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Vascularized tissue-engineered chambers promote survival and function of transplanted islets and improve glycemic control

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ABSTRACT

We have developed a chamber model of islet engraftment that optimizes islet survival by rapidly restoring islet-extracellular matrix relationships and vascularization. Our aim was to assess the ability of syngeneic adult islets seeded into blood vessel-containing chambers to correct streptozotocin-induced diabetes in mice. Approximately 350 syngeneic islets suspended in Matrigel® extracellular matrix were inserted into chambers based on either the splenic or groin (epigastric) vascular beds, or, in the standard approach, injected under the renal capsule. Blood glucose was monitored weekly for 7 weeks, and an intraperitoneal glucose tolerance test performed at 6 weeks in the presence of the islet grafts. Relative to untreated diabetic animals, glycemic control significantly improved in all islet transplant groups, strongly correlating with islet counts in the graft ($P<0.01$), and with best results in the splenic chamber group. Glycemic control deteriorated after chambers were surgically removed at week 8. Immunohistochemistry revealed islets with abundant insulin content in grafts from all groups, but with significantly more islets in splenic chamber grafts than the other treatment groups ($P<0.05$). It is concluded that hyperglycemia in experimental type 1 diabetes can be effectively treated by islets seeded into a vascularized chamber functioning as a "pancreatic organoid."

Key words: streptozotocin • diabetes • islet transplantation • vascularized chambers • renal capsule

There is a chronic shortage of suitable organ donors for islet transplantation in human type 1 diabetes (1). It is usually necessary to harvest multiple pancreata to obtain sufficient islets for transplantation (2, 3). This has prompted the search for more efficient techniques

of islet transplantation and engraftment and is the driving force behind the present study using vascularized tissue engineering methodology to promote organ function and insulin production.

The current clinical method of islet transplantation is injection into the portal vein (3, 4). Experimentally, islets are injected into the portal vein, into the omental pouch (5), or under the renal capsule (6), or microencapsulated prior to their injection into one of these sites (7). Of the major factors affecting the long-term viability of transplanted islets, hypoxia is a significant problem because islets are particularly susceptible to ischemia-reperfusion injury in the first 48 h after transplantation (8,9). In clinical trials, vascular thrombosis (10) and irreversible metabolic changes such as liver steatosis (11) have been identified as long-term complications of repeated portal vein injection of islets. With microencapsulation, the functional longevity of encapsulated islets in vivo may be eventually compromised by cellular overgrowth, despite ongoing attempts to maintain long-term membrane porosity (12).

An isolated but readily accessible chamber with an independent blood supply could improve in vivo culture of islets and extend our understanding of the signals required for expansion of insulin-producing tissue. Our laboratory has developed several three-dimensional in vivo models that can sustain transplanted cells. In the rat, an arteriovenous (AV) loop created by anastomosing the divided ends of the femoral artery and vein was sandwiched between layers of dermal collagen and the construct contained within a subcutaneously implanted polycarbonate chamber in the groin (13). Spontaneous outgrowth from the loop resulted in new fibrovascular tissue, which invaded the collagenous scaffold and filled the chamber. A similar model in the mouse, in which a flow-through AV pedicle was enclosed in a subcutaneously inserted cylindrical silicone chamber, also resulted in angiogenesis and spontaneous generation of connective tissue (14). Vascularized tissue growth occurred even in the absence of added extracellular matrix (ECM) or cells, substantially filling the chamber within 2–4 weeks (15). By 4–12 weeks, we found further expansion and maturation of this tissue, with progressively greater collagen fibril alignment (16). The addition of various ECM scaffolds within the chamber increased the rate of new tissue growth (16). Interestingly, the addition to the mouse chamber of specific components, such as Matrigel® and fibroblast growth factor-2 (FGF-2), promoted differentiation of adipose tissue (14). In vascularized rat chambers we have also observed the growth and differentiation of myoblasts after 2–6 weeks into myotubes and myofibers in the new tissue, staining positively for desmin and dystrophin, respectively (17). Theoretically, any combination of ECM, cells, and specific growth or differentiation factors may be added to these chambers to engineer new tissue. Furthermore tissue can be retrieved easily or further components added at any stage.

Initial experiments with an in vivo vascularized rat chamber seeded with syngeneic islets revealed maintenance of immunohistochemical insulin and glucagon expression after 3 weeks (18). Similarly, adult mouse islets and fetal mouse pancreata have been shown to survive in vascularized chambers in the groin of non-diabetic mice for up to 10 weeks (Cronin, K.J. et al., unpublished data). In this study, we used mice with streptozotocin-induced diabetes to evaluate islet grafts grown in chambers maintained around the epigastric artery and vein (groin chambers) or around the splenic artery and vein adjacent to the pancreas (splenic chambers), compared with the "benchmark" renal subcapsular site. Our aim was to demonstrate that the chamber model allows the development of an insulin-producing graft containing viable islets, suitable for the treatment of diabetes.

MATERIALS AND METHODS

Animals and ethics considerations

Experiments performed on adult male C57BL/6j mice (22–28 g) (Animal Resource Centre, Perth, Western Australia) were conducted under NHMRC Australia guidelines and as approved by the Animal Ethics Committee, St. Vincent's Hospital Melbourne. Mice were housed in an approved facility, on a 12 h day/night cycle, and given food and water ad libitum.

Streptozotocin-induced diabetes

Freshly weighed streptozotocin (STZ; Sigma Chemical Co., St. Louis, MO) was dissolved in 0.01 mol/l tri-sodium citrate buffer, pH 4.5, at 20 mg/ml and administered within 5 min via tail vein injection at an initial dose of 0.16 mg/g. Over the next 3 days, mice were checked for excess urination and water consumption. At day 5, a saphenous vein bleed was performed under 2% flurothane anesthesia and blood glucose (BG) level measured with a glucose oxidase-based monitor (MediSense Precision Plus, Abbott Laboratories, Doncaster, Victoria, Australia). Only mice with non-fasting BG>17 mmol/l and polyuria were defined as diabetic and included in the study. Mice that did not initially develop diabetes (~50% of all mice) were re-injected with half the initial dose of STZ (0.08 mg/g) and BG measured again at day 5. Using this protocol, ~70% of STZ-injected mice became diabetic.

Islet isolation

Islets were isolated from C57BL/6 mice as described previously (19). The common bile duct was cannulated, and the pancreas was distended with 3 ml RPMI medium (Life Technologies, Gaithersburg, MD) containing 1.3 U/ml of collagenase P (Roche Molecular Biochemicals, IN). Pooled pancreata were digested at 37°C for 20 min then disrupted with shaking. Islets purified on a Histopaque-1077 density gradient (Sigma) were washed and cultured at 37°C in 5% CO_2 in CMRL medium-1066 (Life Technologies) containing 10% FCS antibiotics, and glutamine for no more than two days prior to transplantation. The yield was usually 150–250 islets per mouse, and islets of variable size were noted. The islets maintained their morphological integrity and viability during culture in complete CMRL medium prior to transplantation.

Immediately prior to surgery, 400 hand-picked islets were collected into a sterile Erlenmeyer tube, washed three times with PBS, and centrifuged to remove FCS. The islets were finally re-suspended in 50 μl Matrigel® (BD Biosciences, Bedford, MA) at 4°C for seeding into one chamber or injecting into the right kidney. On average, ~45 μl of the islet suspension, corresponding to ~350 islets, was injected into the chambers or the kidney.

Surgery

Vascularized splenic chamber

Mice were anaesthetized with chloral hydrate (4 mg/g body weight, i.p.), and surgery was performed under aseptic conditions. Cylindrical silicone chambers made from laboratory tubing (Dow-Corning Corporation, Midland, MI) were cut into segments (5.5 mm in length, 3.35 mm internal diameter, volume approx 50 μl) each having a lateral slit. The abdominal hair was removed using depilatory cream, and the skin was decontaminated with alcohol and

chlorhexidine. A midline abdominal incision exposed the abdominal contents. The branch of splenic artery, where it exited the pancreas, was identified, and adherent adipose tissue was dissected from the vessels to create a 1 cm length of splenic pedicle. The chamber was inserted around this pedicle. The distal end and the lateral split were sealed with Ethicon Bone Wax™ (Johnson and Johnson International, European Logistics Centre, Brussels, Belgium). The islets in Matrigel® (50 μl) in a cooled syringe were injected into the open (proximal) end of the chamber then sealed with bone wax, taking care not to damage the pedicle (Fig. 1*A*). The splenic chambers were kept in position by the pressure exerted from surrounding organs and suture stabilization was not therefore required. The abdomen was closed in layers, with 6/0 nylon sutures for the abdominal wall and stainless steel clips for the skin.

Vascularized groin chamber

This model has been described previously in detail (14). In outline, using mice anesthetized with chloral hydrate as described above, hair on the groin and upper legs of mice was removed using a depilatory cream, and the skin was decontaminated using alcohol and chlorhexidine. An incision was made above the groin fat pad, from the groin crease to the knee. Superficial epigastric vessels were dissected free of surrounding fat from their origin at the femoral vessels for a distance of ~1 cm to their point of entry into the groin fat pad. The entire fat pad was mobilized free of skin so that the epigastric vessels (free of fat) could be embodied into the cylindrical chamber via a lateral split. The whole chamber was anchored to underlying muscle using 10/0 nylon microsutures. A small amount of the fat pad was used to plug the distal end of the chamber. The lateral slit was sealed with bone wax and the chamber filled with ~50 μl of islets suspended in Matrigel® from a pre-cooled syringe with a 25-gauge needle. The proximal end of chamber was sealed with bone wax (Fig. 1*B*), and the skin was closed with stainless steel clips.

Renal subcapsular injection

Using mice anesthetized as described above, we made a midline abdominal incision, the right kidney was identified and islets suspended in 50 μl of Matrigel® were injected slowly through a 26-gauge needle, with the needle tip close to the surface of the capsule. During injection, care was taken so that the needle passed only through the renal capsule without penetrating the body. The needle was removed without apparent fluid loss. The abdomen was closed as per the splenic chamber operation.

Sham operation

All procedures were performed as above, except that Matrigel® minus islets was used to fill the chamber.

Daily care of diabetic mice, weekly blood glucose monitoring

Mice in the study groups had daily Clinistix™ urine glucose tests (Bayer). One unit of long-acting insulin (Monotard; diluted 1 in 10, 0.1 ml) was injected subcutaneously if urine glucose was positive. The injection sites were rotated between the back of neck and two lumbar regions. After operation, non-fasting BG levels were determined weekly on saphenous vein samples. In weeks 6, 8, and 10, glucose tolerance tests, chamber harvesting and fasting BG, respectively, were performed.

Glucose tolerance test and fasting blood glucose determination

An intraperitoneal (i.p.) glucose tolerance test (GTT) was performed 6 weeks after surgery. Mice were fasted for 6–8 h, with only water provided ad libitum. BG was measured by right saphenous vein sample immediately prior to i.p. injection of 0.2 ml of a 250 mg/ml solution of D-glucose dissolved in saline (2 mg/g body weight). BG was again measured 30, 60, 90, 120, and 180 min after glucose injection. The left saphenous vein was sampled if there was any difficulty obtaining a drop of blood from the right vein. "Glucose tolerance" was defined as the insulin-dependent glucose uptake in 1 h (20). In the context of the GTT, the BG level at 90/120 min minus BG level at time 0 should be ≤50% of the maximal BG level at 30/60 min minus BG level at time 0, to be regarded as an "effective" treatment (20).

Ten weeks after surgery, once splenic and groin chambers had been harvested, fasting BG levels were determined for these two groups only.

Chamber harvest and graft evaluation

Mice were anesthetized as described earlier, and the chambers or kidneys were removed via a minimal access incision, 8 weeks after the initial operation. At the time of harvest, the size of tissues and patency of the pedicle were assessed qualitatively. Mice from the splenic and groin chamber groups were allowed to recover for the two weeks prior to the fasting BG determination, before being killed by an i.p. injection of Lethobarb (0.3 ml, pentobarbitone sodium; Virbac, Sydney, NSW, Australia) at week 10. In the renal subcapsular group, both kidneys were removed for histology and the mice were killed at week 8.

Histology and immunohistochemistry

Tissue was fixed overnight in 4% formaldehyde, before being processed and embedded into paraffin. Serial sections (5 μm) were placed on slides coated with 3-aminopropyltriethoxysilane (Sigma), dried overnight in a 37°C incubator, and stained with hematoxylin and eosin (H&E).

Immunohistochemical staining to identify α (glucagon), β (insulin), and delta (somatostatin) cells in islets was performed on a commercial autostainer (DAKO, Carpinteria, CA). Slides were washed with PBS, incubated for 5 min with 3% hydrogen peroxide, then for 30 min with primary antibodies: guinea pig anti-insulin antibody diluted 1:10, rabbit anti-glucagon antibody diluted 1:200 or rabbit anti-somatostatin antibody diluted 1:100 (DAKO). The components of the rabbit EnVision plus detection system (DAKO) were then applied for 30 min, followed by diaminobenzaldehyde reagent for 5 min. The slides were counterstained with hematoxylin. Rabbit non-immune serum was substituted for negative controls, and normal pancreas sections were tested as positive controls.

Morphological assessment

Sections were selected every 100 μm throughout each tissue graft, assuming an average islet diameter of 100–130 μm. Specimens harvested from splenic or groin chambers were stained with H&E, whereas those from the kidney were immunostained with insulin antibody. With the observer blinded to the origin of the specimen, each slide was observed at ×2 and then ×20 magnification. Islets were quantified and their relative positions in the specimen were recorded,

taking care not to duplicate counting of the same islet in consecutive sections. The total islet count for each specimen was compiled.

To assess the degree of vascularization of the surviving islets in the grafts, the distance between each islet and the nearest blood vessel (of >20 μm diameter) was determined using the AxioVision, version 4.2, image processing and analysis system based on the Zeiss Axioscope 2 microscope (Carl Zeiss Vision, Munich, Germany).

Statistics

Data are reported as mean ± standard deviation (SD) for all observations, where *n* equals the number of mice. Statistical comparisons between groups, using ANOVA and a post-hoc Tukey's multiple comparison test, were considered significant at $P \leq 0.05$ (GraphPad Prism software, San Diego, CA). The relationship between the "glycemic control index" and total islet counts per specimen was determined by least-squares regression analysis using SPSS for Windows version 12.01.

RESULTS

Evaluation of grafts in chambers

At the time of harvest, the vascular pedicle was patent in all chambers. After 6 weeks' growth, tissue occupied ~50% of the chamber volume for splenic chambers and ~90% of the chamber volume for groin chambers, most of the latter appearing to be vascularized fat.

Weekly BG levels

The mean weekly BG levels for the control and groups over 10 weeks are shown in Table 1. The mean BG for non-diabetic mice was in the range 7–10 mmol/l and for STZ-induced diabetic mice 18–22 mmol/l. BG levels for sham-operated mice were indistinguishable from non-operated counterparts. BG in splenic chamber, groin chamber, and renal subcapsule groups exhibited a gradual fall in the first 4 weeks, stabilizing between weeks 4 to 7, and reaching minimal levels of 10.9, 14.1, and 12.1 mmol/l, respectively, by week 7. Compared with untreated diabetic controls, these decreases in BG of 8.1, 8.2, and 7.2 mmol/l were significant in all three groups ($P<0.001$). However, only the splenic chamber group reached the threshold BG level of 11.1 mmol/l, regarded as a significant reversal of hyperglycemia (21). The decrease in BG achieved by the splenic chamber group was significantly better than that of the groin chamber group ($P<0.01$). However, no significant differences were found in BG between the splenic chamber and renal subcapsule groups, nor between the groin chamber and renal subcapsule groups. Once the islet-containing chambers had been harvested, the mean BG returned to near pre-operative levels in both splenic and groin chamber groups ($P<0.05$ in BG at week 9 vs. BG at week 7).

Glucose tolerance test

An i.p. GTT was performed 6 weeks after islet grafting (Fig. 2). Fasting BG in normal or sham-operated mice was around 7.0 mmol/l. Following i.p. glucose BG typically peaked at 12 mmol/l after 30–60 min and returned to near-normal fasting levels of 7.7 mmol/l by 120 min (mean hourly glucose uptake being 87% of the i.p.-injected glucose bolus). By contrast, diabetic control

mice had a fasting BG of 13.8 mmol/l, which peaked at 22.2 mmol/l at 30–60 min after i.p. glucose, and was still significantly elevated at 20.3 mmol/l after 120 min (mean hourly glucose uptake being 22% of the i.p.-injected glucose). The splenic chamber, groin chamber, and renal subcapsule groups had mean hourly uptakes of 40, 27, and 28% of the i.p.-injected glucose, respectively, which is less than the 50% hourly glucose uptake regarded by Abel et al. (20) as "effective" treatment of hyperglycemia. All three groups had diminished capacity for glucose tolerance compared with normal/no-operation control mice ($P<0.01$).

Morphological evidence of islet survival, hormone production, and vascularization

After 8 weeks' growth H&E staining revealed isolated surviving islets in the chamber tissue. Each cross section studied had between 0 and 7 islets, an average of 1 islet in every 2 sections examined. Patent pedicles and well-dispersed small blood vessels were observed in tissue from both splenic and groin chambers. Fibrous responses were seen at the edge of tissues. They were generally free of inflammatory infiltrates (Figs. 3*A* and 4*A*). At higher magnification, we observed that islets had maintained their rounded morphology in the chamber tissue. Vascularized adipocytes were seen in the middle of the chamber tissue (Figs. 3*C* and 4*B–E*), and more numerous small blood vessels were seen in the fibrous zone (Figs. 3*C* and 4*A*, *D*). Islets were commonly located in vascularized connective tissue or adipose tissue at the periphery of the graft (Fig. 4*B–D*) and were readily identified by their immunochemical staining for insulin, glucagon, and somatostatin (Figs. 3*B–D* and 4*C–E*). Islets injected under the renal capsule were more difficult to identify due to the similar histological appearance of the glomeruli (Fig. 5*A*). One islet, identified by insulin immunostaining, had a flattened morphology (Fig. 5*B*), but others had a more rounded in appearance (Fig. 5*C*).

Islets surviving after 8 weeks' growth were commonly located adjacent to a large artery or vein. Morphological analysis of islets in graft tissue harvested from splenic chambers and groin chambers revealed that, on average, islets were 41.7 ± 27.7 μm (n=29) and 47.7 ± 34.8 μm (n=40), respectively, from a major blood vessel. Interestingly some islet clusters had capillaries passing directly through them (Fig. 4*B*). Compared with the rich vasculature in the chamber tissue, blood vessels were difficult to identify in the kidney specimens, so this parameter was not determined for islets in the renal subcapsule group.

Quantification of islet survival in grafts after 8 weeks and correlation with BG

The mean numbers of islets in grafts were 70 ± 22 (range 4–120) in the splenic chamber group, 23 ± 5 (range 7–42) in the groin chamber group, and 19 ± 11 (range 0–66) in the renal subcapsule group. The splenic chamber group had significantly more surviving islets than the other two groups ($P<0.05$). Given that on average 350 islets were seeded, the mean islet survival was 22, 7, and 6%, respectively (range 0 to 37%).

Glycemic control during islet transplantation in vascularized chambers was estimated by plotting the minimal BG in weeks 1–8 against total islet counts in the graft specimen harvested at week 8. Regression analysis showed a strong correlation between these two parameters (r=–0.68, $P<0.01$) (Fig. 6).

DISCUSSION

In this study, we have shown that islets seeded into vascularized chambers reduce hyperglycemia in STZ-induced diabetic mice just as effectively as islets injected under the renal capsule, the "benchmark" procedure (6).

Properties of vascularized chambers that promote islet survival

Several factors influence the viability of transplanted islets. Hypoxia is likely to be one of the major causes of islet loss soon after transplantation. Folkman and co-workers determined that, ideally, cells need to be within 150–200 μm of a blood vessel in order to receive adequate oxygen and nutrition for survival (22). Our morphological study on tissue at 8 weeks demonstrated that surviving islets were, on average, 45 μm from a major blood vessel (range 6–118 μm), well within the survival limits defined by Folkman. Islets injected under the renal capsule also become hypoxic, because normoxia is limited to a few cell layers before critical ischemia occurs (23, 24). During islet isolation, islet-basement membrane interactions are disrupted by collagenase digestion. In vitro studies have shown that laminin-1 promotes survival and differentiation of β cells (25). The use of laminin-rich Matrigel® as a carrier for islets in the vascularized chambers is designed to restore islet-laminin interactions and improve islet survival. Once seeded into the chamber, islets should benefit from both the angiogenic and adipogenic properties of Matrigel® (26). However, the time spent at 4°C (necessary for keeping Matrigel® in its liquid form prior to islet seeding) may be critical, as this temperature is considered too low for optimal β cell survival (27).

The lag-time before the decline of BG after islets are seeded into vascularized chambers or injected under the renal capsule may reflect the time taken for islet engraftment within a new vascular bed. Patency of the vascular pedicle was maintained in all chambers and, histologically, marked angiogenesis was observed throughout the entire tissue. Quantitative morphological studies after 8 weeks showed that glycemic control was improved with as few as 50 (~14%) of the 350 islets surviving in chambers or under the renal capsule. Importantly, a strong correlation was also found between the minimal BG achieved by islet transplantation in chambers and the number of islets found in the specimen by morphological analysis ($P<0.01$). Endocrine function was confirmed by the increase in glycemia after the chambers were surgically removed at 8 weeks, and by immunohistochemical staining for insulin, glucagon and somatostatin.

Optimal site of chamber for islet transplantation

The lower mean BG over time, lower fasting glucose levels, improved glucose uptake during the GTT, and the higher islet counts per graft demonstrate that the splenic chamber group had better glycemic control than the groin chamber group. Splenic chambers inserted in the pancreatic bed use the same portal drainage as native islets, whereas chambers inserted in the groin have systemic drainage. Portal drainage has been shown to provide more effective and physiological function of islets (28). Groin chambers, however, may have an advantage of maintaining long-term patency of the pedicle because superficial epigastric vessels, branching from femoral vessels, form anastomoses with ilio-inguinal vessels. This may result in arterial input and venous drainage from both sides (13). We would expect the angiogenic capacity of the two locations to be similar. Relatively more tissue formed in groin chambers compared with splenic chambers, and qualitatively a greater percentage of adipose tissue was found in the groin chambers. Islets

were located in both the connective tissue and the adipose tissue, with no apparent preference. In all cases the islets were close to blood vessels, indicating that vascularization is crucial for their survival and function. Of particular interest was the finding of multiple capillaries in some revascularized islets, a typical example is shown in Fig. 4*B*. This intimate association between islet and the bloodstream is vital for the islet's ability to sense hyperglycemia, to respond by producing insulin then to secrete the hormone efficiently into the bloodstream.

The renal subcapsule group had BG levels intermediate between the splenic and groin chamber groups but were not statistically different from either. These BG levels were significantly better than untreated diabetic controls ($P<0.001$). The renal subcapsular site has been shown previously to maintain effective islet function compared with intrahepatic and other sites in rats and mice (23, 24) due, in part, to the ready access of a blood supply (28, 29) and rapid revascularization rate (24). Most parameters suggest that vascularized chambers are at least comparable with this "benchmark" islet transplantation procedure. Furthermore, data from the islet counts per specimen demonstrated the survival of significantly more islets on average in vascularized splenic chambers compared with either the renal subcapsule or the vascularized groin chamber groups ($P<0.05$).

Potential clinical application of this technique

The clinical method of choice for islet transplantation is portal vein injection, although thrombosis after repeated injections and long-term metabolic disturbances in the liver such as steatosis are significant complications of this procedure (10, 11). Whole pancreas transplantation is another alternative but, as with islet transplantation, suffers from a chronic lack of pancreata (2, 3).

The chamber model is a useful experimental tool, but would need to undergo revision before it could become the clinical method of choice. The surgery involved requires considerable expertise and may be too complex to be performed routinely by a general surgeon. Surgical insertion of chambers is an invasive procedure and the introduction of infection is a further complication. In its present form, the chamber technique cannot effectively protect islets from allorejection or recurrence of autoimmunity, but it could be combined with cell protection techniques such as islet microencapsulation (12, 30) to overcome this problem and improve the outcome. The islets most distant from the pedicle are subject to hypoxia in the early days after engraftment (8, 9, 31). It may be advantageous in the future to attempt seeding islets directly onto the pedicle in a smaller volume of carrier solution (e.g., 10–20 μl) to minimize islet loss. The strategy of delaying the introduction of islets into chambers by 1 to 2 weeks, the time taken to establish an adequate angiogenic response in the mouse model (14), could ultimately improve islet engraftment and viability. Nevertheless, vascularized chambers have some distinct advantages. Chambers can be located on blood vessels of choice, such as the splenic vessels, offering portal vein drainage of the islet-containing graft. Furthermore, the contents of the chamber could theoretically be replenished with fresh islets if the original islets lose their ability over time to produce insulin. Only time and further refinement of the technique will prove whether it has a future as a viable clinical method of islet transplantation.

The use of the tissue-engineered, insulin-producing "organoid," with its intrinsic vascular supply, the use of autologous cells and biomaterials (where possible), and the ability to retrieve or even replenish seeded islets, could overcome several deficiencies in the currently available methods of

islet transplantation. In this novel model the graft environment can be altered via ECM substitution, cellular co-culture, and concurrent growth factor or drug administration. Furthermore, the transplanted tissues are recoverable without animal sacrifice and are microsurgically transferable within and between individuals. Due to its independent vascular supply, manipulation, analysis, and evaluation are simplified; for example, blood sampling from the afferent and efferent pedicle to assess insulin production by the transplanted islets is theoretically feasible. The model provides an in vivo culture system for the study of islet transplantation and lends itself to the introduction of pancreatic stem cells and islet precursor cells.

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TABLE 1. *Weekly nonfasting blood glucose (mmol/l; mean±SD) for splenic chamber, groin chamber, and renal subcapsule groups, normal and diabetic controls with/without sham operation*

Group	Splenic chambers (n=7)	Groin chambers (n=7)	Renal capsule (n=7)	Normal No operation (n=4)	Normal Sham operation (n=4)	Diabetic[a] No operation (n=4)	Diabetic sham operation (n=3)
Week							
0	19.0 ± 1.9	22.3 ± 4.8	19.3 ± 3.4	—	—	19.2 ± 2.3	18.1 ± 0.6
1	16.0 ± 2.4	17.5 ± 3.9	15.0 ± 4.1	10.1 ± 0.6	7.8 ± 1.1	18.6 ± 2.8	17.9 ± 2.1
2	12.5 ± 2.0	19.0 ± 4.3	15.1 ± 4.6	9.7 ± 0.9	9.8 ± 1.4	18.1 ± 3.6	20.6 ± 7.9
3	11.6 ± 1.8	16.5 ± 3.8	14.0 ± 4.5	8.5 ± 0.8	9.4 ± 1.4	18.1 ± 2.0	19.0 ± 5.0
4	11.4 ± 1.4	15.4 ± 3.8	12.1 ± 2.7	9.7 ± 1.0	8.8 ± 1.2	20.2 ± 5.0	18.4 ± 3.6
5	11.8 ± 2.2	14.4 ± 3.5	12.8 ± 3.4	8.8 ± 0.4	6.8 ± 0.8	18.5 ± 1.2	18.8 ± 0.3
6				GTT			
7	10.9 ± 1.0	14.1 ± 3.1	12.6 ± 3.7	NM[b]	NM	20.6 ± 2.0	20.0 ± 3.3
8				Chamber harvest			
9	14.4 ± 2.4	20.1 ± 5.7	NM	NM	NM	NM	NM
10				Fasting BG			

[a] Diabetes was confirmed in week 0. [b] NM, not measured.

Fig. 1

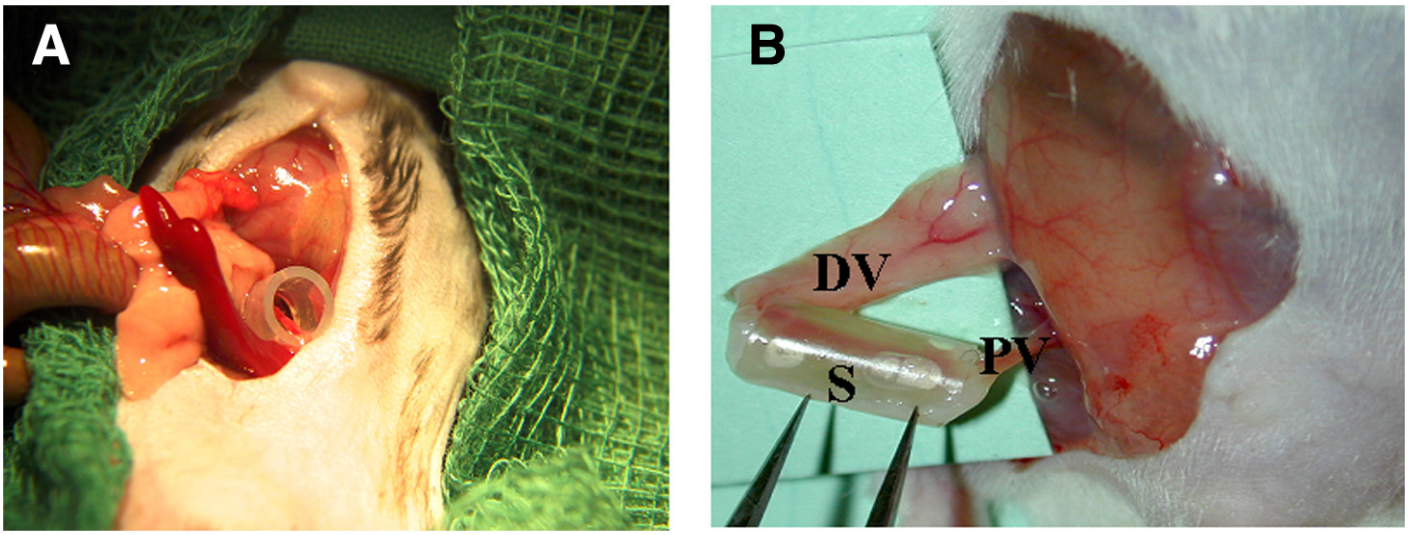

Figure 1. Vascularized chamber models. ***A)*** Splenic chamber with blood vessels, Matrigel® and islets, immediately prior to sealing the end of the chamber with bone wax. ***B)*** Groin chamber, showing the sealed silicone tube (S) encasing the inferior epigastric arteriovenous pedicle, Matrigel® and islet tissue. The proximal epigastric vessels (PV) are seen to the right of the silicone tube and the distal vessels (DV) still encased in adipose tissue on the left.

Fig. 2

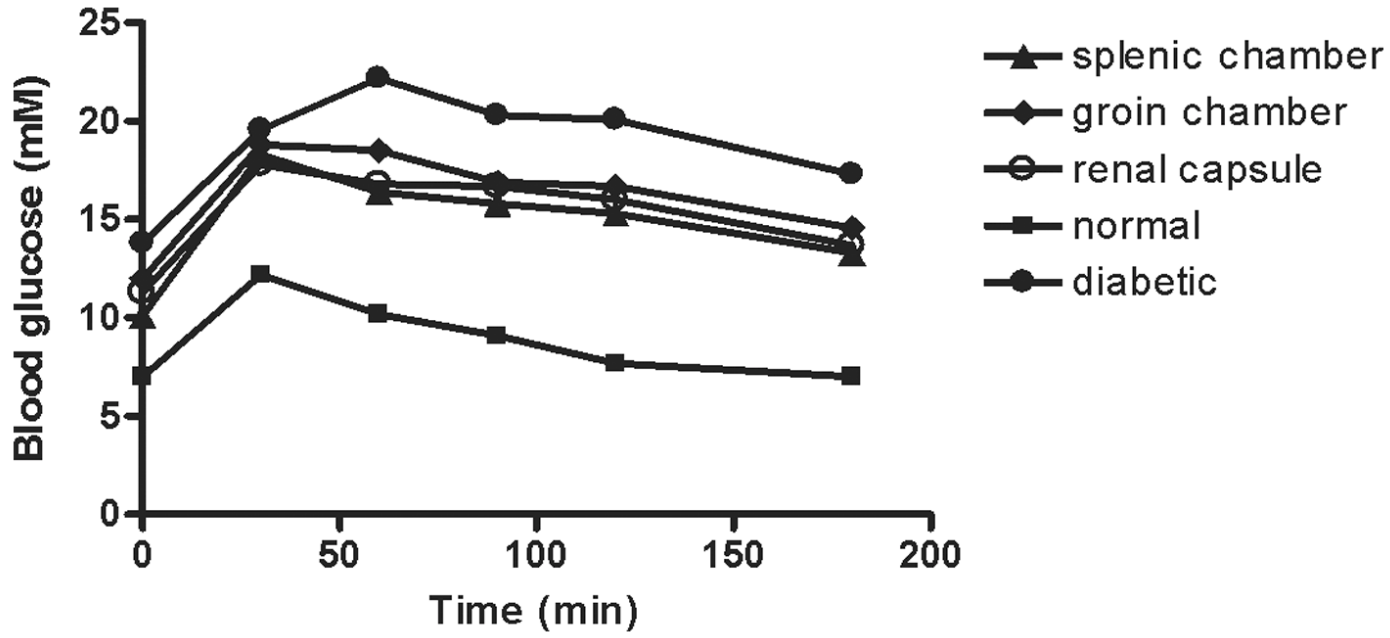

Figure 2. Mean blood glucose levels in the intraperitoneal glucose tolerance test (GTT) performed 6 weeks after transplantation of islets.

Fig. 3

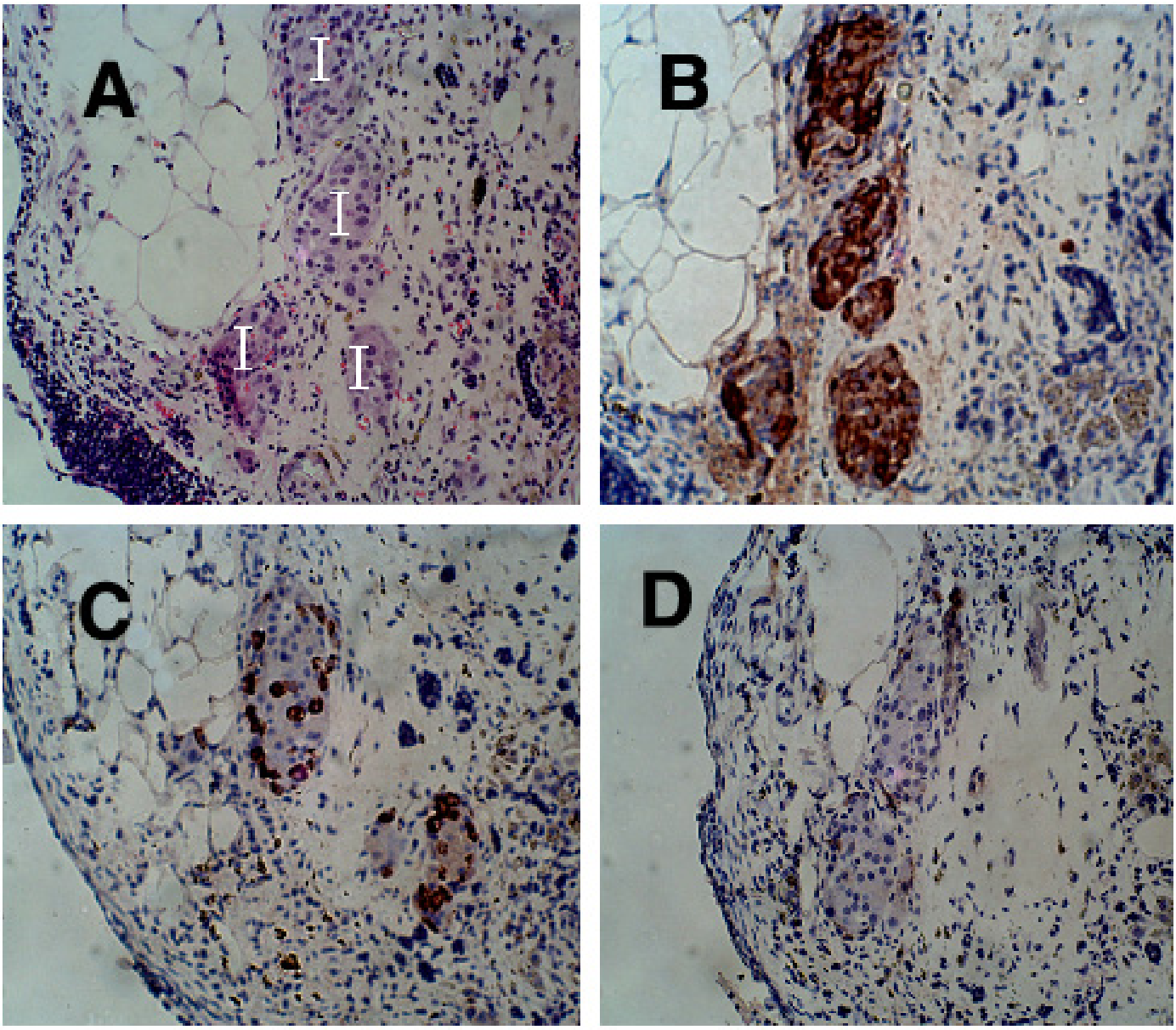

Figure 3. Histological sections of islets in a splenic chamber 8 weeks after transplantation. ***A)*** Islets (I) grouped together in a splenic chamber (H&E, ×100). ***B)*** An adjacent section of the same islets as in (***A)*** stained with insulin antibody (×100). ***C)*** Islets in splenic chamber stained with glucagon antibody (×100). ***D)*** Islets in splenic chamber stained with somatostatin antobody (×100).

Fig. 4

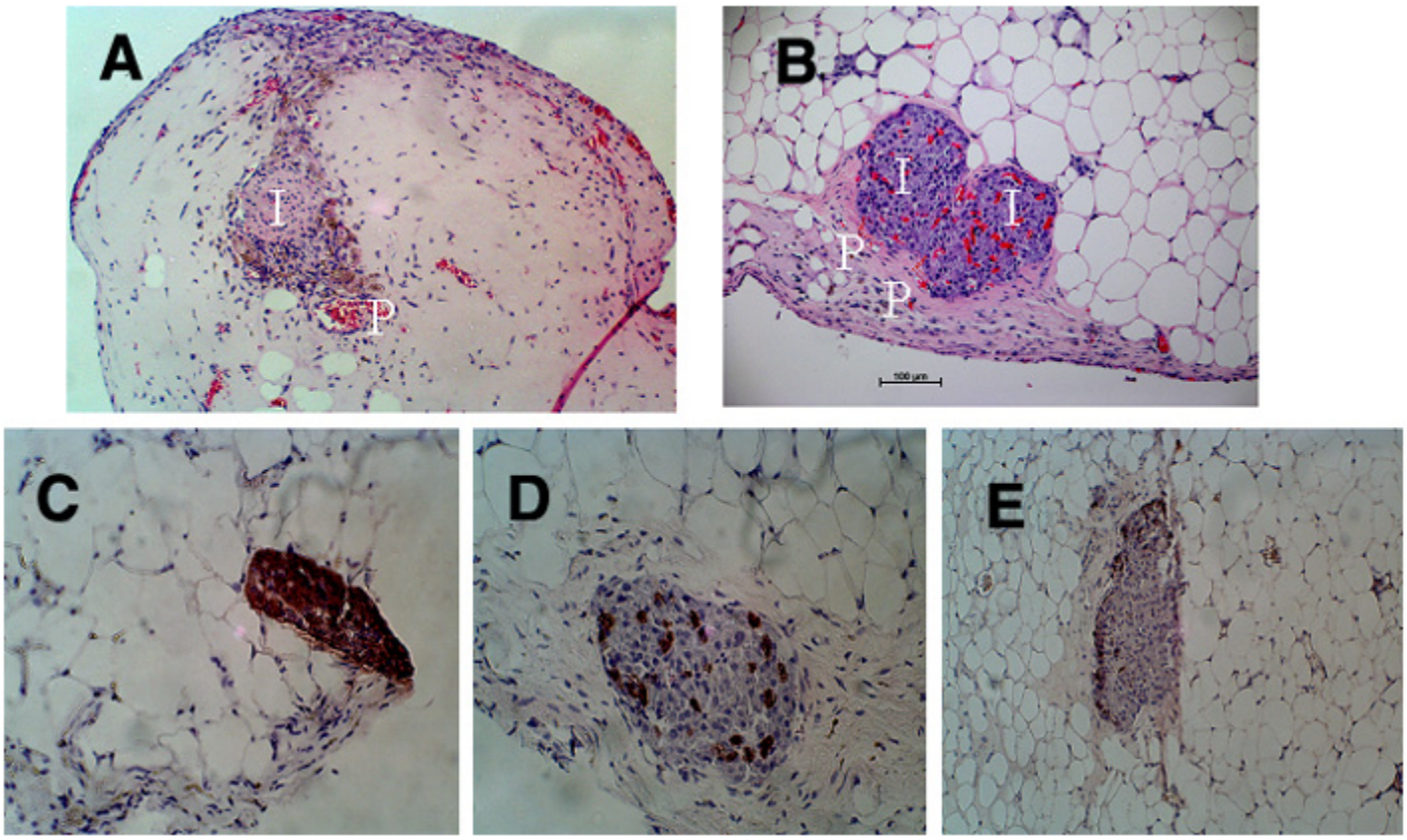

Figure 4. Histological sections of islets in a groin chamber 8 weeks after transplantation. ***A)*** An islet (I) in vascularized Matrigel® adjacent to the patent pedicle (P) (H&E, ×50). ***B)*** Two adjacent islets (I) close to blood vessels (P) in the tissue and containing numerous capillaries within each islet (H&E, ×100). ***C; left)*** An islet stained with insulin antibody (×100). ***D; middle)*** An islet stained with glucagon antibody (×100). ***E; right)*** An islet stained with somatostatin antibody (×100).

Fig. 5

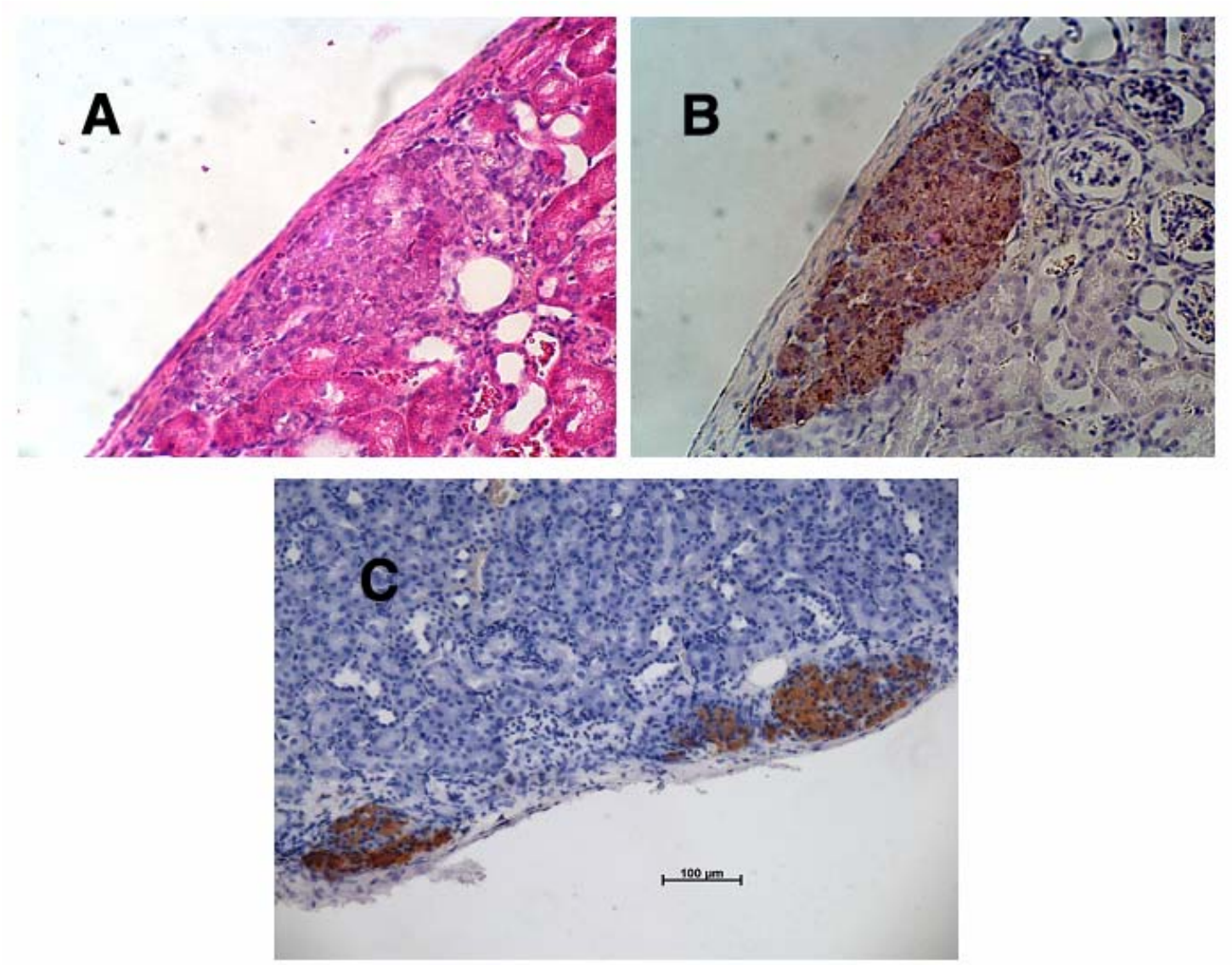

Figure 5. Histological sections of islets injected under the renal capsule after 8 weeks. ***A)*** An islet under the renal capsule (H&E, ×200). Note the difficulty in distinguishing this islet from the surrounding tissue of the kidney. ***B)*** The same islet depicted in (***A***) stained with insulin antibody (×200), allowing its ready identification compared with surrounding tissue. ***C)*** A group of islets stained with insulin antibody (×100) close to the surface of the renal capsule.

Fig. 6

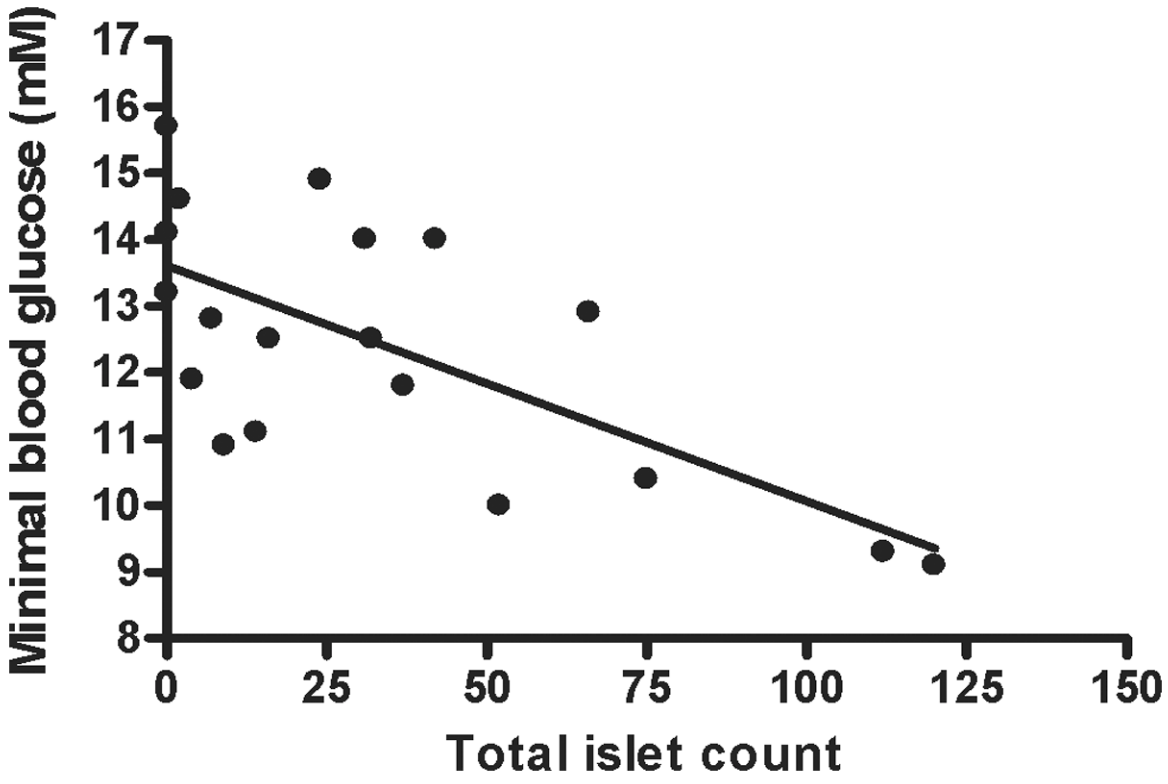

Figure 6. Relationship between minimal blood glucose during islet transplantation and total islet count per graft. The data is from all three treatment groups.