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ARTICLE

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Better vascular engraftment and function in pancreatic islets transplanted without prior culture

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Abstract *Aims/hypothesis:* Recent studies suggest that donor endothelial cells may contribute to islet graft revascularisation. Since islet endothelial cells disappear during culture, we hypothesised that transplantation of islets without prior culture is beneficial for their engraftment. *Methods:* Cultured (4–7 days) or freshly isolated islets (<4 h after donor pancreas extirpation) were syngeneically transplanted into Wistar–Furth rats and C57Bl/6 mice beneath the renal capsule. Islet graft revascularisation was evaluated by measuring vascular density, blood flow and tissue oxygen tension. Islet graft function was investigated by a minimal islet mass model in inbred mice (C57Bl/6). *Results:* Four days after implantation, the partial pressure of oxygen (pO_2) in the transplanted cultured islets was less than 10 mmHg (1.33 kPa), but tended to be higher in grafts composed of freshly isolated islets. The pO_2 in the grafts of freshly isolated islets had more than doubled 4 weeks later, whereas the pO_2 in the grafts of cultured islets remained at values similar to those recorded 4 days after transplantation. Transplanted freshly isolated islets also had a higher vascular density than transplanted cultured islets (~40 vs ~25% of that in endogenous islets) when investigated 1 month post-implantation. When applying a minimal islet mass model in inbred mice, 200 freshly isolated islets cured alloxan-diabetic mice in all cases, whereas only 33% of the group receiving similar numbers of cultured islets were cured. *Conclusions/interpretation:* Transplantation of pancreatic islets without prior culture is beneficial for their vascular engraftment and function.

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Keywords Engraftment · Islet culture · Islet transplantation · Minimal islet mass · Revascularisation · Tissue oxygen tension · Vascular density

Abbreviations bFGF: basic fibroblast growth factor · pO_2: partial pressure of oxygen · TBS: Tris buffered saline · TPU: tissue perfusion units · VEGF: vascular endothelial growth factor

Introduction

For many years, the clinical outcome of allogeneic islet transplantations was very poor, with less than 10% of the recipients being insulin-independent after 1 year [1]. However, the recently introduced Edmonton Protocol, which applies a steroid- and cyclosporine-free immunosuppressive regime, has improved insulin independence 1 year post-transplantation to approximately 80% [1–3]. The Edmonton Protocol used freshly isolated islets for transplantation [2]. In most former, less successful islet transplant protocols, cultured islets have been used for transplantation, since in vitro culture is often practical from a logistic point of view [4] and may reduce islet immunogenicity by depletion of viable haematogenous and lymphoid cells [5]. Concerns have also been raised regarding the common pronounced exocrine contamination of freshly isolated islet transplants [6].

Endogenous islets normally have a complex architecture of capillaries, which ensures that no portion of the islet is more than one cell away from arterial blood [7]. This unique capillary network is crucial for the supply of oxygen and nutrients to the islet cells and for the dispersal of islet hormones to the systemic circulation [8]. The islet capillary system is, however, disrupted by isolation, and during in vitro culture islet endothelial cells de-differentiate or die [9–11]. The revascularisation process of islets is generally thought to occur over a period of 7–14 days post-transplantation [12–14]. The newly formed blood vessels were earlier considered to originate from recipient blood ves-

sels [15]. However, recent studies suggest that endothelial cells originating from the donor may also contribute significantly, and be important for the revascularisation process [16, 17]. In our previous studies, we have observed that grafts composed of cultured rodent or human islets are not sufficiently revascularised, which results in a low graft oxygen tension and tissue acidosis [18–22]. The present study tested the hypothesis that syngeneic islet grafts composed of freshly isolated rodent islets become more efficiently revascularised than islets transplanted after culture, and that this might result in an improved islet graft function.

Materials and methods

Animals Male inbred Wistar–Furth rats weighing 300–350 g, and male inbred C57Bl/6 mice weighing 25–30 g were purchased from B&K, Sollentuna, Sweden. The rats and mice had free access to water and pelleted food, and were housed in a room with a 12-h light/dark cycle and 70% humidity throughout the course of the study. All experiments were approved by the local animal ethics committee of Uppsala University, Uppsala, Sweden.

Islet isolation and transplantation Pancreatic islets were isolated by collagenase (Boehringer-Mannheim; Mannheim, Germany) digestion, as previously described [23]. Some of the islets were cultured free-floating in groups of ~150 islets in RPMI 1640 medium (Sigma-Aldrich, Irvine, UK) supplemented with 10% (vol/vol) fetal calf serum (Sigma-Aldrich) prior to transplantation. The medium was changed every second day. At the time of transplantation, 250 (rat) or 200 (mouse) freshly isolated (<4 h after pancreas extirpation) or cultured (4–7 days of culture) islets were packed in a braking pipette and implanted beneath the renal capsule on the dorsal side of the left kidney of syngeneic normoglycaemic rats that had been anaesthetised with pentobarbital (60 mg/kg, i.p., Apoteket, Umeå, Sweden) or of syngeneic alloxan-diabetic mice that had been anaesthetised with Avertin (0.02 ml/g i.p, of a 2.5% [vol/vol] solution of 10 g 97% [vol/vol] 2,2,2,-tribromoethanol [Sigma-Aldrich] in 10 ml of 2-methyl-2-butanol [Kemila, Stockholm, Sweden]). For practical reasons, normoglycaemic rats were, used as recipients in the engraftment studies, since there is no difference in revascularisation and oxygenation of transplanted islets, compared to when the islets are implanted to cure diabetic recipients instead [19, 24]. In all cases exocrine contamination of the transplanted islets was avoided as much as possible, since this has previously been observed to have a negative influence on the engraftment process of transplanted islets [25].

Analysis of blood glucose concentration Glucose reagent strips were used to measure blood glucose concentrations in venous blood obtained from the cut tip of the tail of the animals (MediSense; Baxter Travenol, Deerfield, IL, USA). Blood glucose values above the detection range were set to 27.4 mmol/l, which according to the technical data supplied by the manufacturer is the highest reliable blood glucose concentration evaluable by this device.

Oxygen tension in endogenous islets Non-transplanted rats were anaesthetised by thiobutabarbital (Inactin, 120 mg/kg i.p.; Research Biochemicals International, Natick, MA, USA), placed on an operating table maintained at body temperature (37°C), and tracheotomised. Polyethylene catheters were inserted into the left femoral artery and the left femoral vein. The arterial catheter was connected to a Statham P23dB pressure transducer to continuously monitor the mean arterial blood pressure throughout the experiment. The femoral vein catheter was used to infuse saline (5 ml kg^{-1} h^{-1}) to substitute for body fluid loss. The abdomen was opened by a mid-line incision, and the pancreas was immobilised over a hollow cylindrical plastic block attached to the operating table. The pancreas was then continuously superfused with mineral oil (Apoteket) at body temperature to prevent desiccation of the tissue. After allowing the mean arterial pressure to stabilise, 0.8-ml sterile-filtered 2% (wt/vol) neutral red (Kebo Grave, Stockholm, Sweden) was injected intravenously to selectively stain the islets within the pancreas. This dye has previously been evaluated and has been shown not to affect pancreatic oxygenation, whole pancreatic and islet blood flow, or glucose homeostasis [26]. The rats were then allowed to rest for 30 min to minimise the influence of surgical stress and neutral red administration on the measurement of partial pressure of oxygen (pO_2).

The pO_2 was measured by modified Clark microelectrodes (outer tip diameter 2–6 μm; Unisense, Aarhus, Denmark) [18, 27]. The microelectrodes were calibrated in water saturated with $Na_2S_2O_5$ or air at 37°C before and after the experiment. The drift of the microelectrode recordings was <0.5%/h. The microelectrode tip was inserted into the islets and the exocrine pancreas with a micromanipulator under a stereo microscope. Measurements of pO_2 were performed in three to six islets in each animal. Multiple measurements (≥3) were frequently performed in each islet, and the mean of these measurements was then calculated to obtain the pO_2 in one islet. The mean of the pO_2 values in one animal was treated as one experiment in the subsequent statistical analysis.

Oxygen tension and blood flow in islet grafts Four days or 1 month post-transplantation, the transplanted rats were anaesthetised by thiobutabarbital (Inactin, 120 mg/kg i.p.; Research Biochemicals International) and surgically prepared similarly to the non-transplanted rats (see above). However, in this case the abdomen was opened by a left subcostal flank incision, and the left kidney, bearing the islet graft, was immobilised in a plastic cup attached to the operating table. The kidney and the islet graft were embedded in cotton and mineral oil (Apoteket) to prevent heat loss and desiccation. The animals were then allowed to rest for 30 min to minimise the influence of surgical stress on the subsequent measurements.

Repeated measurements (n≥10) of pO_2 were conducted in the transplanted islets and adjacent renal parenchyma,

and the mean was treated as one experiment. In conjunction with the pO_2 measurements, the blood flow in the islet graft and the adjacent renal cortex was recorded by laser-Doppler flowmetry (PF 4001-2, Perimed, Stockholm, Sweden). The blood flow in the islet graft and the adjacent renal cortex was determined by repeated measurements ($n \geq 3$) in each animal, and again the mean was treated as one experiment.

Endothelial staining Following blood flow and pO_2 measurements, the 1-month-old islet grafts were retrieved for histological examination. The pancreas was also retrieved from the age-matched non-transplanted rats. The pancreas and the islet grafts were fixed in 10% (vol/vol) formalin and embedded in paraffin. Sections (5 μm thick) were stained for endothelial cells by the lectin *Bandeiraea simplicifolia* [28]. In brief, the sections were incubated with normal goat serum (NGS; Dakopatts, Glostrup, Denmark), diluted 1:20 with Tris buffered saline (TBS), and kept for 1 h in a moist chamber at room temperature (20°C). Biotinylated forms of lectin from *Bandeiraea simplicifolia* (Sigma-Aldrich), diluted 1:100 in TBS, were applied to the sections overnight at 4°C. The sections were then washed and incubated with Vectastain ABC-AP kit (Vector Laboratories, Burlingame, CA, USA) for 30 min in a moist chamber at room temperature. They were then washed again and the chromogen Vector Red (Vector Laboratories) was applied to the sections and left for 30 min to develop in a moist chamber at room temperature. Thereafter, the slides were washed in TBS, counterstained with haematoxylin, dehydrated and mounted with Mountex (Histolab Products, Gothenburg, Sweden).

Evaluation of vascular density In each rat, more than 15 endogenous islets or more than 2 mm^2 of islet grafts were randomly chosen from the histological sections and evaluated. The blood vessels in endogenous and transplanted islets were counted in a light microscope at a magnification of 600× and by an examiner unaware of the origin of the sections. Stroma surrounded the individual islets in the grafts. The microvessels in the transplanted islets and stroma were counted separately. The respective fractions of islets and connective tissue in the islet grafts were determined by a direct point counting technique [29]. For this purpose, the intersections overlapping the stroma and endocrine cells within the islet grafts were counted (magnification 600×). At least ten fields (corresponding to ~1,200 intersections) were counted in each islet graft. The areas of the investigated endogenous islets and grafted islets were determined using a computerised system for morphometry (MOP-Videoplan; Carl Zeiss, Stockholm, Sweden). Vascular density, i.e. the number of blood vessels per measured islet or graft area (mm^2), was then calculated.

Evaluation of graft volume Renal subcapsular islet grafts, composed of 250 freshly isolated or 250 cultured rat islets, were retrieved 1 month post-transplantation and prepared for histological evaluation. The islet grafts were formalin-fixed, paraffin-embedded, consecutively sectioned (5 μm) and stained with haematoxylin and eosin. The total graft volumes and the fractions constituting endocrine cells were estimated using a computerised system for morphometry (MOP-Videoplan; Carl Zeiss, Stockholm, Sweden), as previously described [30].

Insulin, vascular endothelial growth factor and basic fibroblast growth factor content in freshly isolated and cultured rat islets Groups of 125 freshly isolated or cultured rat islets (5 days of culture) were placed in 500-μl Hanks and sonicated. Homogenates were then stored at −70°C until analysis. The insulin content of the homogenates was measured with a rat insulin ELISA (Mercodia, Uppsala, Sweden), whereas the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) contents were analysed with a mouse VEGF ELISA and human bFGF ELISA respectively (R&D Systems, Minneapolis, MN, USA). Amino acid homology greater than 90% between species is recommended by the manufacturer for their ELISAs to ascertain specific cross-reactivity. The amino acid homology for rat and mouse VEGF is 98%, and for rat and human bFGF 95.5%.

Insulin, vascular endothelial growth factor and basic fibroblast growth factor content in rat islet grafts Four-day-old or 1-month-old islet grafts were dissected free from the surrounding renal parenchyma, placed in 1 ml acid ethanol (0.18 mol/l HCl in 70% vol/vol ethanol) and sonicated to disrupt the islet cells. The samples were left to extract overnight at 4°C, and then stored in a freezer until analysed by ELISA (see above).

Minimal islet mass Mice were given an intravenous injection of alloxan (75 mg/kg; Sigma-Aldrich) 5 days prior to transplantation and were considered diabetic if they had non-fasting blood glucose concentrations above 16.7 mmol/l at this time. The number of transplanted islets (200) was chosen on the basis of our previous studies in this strain, e.g.

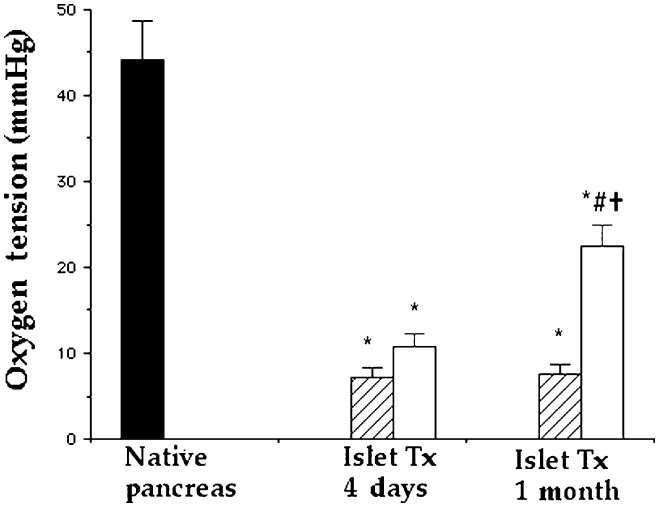

Fig. 1 Oxygen tension in endogenous rat islets (*closed bar*) and in 4-day-old and 1-month-old syngeneic islet grafts composed of cultured (*hatched bar*) or freshly isolated (*open bar*) rat islets. All values are expressed as means±SEM for seven to eight experiments in each group. *$p<0.05$ when compared to native islets; $^{\#}p<0.05$ when compared to corresponding 4-day-old grafts; $^{\dagger}p<0.05$ when compared to corresponding 1-month-old grafts of cultured islets

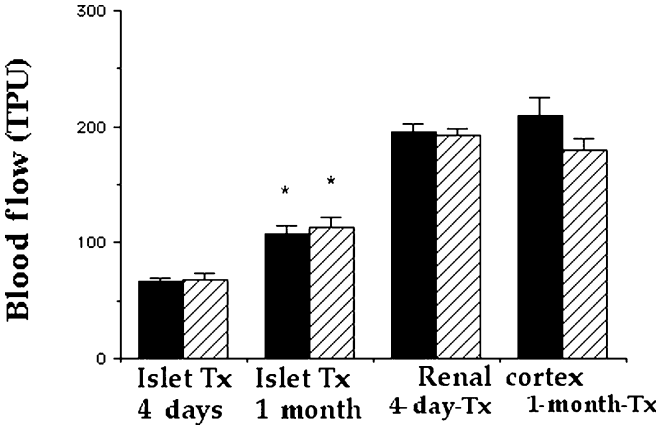

Fig. 2 Blood flow in 4-day-old and 1-month-old syngeneic grafts composed of cultured (*closed bar*) or freshly isolated rat islets (*hatched bar*), and in the adjacent renal cortex. All values are expressed as means±SEM for seven to eight experiments in each group. *p<0.05 when compared to corresponding 4-day-old islet grafts

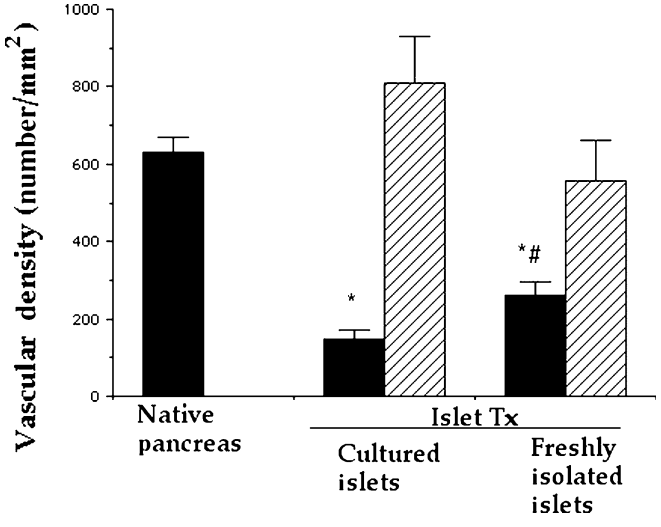

Fig. 3 Vascular density in endogenous rat islets and endocrine parts of 1-month-old syngeneic islet grafts composed of cultured or freshly isolated rat islets (*closed bars*), and in the stroma of the same islet grafts (*hatched bars*). All values are expressed as means±SEM for seven to eight experiments in each group. *p<0.05 when compared to native islets; #p<0.05 when compared to grafts composed of cultured islets

[24], and intended to reach an islet mass insufficient for full reversal of hyperglycaemia in most of the diabetic recipients receiving cultured islets. The body weights and blood glucose concentrations of the transplanted animals were measured every fifth day up to 1 month post-transplantation. Animals cured from diabetes were defined as those with non-fasting blood glucose concentrations lower than 11.1 mmol/l. The graft-bearing kidneys were removed on all the cured animals 1 month post-transplantation to ascertain that they would subsequently return to hyperglycaemia (>16.7 mmol/l) and that the improved blood glucose concentrations were not merely the result of regained function in the endogenous pancreas.

Statistical analysis Values are expressed as the mean±SEM. Multiple comparisons between data were performed using ANOVA and Fisher's protected least significant difference test (SigmaStat 2.0, SPSS, Chicago, IL, USA). When only two groups were compared, probabilities (p) of chance differences between the experimental groups were calculated using Student's unpaired two-tailed t-test. For all comparisons, p values of less than 0.05 were considered statistically significant.

Results

Animal characteristics All rats allocated to the islet graft blood flow and pO_2 measurements weighed ~300 g and were normoglycaemic with blood glucose concentrations of ~6.0 mmol/l. The mean arterial blood pressure was similar in all groups of rats and ranged between 100 and 130 mmHg.

Oxygen tension in endogenous and transplanted rat islets The pO_2 in endogenous islets was ~45 mmHg (6.0 kPa) (Fig. 1), whereas the pO_2 in the surrounding exocrine parenchyma was 28.8±2.0 mmHg (3.83±0.27 kPa; n=7). All transplanted islets, irrespective of whether investigated 4 days or 1 month post-transplantation, had a markedly lower pO_2 (Fig. 1). However, already 4 days after transplantation, islet grafts composed of freshly isolated islets tended (p=0.09) to have higher pO_2 than corresponding grafts composed of cultured islets. One month post-transplantation, the pO_2 in the islet grafts composed of freshly isolated islets had more than doubled, whereas the pO_2 in grafts composed of

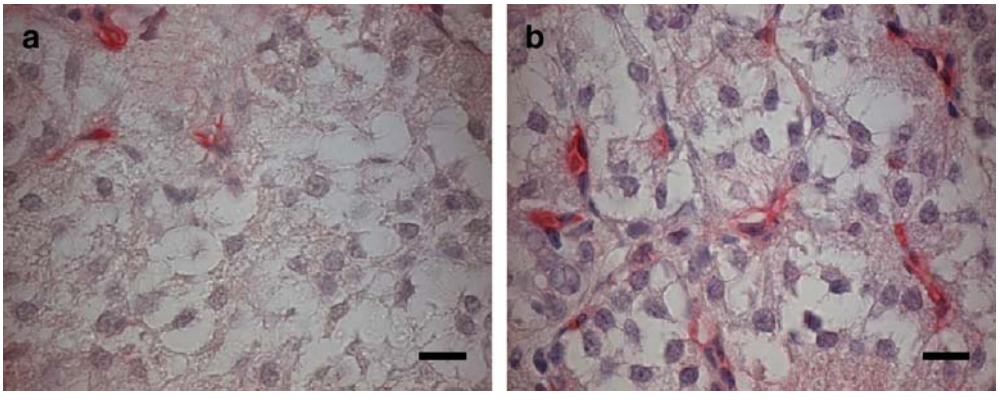

Fig. 4 Micrographs of 1-month-old syngeneic rat islet grafts composed of cultured (**a**) or freshly isolated islets (**b**). All tissues were stained with the lectin *Bandeiraea simplicifolia* (*red*) to visualise blood vessels within the pancreatic islets. Scale bar 10 μm

cultured islets remained at values similar to those recorded 4 days after transplantation (Fig. 1). The pO_2 in the renal cortex adjacent to the islet grafts was similar in all animals [19.1±0.9 mmHg (2.54±0.12 kPa), n=30].

Blood flow in rat islet grafts Islet graft blood flow (Fig. 2) increased from ~35 to ~60% of that in the renal cortex between 4 days and 1 month post-transplantation. The blood perfusion of grafts composed of freshly isolated and cultured islets did not differ at either point of time. Renal cortical blood flow was similar in all groups.

Vascular density and volume of rat islet grafts Figures 3 and 4 show that islet grafts composed of cultured islets had a vascular density only 25% of that in endogenous islets when investigated 1 month post-transplantation, whereas transplanted freshly isolated islets had a vascular density at the same time point after implantation of about 40% of that in endogenous islets. The vascular density in the stroma surrounding the individual islets in the grafts was markedly higher than in the endocrine parts per se, and it was similar in grafts composed of cultured and non-cultured islets. The percentage of stroma did not differ between islet grafts composed of cultured or non-cultured islets (25±3 vs 22±3%; n=7 in both groups). Neither did the endocrine volume of the 1-month-old grafts composed of cultured and freshly isolated islets differ (375±28 nl, n=8 and 368±16 nl, n=7, respectively).

Insulin, vascular endothelial growth factor and basic fibroblast growth factor content in isolated rat islets and rat islet grafts The insulin content in isolated islets decreased during 5 days of culture (Fig. 5a). Four days after transplantation, the insulin content in the islets was even lower than during in vitro culture, and was similar in grafts composed of non-cultured and cultured islets. The insulin content of grafts composed of freshly isolated islets, but not of those composed of cultured islets, decreased further between 4 days and 1 month post-transplantation. However, the insulin content did not differ statistically between the 1-month-old grafts composed of freshly isolated and cultured islets.

The VEGF content of isolated islets increased sixfold during 5 days of culture (Fig. 5b). Four days after transplantation, the VEGF content was ~0.15 pg/islet in grafts composed of freshly isolated islets and in grafts composed of cultured islets. One month post-transplantation, VEGF in the transplanted islets was no longer detectable (values <0.02 pg/islet for all grafts).

Islet bFGF content was not effected by culture, but decreased soon after transplantation (day 4) in islets transplanted as freshly isolated specimens and in islets transplanted after a culture period (Fig. 5c). In 1-month-old grafts, the bFGF content was again higher, and there was a tendency (p=0.07) for islet grafts composed of freshly isolated islets to have a higher bFGF content than grafts of cultured islets.

Minimal islet mass in mice Treatment with alloxan rapidly increased blood glucose concentrations in treated mice to

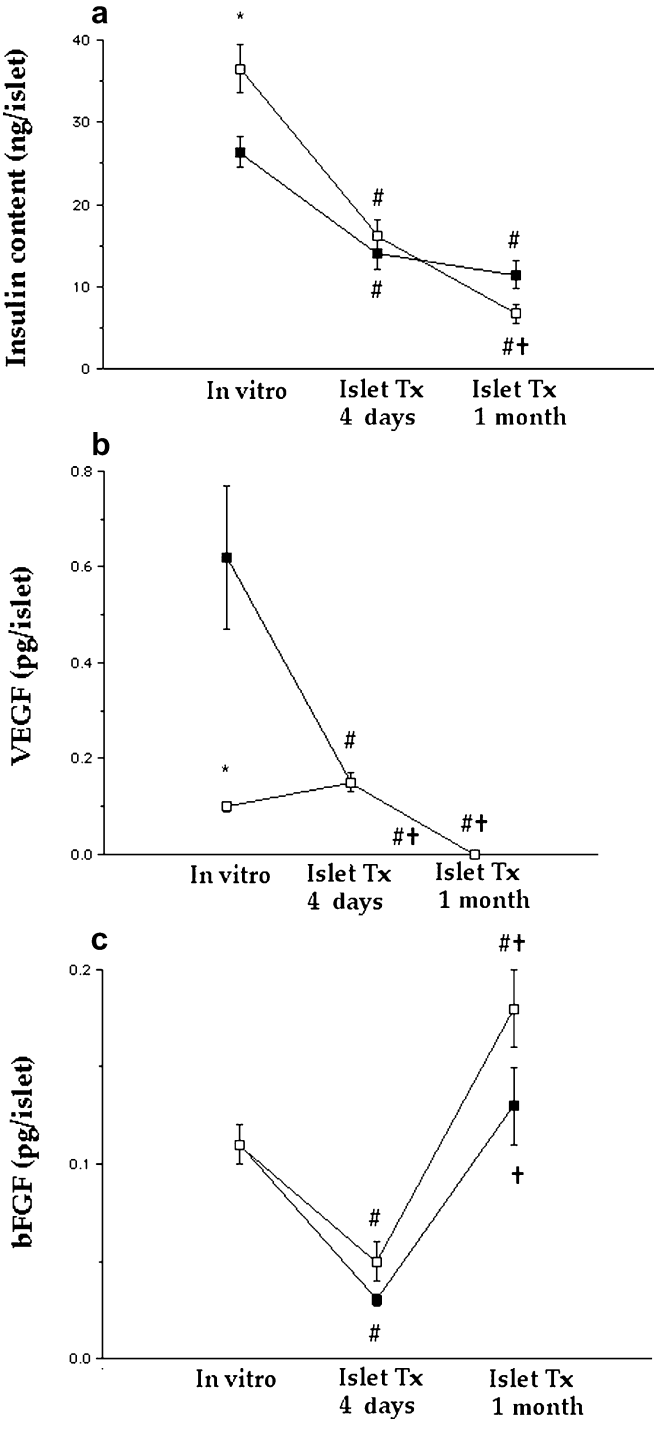

Fig. 5 Insulin (**a**), vascular endothelial growth factor (*VEGF*) (**b**) and basic fibroblast growth factor (*bFGF*) (**c**) content of cultured (*closed boxes*) and freshly isolated (*open boxes*) rat islets in vitro, 4 days or 1 month post-transplantation. All values are expressed as means±SEM for seven to eight experiments in each group. *p<0.05 when compared to corresponding cultured islets; $^{\#}p$<0.05 when compared to corresponding islets in vitro; $^{\dagger}p$<0.05 when compared to corresponding islets 4 days post-transplantation

Table 1 Body weights and blood glucose concentrations of alloxan-diabetic mice transplanted with a renal subcapsular graft composed of 200 cultured or freshly isolated islets

	Animals receiving cultured islets		Animals receiving freshly isolated islets	
	Pre-transplantation	1 month post-transplantation	Pre-transplantation	1 month post-transplantation
Body weight (g)	22.0±0.7	28.1±0.8[a]	25.7±0.6[b]	30.3±0.7[a]
Blood glucose (mmol/l)	26.8±1.0	15.1±0.6[a]	25.3±0.9	9.2.±0.5[ab]

All values are given as means±SEM for six to eight experiments in each group
[a] $p<0.05$ when compared to pre-transplantation in the same experimental group
[b] $p<0.05$ when compared to animals receiving cultured islets

above 20 mmol/l (Table 1). At the time of transplantation, all mice had similar blood glucose concentrations, but those randomly assigned to receive cultured islets weighed slightly less than those receiving freshly isolated islets (Table 1). Transplantation of 200 freshly isolated mice islets fully reversed the hyperglycaemia in all (n=8) alloxan-diabetic recipients within 1 month of transplantation (Fig. 6). In contrast, only one-third (n=6) of the alloxan-diabetic mice that received 200 cultured islets was cured. One month post-transplantation, animals receiving cultured islets had higher mean blood glucose concentrations than those receiving freshly isolated islets (Table 1). Due to overt hyperglycaemia, two animals receiving cultured islets died during the course of the study and had to be excluded, whereas all mice receiving freshly isolated islets survived the study period. Removal of the kidney bearing the graft 1 month post-transplantation, reversed all cured mice to diabetes (>16.7 mmol/l).

Discussion

In this study, using a syngeneic rat model, we observed a higher vascular density and oxygen tension in 1-month-old islet grafts composed of freshly isolated islets than in grafts composed of cultured islets. This suggests that revascularisation is better in transplanted freshly isolated islets, resulting in a more adequate supply of oxygen to the transplanted islet cells. When applying a minimal islet mass model in inbred mice, we also observed that grafts composed of freshly isolated islets had a higher capacity to cure alloxan-induced diabetes than corresponding numbers of cultured islets. Despite better vascular engraftment, the volume and insulin content did not differ between rat islet grafts composed of freshly isolated or cultured islets. However, the higher vascular density in the 1-month-old grafts of freshly isolated islets is likely to improve the delivery of insulin to the blood stream.

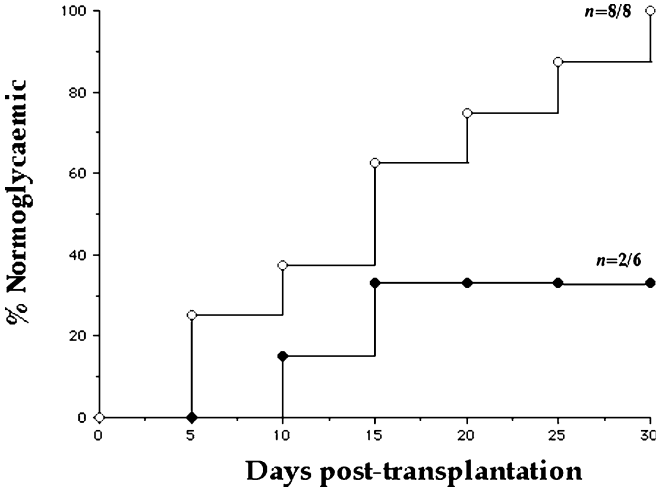

Fig. 6 Cumulative incidence of reversal to normoglycaemia in alloxan-diabetic mice receiving 200 cultured (*closed symbols*) or freshly isolated islets (*open symbols*). Blood glucose concentrations were monitored every fifth day during a month-long period after transplantation. The animals were considered to be cured when blood glucose concentrations were <11.1 mmol/l

There was no difference in the blood perfusion of rat islet grafts composed of freshly isolated and cultured islets either at 4 days or 1 month post-transplantation. It should, however, be noted that the blood flow measured by laser-Doppler flowmetry represents not only the nutritive blood flow to the endocrine cells, but total blood perfusion, i.e. all moving blood cells within the illuminated tissue. A large number of the intragraft capillaries was stroma capillaries, see [20], which are likely to contribute substantially to total graft blood perfusion. In contrast, it could be expected that the blood flow in capillaries in the endocrine parts mainly contributes to the delivery of oxygen to the endocrine cells, due to limitations of oxygen diffusion distance [31]. Indeed, 1-month-old grafts composed of freshly isolated islets had a higher islet vascular density than corresponding grafts of cultured rat islets, whereas the vascular density in the stroma was similar in grafts composed of freshly isolated and cultured rat islets. Moreover, the oxygen tension measurements suggested that the higher number of blood vessels in the endocrine parts of 1-month-old grafts of freshly isolated rat islets were not merely explained by remnant donor endothelial cells, but also by functional blood-perfused capillaries. In 4-day-old rat islet grafts, only blood flow and oxygen tension were recorded, since we deemed it likely that remnant donor endothelial cells, especially in transplanted freshly isolated islets, would confound measurements of vascular density. In contrast to our results, a previous histological study in rats [13] suggested that transplanted cultured islets may become revascularised more slowly than freshly isolated islets, but that all islets become fully revascularised within 1 week. However, the authors [13] did not mention whether endocrine and connective tissue parts were evaluated separately.

In an attempt to explain why freshly isolated islets become more efficiently revascularised than cultured islets following transplantation, we measured concentrations of angiogenic factors in freshly isolated and cultured rat islets, as well as in 4-day-old and 1-month-old rat islet grafts. The process of angiogenesis has been extensively studied in different experimental setups, and the most important growth factors involved seem to be VEGF and bFGF [32–34]. Consistent with previous studies [35, 36], we found that the islet production of VEGF increased markedly during culture. Transplanted islets also seem to have an increased expression of VEGF in the immediate post-transplantation period [37], which is consistent with the fact that the VEGF concentration is elevated during the early angiogenic phase. However, in the present study the VEGF content was similar in islet grafts composed of freshly isolated or cultured rat islets when investigated during active angiogenesis 4 days after transplantation. Thus, differences in VEGF production seem unlikely to explain the better revascularisation of islets transplanted immediately after isolation. This is also indirectly supported by results from a previous study, where in vivo blockage of actions of endogenous VEGF did not impair the revascularisation of freely transplanted islets [38]. Notably, however, the induction of marked VEGF hyperexpression in islets prior to transplantation through gene transfer seems to improve both islet revascularisation and function [39].

Freshly isolated islets have previously been reported to produce bFGF in vitro [40]. In the present study we observed that the bFGF production of isolated rat islets is not affected by subsequent culture. A rapid decline in islet graft bFGF expression has been reported to occur 3–5 days post-transplantation in grafts composed of freshly isolated islets [40]. In our study, the production of bFGF in 4-day-old rat islet grafts was similar in grafts composed of freshly isolated and cultured islets. One-month-old rat islet grafts had a higher production of bFGF than 4-day-old islet grafts. There was also a tendency (p=0.07) for 1-month-old grafts composed of freshly isolated rat islets to have a higher bFGF content than those of cultured islets. This may have contributed to the better revascularisation of grafts composed of freshly isolated islets, since bFGF has been reported to improve blood vessel stability [41].

The formation of a highly vascularised stroma in the grafts is probably due to growth factors excreted by the transplanted islets, whereas the reason(s) for the defective revascularisation of the endocrine tissue per se remain(s) largely unclear. However, some clues were provided in the present study, where transplanted freshly isolated islets were more efficiently revascularised than transplanted cultured islets. This suggests that remnant donor endothelial cells are indeed important in the revascularisation process. It could be argued that the remaining microvessels in freshly isolated islets serve as channels for the migration of new vessels, which may induce the process of revascularisation. Remaining endothelial cells may also attract and become incorporated within the newly formed microvessels [16, 17]. Furthermore, it should be noted that macrophages residing within the pancreatic islets are known to disappear during culture [9]. Monocytes and tissular macrophages seem strongly involved in adult angiogenesis due to their local secretion of metalloelastases, which cause the formation of capillary lumens through local tunnelling in the parenchyma [42, 43]. Monocytes and macrophages also contribute to the local pool of endothelial progenitor cells [44]. The role of these cells in adult angiogenesis, including that in the revascularisation of pancreatic islets, has recently been demonstrated [45, 46]. Moreover, the macrophage-derived factor matrix metalloproteinase-9 has been reported to be pivotal for the angiogenic switch to occur in insulin-producing tumours [47].

In this study, we compared the revascularisation of syngeneic rat islet grafts composed of freshly isolated islets with that of grafts composed of islets cultured for 4–7 days. Since human islets in most cases nowadays are cultured for a shorter period of time prior to transplantation, it cannot be excluded that such islets still possess some of the angiogenic properties of freshly isolated islets. As indicated in early clinical studies, a culture period, at least overnight, may be valuable in the clinical situation to reduce exocrine contamination [48].

In conclusion, the present results imply that immediate transplantation of islets, without preceding culture, may be advantageous in islet transplantation by improving their vascular engraftment and function. These findings are based on syngeneic transplantation models, where revascularisation can be studied in a standardised manner without interference by factors such as immunosuppression and immunological rejection. The clinical importance of the present results needs to be further evaluated in the human allogeneic setting.

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