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A prevascularized subcutaneous device-less site for islet and cellular transplantation

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Transplantation of donor-derived islets into the liver is a successful cellular replacement therapy for individuals with diabetes. However, the hepatic vasculature is not an optimal transplant site for several reasons, including graft attrition and the inability to retrieve or image the islets. Here we describe islet transplantation into a prevascularized, subcutaneous site created by temporary placement of a medically approved vascular access catheter. In mice with streptozotocin (STZ)-induced diabetes, transplantation of ~500 syngeneic islets into the resulting 'device-less' space reversed diabetes in 91% of mice and maintained normoglycemia for >100 days. The approach was also effective in mice with pre-existing diabetes, in another mouse strain that mounts a more vigorous inflammatory response, and across an allogeneic barrier. These results demonstrate that transient priming of a subcutaneous site supports diabetes-reversing islet transplantation in mouse models without the need for a permanent cell-encapsulation device.

Cellular transplantation is an attractive and growing treatment strategy for a variety of disease processes, including diabetes, Parkinson's disease, myocardial ischemia, stroke, metabolic liver disease and hemophilia. A prototypic example of cellular replacement therapy is intrahepatic transplantation of donor-derived pancreatic islets of Langerhans in individuals with type 1 diabetes mellitus who have unstable glucose control. The 'Edmonton protocol' for administering this therapy achieved high rates of insulin independence[1]. Initial long-term analysis indicated that insulin independence was not durable, as most recipients eventually returned to requiring moderate amounts of insulin, although they remained protected from recurrent hypoglycemia[2]. Recent results from six islet centers suggest marked improvement in durable graft function, with insulin independence now seen in more than half of recipients at five years after transplantation[3]. However, the procedure often results in acute or gradual graft attrition, and carries risks of bleeding, thrombosis and localized steatosis. Moreover, intrahepatic transplantation does not permit imaging or retrieval of donor islets. The ability to retrieve the graft is especially important for current efforts to replace donor-derived islets with cells produced from human pluripotent stem cells, which may have unwanted effects. These considerations suggest that the liver is not the optimal site for islet transplantation[4,5].

Islets isolated for transplantation have lost their natural vascularized and specialized extracellular matrix[6,7] and, for successful engraftment, must receive nutritional and physical support from the host through the formation of new blood vessels around and within the graft. The density of newly formed vessels after transplantation is much lower than that in native islets[8,9], irrespective of whether islets are delivered to the human liver, kidney or spleen[9]. Research on the development of alternative transplant sites[9–11] (**Supplementary Tables 1** and **2**) has suggested that an optimal site for islet transplantation should (i) have an adequate tissue volume capacity, (ii) be in close proximity to vascular networks, ensuring a sufficient oxygen supply to the graft before revascularization, (iii) allow for dynamic communication between the graft and the systemic circulation in a physiologically relevant manner, (iv) facilitate minimally invasive methods to transplant, biopsy and retrieve the graft, and (v) elicit minimal inflammation to reduce immunogenicity and promote long-term graft survival[11]. In theory, subcutaneous transplantation should be superior to portal vein infusion as it provides ready access to the graft and the possibility of monitoring function through imaging[12–14]. However, islet transplantation into an unmodified subcutaneous site has never reversed diabetes in animals or humans as the microenvironment is inhospitable to cell survival owing to poor oxygen tension and inadequate vascularization[15]. Stimulation of angiogenesis is critical to successful subcutaneous islet transplantation[9,11,14,16]. Oxygen generators, polymers, meshes, encapsulation devices, matrices, growth factors (including fibroblast growth factor, hepatocyte growth factor and vascular endothelial growth factor) and co-transplantation of mesenchymal stem cells have all been explored with variable success (**Supplementary Table 1**).

Strategies for subcutaneous transplantation that rely on biomaterials often fail because of the foreign-body and inflammatory reaction—a complex, dynamic process that can persist for the lifetime of the implant[17]. Physical contact of the implant with host blood, lymph, exudate or other fluids triggers an instant inflammatory response that leads to spontaneous adsorption to the biomaterial of host blood proteins, including albumin, fibrinogen, complement, fibronectin and γ-globulin[17–19]. Host cells responsible for wound healing encounter this layer and release cytokines, chemokines, reactive oxygen species and other enzyme products that recruit tissue-resident macrophages and undifferentiated monocytes to the wound site[17–19]. As macrophages

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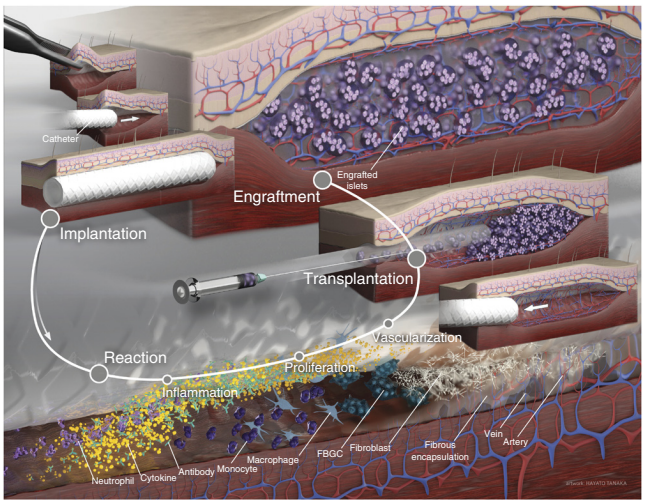


Figure 1 Design and characteristics of the subcutaneous DL cellular transplant site.

engage the biomaterial, they form foreign-body giant cells. These cells secrete signaling molecules (e.g., IL-1, IL-6, IL-10, IL-12, TNF-α, TGF-β), attracting fibroblasts, which secrete collagen during cellular proliferation and neovascularization[17,20]. In the early stages of the foreign-body reaction, neovascularization may be driven by macrophage-derived hypoxia-inducible factors that induce expression of pro-angiogenic factors[21]. Within several weeks, a dense collagenous fibrotic capsule forms around the implant that isolates it from the host[17,22], hinders metabolic exchange, cell signaling, healing and tissue-device integration, and increases the risk of bacterial infection[17,22].

Here we report a subcutaneous device-less (DL) transplant technique that enables successful transplantation of mouse or human islets in mice. The approach was designed to harness the innate foreign-body response in a controlled manner so as to induce local neovascularization favorable to islet survival and function. A hollow nylon catheter is implanted subcutaneously, inducing a foreign-body response, and withdrawn after one month. Removal promptly extinguishes the foreign-body response, leaving a space lined with neovessels (**Fig. 1** and **Supplementary Fig. 1**). Transplantation of islets into this space enables reversal of diabetes without need of a permanent encapsulation device or exogenous growth factors.

RESULTS

Design and testing of the subcutaneous DL site

Initial studies summarized in **Supplementary Table 3** describe how we chose the biomaterial used in the present study. Briefly, we compared a range of hydrophilic and hydrophobic catheters of different diameters for their ability to create a space that supported transplantation of syngeneic BALB/c mouse islets. Reversal of diabetes rates and intraperitoneal glucose tolerance tests (IPGTTs) were measured (**Supplementary Figs. 2** and **3**). Of the catheters tested, the 5-French (Fr.) nylon catheter induced the best thin, supporting collagen matrix (**Fig. 2a**) and led to extensive neovascularization of transplanted islets (**Fig. 2b–f**). We also compared implant periods of 2–4 weeks. We chose the 4-week period for pre-islet catheter implantation as the collagen space appeared to be more favorable histologically for neovascularization (data not shown). These results suggested that a time-limited induction of the foreign-body response converts the subcutaneous space into a favorable site for islet engraftment, although direct causality was not proven.

We observed extensive differences in tissue cytokine expression, in BALB/c mice, in response to the nylon catheter compared to the silicone catheter and unmodified subcutaneous controls (**Fig. 3a–r**). Both nylon and silicone elicited strong interleukin (IL-) IL-1β, IL-10 and keratinocyte growth factor (KC/GRO) responses, but the responses to nylon were much faster (24 h versus 1 week). IL-6 expression was elevated within 24 h, and remained mildly elevated during the 2-week experiment in both groups. In contrast, tumor necrosis factor α (TNF-α) expression became elevated in both groups by 1 week after implant. Interferon (IFN)-γ was analyzed but was not detected in any samples. These data suggest that the temporary presence of the nylon catheter induces strong cytokine and chemokine responses that contribute to host inflammatory cell recruitment (e.g., neutrophils, macrophages and fibroblasts) and neovascularization. We do not, however, have causative data demonstrating that the degree of inflammatory marker response relates directly to the degree of diabetes reversal.

We compared the efficacy of the subcutaneous space generated by various biomaterials supporting islet engraftment in BALB/c recipients (**Supplementary Table 3**). The nylon- and silicone-based catheters appeared to be the most effective and were studied in more

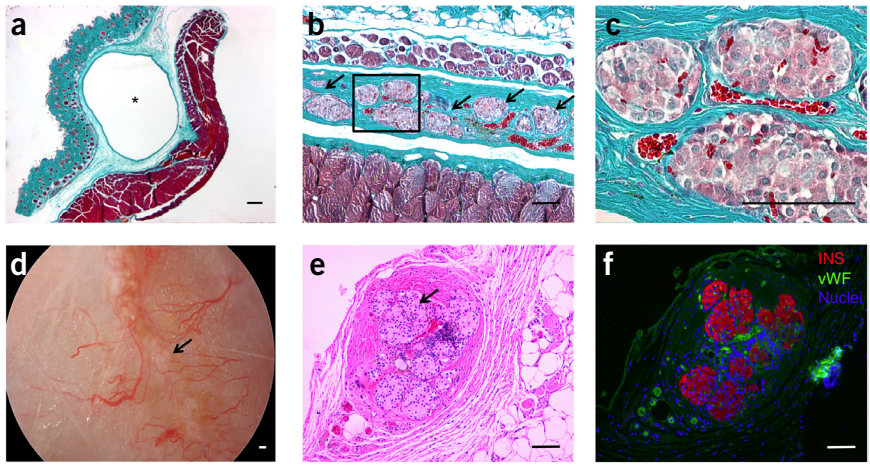


Figure 2 Histological analysis of islets transplanted long-term into the DL space. (**a**) Masson's trichrome staining of the cross-section of the DL site after removal of a catheter that had been implanted for 4 weeks. Islets were infused into the resulting lumen (*). Collagen (blue), smooth muscle and erythrocytes (red) at 2×. (**b**,**c**) Masson's trichrome staining at 10× (**b**) and 40× (**c**) of an islet graft in the DL site at >100 days after transplant, surrounded by collagen and blood vessels. Arrows indicate engrafted islets in a vascularized collagen scaffold. (**d**) Macroscopic image of the neovascularization penetrating the length of an islet graft in the DL site, >100 days after transplant. Arrow indicates islets within vascularized graft. (**e**) Hematoxylin and eosin staining of an islet graft cross-section, 100 days after transplant at 10×. Arrow indicates islets with the DL transplant site. (**f**) Fluorescent staining of the same cross-section stained for insulin (red), blood vessels (green) and nuclei (blue) at 10×. Scale bars, 100 μm.

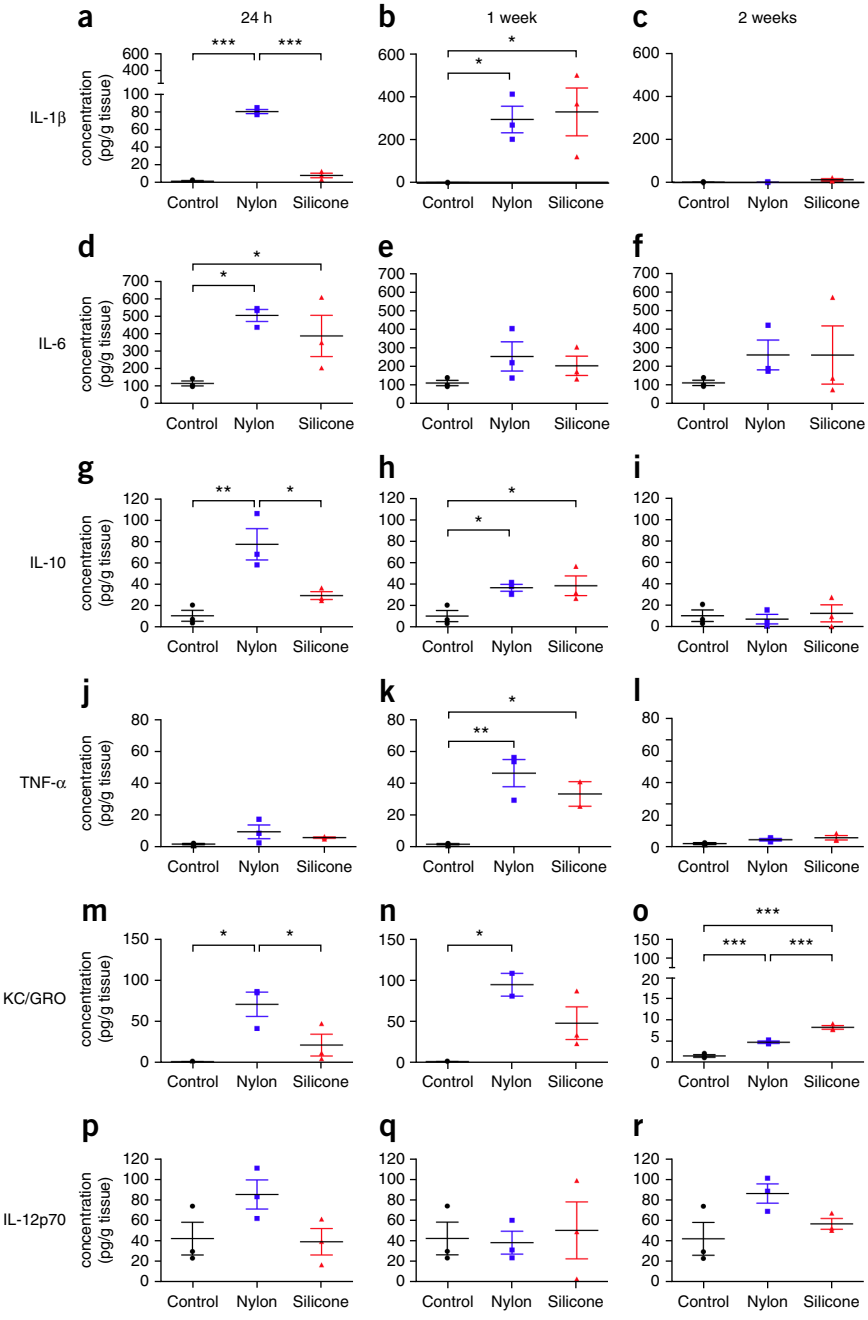


Figure 3 The proinflammatory response elicited by angiocatheters composed of nylon (blue) or silicone (red) when implanted subcutaneously for 24 h, 1 week and 2 weeks. (**a**–**r**) The peri-implant concentrations of IL-1β (**a**–**c**), IL-6 (**d**–**f**), IL-10 (**g**–**i**), TNF-α (**j**–**l**), KC/GRO (**m**–**o**) and IL-12p70 (**p**–**r**). Data points represent mean ± s.e.m. for pg/g tissue, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ ($n = 3$/time point). One-way ANOVA was calculated with Newman-Keuls *post-hoc* testing for multiple comparisons between controls and individual biomaterials tested. *Y* Axis labels for all graphs indicated in left margin.

depth. Glycemic control was monitored over time, and IPGTTs were conducted at 60 days. In kidney capsule (KC) controls, by 3 weeks, 95% of mice (19/20) became euglycemic and remained so for >60 days (**Supplementary Fig. 2**). In controls using unmodified subcutaneous transplantation (SubQ), no mice (0 of 10) showed diabetes reversal. In the silicone-DL group, 35% of the mice (6/17) became normoglycemic by 3 weeks, and by day 60, 65% (11/17) were normoglycemic. In contrast, in the nylon-DL group, 57% of the mice (12/21) became normoglycemic by 3 weeks, and by day 60, 91% (19/21) were normoglycemic, similar to the KC control group (**Supplementary Fig. 2**).

IPGTT showed that mice in the KC (n = 14) and nylon DL (n = 15) groups rapidly returned to normoglycemia (**Supplementary Fig. 3a**). There was no difference as measured by mean area under the curve (AUC) ± s.e.m. (AUC KC: 2,101 ± 168 mmol/L/120 min versus nylon-DL: 2,219 ± 93 mmol/L/120 min, $P > 0.05$, ANOVA, **Supplementary Fig. 3b**). Silicone-DL mice (n = 13) were more glucose intolerant than nylon-DL mice (AUC silicone-DL: 2,764 ± 149 mmol/L/120 min versus nylon-DL, $P < 0.01$, ANOVA, **Supplementary Fig. 3b**), and all mice in the unmodified subcutaneous group (n = 5) had diabetic profiles (AUC SubQ 3,635 ± 95 mmol/L/120 min, compared to nylon-DL, $P < 0.001$, ANOVA, **Supplementary Fig. 3b**). These data demonstrate that the choice of biomaterial affects the rates of islet engraftment *in vivo* and further support the superiority of nylon over silicone.

Long-term function and vascularization of DL islet grafts

We followed graft function for >100 days to measure long-term performance. Grafts were subsequently retrieved and mice promptly

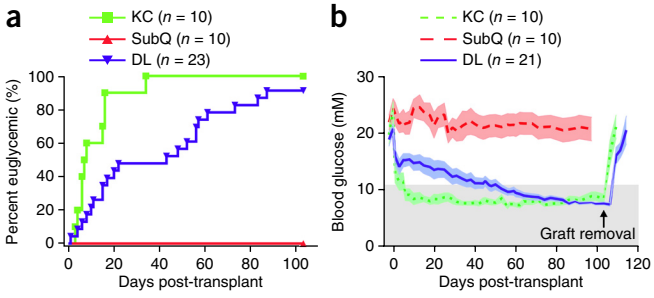


Figure 4 Long-term function of syngeneic islet grafts transplanted into the DL space. (**a**) The proportion of animals that achieved euglycemia was similar in KC recipients (n = 10) and DL space recipients (n = 21) 100 days after transplant, with KC recipients reversing diabetes earlier (P = 0.001, log-rank, Mantel-cox test). (**b**) Nonfasting blood glucose measurements showed that both KC and DL space recipients maintained normoglycemia until the graft was retrieved (arrow), at which point they reverted to their pretransplant hyperglycemic state. Islets transplanted in the unmodified subcutaneous space did not provide glycemic control (SubQ-red, n = 10). Shaded area represents a nonfasting physiological range (<11.1 mM). Data points represent blood glucose mean ± s.e.m. Islets transplanted were from ten separate islet isolations (n = 20 pancreata per isolation).

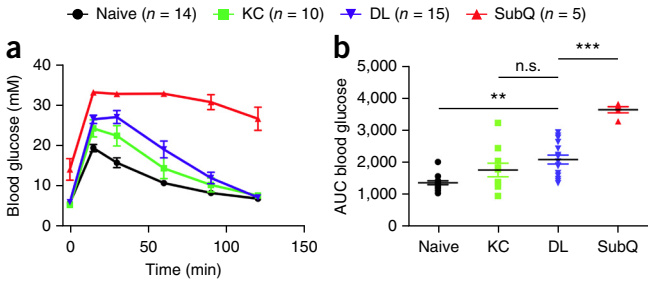
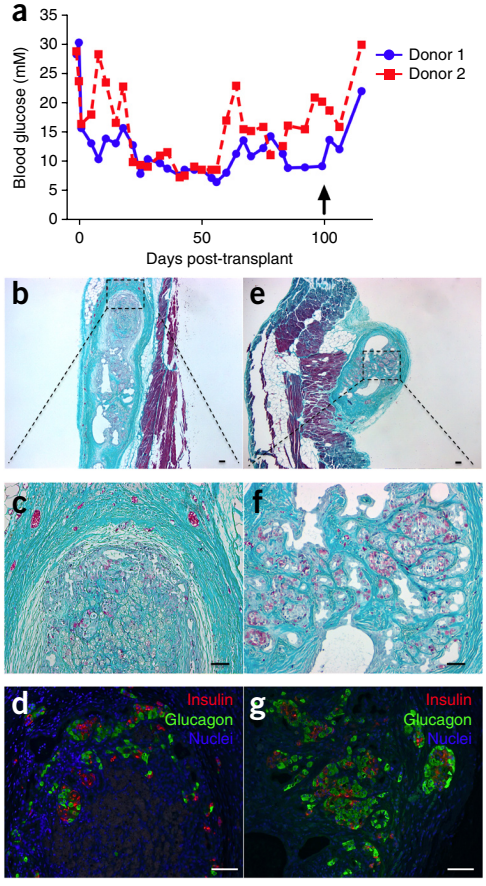


Figure 5 IPGTTs of syngeneic mouse islets transplanted under the KC or into the DL site, 100 days after transplant. (**a**,**b**) Blood glucose after dextrose bolus (**a**) AUC analysis (**b**) did not differ between the KC ($n = 10$) and DL space ($n = 15$) recipients ($P > 0.05$, one-way ANOVA with Newman-Keuls *post-hoc* testing for multiple comparison between transplant groups). Naive represents nondiabetic, nontransplanted BALB/c mice (black, $n = 14$), which were more tolerant to the metabolic test than the DL space recipients (**$P < 0.01$, $P > 0.05$, one-way ANOVA with Newman-Keuls *post-hoc* testing for multiple comparison to naive controls). Animals receiving islets in the unmodified SubQ ($n = 5$), were intolerant to the glucose challenge compared to DL space recipients (***$P < 0.001$ one-way ANOVA with Newman-Keuls *post-hoc* testing for multiple comparison between transplant groups). Mice were administered 3 g/kg 50% dextrose i.p. Blood glucose measurements were monitored at $t = 0$, 15, 30, 60, 90 and 120 min. Data points represent blood glucose mean ± s.e.m. Islets transplanted were from ten separate islet isolations ($n = 20$ pancreata per isolation). n.s., not significant.

reverted to hyperglycemia. Recipient BALB/c mice were rendered diabetic with STZ, and ~500 syngeneic mouse islets were transplanted in the DL space, under the KC, or in the unmodified subcutaneous space. In addition, ~2,000 human islet equivalents were transplanted in the DL space in diabetic, immunodeficient Rag$^{-/-}$ mice to test compatibility of the DL approach with clinical-grade human islets.

Syngeneic islets transplanted under the KC reversed diabetes in 100% of recipients (10/10) (**Fig. 4a**) within 11.5 ± 2.9 days (**Fig. 4b**). Transplants in the unmodified subcutaneous space did not reverse diabetes (0%; 0 of 10) at any time point (**Fig. 4a**,**b**). Transplants in the DL space reversed diabetes in 91.3% of the mice (21/23) (**Fig. 4a**), with marked improvement compared with the unmodified subcutaneous group ($P < 0.0001$, log-rank). Engraftment in the DL space was delayed, with mean diabetes reversal at 35.4 ± 6.0 days after transplant ($P < 0.05$, Student's *t*-test), compared to KC engraftment (**Fig. 4b**).



After 100 days, we conducted IPGTT (**Fig. 5a**). Both KC ($n = 10$) and DL ($n = 15$) groups had well-preserved glucose clearance profiles that were not significantly different. AUCs ± s.e.m. for glucose clearance were similar (**Fig. 5b**; KC: 1,771 ± 212 mmol/L/120,min versus DL: 2,095 ± 138 mmol/L/120 min, $P > 0.05$, ANOVA). By contrast, glucose profiles in the unmodified subcutaneous group were significantly worse (AUC 3,635 ± 95 mmol/L/120 min, $P < 0.001$, ANOVA) (**Fig. 5a**,**b**). Normal, nondiabetic, nontransplanted BALB/c mice demonstrated the best glycemic profiles (AUC naïve: 1,375 ± 62 mmol/L/120 min, versus DL recipients, $P < 0.01$, ANOVA) (**Fig. 5b**). The glycemic profiles of Rag$^{-/-}$ diabetic mice that received human islets in the DL space were similar to those of BALB/c mice that received syngeneic islets (**Fig. 6a**). To confirm graft-dependent euglycemia, we explanted the grafts and in all cases saw prompt return of diabetes (**Figs. 4b** and **6a**).

Histological analysis of explanted grafts from the DL site revealed islets enveloped in a vascularized collagen scaffold between skin and musculature (**Figs. 2b–f** and **6b–g**). Extensive vascular networks were visible macroscopically and penetrated the islet tract created by the nylon catheter (**Fig. 2d**). Of note, capillary networks were localized to the DL area, whereas outside of the tract margins, planes were relatively avascular. Grafts in the DL space stained positive for insulin, glucagon and for the presence of endothelial cells in new intra-islet microvessels (**Figs. 2e**,**f** and **6b–g**). In contrast, islets transplanted into the unmodified subcutaneous space underwent extensive necrosis and a destructive inflammatory response, resulting in graft failure (**Supplementary Fig. 4a**,**b**).

We compared vascular density in DL and unmodified subcutaneous islet grafts at 100 days after transplant (**Supplementary Fig. 5a–c**). DL grafts showed a marked increase in neovascularization as measured by the percentage of the graft staining positive for von Willebrand (vWF) ± s.e.m. (2.30 ± 0.38% of the entire graft vWF$^+$ in DL grafts versus 0.56 ± 0.07% vWF$^+$ in SubQ controls, $P < 0.01$, Student's *t*-test).

Efficacy of DL transplantation in additional mouse models

To confirm that the potential of the prevascularized DL space is not unique to BALB/c mice, we studied C57BL/6 mice, a strain that, unlike BALB/c mice, has a vigorous foreign-body response.

Figure 6 Long-term function of human islets transplanted into the DL space. (**a**) Nonfasting blood glucose measurements showed maintenance of normoglycemia until the time of graft retrieval (arrow), at which point recipients reverted to the pretransplant hyperglycemic state ($n = 2$ human donors). (**b**–**f**) 2× (**b**,**e**) and 20× (**c**,**f**) Masson's trichrome staining of a long-term (>100 days) human islet graft in the DL site, surrounded with collagen and blood vessels. (**d**,**g**) Fluorescent staining of the same cross-section staining for insulin, glucagon and nuclei at 20×. Scale bars, 100 µm.

Transplantation of ~500 syngeneic islets in the DL space reversed diabetes more quickly in C57BL/6 mice than in BALB/c mice, as calculated by mean days after transplant ± s.e.m. (11.3 ± 3.1 days versus 35.5 ± 6.1 days, $P < 0.05$, t-test) (**Supplementary Fig. 6**). Proportional rates of diabetes reversal were similar in the two strains by day 50 after transplant (C57BL/6: 75% (9/12) versus BALB/c: 62% (13/21), $P > 0.05$, Student's t-test).

As diabetes may be associated with impaired wound healing, we studied mice with pre-existing diabetes. BALB/c and C57BL/6 mice were rendered diabetic with STZ 1 week before placement of the DL catheter and compared with similar mice that were not diabetic at the time of catheter placement. All mice were maintained for 4 weeks before catheter withdrawal and transplantation of syngeneic islets in the DL space. The prediabetic state did not inhibit diabetes reversal, as similar rates were observed in the two groups by day 50 (78% (7/9) prediabetic versus 62% (13/21) nondiabetic at time of catheter placement; $P > 0.05$, Student's t-test; **Supplementary Fig. 7**).

To determine whether the DL transplant technique is efficacious across an alloimmune barrier, we transplanted ~500 BALB/c islets into the DL space of C57BL/6 recipients rendered diabetic after formation of the DL site. Rejection occurred within 7 days in mice that did not receive immunosuppression therapy, whereas tacrolimus therapy (0.5 mg/kg for 28 days) led to prolonged allograft survival (**Supplementary Fig. 8**). These results show that the DL space is not immunoprotective but can still provide an effective microenvironment that supports allogeneic islet survival, despite the presence of calcineurin inhibition (**Supplementary Fig. 8**).

DISCUSSION

Our results indicate that a controlled foreign-body response can be used to generate a prevascularized subcutaneous site that supports islet engraftment. Withdrawal of an implanted catheter after 1 month terminates the foreign-body reaction at a stage where a rich vascularized collagen network has formed but before the development of a mature fibrotic scar. An advantage of the DL approach is that it avoids the need for a permanent encapsulation device, which often generates an avascular fibrotic granular capsule[17,19,22–25] and a chronic inflammatory response that contribute to graft failure. We showed that the neovascularization response and successful islet engraftment occurred in different mouse strains, in the presence of pre-existing STZ-induced diabetes, and with islet allografts. Notably, human islets transplanted into immunodeficient $Rag^{-/-}$ mice reversed diabetes to the limited extent tested. Innate immunity in $Rag^{-/-}$ mice remained intact, and the foreign-body neovascularization response was preserved. We also demonstrated return to the diabetic state in all cases promptly after excision of the subcutaneous implant tract, indicating that euglycemia was dependent on graft function.

To discover conditions for generating a favorable DL site, we compared inflammatory responses to a selection of catheter materials with different surface properties and diameters and tested implant periods of 2–4 weeks. We chose catheter materials that are in routine clinical practice so that off-label clinical application would be relatively straightforward. Hydrophilic nylon induced stronger pro-inflammatory responses than did silicone, which translated into more effective diabetes reversal. Four-week implantation of a 5-Fr. nylon catheter was used in subsequent studies. We were concerned that longer catheter implantation times could promote an excessive collagen scar response but did not test these. Our observations are consistent with previous studies of subcutaneous devices in rodents[26]. However, the tempo of the inflammatory response and therefore the optimal period of catheter indwell may vary between species. Clearly, biomaterials could be engineered to further improve neovascularization responses in a DL subcutaneous site while minimizing fibrotic capsule formation. For example, zwitterionic polymers hinder the foreign-body response and increase vessel density around an implanted biosensor[22,27].

We tested pro-inflammatory markers in tissue at 24 h, 1 and 2 weeks after catheter implantation but not immediately before or after islet transplantation. We found that catheter implantation led to marked local elevation in an array of proinflammatory cytokines, maximally observed by 1 week. In future studies it would be interesting to compare the levels of pro-inflammatory markers with the extent of islet engraftment. It is possible that withdrawal of the catheter fragment after prevascularization could impose mechanical or other stress responses that either promote or inhibit islet engraftment.However, we did not observe tract adhesion, bleeding or apparent tissue trauma after catheter withdrawal. We further acknowledge that the ~500 mouse islets used here represents a nonmarginal graft. Had we transplanted fewer islets, we may have uncovered differences in engraftment efficiency between the KC and DL sites. Although the KC site has a track record of efficacy in mouse islet transplantation, it has routinely failed to result in insulin independence in large animals or humans.

After intraportal transplantation, islets are initially avascular, denervated and isolated from contact with endogenous cells or extracelluar matrix, resulting in delayed engraftment[28]. Neovascularization is initiated within the first 2 weeks and remodels extensively over months[29–31]. Several previous studies have shown that islet neovessels are chimeric, consisting of both donor and host cells[32]. Although we did not address this question here, we expect a similar chimeric vascular ingrowth/outgrowth response in the DL site.

If the DL approach proves successful in humans, it may facilitate transplantation of insulin-producing cells or islet progenitor cells derived from stem cells, as the graft could be easily retrieved in the event of a local complication such as a teratoma, malignant transformation or unchecked hormone release. Compared with insulin-producing cells, islet progenitor cells may be more tolerant of hypoxia and may induce greater neovascularization. The DL approach also opens up the possibility of real-time, noninvasive imaging, including islet labeling and photoacoustic ultrasound[33], to monitor graft survival and immunological response. This is a clear limitation of clinical intraportal islet transplantation as practiced currently.

The relevance of our results to clinical transplantation remains to be determined. In BALB/c mice, which do not mount a strong foreign-body response, islet engraftment in the DL space was delayed compared with the KC site, but in C57BL/6 mice, which have a stronger foreign-body response, the time to normoglycemia was similar to that of the KC controls. By contrast, permanently implanted devices have traditionally failed in C57BL/6 mice but have worked well in alternative strains. We also showed that the DL site can support islet engraftment across a strong allogeneic barrier in mice, but only when tacrolimus immunosuppression is given to avert rejection. Thus, the DL site is not immunoprivileged. Notably, tacrolimus immunosuppression facilitated long-term islet engraftment despite the known diabetogenic side effects of this calcineurin inhibitor. Additionally, the DL site offers the possibility of co-transplanting immunoregulatory cells (e.g., mesenchymal stem cells or regulatory T cells) in a manner that is not currently possible with a dispersed intrahepatic islet graft. Our results indicate that a stronger early inflammatory response favors rather than hinders neovascularization and islet engraftment in our model.

We further showed that a pre-existing diabetic state does not interfere with diabetes reversal rates, at least in mice. We cannot infer that a neovascularization response will occur to a similar

degree in humans with longstanding diabetes, but it is of note that neovascularization remains a hallmark response to ischemia in end-stage secondary complications of clinical diabetes[34–36]. Although we do not anticipate major limitations in neovascularization responses in humans with longstanding diabetes, this remains to be tested in first-in-human studies planned at the University of Alberta.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

A.R.P. and A.M.J.S. initiated and designed the experiments. A.R.P., B.G.-L., S.M. and R.P. performed the experiments. T.K. isolated and provided human islets. A.R.P. and A.M.J.S. analyzed the data. All authors provided input for the manuscript writing and discussion.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Creation of the DL subcutaneous transplant site. Our method was designed to harness the natural foreign-body response elicited by medically approved vascular catheters to transform the tissue under the skin from a hypoxic, avascular space into a densely vascularized cellular graft–supporting matrix. Three to 6 weeks before islet transplant, 2-cm segments of a 5-French (Fr.) textured nylon radiopaque angiographic catheter (Torcon NB Advantage Beacon tip Cook Medical, Indiana, USA) or a 6.5-French smooth silicone catheter (Cook Medical, Indiana, USA) were implanted subcutaneously into the lower left quadrant of 20–25 g male BALB/c or C57BL/6 mice (Jackson Laboratories, Canada) for mouse syngeneic islet transplantation, (**Supplementary Fig. 1a**) or B6.129S7-Rag1^{tm1Mom} immunodeficient mice for human islet transplantation. A 4-mm lateral transverse incision was made caudal to the rib cage allowing for a small pocket to be created inferior to the incision line using blunt dissection. An adequate void (1 cm by 3 cm) was created. The catheter segment was implanted into the space such that the catheter laid parallel to the midline. The incision was closed with a surgical staple (Autoclip, Becton Dickinson, Sparks, MD) (**Supplementary Fig. 1b**). Once implanted, the catheter became adherent with blood proteins, leading to the formation of densely vascularized tissue, which exhibited a minimally visible profile (**Supplementary Fig. 1c**,**d**). At the time of transplant, removal of the catheter revealed a vascularized lumen allowing for cellular transplant infusion (**Figs. 1** and **2a**).

Proinflammatory cytokine and chemokines measurements. A 1-cm segment from either source catheter material was placed subcutaneously into the left lower quadrant of 20–25 g male BALB/c mice. Implanted catheters with surrounding skin and muscle tissue margins were explanted 24 h, 1 week and 2 weeks after implantation. Similarly, tissue dissections were retrieved from the abdomen of nonimplanted mice, serving as background cytokine and chemokine control specimens. The respective catheter segments were carefully removed from the surrounding tissue, yielding a hollow void encompassed by a vascularized matrix. Tissue samples were immediately placed in preweighed microcentrifuge tubes. The tissue weights were recorded, then subsequently flash frozen with liquid nitrogen and stored at −80 °C before conducting the cytokine and chemokine proinflammatory analysis. Once all tissue samples from respective implantation period were collected and frozen, 1 ml of lysis buffer (0.15 M NaCl, 1 mM Tris-HCl, 0.1% SDS, 0.1% Triton X-100, 20 mM sodium deoxycholate, 5 mM EDTA) per 200 mg of tissue was added to the tissue containing microcentrifuge tube. Each tissue sample was homogenized (PowerGen, Fisher Scientific, Ontario, Canada) on ice for 30 s × 2 replications. Samples were then sonicated (VirSonic, VirTis, NY, USA) with ten quick pulses while on ice. Lysed tissue samples were centrifuged at 14,000 r.p.m. for 10 min at 4 °C to remove cellular debris. The resulting supernatant was collected and placed in a microcentrifuge tube containing 10 µl of a protease inhibitor cocktail (Sigma-Aldrich Canada Co., Oakville, ON, Canada) per 1 ml of lysate (1:100). Peri-implant cytokine and chemokine (IL-1β, IL-12p70, IFN-γ, IL-6, KC/GRO, IL-10 and TNF-α) measurements were conducted using a Multi-Spot Mouse ProInflammatory 7-Plex Ultra-Sensitive kit (Meso Scale Discovery, Gaithersburg, MD, USA) requiring 25 µl of lysate/replicate and analyzed on a SECTOR Imager (Meso Scale Discovery, Gaithersburg, MD, USA).

Mouse pancreatectomy and islet isolation. Pancreatic islets were isolated from 8- to 12-week-old male BALB/c or C57BL/6 mice. Animals were housed under conventional conditions having access to food and water *ad libitum*. The care for all mice within the study was in accordance with the guidelines approved by the Canadian Council on Animal Care. Before pancreatectomy, the common bile duct was cannulated with a 27 G needle and the pancreas distended with 0.125 mg/ml cold Liberase TL Research Grade enzyme (Roche Diagnostics, Laval, QC, Canada) in Hanks' balanced salt solution (Sigma-Aldrich Canada Co., Oakville, ON, Canada). Islets were isolated by digesting the pancreata in a 50-ml tube placed in a 37 °C water bath for 14 min with gentle shaking. Subsequent to digestion, islets were purified on histopaque-density gradients (1.108, 1.083 and 1.069 g/ml, Sigma-Aldrich Canada Co., Oakville, ON, Canada).

Human islet isolation. Pancreata were procured from multi-organ donors and shipped to the isolation center in cold preservation solution. Consent for islet isolation had been granted in all cases. Islets from two separate human islet preparations were isolated implementing a modified Ricordi technique[37,38]. In short, the pancreas was distended with collagenase blend solution and digested in a Ricordi chamber. When islets were adequately dissociated from surrounding acinar tissue, the pancreatic digest was collected. Islets were purified using a continuous density gradient on a cell processor centrifuge (Model 2991, Cobe, Lakewood, Co, USA). All human islet preparations were processed for clinical transplantation and were made available for research only when the islet yield fell below that of the minimal mass required for clinical transplantation. Permission for these studies was granted by the Health Research Ethics Board of the University of Alberta, Edmonton, Alberta, Canada. Human islets were cultured overnight in CMRL-1066 media supplemented with insulin selenium-transferrin and insulin-like growth factor-1 at 22 °C before transfer to the laboratory for experimentation.

Diabetes induction and islet transplantation. Three to 5 days before transplantation, implanted mice were rendered diabetic through administration of an intraperitoneal injection of STZ at 185 mg/kg in acetate phosphate buffer, pH 4.5 (Sigma-Aldrich Canada Co., Oakville, ON, Canada). Animals were considered diabetic when their blood glucose levels exceeded a pre-established value of 15 mmol/L (350 mg/dl) for two consecutive daily readings. At the time of transplantation, ~500 mouse islets ± 10% with purity of 90% ± 5% or 2,000 human islet equivalents were aspirated into polyethylene (PE-50) tubing using a micro-syringe, and centrifuged into a pellet suitable for transplantation. Islet preparations were distributed randomly to all three transplant recipient groups: DL, KC or subcutaneous alone. DL-recipient mice were maintained under anesthesia with inhalant isoflurane and placed in a supine position. A field surrounding the implanted catheter was prepared by shaving and disinfecting the surface with soap scrub, povidone-iodine (Betadine, Purdue Pharma, Oakville, ON, Canada) and isopropyl alcohol. Cranial to the superior edge of the implanted catheter, a small (4 mm) incision was made to gain access to the catheter. The tissue matrix surrounding the superior margin of the catheter was dissected to withdraw and remove the catheter (**Fig. 1** and **Supplementary Fig. 1e**). The PE-50 tubing and islet preparation was delivered within the vascularized lumen, and islets expelled into the void using a microsyringe (**Figs. 1** and **2a** and **Supplementary Fig. 1f**). The incision was closed with a surgical staple (Autoclip, Becton Dickinson, Sparks, MD) (**Supplementary Fig. 1g**). Prior to recovery, recipients received a 0.1 mg/kg subcutaneous bolus of buprenorphine. Control animals were rendered diabetic and transplanted with ~500 mouse islets/recipient as described above, however, the islets were infused into the subcutaneous space alone (no prevascularization or catheter implant).In addition, a subset of diabetic animals was transplanted with ~500 mouse islets/recipient under the KC, the standard site for rodent islet transplantation. For all experiments islets were pooled, batched and transplanted in random allocation to either the DL or KC sites. To facilitate the KC transplants, a left lateral paralumbar subcostal incision was made and the left kidney was delivered into the wound. The renal capsule was incised and space was made under the capsule to allow transplantation of the islets using PE-50 tubing (Instech Laboratories, Boston, USA). The subcostal incision was closed in two layers. The efficacy of mouse islets transplanted into the DL site to reverse diabetes was compared to the engraftment efficacy of islets transplanted in the unmodified subcutaneous site or under the renal subcapsule.

Evaluation of islet graft function. Islet graft function was assessed through nonfasting blood glucose measurements, using a portable glucometer (OneTouch Ultra 2, LifeScan, Canada) three times per week following islet transplantation, in all groups transplanted. Reversal of diabetes was defined as two consecutive readings <11.1 mmol/L (in accordance with the American Diabetes Association) and maintained until study completion.

In addition, glucose tolerance tests were conducted at 60 or 100 days after transplant, as a means to further assess metabolic capacity in response to a glucose bolus, mimicking postprandial stimulus. Animals were fasted overnight before receiving an intraperitoneal glucose bolus (3 g/kg). Blood glucose levels were monitored at 0, 15, 30, 60, 90 and 120 min after injection, allowing for AUC-blood glucose to be calculated and analyzed between transplant groups.

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Long-term islet graft retrieval. To confirm graft dependent euglycemia, and to eliminate residual or regenerative native pancreatic beta cell function, animals with functional grafts had their islet transplants explanted either by nephrectomy or subcutaneous graft excision. Renal subcapsular islet transplant recipients were placed under anesthesia, and the graft-bearing kidney exposed. A LT200 Ligaclip (Johnson & Johnson, Inc., Ville St-Laurent, QC, CA) was used to occlude the renal vessels and the ureter at the pedicle. The left kidney was dissected, and the explanted graft preserved for immunohistochemistry in 10% formalin. Likewise, the subcutaneous islet grafts within the DL-transplanted animals, which exhibited no visible profile after transplant (**Supplementary Fig. 1h**), were excised with a margin of surrounding abdominal skin containing the islet graft. Following islet-graft removal nonfasting blood glucose measurements were monitored for the subsequent 7 days to observe a return to hyperglycemia, confirming post-transplant graft function.

Histological assessment. Immunofluorescence was used to identify endothelial cells for assessment of vascularization using anti-vWF antibody and anti-insulin and anti-glucagon antibodies to identify the presence of pancreatic β-cells and α-cells, respectively. Briefly, following deparaffinization and antigen heat retrieval, the graft sections were washed with phosphate buffered saline supplemented (PBS) with 1% goat serum, followed by blocking with 20% goat serum in PBS for 30 min. The sections were treated with a primary antibody of guinea pig anti-pig insulin (Dako A0564) diluted 1:100 (PBS with 1% goat serum), rabbit anti-pig von Willebrand factor (Dako A0082) diluted 1:400 (PBS with 1% goat Serum) and or rabbit anti-glucagon (Abcam) diluted 1:200 (PBS with 1% goat serum) for 2 h at 4 °C. Samples were rinsed with PBS with 1% goat serum followed by secondary antibody treatment consisting of goat anti-guinea pig (Alexa 568) diluted 1:500 (PBS with 1% goat serum), and goat anti-rabbit (Vector Fl-1000) diluted 1:500 (PBS with 1% goat serum) for 30 min at room temperature. Samples were rinsed with PBS and counter stained with DAPI in anti-fade mounting medium (ProLong, LifeTechnologies). Using a fluorescent microscope, the resulting microphotographs were taken using the appropriate filter with AxioVision imaging software. Vascular density was quantified by ImageJ software (National Institute of Health, Bethesda, MD), and reported as the percentage of the islet graft staining positive for von Willebrand staining. In addition, to assess the incorporation of vascularized collagen tissue into surrounding the DL islet-grafts, representative sections were stained with hematoxylin/eosin and Masson's trichrome.

Statistical analysis. Nonfasting blood glucose and proinflammatory data are represented as the mean ± s.e.m. Sample size calculations were based on reversal of glycemia rates in control mice with islets placed subcutaneously (0%) versus a projected estimate of 60% engraftment in the DL site (sample size $n = 20$ or more per group; alpha 5%, power 100%). Blood glucose AUC analysis for glucose tolerance test data was conducted through parametric one-way ANOVA using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Newman-Keuls post-hoc tests were used following the analysis of variances for multiple comparisons between study groups. Kaplan-Meier survival function curves were compared using the log-rank statistical method. $P < 0.05$ was considered significant.

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