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RESEARCH ARTICLE

Tracing of islet graft survival by way of *in vivo* fluorescence imaging

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Abstract

Background To increase the success rate in xenogeneic islet transplantation, proper assessment of graft mass is required following transplantation. For this reason, we aimed to develop a suitable fluorescence imaging system to monitor islet xenograft survival in diabetic mice.

Methods Adenovirus vector encoding enhanced green fluorescent protein-transduced rat pancreatic islets were transplanted under the renal capsule of streptozotocin-induced diabetic mice and the fluorescence signal was quantified over time using a cooled charge-coupled device. Non-fasting blood glucose levels were recorded during the same period. Insulin release from transduced and control islets was detected via enzyme-linked immunosorbent assay.

Results Adenovirus vector encoding enhanced green fluorescent protein infection did not alter the function or survival of pancreatic islets post transduction. A direct correlation was found between the number of islets (250–750) transplanted under the kidney capsule and the blood glucose recovery.

Conclusions Fluorescence imaging appears to be a useful tool for quantitative assessment of islet cell viability post transplantation and could permit earlier detection of graft rejection. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords EGFP; *in vivo* imaging; adenovirus; type 1 diabetes; xenogeneic islet transplantation

Introduction

Early clinical trials involving islet transplantation were disappointing, with 1-year insulin independence rates reaching only 8%. Subsequent improvements in islet isolation and preparation resulted in an increase in the number of patients becoming insulin independent (~50%) after 1 year [1]. A pioneering study involving the use of the 'Edmonton protocol', consisting of the transplantation of freshly isolated islets accompanied by a steroid-free sirolimus-based immunosuppressant therapy, demonstrated that all seven patients enrolled in the study acquired insulin independence for at least 1 year following transplantation [2]. A larger study with 50 additional patients treated with the same protocol confirmed the high insulin independence rate (80%) at 1 year [3]. Because transplantation of large numbers of pancreatic islets can overcome diabetes-related complications, islet transplantation is a promising method to achieve insulin independence in patients with type 1 diabetes [4].

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Compared to full pancreas transplantation, the transplantation of pancreatic islets is much less invasive. Despite these advantages, islets are very sensitive to mechanical and chemical treatment and islet isolation from a cadaveric pancreas is a laborious and difficult procedure. Furthermore, the number of islets isolated from one cadaveric pancreas is insufficient to ameliorate the diabetic state in an immunosuppressed recipient. At least 2–4 donor pancreata must then be used to obtain the ideal number of islets needed for transplantation, but the non-availability of suitable donors can make this a difficult task to accomplish. Thus, significant effort is being made to find new ways to increase the number of transplantable islets, even if this requires xenotransplantation. Although conceptually feasible, the intense immune reaction that can follow xenogeneic islet transplantation represents a major hurdle, preventing this procedure from more widespread use [5,6].

Despite the implementation of immunosuppressive protocols, the majority of transplanted islets undergo a gradual rejection [7]. For example, 5-year follow-up of islet transplant recipients treated with the Edmonton protocol revealed that the insulin independence rate decreased to 10% after 5 years [8]. These trials were extended and expanded to include international sites for the testing, and confirmatory results were attained [9,10]. Currently, graft function is assessed by patient's blood glucose, C-peptide and insulin levels after islet transplantation. However, alterations in these parameters may only occur after the loss of most of the graft mass, making it difficult, if not impossible, to medically intervene on time. Thus, specific molecular markers are needed in clinical islet transplantation studies to provide 'real-time' information about the survival and function of the engrafted islets [11], and earlier recognition of islet graft rejection by non-invasive imaging methods is proving to be essential for the early implementation of additional methods to delay graft rejection [12,13].

Imaging methods, such as magnetic resonance imaging [14], positron emission tomography [15] and optical imaging techniques such as bioluminescence [15–17] or fluorescence, have been tested to quantify transplanted islet cells *in situ*. Among these, optical imaging is known to have significant advantages, such as being relatively of low cost, and not depending on the use of radioactive substrates or contrast agents. Although bioluminescence is an effective technique for optimal imaging, it requires the use of a relatively expensive substrate luciferin, which has a short half-life. This creates the need for immediate visualization, usually within half an hour of application [18]. Fluorescence imaging, on the other hand, does not require any substrate use. Green fluorescent protein (GFP) is commonly used in fluorescence imaging, which relies on the visualization of fluorescent emission from fluorophores within cells or tissues by a specialized cooled charge-coupled device (CCD) camera [19]. Besides being non-toxic, it has no negative effects on normal cellular activites. With this in mind, we used a recombinant adenovirus vector encoding enhanced GFP (AdEGFP) to monitor islet cell fate after xenotransplantation. It is well acknowledged that the route of administration of a vector to provide tracing of the pancreatic islet fate should not have any negative effects on any functional features of the islets. Thus, we demonstrated that AdEGFP transduction had no negative effects on the function and survival of the pancreatic islets. Overall, the goal of our study was to test the extent to which repetitive *in vivo* fluorescence imaging could be used to monitor xenogeneic graft survival and function over time.

Materials and methods

Production of recombinant adenovirus vectors

E1/E3-deleted first-generation recombinant adenovirus (AdEGFP)-expressing EGFP gene [20,21] via a cytomegalovirus promoter was amplified in HEK293 cells and purified by CsCl banding [22]. Adenoviral vectors were stored at −80 °C in 10 mM Tris-Cl containing 20% glycerol. The titres of adenoviral stocks were determined as 10^{13} DNA particles/mL by spectrophotometric measurements (A_{260} readings) [23]. The functional titres of adenoviral stocks were determined by plaque titration on 293 cells and expression assays for encoded proteins [24]. Typically, the particle/plaque forming unit ratio was 25.

Animals

About 10- to 12-week-old male BALB/c mice and 10- to 12-week-old female Wistar rats were obtained from the Laboratory Animal Care Unit of Akdeniz University Hospitals and Clinics (Antalya, Turkey). Animal experiments were conducted in accord with the Institutional Animal Care Guidelines.

Rat pancreatic islet isolation

Pancreatic islets were obtained from outbred female Wistar rats, as previously described [25]. Briefly, after intraperitoneal ketamine/xylazine anaesthesia, an abdominal V-incision was made and the pancreas was exposed. The pancreas was then distended by direct injection of 10 mL cold Liberase RI solution (0.25 mg/mL; Roche, Indianapolis, IN) through the pancreatic duct with PE50 polyethylene tubing (inner diameter 0.58 mm; outer diameter 0.965 mm, Becton Dickinson, Franklin Lakes, NJ). The inflated pancreas was dissected from external tissues and incubated at 37 °C for 20 min for enzymatic digestion. Then, 40 mL of ice-cold Dulbecco's Modified Eagle Medium with 10% new born calf serum (Sigma-Aldrich) was added to stop enzyme activity. The pancreas was vigorously shaken for mechanical digestion and then filtered through a 424-μm pore size sieve (Thomas Scientific, Swedesboro, NJ) to remove

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fat and exocrine tissues. The islets were separated from the digested pancreas using a Histopaque-1077 density gradient (Sigma-Aldrich). Purified islets were hand-picked under a microscope and then cultured for 48 h in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum at 37 °C and 5% CO_2.

Determination of cell viability and purity of isolated islets

Fifty islets were placed on a 35 mm cell culture plate containing 1 mL sterile phosphate-buffered saline to assess the viability of freshly isolated islets. Then, 2 μL fluorescein diacetate (5 mg/mL; Sigma) and 2 μL propidium iodide (1 mg/mL; Sigma) were added onto the plate. After waiting 5 min, live and dead cells within the islet cell cluster were determined under a fluorescence microscope equipped with a green and a red filter. For the assessment of cell purity, another 50 islets were placed into 1 mL sterile PBS and then 350 μL dithizone (0.25 mg/mL; Fluka) was added. After waiting 15 min, insulin-positive cells were detected under an inverted microscope.

Adenoviral transduction of isolated pancreatic islets

Freshly isolated islets were cultured with AdEGFP in serum-free RPMI 1640 medium for 2 h at 37 °C [20,26]. Fetal bovine serum was then added to reach a final concentration of 10% and islets were further cultured for 48 h at 37 °C before transplantation. Fluorescence microscopy was used to monitor and quantify cell infection. Islet morphology was evaluated under an inverted microscope. Mock-infected islets underwent a similar procedure, but were not transduced with any virus.

Glucose-stimulated insulin secretion assay

To assess insulin secretion, 200 AdEGFP-transduced islets were washed with PBS and incubated in 0.5 mL of RPMI 1640 medium containing low (2.8 mM) and then high (16.7 mM) concentrations of glucose for 1 h in 24-well plates. Supernatants were collected, and the insulin concentration was determined by enzyme-linked immunosorbent assay, as previously described [25].

Diabetes induction

Diabetes was induced in mice by a single intraperitoneal injection of streptozotocin (STZ) (Sigma-Aldrich), freshly dissolved in 0.1 M citrate buffer (pH 4.5). Tail blood was taken from non-fasting animals and whole glucose concentrations were measured with a portable glucose meter (Accu-Check Go; Roche, Nutley, NJ). Diabetes was confirmed when blood glucose levels were greater than 300 mg/dL on 2 consecutive days. Body weight was measured immediately after blood glucose measurement.

Islet transplantation

Rat islets were implanted beneath the left kidney subcapsular space of STZ-induced diabetic recipients 10 days after STZ injection. Islets were aspirated into a 200 μL pipette tip connected with PE50 tubing, and centrifuged for 1 min at 1200 rpm. The left kidney of the mouse, which was under ketamine/xylazine anaesthesia, was exposed through a lumbar incision and a small scratch was made on the capsule. The PE50 tubing was slid under the kidney capsule, and the islets were carefully transferred under the kidney capsule with the aid of a 0.5 mL syringe (Hamilton, Reno, NV). The tubing was then removed and the capsule was cauterized. The kidney was replaced into the peritoneum and the incision was sutured. Reversal of diabetes was defined as blood glucose <250 mg/dL on 2 consecutive days.

Fluorescence imaging of grafts

Diabetic mouse recipients of 750 AdEGFP-transduced rat islets were imaged post transplantation using a CCD camera (Kodak 2000 MM Image Station; Eastman Kodak Company, New Haven, USA), as previously described [27]. Mice were imaged at 3- to 4-day intervals. To acquire the images, mice were anesthetized and the kidney was exposed through a lumber incision. Next, the anesthetized mouse was placed in a sterile cabinet and greyscale photographs were taken using a 0.05 s exposure time with open filters. Keeping the mouse in the same position, fluorescence images were also collected using a 60-s exposure time with 465 excitation/535 emission filters but no binning. Fluorescence images were artificially coloured for depiction purposes. Fluorescence and greyscale images were overlapped to detect signal location. For analysing the fluorescence signal intensity, Kodak 1D Software 3.6.5 (Eastman Kodak Company, New Haven, USA) was used. Regions of interest were defined using a standard area in each image and fluorescence signal intensity was recorded as a net intensity reflecting the sum of the background subtracted pixels within the region of interest.

Immunohistochemistry and histology

Kidneys bearing xenografts were collected at three different time points (0, 7 and 15 days after transplantation), fixed in 10% formalin and embedded in paraffin. Graft samples were serially cut at 5 μm and placed on positive charged slides. Sections were deparaffinized in xylene and alcohol, then stained with haematoxylin and

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eosin for histological analysis of infiltrating mononuclear cells. Deparaffinized and rehydrated sections from islet xenografts were also stained overnight with a mouse anti-rat insulin antibody (diluted 1:500; Abcam) followed by incubation for 1 h with a peroxidase-labelled goat anti-mouse immunoglobulin G antibody (diluted 1:1000; Bio-Rad, Hercules, CA). Peroxidase activity was visualized by incubating the slides for 1–2 min in diaminobenzidine solution (Lab Vision/Thermo Fisher Scientific). Counterstaining was performed with Mayer's haematoxylin (Sigma) for 10 s. As a negative control, serial sections were stained only with secondary antibody.

Statistical analysis

Values were given as standard error of the mean and compared using Mann–Whitney *U*-test or one-way analysis of variance followed by Bonferroni's multiple comparison test, where appropriate. Islet xenograft survival was analysed by log-rank (Mantel–Cox) test (Graph Pad Prism 5 Software, Inc, San Diego, CA). Statistical significance was accepted at $p < 0.05$.

Results

Quality assessment of freshly isolated pancreatic islets

Isolated pancreatic islets from healthy Wistar rats (Figure 1A), were initially checked for purity and viability. To assess islet cell purity dithizone was used, which binds to the zinc ions present within the insulin molecule. Dithizone staining indicated that the isolated pancreatic islets were highly pure and expressed insulin (Figure 1B). Live and dead cells were assessed using two fluorescent dyes, fluorescein diacetate and propidium iodide. Fluorescein diacetate/propidium iodide double staining showed that most of the isolated islets were alive (Figure 1C and D).

Induction of diabetes in BALB/c mice

We next induced diabetes in mice via intraperitoneal administration of the pancreatic beta cell toxin STZ. Previous studies suggested that intraperitoneal injection of 175–200 mg/kg STZ successfully produced hyperglycaemia in BALB/c mice [28]. In our experience, a single intraperitoneal injection of 175 mg/kg STZ induced hyperglycaemia in 1 day, and high blood glucose levels were maintained for the next 3 months (Figure 2B). However, the majority of the mice (42%) treated with this dose of STZ died within the first week (Figure 2A) following a major loss of body weight (Figure 2C). Because of the high mortality rate observed with 175 mg/kg STZ, we reduced the amount of STZ to 150 mg/kg. Using this STZ dose, blood glucose levels gradually increased during the first week after STZ injection and remained elevated throughout the observation period of 3 months (Figure 2B). Importantly, the 150 mg/kg STZ dose did not lead to a significant loss in survival or body weight (Figure 2A and C). Thus, after considering the STZ dose effect on morbidity and mortality, all subsequent experiments used the 150 mg/kg STZ dose to induce diabetes in mice.

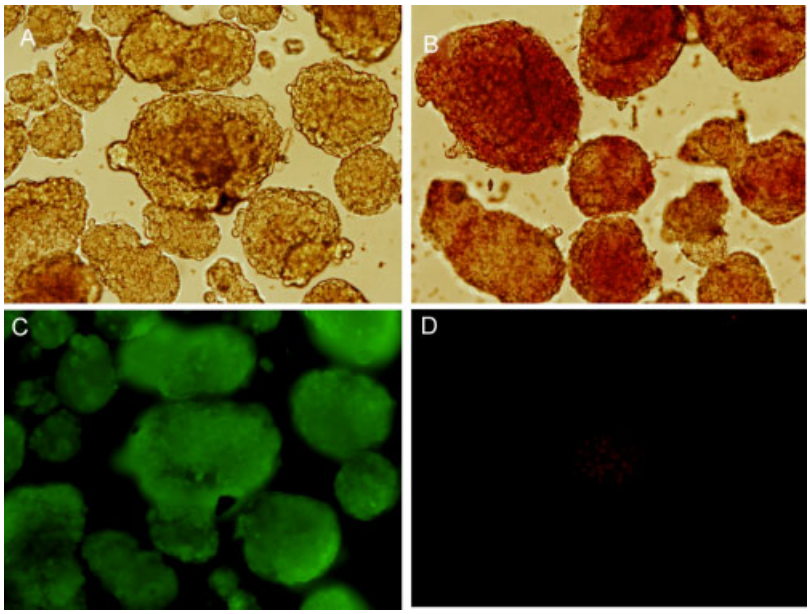

Figure 1. Viability and purity of isolated rat islets. Panel A shows a bright field microscopic view of isolated islets. The islets appear reddish after dithizone staining (B). Live cells exhibit green fluorescence after fluorescein diacetate staining (C) and dead cells emit red fluorescence after propidium iodide staining (D) (original magnification is 320×)

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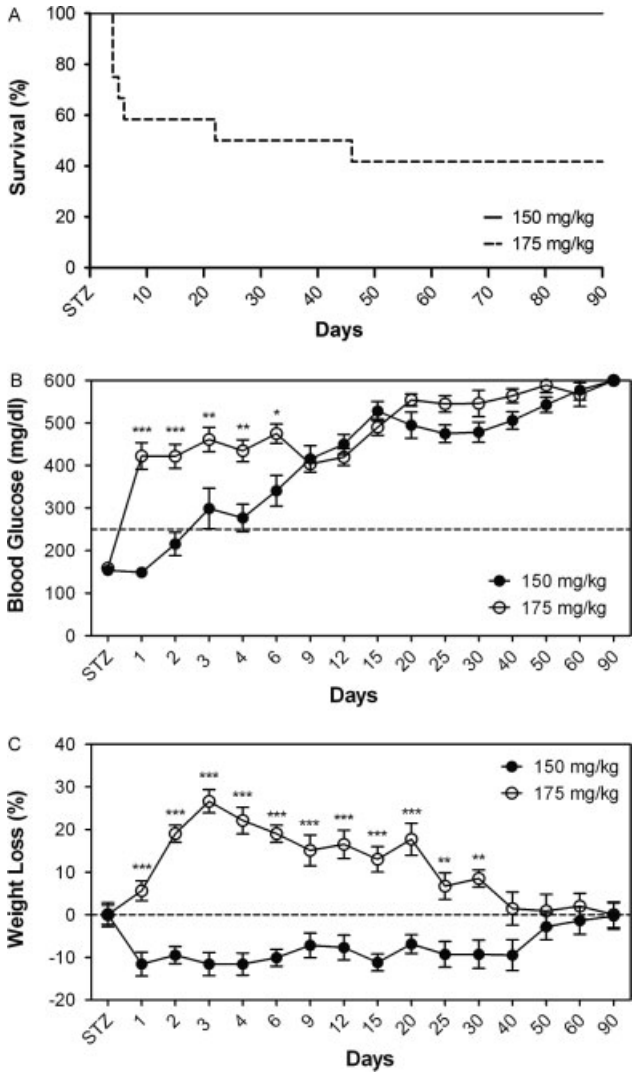

Figure 2. Diabetes induction in BALB/c mice. Changes in survival (A), blood glucose levels (B) and body weight (C) in mice after 175 mg/kg ($n = 12$) or 150 mg/kg ($n = 10$) of streptozotocin administration. Data are expressed as means ± standard error of the mean. $^{*}p < 0.05$; $^{**}p < 0.001$; $^{***}p < 0.0001$

Xenograft longevity is related to the number of islets transplanted

Before the initiation of the *in vivo* imaging experiments, we next determined the number of islets required to achieve euglycaemia for at least 7 days. Various numbers of non-transduced islets (250, 500 and 750 islets) were transplanted into diabetic mice, and blood glucose levels were monitored as described in the section on Materials and Methods. Diabetes was reversed in all groups within 3 days of transplantation (Figure 3). One-way analysis of variance followed by Bonferroni's multiple comparison test indicated that although blood glucose recovery levels were similar in three groups, the number of transplanted islets directly correlated with xenograft survival – such that mice transplanted with the highest number of islets maintained normoglycaemia for the longest period of time.

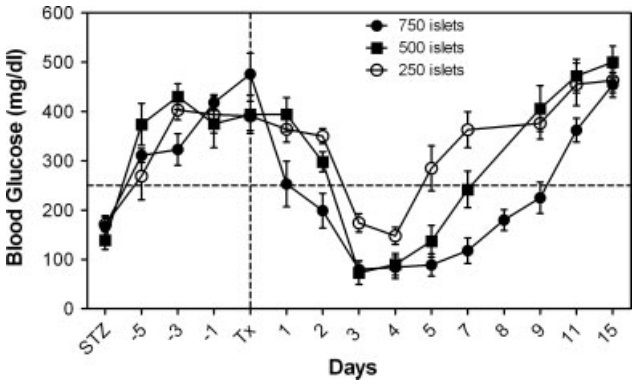

Figure 3. Changes in blood glucose levels in diabetic mice transplanted with 250, 500 or 750 non-transduced islets ($n = 6$). Islets were transplanted 10 days after streptozotocin injection. Data are expressed as means ± standard error of the mean

AdEGFP transduction neither alters the morphology nor function of rat pancreatic islets *in vitro*

The next step was to visualize the islet cells *in vivo* using AdEGFP [29]. However, we first had to determine the extent to which the adenoviral infection and/or EGFP synthesis and expression affected islet morphology and function. For this purpose, islets were isolated from healthy Wistar rats and then transduced with AdEGFP at a multiplicity of infection of 1000 DNA particles/cell [25]. Visual assessment of AdEGFP-transduced islets was performed 48 h after the transduction. Both bright field and fluorescent microscopic analyses suggested that the transduced islet cells looked healthy and expressed EGFP (Figure 4A). To exclude the possibility of any toxic effects of adenovirus infection or EGFP expression on islet cell function, a glucose-stimulated insulin secretion assay was performed. As shown in Figure 4B, based on the Mann–Whitney *U*-test, no significant differences in insulin secretion was found between AdEGFP-infected and uninfected islets.

In vivo xenogeneic graft survival and function were not affected by AdEGFP transduction

For *in vivo* imaging of xenogeneic grafts, 750 AdEGFP-infected or uninfected islets were transplanted separately under the kidney capsule of diabetic mice 48 h after transduction. To examine the effect of AdEGFP transduction on xenograft survival and/or function *in vivo*, changes in blood glucose levels were compared in mice transplanted with either AdEGFP-infected or uninfected control islets (Figure 5). There were only subtle differences between the two groups, such that mice transplanted with uninfected islets rapidly reversed diabetes within a day of

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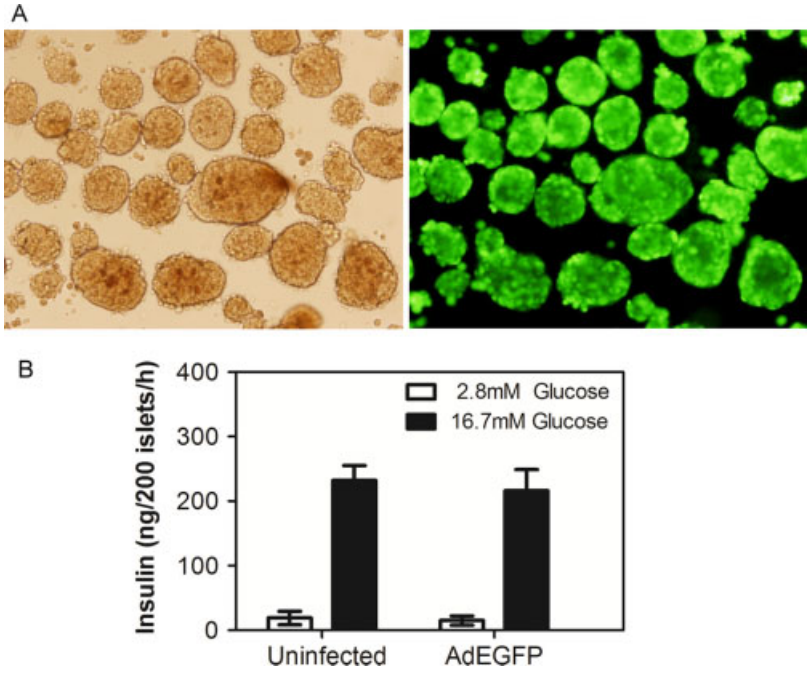

Figure 4. Visual and functional assessment of rat pancreatic islets before and after enhanced green fluorescent protein gene delivery. (A) Bright field (left) and fluorescence microscopic (right) images of rat pancreatic islets are shown at the upper panel (magnification 160×). Photomicrographs were taken 48 h after transduction. (B) Non-stimulated or glucose-stimulated insulin secretion of adenovirus vector encoding enhanced green fluorescent protein-infected and uninfected islets. Forty-eight hours after the infection, 200 islets were exposed to 2.8 or 16.8 mM glucose for 1 h, and the insulin content of the medium was determined by enzyme-linked immunosorbent assay

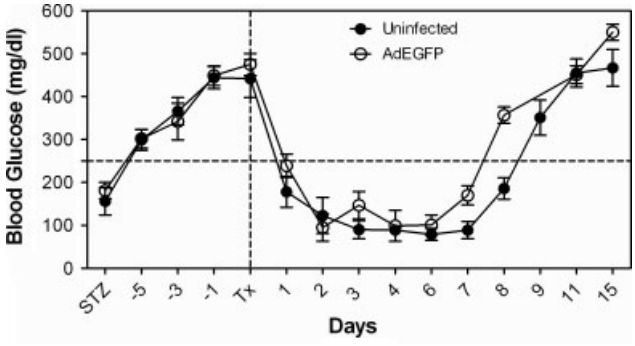

Figure 5. Blood glucose status of diabetic mice transplanted either with adenovirus vector encoding enhanced green fluorescent protein-infected or uninfected islets. Seven hundred and fifty rat islets, either transduced with adenovirus vector encoding enhanced green fluorescent proteinor untransduced, were transplanted under the kidney capsule of diabetic mice 10 days after streptozotocin administration ($n = 6$)

transplantation and remained normoglycaemic for nearly 8 days. Recipient mice that received AdEGFP-infected islets became normoglycaemic within 2 days of the transplant, and remained normoglycaemic for at least a week. Overall, based on the Mann–Whitney *U*-test, the mean survival time or function of AdEGFP-infected xenogeneic islet grafts was not significantly different from that of uninfected grafts. These findings suggest that adenoviral infection did not alter xenogeneic graft function or survival compared with control islet grafts.

In vivo fluorescence imaging of xenogeneic islet grafts as a marker to follow graft failure

Xenografts transduced with AdEGFP were monitored over time, using a CCD camera to follow islet cell fate after transplantation. A small lumbar incision was made to expose the kidney for fluorescent imaging, which was done at different time points (0, 4, 7, 11 and 15 days) post transplantation (Figure 6A). A strong fluorescent signal was detected from grafts indicating engraftment of islets. Intriguingly, the signal intensity was further increased 4 days after transplantation. This was attributed to the dispersion of the islets over the surface of the kidney and the eventual clearance of blood from the implantation region. An inverse correlation was detected between the fluorescent signal intensity and the duration of normoglycaemia, suggesting that the fluorescent signals emitted from engrafted islets might serve as an early marker for graft rejection (Figure 6B).

Infiltration of mononuclear cells results in the loss of fluorescent signal from xenogeneic grafts

Immunohistochemical analysis of islet graft sections was performed to determine any cellular changes within the transplanted region. As seen in Figure 7, islet morphology was intact and grafts were all positive for insulin staining

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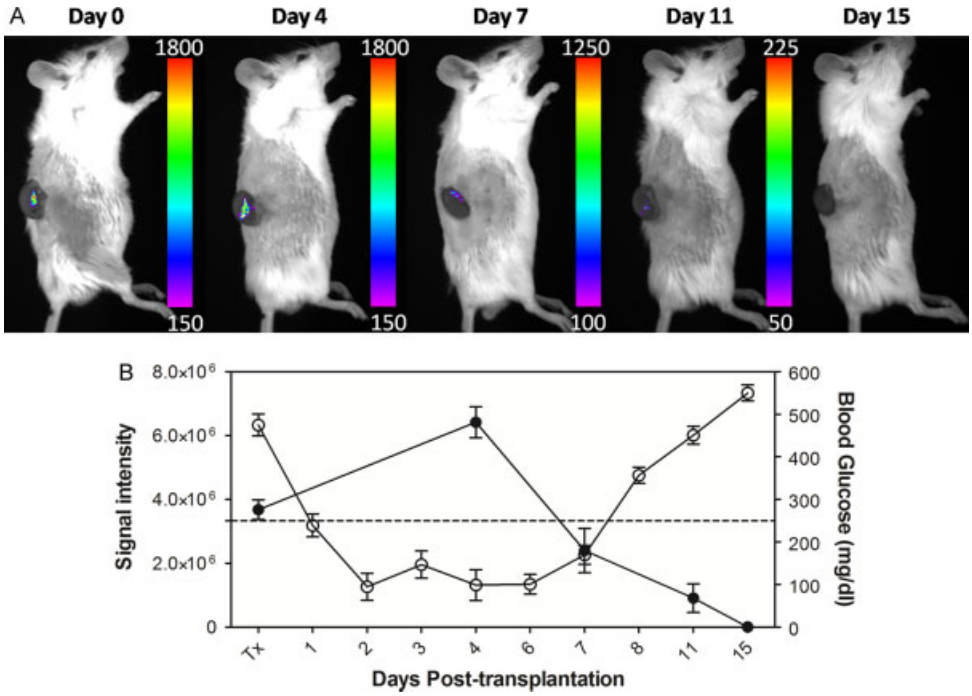

Figure 6. *In vivo* **fluorescence imaging of xenogeneic islet grafts over time. (A) Streptozotocin-induced diabetic mice were transplanted with 750 transduced islets under renal capsule ($N = 5$). Graft was monitored using a charge-coupled device camera after transplantation (days 0, 4, 7, 11 and 15). (B) The relationship between signal intensity and blood glucose. Fluorescent signal intensities are shown as black circles, while blood glucose measurements are shown as open circles**

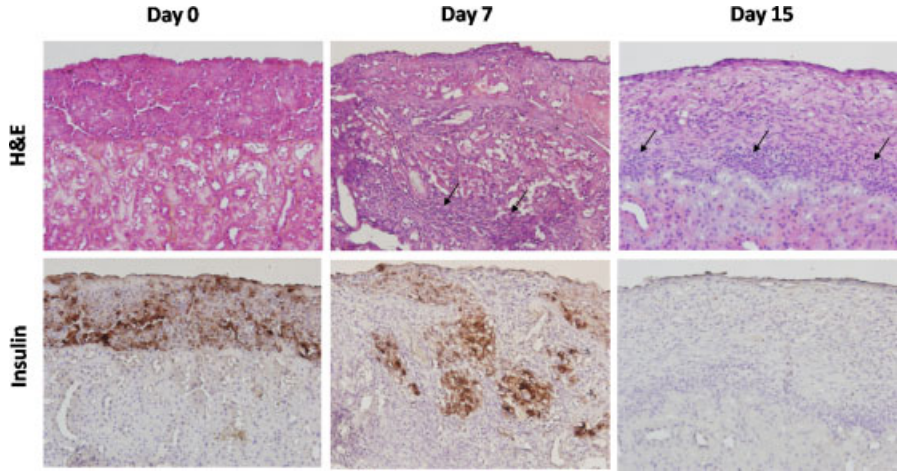

Figure 7. Immunohistochemical and histological analysis of xenogeneic islet grafts. Xenograft-bearing kidneys were removed at certain time points indicated above the panel. Insulin staining (lower panel) and haematoxylin and eosin staining (upper panel) were performed on serial sections. Arrows show mononuclear cell infiltration sites. Images were taken at an original magnification of 400×

on the day of transplantation (day 0). However, islet architecture was compromised and insulin-positive cell content was reduced 7 days after transplantation. At this time point, despite the decrease in signal intensity, mice were still normoglycaemic (Figure 6B). In accord with reduced insulin content, haematoxylin and eosin staining showed the presence of infiltrating cells in the region. When the fluorescent signal became undetectable 15 days post transplantation, all islets were destroyed and no insulin positive cell was detectable (Figure 7). Instead, the area where the graft had been was now heavily infiltrated with mononuclear cells. Similar results were obtained in mice transplanted with uninfected control islets (data not shown).

Discussion

Intrahepatic islet transplantation has been a promising approach for the treatment of patients with type 1 diabetes, but the success of this technique has been questioned because of the high frequency of non-functioning

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grafts and secondary graft failure leading to the majority of recipients resuming insulin replacement therapy within 5 years [30,31]. Thus, monitoring the islet cell mass is necessary for early intervention to prolong normoglycaemia. Development of fluorescent imaging techniques combined with gene transfer has enabled *in vivo* tracking of fluorescent-labelled cells in living organisms. Unlike bioluminescent imaging, which is considered the strongest alternative to fluorescent imaging strategies, fluorescence analysis is more cost-effective, and enables analysis within a much longer period of time because of the longer half-lives of the probes currently in use [18]. Furthermore, the non-oxygen dependence of the fluorescent probes renders them suitable for use in both aerobic and non-aerobic conditions. GFP and its mutant variants are the only fully genetically encoded fluorescent probes currently available [32]. *In vivo* 'real-time' imaging of GFP-expressing cells has been used in diverse research fields of science, such as cancer cell imaging [33,34], metastasis [35], tumour angiogenesis [36], bacterial infection [37] and gene therapy [27,38]. This study was conducted to determine the extent to which transplantation could be added to this list, by visualizing the persistence of EGFP-expressing pancreatic islets. Although there are convincing results about the suitability of bioluminescent imaging of transplanted islets [13,39,40], we believe that fluorescent imaging has significant advantages over bioluminescence for imaging of transplanted islets as well, mainly due to the above mentioned facts.

For *in vitro* fluorescent imaging purposes, the EGFP gene was successfully delivered via adenoviral vectors to isolated pancreatic islets as in one of our previous studies [29]. However, this previous report was carried out *in vitro* where host immunity against the viral vector and/or the transgene was not a concern. For an *in vivo* fluorescence imaging study, though, it was essential to determine the extent to which the gene-transfer vector and/or the reporter gene of interest had any toxicity on the pancreatic islets before and after transplantation. The glucose-stimulated insulin secretion assay showed that adenovirus infection and/or EGFP expression did not cause any adverse effects on the function of the isolated islets *in vitro*. For the *in vivo* analysis, monitoring the blood glucose levels of diabetic mice transplanted with AdEGFP-transduced rat pancreatic islets suggested that viral gene delivery and/or EGFP expression did not yield any deleterious adverse effect on islets compared with animals transplanted with uninfected control islets.

Because haemoglobin is the primary light-absorbing molecule in mammalian tissues [41], blood dispersed in the graft area may have initially absorbed the fluorescent signal and reduced its detection by the CCD camera. Thus, the increase in the fluorescent signal intensity on the fourth day of transplantation could be explained by the eventual resolution of erythrocyte mass around the graft. To further enhance the detection of the fluorescent signal, a small incision over the left kidney area had to be made. Live tissue can absorb and scatter light, especially light emitted from EGFP, so making an incision along the light path was needed to decrease the signal interference by live tissues [35,42]. Comparing fluorescence imaging and bioluminescence at this point, as Caceres *et al*. did, although in a different setting, may be meaningful [35]. Caceres *et al*., in their study where they compared imaging of luciferase- and GFP-transfected human tumours in nude mice, referred to the fact that GFP-transfected cells may be useful for imaging studies of superficial tumours, whereas luciferase-transfected cells are defined as superior for imaging studies of primary and metastatic tumours in distant sites and deep tissues. With regard to islet monitoring, using red-shifted fluorescent proteins [41,43] instead of EGFP may alleviate the incision procedure and overcome the above-mentioned handicap related to GFP fluorescence.

Regardless of this aspect, the fluorescent signals emitted from EGFP-expressing xenogeneic islets suggest the potential utility of using this technique as a quantitative methodology [27] to monitor the survival and function of the transplanted tissue, as the fluorescent signal intensity inversely correlated with blood glucose levels. Consequently, our results suggested that fluorescent imaging could successfully be used to monitor engrafted xenogeneic islet cell mass following transplantation.

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Conflict of interest

The authors have no conflicts of interest.

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