Clinical and Experimental Pancreatic Islet Transplantation to Striated Muscle: Establishment of a Vascular System Similar to that in Native Islets

Gustaf Christoffersson¹, Johanna Henriknsä¹, Lars Johansson², Charlotte Rolny³, Håkan Ahlström², José Caballero-Corbalan², Ralf Segersvärd⁴, Johan Perment⁴, Olle Korsgren², Per-Ola Carlsson¹,⁵, Mia Phillipson¹

¹ Department of Medical Cell Biology, Box 571, SE-75123 Uppsala University, Uppsala, Sweden, ²Department of Oncology, Radiology and Clinical Immunology, Uppsala University, Uppsala, Sweden, ³Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden ⁴Department for Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden, ⁵Department of Medical Sciences, Uppsala University, Uppsala, Sweden

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Correspondence:
Dr Mia Phillipson
E-mail: mia.phillipson@mcb.uu.se

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**Objective:** Curing type 1 diabetes by transplanting pancreatic islets into the liver is associated with poor long-term outcome and graft failure at least partly due to inadequate graft revascularization. The aim of the current study was to evaluate striated muscle as a potential angiogenic site for islet transplantation.

**Research Design and Methods:** The current study presents a new experimental model which is found applicable to clinical islet transplantation. Islets were implanted into striated muscle where after intra-islet vascular density and blood flow were visualized with intravital and confocal microscopy in mice, and by magnetic resonance imaging in three auto-transplanted pancreatectomized patients. Mice were rendered neutropenic by repeated injections of Gr-1 antibody and diabetes was induced by alloxan treatment.

**Results:** Contrary to liver-engrafted islets, islets transplanted to mouse muscle were revascularized with vessel densities and blood flow entirely comparable to islets within intact pancreas. Initiation of islet revascularization at the muscular site was dependent on neutrophils, and the function of islets transplanted to muscle was proven by curing diabetic mice. The experimental data were confirmed in auto-transplanted patients where higher plasma volumes were measured in islets engrafted in forearm muscle compared to adjacent muscle tissue through high-resolution magnetic resonance imaging.

**Conclusions:** This study presents a novel paradigm in islet transplantation whereby recruited neutrophils are crucial for the functionally restored intra-islet blood perfusion following transplantation to striated muscle under experimental and clinical situations.

Transplantation of pancreatic tissue is today the only curative treatment for type I diabetes mellitus. Clinically, either the entire pancreas is transplanted into the abdominal cavity or isolated insulin producing islets of Langerhans are implanted into the liver through infusion via the portal vein. The former procedure is highly successful as the graft functions well following transplantation. However, it requires extensive surgery, while the latter procedure is attractive as only minor surgery is required in conscious recipients. Unfortunately, both function and survival of intrahepatically transplanted islets deteriorate with time (1). Delayed and insufficient islet revascularization (2), gluco- and lipotoxicity (3), presence of an instant blood mediated inflammatory reaction (4; 5), as well as toxicity of the immunosuppressive drugs present in high concentrations in the portal blood (6; 7), are all factors believed to contribute to graft failure at this site. Native islets are highly vascularized, with blood perfusions ten times higher than the exocrine pancreas (8; 9). The islet microvasculature consists of a dense glomerular-like capillary network. A specific perfusion order of the different endocrine cell types has been shown in islets of Langerhans (10-15), enabling intra-islet cell communication and further demonstrating the importance of a refined and adequate intra-islet blood flow for normal islet function.

Many of the factors contributing to poor islet function are associated with the liver as the site for engraftment (3; 5; 6; 16; 17), and for this reason other sites are now being investigated. The intramuscular site has attracted recent interest due to positive long-term outcome of auto-transplantation of parathyroid glands to the brachioradialis muscle (18). Indeed, in a recent case report
we documented a successful two-years-follow up of a child receiving auto-transplanted islets into muscle (19).

Myeloid derived leukocytes have recently been shown to be involved in muscle healing and regeneration including angiogenesis (20; 21), and we therefore hypothesized that leukocytes may be involved also in the engraftment and revascularization of transplanted islets to muscle. To address this hypothesis, in addition to evaluating striated muscle as a potential angiogenic site for islet transplantation, an in vivo mouse model was developed that enables studies of leukocyte-endothelial cell interactions and blood flow during revascularization of transplanted islets by intravital and confocal microscopy. Long-term islet function and survival following transplantation to muscle were evaluated in diabetic recipients. The results achieved in the experimental model were thereafter validated in patients receiving islets auto-transplanted to muscle following pancreatectomy with a sophisticated magnetic resonance imaging technique.

RESEARCH DESIGN AND METHODS


Animals. Male C57Bl/6 mice [25–30 g (B&K Universal)], and C57Bl/6 nu/nu mice [25–30 g (Taconic M&B)] were used. Experiments were approved by the Uppsala Laboratory Animal Ethical Committee.

Islet isolation and transplantation to mice. Mouse islets of Langerhans were isolated and cultured over night as previously described (22). Human islets from five heart-beating female donors (age 57±7 years) were isolated and cultured as previously described (23).

Islets were fluorescently labeled immediately before transplantation with the intracellular probes Celltracker Blue CMAC or Celltrace Far Red DDAO (Invitrogen), depending on type of imaging performed later. For transplantation into the cremaster muscle (surrounding the testis) of non-diabetic mice, 5-20 islets in suspension were, by the use of a butterfly needle (25G), repeatedly injected subfascially at different spots to allow for single islet engraftment. For transplantation to the liver of non-diabetic mice, 200 islets were infused via the portal vein.

Alloxan-diabetic mice (iv., 75 mg/kg, Sigma-Aldrich) had plasma glucose concentrations of 25.8±1.0 mmol/l on the day for transplantation. A suspension with 300 islets was injected superficially between abdominal external oblique muscle fibers (on the side of the abdomen) or infused into the portal vein to the liver. Plasma glucose levels were deemed normalized <11.1 mmol/l, and mice were euthanized if blood glucose levels were >20 mmol/l seven days post-transplantation.

Intravital microscopy. The cremaster muscles of isoflurane-anaesthetized (Abbott) mice with implanted islets were prepared for intravital microscopy as previously described (24). A catheter in the femoral artery allowed retrograde infusion close intra-arterially to the muscle. An intravital microscope (Leica Microsystems DM5000B, equipped with a Hamamatsu Orca-R2 CCD camera and HCX Apo L 20X/0.50W and 40X/0.80W objectives, Volocity software) was used to visualize the microcirculation of transplanted islets and surrounding muscle. Recordings were made for analysis of adherent (stationary for >30 s within 100 μm length of venule in 3 min) and emigrated leukocytes (cells in the extravascular space per field of view, 0.05 mm²).

Laser scanning confocal- (Nikon C-1 with Plan Fluor ELWD 20X/0.45, 40X/0.60 objectives, Nikon EZ-C1 software), spinning disk confocal- (Olympus BX51/Quorum WaveFx, with a Hamamatsu C9100–13 EMCCD camera, an XLUM Plan F1 20X/0.95W objective, Volocity software) or multiphoton- (Olympus FV300/Chameleon...
ti:sapphire laser with a 40X/0.80W objective and Olympus FluoView and ImageJ software or Zeiss 710 NLO with a Plan-Apo 20X/1.0W objective, Zeiss Zen software) microscopy was performed after intra-arterial injection of 25 µg Alexa Fluor 555 or 594-anti-CD31-monoclonal antibody (mAb, clone 390, eBioscience) to stain endothelium and 15 µg FITC-anti-Gr-1-mAb (clone RB6-8C5, eBioscience) to stain neutrophils. 

*Vessel density and functionality.* Paraffin sections of transplants were stained for insulin with anti-insulin antibody (Fitzgerald) and for endothelium with the lectin BS-1 (Sigma-Aldrich, Supplemental Fig. 1 and 2). Vessel functionality within grafts was studied through intra-jugular injection of 100 µg SBA lectin, which stains endothelium and allows for detection of perfused vessels. Endothelium was stained in cryosections with anti-CD31-mAb (conjugated to Alexa Fluor 555) and nuclei were stained with Hoechst 33342 (Invitrogen).

*Neutrophil depletion.* Mice were rendered neutropenic by intraperitoneal injections of anti-Gr-1-mAb (clone RB6-8C5, eBioscience). IgG isotype antibody (eBioscience) was injected in controls. One day prior to transplantation (day zero) 150 µg was injected, and an additional 150 µg on day three. Determination of treatment efficiency was made by blood analysis and differential count. Total leukocyte counts were down by 50% (8.2±1.0*10⁹/l vs 4.1±0.6*10⁹/l) and neutrophil counts were reduced with 80% (19.2±5.2*10⁸/l vs 4.2±1.2*10⁸/l). No Gr-1-positive cells were found nearby the grafts in Gr-1-antibody treated animals.

*Clinical intramuscular islet autotransplantation.* The study was approved by the Regional Ethics Board, Uppsala, Sweden and was performed in accordance with local institutional and Swedish national rules and regulations. Three patients (2 men, 1 woman) with intraductal papillary mucinous neoplasm underwent total pancreatectomy with Whipple procedure and autologous intramuscular islet transplantation (Table 1). *Ex vivo* the pancreas was immediately perfused with cold (4°C) University of Wisconsin solution, and shipped to the islet isolation laboratory of the Nordic Network for Clinical Islet Transplantation (cold ischemic time less than two hours). Islet isolation was performed as previously described (23) and islets maintained in culture for 24 hours before characterization (Supplemental Table 1).

Absence of functioning endocrine pancreatic tissue before islet transplantation was confirmed in all patients by lack of C-peptide (25) (Table 1). Under brachial plexus anesthesia islets in volumes of 50-100 µl where injected into the muscle fibers of the brachioradialis muscle (in the forearm) with the help of a central venous catheter (Secalon-T, 16G, 130 mm, BD). Islet graft function after transplantation was assessed by circulating C-peptide (Table 1).

The subjects were scanned three to six months post-transplantation using a 1.5 T clinical MR-scanner (Gyroscan Intera, Philips Medical Systems). The body coil was used for RF-transmission and a 45 mm circular linear coil for RF-reception. High resolution axial images were obtained using a T1-weighted 3D gradient echo acquisition with 32 slices and an in-plane resolution of 200 x 200 µm² and a slice thickness of 1.0 mm (TR/TE/flip=16/5/10).

**Statistics.** Values are expressed as mean±SEM. Paired and unpaired two-tailed Student’s t-tests were used to compare between groups.

**RESULTS**

This study uses a novel experimental model where isolated pancreatic islets are transplanted into the mouse cremaster muscle for ease of visualization. The reestablished circulation to the transplanted tissue was studied by transillumination, epifluorescence,
spinning disk, laser scanning confocal and multiphoton microscopy, and the functionality of intra-muscularly engrafted islets was tested in diabetic recipients. For translational purposes, ultra-high resolution magnetic resonance imaging was performed on auto-transplanted islets engrafted in the brachioradialis muscle of patients in order to evaluate islet revascularization through measurements of plasma volume in transplanted islets versus surrounding muscle tissue.

Normalized intra-islet vascular density following islet transplantation to striated muscle. Islets syngeneically transplanted to the cremaster muscle of non-diabetic mice rapidly became vascularized and showed functional intra-islet blood vessels already at three to five days after transplantation, as confirmed by close intra-arterial injection of fluorescently labeled anti-CD31-mAb (Fig. 1A), with vessel densities of 407±94 vessels per mm² (Fig. 1D). Following these early events, the vasculature continued to develop and had two weeks after transplantation the characteristic glomerular-like vascular network (Fig. 1B) seen in native islets (Fig. 1C). Two to four weeks post-transplantation, intra-islet vascular densities had increased threefold (1187±187 and 1162±120 vessels/mm², respectively) and were similar to those observed in islets in intact pancreas (1074±174 vessels/mm², Fig. 1D). Imaging of islet vasculature with deeply penetrating multiphoton microscopy two weeks post-transplantation revealed a complete vascular network throughout the islet (Supplemental Video 1). When intra-islet vessel densities were analyzed in islets transplanted intraportally to the liver of non-diabetic mice, vessels surrounding the whole islets were detected but no intra-islet vessels could be found at two or four weeks after transplantation (n=7 mice, Supplemental Fig. 1). Transplanted islets at both sites stained positive for insulin (Supplemental Fig. 1).

The intra-islet capillary diameter was enlarged three to five days post-transplantation to muscle, but was normalized to diameters found in islets in intact pancreas after two weeks (Fig. 1E), as previously reported (26).

High vascular densities following human islet transplantation to mouse striated muscle. To investigate if the improved vascularization of mouse islets transplanted to muscle also applied to human islets, human islets were implanted into the cremaster muscle of immune incompetent mice (nu/nu). For logistic reasons, the human islets were cultured for an average of four days prior transplantation and for comparison mouse islets were also cultured for an equal period of time. Four weeks post-transplantation, human islets were highly vascularized with an intra-islet vascular density comparable to that of cultured mouse islets (Fig. 1F). Intravital and confocal microscopy showed functional vessels in transplanted human islets, although the vessel structure was somewhat different than what was observed for transplanted mouse islets, as intra-islet capillary diameters were more heterogeneous (9-20 µm) and had a differently organized, less glomerular-like vasculature (Fig. 1G, and Supplemental Video 2) than what was observed in mouse islets (Fig. 1, B and C). The number of remnant endothelial cells has been reported to decrease with time in culture (27). However, the vascular densities of mouse islets cultured for four days were not significantly lower compared to mouse islets cultured over-night (942±50 and 1162±120 vessels/mm², Fig. 1, F and D, respectively), suggesting that the decreased number of remnant donor endothelial cells do not affect the vascularization four weeks after transplantation.

Functionally restored vessels following islet transplantation to striated muscle. The functionality of the newly formed vessels within transplanted islets was investigated by
intravenous injections of fluorescent SBA lectin (Fig. 2, A and B), which ensures endothelial staining of perfused vessels only. After termination of experiments, muscle cryosections were stained for endothelial cells with anti-CD31-mAb, and the numbers of lectin and CD31 positive vessels were analyzed. In islets transplanted to striated muscle as well as in islets in the intact pancreas, close to 100% of the CD31 positive vessels were also positive for lectin (Fig. 2, C, D and E) indicating that nearly all vessels were indeed perfused. Contrary, the very few CD31-positive cells that could be found within islets transplanted to liver showed low staining overlap with intravenously administered lectin, suggesting mainly non-functional vessels, i.e. possibly remnant donor endothelial cells (Fig. 2F).

**Blood flow organization in transplanted mouse and human islets.** In rodents, the β-cell core of the islet receives blood before the endocrine cell types in the islet mantle, the α-, δ- and PP-cells (i.e. β-α-δ blood flow pattern) (14; 28). To investigate the blood flow pattern, following transplantation to muscle, fluorescently labeled dextran was injected close intra-arterially and islet blood flow was recorded by epifluorescence microscopy. Indeed, four weeks after transplantation mouse islets in muscle were perfused from the core outwards, since the fluorescent signal was initially observed in the central part of the islet (Supplemental Fig. 3, A, C and E). This indicates that transplantation and revascularization of islets at the intramuscular site do not change the inherent perfusion pattern.

Contrary to the mouse islet organization described above, human islets have a differently organized distribution of endocrine cells throughout the islet with clusterized subunits (29-32). Blood flow recordings of human islets transplanted to the cremaster muscle of nu/nu mice during fluorescent dextran injections revealed a differently organized perfusion pattern (four weeks post-transplantation, Supplemental Fig. 2, B, D and F). While the blood perfusion of mouse islets was directed from the core and outwards, human islets were observed to be perfused from one side to the other in a more polarized manner, and no core-to-mantle perfusion could be detected. This implies that the blood flow of pancreatic islets differs between species, and that the inherent organization of different endocrine cell-types within the islet influences revascularization and thereby gives the perfusion order.

**Increased leukocyte-endothelial cell interactions in grafts.** Syngeneic transplantation is not associated with acquired immune cell activation and concomitant graft rejection, as donor and recipient share the same genetic background. However, intravital microscopy of newly formed vasculature in these grafts exposed interactions between circulating leukocytes and endothelial cells within the islet capillaries as well as in the venules draining the islets, but not at sites receiving sham injections of islet culture medium. Significantly more leukocytes adhered in venules draining islets compared to venules draining adjacent muscle tissue three to five days post-transplantation (Fig. 3A), and emigrated leukocytes were found in the perivascular tissue in the proximity of recently transplanted islets (Fig. 3B). In vivo fluorescent staining of leukocytes with an acute injection of low-dose anti-Gr-1-mAb intra-arterially revealed that the majority of recruited leukocytes were Gr-1-positive leukocytes (Fig. 3C). The number of recruited leukocytes decreased with time after transplantation, indicating a role of leukocyte recruitment during the initial stages of reestablishment of circulation (Fig. 3B). Gr-1-positive leukocytes have been shown to be involved in muscle healing and regeneration (20; 21), and we therefore investigated their involvement in the revascularization of transplanted islets. Transplantation of islets to
Engraftment in Muscle Restores Islet Vasculature

neutropenic mice resulted in complete inhibition of revascularization of islets three to five days post-transplantation (Fig. 3, D and E), as no intra-islet vessels could be detected.

Alloxan-induced diabetes is reversed by islets transplantation to muscle or liver, but only muscle engrafted islets show an unaffected response to glucose load. To investigate the ability of islets transplanted to muscle to regulate blood glucose, recipient mice were rendered diabetic through alloxan treatment, resulting in plasma glucose levels of 25.8±1.0 mmol/l. Thereafter 300 islets were either transplanted into the abdominal external oblique muscle (n=8) or into the liver (n=6). There was no difference in time to normalization of blood-glucose levels between the two sites as both groups were below 11.1 mmol/l at just over a week post-transplantation (Fig. 4A). Three mice with plasma glucose levels >20 mmol/l one week post-transplantation were euthanized due to ethical requirements (two with grafts in muscle and one with grafts in the liver). Plasma glucose levels in the successful recipients remained <11.1 mmol/l for at least two months. Islet functionality was tested by an intraperitoneal glucose tolerance test. Animals with islets transplanted to muscle did not significantly differ in glucose handling from non-diabetic control mice, but animals with islets transplanted to liver had a significant delay in lowering blood glucose after glucose load (Fig. 4B) resulting in an increased area under the curve (Fig. 4C). Islets transplanted to the abdominal external oblique muscle of diabetic mice had similar vascular densities as islets transplanted to the cremaster muscle of non-diabetic mice (967±65 vs 1162±120 vessels/mm², Fig. 4D). Insulin staining of pancreata after termination of experiments confirmed maintained low densities of β-cells in all alloxan-treated mice, excluding the possibility of normalization of blood glucose levels due to β-cell regeneration. The intramuscular site was visualized three days after transplantation of fluorescently labeled islets through IVIS-imaging (Fig. 4E).

Auto-transplanted islets to striated muscle of pancreatectomized patients exhibit higher blood flow compared to adjacent muscle. Three pancreatectomized patients receiving auto-transplantation of isolated islets to the brachioradialis muscle were scanned using magnetic resonance imaging three to six months after surgery. The used MRI-technique yields a very high in-plane resolution of 200 μm in vivo (33), which is required to accurately visualize the islet grafts. Islets could indeed be detected in muscle tissue at the site of injection in all three patients (Fig. 5, A, C, E, F, and Supplemental Fig. 5). Therefore, fractional plasma volume, which corresponds to capillary density of the scanned area, was investigated through kinetic modeling. In two of the subjects, the capillary density was assessed through dynamic contrast enhancement of the equivalent parameter, the plasma volume (Vp), and was found to be 2.5 and 3.4 times higher in the implanted clusters of islets when compared to the surrounding muscle tissue. The center of the larger islet grafts (Fig. 5, C and D) had a non-perfused area, which most likely corresponds to central fibrosis due to a too large volume of islets implanted at the same site. Hepatic steatosis has been reported following islet transplantation to the liver (34; 35), but no focal lipid accumulation surrounding the muscle implanted grafts in the first two subjects could be detected (Fig. 5, A and C). Even when using high resolution morphological imaging on the third subject, no lipid deposits could be detected close to engrafted islets (Fig. 5, E and F).

**DISCUSSION**

Transplantation of isolated pancreatic islets to patients suffering from diabetes is limited by...
Engraftment in Muscle Restores Islet Vasculature

loss of function of the grafts, which appears to be related to the site of engraftment (36) including poor revascularization of the islets (17). In the present study we report that vasculature within islets engrafted in striated muscle are functionally and morphologically restored within two weeks following transplantation. The results were obtained using intravital and confocal microscopy as well as by immunohistochemical evaluation and curative transplantations of diabetic mice. Initiation of revascularization was dependent on Gr-1-positive leukocytes recruited to the implantation site, since no revascularization of transplanted islets could be observed after depletion of this leukocyte subset. Ultimately, an increased capillary density was detected in islet grafts compared to adjacent tissue in pancreatectomized patients with isolated islets auto-transplanted to muscle using an ultra-high resolution acquisition technique for the first time applied to the study of transplanted islets. This technique enables registration of plasma volumes proportional to capillary densities in small superficial, graft-containing tissue fractions corresponding to transplanted islets. In addition to verifying the experimental observations, this technique will be instrumental during our forthcoming development and evaluation of clinical islet transplantations to the promising muscle site. Pancreatic islets are highly organized miniature organs with the primary function of regulating blood glucose levels through efficient sensing of present blood glucose concentration and concomitant release of the appropriate hormone (i.e. insulin, glucagon) from each specialized cell type. Justifiably, high blood perfusion and minimal diffusion barriers between the blood, the sensor and the hormone releasing cells are prerequisites for efficient blood glucose control (8; 9). During isolation, all vascular connections to the islets are interrupted and, contrary to solid organ transplantations, are not reconnected during surgery. Transplanted islets therefore depend completely on revascularization to occur. When islets are grafted into the liver, blood vessels surround but only few penetrate the islets (2; 17) (Supplemental Fig. 1), which may explain the poor long-term function of the grafts. Experimental islet transplantation to other commonly used implantation sites such as the kidney and spleen also results in impaired revascularization (2). Indeed, the present study where islets were engrafted into striated muscle, reports for the first time equal islet vascular density, vessel morphology and blood perfusion to that of islets in the intact pancreas. These findings were not restricted to the cremaster muscle, but could also be seen in islets transplanted to abdominal external oblique, gluteal and psoas muscles (Fig. 4D, Supplemental Fig. 4). In addition, the experimental data were confirmed in auto-transplanted patients where higher plasma volumes were measured in islets engrafted in forearm muscle compared to adjacent muscle tissue, indirectly demonstrating higher capillary densities in the grafts compared to muscle. Hepatic steatosis of tissue surrounding the grafts has been reported following islet transplantation to the liver (34; 35), due to paracrine insulin effects on adjacent tissue. In the present study, no fat deposits could be detected after auto-transplantation of pancreatectomized patients to muscle. Striated muscle has successfully been used for auto-transplantation of parathyroid glands, where these endocrine glands engraft and become vascularized (18). Muscle has also been investigated under experimental settings as a site for pancreatic islet transplantation, but with varied success (37-39). The variations in outcome may relate to the techniques used to implant the islets. Indeed, the negative influence of large clusters of islets were evident in the pancreatectomized patients receiving auto-transplanted islets to striated muscle as fibrosis could be detected in the center of large number of islets grouped.
together. In the experimental part of this study, a small volume of 300 non-packed islets was injected in a pearls-on-a-string fashion (39) superficially into abdominal muscle. This resulted in a complete cure of diabetic mice within approximately eight days following transplantation.

The increased revascularization of islets engrafted in muscle observed in the present study might be dependent on the specific plasticity of the endothelial cell population in muscle. Endothelial cells are heterogeneous with specialized properties depending on the organ they reside in and their order in the vascular tree. For instance, angiogenesis is induced during a variety of pathologies, but during physiological conditions in adults it is only provoked in ovaries (during ovarian cycling), in the placenta (during placental development) and in muscles (during exercise) (40). Mechanical stretch and increased vessel wall tension during exercise is reported to result in increased capillary supply due to increased local levels of VEGF, HIF-1β and MMP-2 (41). Thus, a clear difference between striated muscle and other implantation sites investigated for islet vascular densities is that muscle endothelium is programmed to proliferate during muscle overload and concomitant hypoxia (40).

Leukocytes have recently been described to contribute to angiogenesis occurring during remodeling and healing of muscle tissue (20; 21; 42). Hypoxic mediators are known to recruit leukocytes (43; 44) and might account for the leukocyte-endothelial cell interactions observed early after islet implantation in this study, but not after sham injections. The number of recruited leukocytes decreased with time after transplantation, which parallel islet revascularization and associated reduced hypoxia (45). No revascularization of islets three to five days after transplantation could be detected when Gr-1-positive leukocytes had been depleted in recipients, indicating a crucial role of these cells in angiogenic switch. Gr-1 is reported to be expressed on neutrophils as well as inflammatory monocytes but not on the monocyte population involved in tissue repair (20; 42; 46). The Gr-1-positive leukocytes recruited to the site of engraftment in the current study that were responsible for initiation of revascularization are therefore most likely neutrophils, while the Gr-1-negative monocyte population that has been shown to be involved in tissue remodeling (21) and tumor angiogenesis (42) does not seem to contribute at these early time-points. The mechanism by which neutrophils induce angiogenesis might be through release of VEGF, either from intracellular storages, or through secretion of matrix metalloproteinase-9 (MMP-9) that has been shown to contribute to angiogenesis through digesting extracellular matrix and release of immobilized VEGF (42).

This study presents a novel paradigm for islet transplantation whereby neutrophils recruited to the site for engraftment were crucial for the functionally restored intra-islet blood perfusion following transplantation to striated muscle. In the clinical setting, this site proved to promote islet revascularization and also permitted longitudinal graft-monitoring by high-resolution magnetic resonance imaging.

**Author contributions.** Conceived and designed the experiments: mp, gc, poc, ok, lj, cr. Performed the experiments: gc, lj, jh, mp, jcc, rs, jp, ha. Analyzed data: gc, mp, lj. Wrote the paper: mp, gc, poc, ok.

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Engraftment in Muscle Restores Islet Vasculature


**FIGURE LEGENDS**

**Figure 1.** Islets transplanted to mouse muscle revascularize rapidly and completely. (A) Three to five days after transplantation, islets in the cremaster muscle have functional intra-islet blood vessels: Confocal image of a mouse islet (blue, Celltracker) in the cremaster muscle five days post-transplantation (post-tx), with ingrowing blood vessels (red, anti-CD31-mAb
Engraftment in muscle restores islet vasculature. (B) Two weeks after transplantation a dense, glomerular-like vascular system has developed: Multiphoton microscopy image of the vasculature of a transplanted mouse islet in muscle (anti-CD31-mAb). (C) The vascular density and architecture are at two and four weeks post-transplantation comparable to native islets in the pancreas: Spinning disk confocal image of islet vasculature in mouse pancreas (anti-CD31-mAb). (D) Vessel density in islets transplanted into mouse muscle at three to five days (n=4), two weeks (n=4) and four weeks (n=6) after transplantation, compared to the density observed in islets in intact pancreas (n=4). Islets transplanted into the liver did not have any intra-islet vessels at these time-points (n=7). (E) Intra-islet capillary diameter of transplanted mouse islets is increased shortly after transplantation (three to five days, n=5, 3 capillaries/islet), but reaches values comparable to native islet capillaries (n=3, 3 capillaries/islet) at two weeks post-transplantation (n=4, 3 capillaries/islet). (F) The vascular densities of human islets transplanted to mouse muscle (n=4) were at the same high level as mouse islets cultured for the same number of days (n=3). (G) Human islets transplanted to muscle of nu/nu mice had a well-developed intra-islet vascular system four weeks after transplantation: Multiphoton microscopy image of human islet vasculature (anti-CD31-mAb). All values are given as mean±SEM. Bars in A, B, C and G are 50 µm.

Figure 2. Newly formed vessels in islets transplanted to cremaster muscle are functional. (A) Confocal image of the vasculature of one small and one larger SBA lectin perfused mouse islet in muscle. (B) Confocal image of the vasculature of an SBA lectin perfused mouse islet in pancreas. (C) Functionality of newly formed islet vasculature was evaluated by injections of fluorescent endothelium-binding SBA lectin and quantified as percentage of lectin positive vessels over CD31-positive vessels. SBA lectin (green) was found to overlap with anti-CD31-mAb (red) staining on cryosections in (D) islets (dashed lines) transplanted to muscle (n=5, 11 islets) (blue, nuclei, Hoechst 33342) and (E) in native islets (n=4, 7 islets). (F) Islets transplanted to the liver (n=4, 8 islets) had no functional intra-islet vessels. All values are given as mean±SEM. Bars in A, B, D, E and F are 50 µm.

Figure 3. Islet revascularization in muscle is dependent on neutrophils. (A) Leukocyte adhesion in venules from mouse cremaster muscle. Three to five days post-transplantation, more adherent leukocytes were found in venules draining islets (n=5) than in venules draining only muscle tissue (n=5), *P=0.049. (B) Leukocyte emigration in the proximity of the transplanted islet was increased at three to five days post-transplantation (n=5) compared to four weeks post-transplantation (n=5), *P=0.042. (C) Confocal image of a transplanted mouse islet (blue, Celltracker) four days after transplantation to a mouse treated with IgG isotype control antibody, with ingrowing blood vessels (red, anti-CD31-mAb given intra-arterially in vivo) surrounded by leukocytes (green, anti-Gr-1-mAb and red, anti-CD31-mAb given intra-arterially in vivo) in the peritransplant area. (D) Confocal image of two islets (blue, Celltracker) at four days after transplantation transplanted to a neutropenic mouse with no ingrowing blood vessels (red, anti-CD31-mAb given intra-arterially in vivo). All values are given as mean±SEM. Bars in C and D are 50 µm.

Figure 4. Islets transplanted to muscle can reverse alloxan-induced diabetes. (A) Plasma glucose levels of mice made diabetic with alloxan, then transplanted with pancreatic islets into the abdominal wall (circles/continuous line, n=6) or into the liver (triangles/dashed line, n=5) at day 0. Plasma glucose levels fell to a stable, normoglycemic level (<11.1 mmol/l, dashed grey line) within 8±2 days. (B) Intraperitoneal glucose tolerance test of transplanted mice reaching normoglycemia with islets in muscle (empty circles, n=6), with islets in the liver (empty
triangles/dashed line, n=5) and non-diabetic control mice (filled squares, n=4) (*P<0.05 control vs tx to liver, #P<0.05 vs tx to muscle). (C) Calculated area under the curve from the intraperitoneal glucose tolerance test (*P=0.047 vs control, #P=0.048 vs tx to muscle). (D) Tissue sections showing islets engrafted in abdominal muscle with beta cells in red (insulin antibody) and blood vessels in brown (BS-1 lectin). Bars are 50 µm. (E) Image showing emitted fluorescent light from 300 islets, stained with an intracellular fluorescent probe, transplanted to the abdominal wall.

**Figure 5. Higher vessel densities in transplanted islets compared to adjacent muscle in pancreatectomized patients.**
Axial images of the forearm in three different subjects after islet transplantation. (A) is subject 1 post-contrast and (B) the corresponding plasma volume (Vp) map calculated from the first pass response. The bright spot in (B) is a superficial vein showing very high blood volume. (C) is subject 2 post-contrast and (D) the corresponding Vp map. The increased Vp in the islet grafts compared to the surrounding muscular tissue in subject 1 and 2 are visualized through brighter color markings corresponding to the position of the islets. (E) and (F) shows two slices from the high resolution acquisition of subject 3, note the multiple islet grafts. For reference, the graft indicated by the red arrow has a diameter of 2.4 mm.
<table>
<thead>
<tr>
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<th>Subject</th>
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<td>F-PG (mM)</td>
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<td>Stimulated CP (ng/ml)</td>
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<td>Stimulation index (16.7/1.67 mM)</td>
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<td>Content (ng/ng DNA)</td>
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<td><strong>Post-transplantation</strong></td>
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<td>F-PG (mM)</td>
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<td>Basal CP (ng/ml)</td>
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<td>Stimulated CP (ng/ml)</td>
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HbA1c, hemoglobin A 1C; F-PG, fasting plasma glucose; CP, C-peptide; IE, islet equivalent; bw, body weight.
Engraftment in Muscle Restores Islet Vasculature

Figure 1

A, B, C: Images showing vasculature in muscle. Scale bars indicate 100 μm.

D: Bar graph showing vessel density (vessels/mm²) for different groups. Error bars indicate standard error.

E: Bar graph showing intra-islet capillary diameter (μm) for different groups. Error bars indicate standard error.

F: Bar graph comparing vessel density (vessels/μm²) between human islet in mouse islet and mouse islet in mouse islet. Error bars indicate standard error.

G: Image of a blood vessel in a mouse muscle. Scale bar indicates 100 μm.
Engraftment in Muscle Restores Islet Vasculature

Figure 2

A. Islets transplanted to mouse muscle

B. Islet in intact pancreas

C. Lectin-positive vessels (%)

D, E, F. Immunofluorescent staining

Muscle
Pancreas
Liver
Figure 3

A. Adherent leukocytes in graft-draining venules 3-5 days post-tx

B. Emigrated leukocytes in adjacent muscle

C. IgG isotype control

D. Gr-1-mAb neutropenic
Engraftment in Muscle Restores Islet Vasculature

Figure 4

A

Blood glucose concentration (mmol/l)

30
25
20
15
10
5
0

Time after transplantation (days)

0 5 10 15 20 25 30 35 40 45 50

- x to muscle
- x to liver

B

Blood glucose concentration (mmol/l)

25
20
15
10
5
0

Time (min)

0 20 40 60 80 100 120

- x to liver
- x to muscle
- control

C

AUC (mmol/l x 120 min)

2100
1900
1700
1500
1300
1100
900
700
500
300
100
0

- control
- x to muscle
- x to liver

D

Beta cells

Endothelial cells

E

Mice

Image

Min=0
Max=6407
600
400
200
100
0

Color Bar
Min=5100
Max=5641
Figure 5

Subject 1

A

Subject 2

C

Subject 3

E

B

D

F

Engraftment in Muscle Restores Islet Vasculature