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned by Cornell Histology Core Facility. Paraffin sections 10 mm thick were stained with hematoxylin/eosin. For staining of insulin with immunofluorescence, paraffin-embedded sections were rehydrated by sequential washing in xylene; 100%, 95%, and 75% ethanol; and water. Then slides were boiled in 1 mM EDTA for antigen exposure. After blocking, primary guinea pig anti-rat insulin antibody (Linco, 1:200) was applied and incubated overnight at 4 °C, followed by a wash and incubation with FITC-conjugated donkey anti-guinea pig IgG (Jackson ImmunoResearch, 1:200). Slides were washed twice with water, applied with antifade/DAPI, and covered with coverslips. Fluorescence images were captured under a Zeiss LSM710 confocal microscope. The histologic analysis of human islets is as follows: Devices and grafted kidneys were fixed in 10% natural buffered formalin, processed to paraffin, and sectioned (3 μ m). Islet morphology was visualized with hematoxylin and eosin staining. Alpha and beta cells were visualized in a triple immunohistochemical stain with guinea pig anti-insulin (1/75 in 0.05% TNB; A0564; DAKO), rabbit anti-Nkx6.1 (1/1,000 in 0.05% TNB; HPA036774; Sigma-Aldrich), and mouse anti-glucagon (1/7800 in 0.05% TNB; Glu-001; Novo Nordisk). Insulin was visualized with goat-anti-guinea pig-488 (A11073; Invitrogen) and donkey-anti-goat-488 (705-545-147; Jackson). Nkx6.1 was visualized with donkey anti-rabbit-biotin (711-065-152; Jackson) and TSA cy3 (NEL704A001KT; Perkin-Elmer). Glucagon was visualized with donkey anti-mouse-Cy5 (715-175-151; Jackson). Antigens were first retrieved with TEG buffer (AMPQ17020; Ampliqon), after which slides were blocked with 1% hydrogen peroxide, avidin, biotin, and TNB buffer (0.05%). The slides were then incubated with primary antibodies for 45 min, secondary antibody and DAPI for 30 min, Streptavidin-PO (1:500) and tertiary antibody for 30 min, and TSA-cy3 substrate for 15 min, and finally the slides were mounted with a fluorescent mount (DAKO). The stains were visualized on an Olympus VS120 slide scanner.

Statistical Analysis. Results are expressed as mean \pm SEM. The data were analyzed via Student's *t* tests and analysis of covariance (ANCOVA) followed by a Tukey post hoc test where appropriate. Treatment (i.e., TRAFFIC vs. diabetic control, vs. nondiabetic control, etc.) was considered as a factor, while time was treated as a continuous covariate. In many experiments, there were data collected before the treatment was applied and/or after the treatment was removed (e.g., before implantation of TRAFFIC and after retrieval). In these cases, only the data collected during the treatment period were analyzed. For Fig. 2I, rather than treating time as a continuous covariate, the area under the curve (AUC) was calculated for each time period during which the cells were exposed to a specific glucose concentration (i.e., either 2 mM or 20 mM glucose). Time period (from 1 through 5) was treated as an additional factor; thus, the ANOVA model consisted of AUC as the response variable with treatment (TRAFFIC vs. islets) completely crossed with time period in a full factorial design. ANCOVA assumes that the relationship between the response variable and each covariate is linear. While this is a good assumption for the majority of the data presented in this paper, in Fig. 3C, the initial spike in BG during the first 30 min of the experiment introduces some nonlinearity. To account for this, we analyzed the data from this figure twice; first, we included all data, and then we analyzed only the BG measurements taken after the first 30 min (when the response varied linearly with time). The statistical significance of the treatments did not change regardless of whether the first 30 min of data were included in the analysis. Therefore, the *P* values presented are from the analysis of all data, including the first 30 min. The sample sizes for the data presented in Fig. 4F and G are not balanced. To ensure that this imbalance did not affect the validity of our analysis we followed a resampling procedure (63), taking a random sample of four animals (the size of the smallest treatment group) from each treatment group and recalculating both the ANCOVA and the Tukey post hoc test for each sample. This process was repeated 1,000 times. The reported *P* values are the median values from these 1,000 trials. It should be noted that while the *P* values from the resampling procedure are not identical to the *P* values obtained without the resampling procedure, the conclusions regarding statistical significance did not change.

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