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Long-term function of islets encapsulated in a re-designed alginate microcapsule construct in omentum pouches of immune-competent diabetic rats

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Abstract

Objectives—Our study aim was to determine encapsulated islet graft viability in an omentum pouch and the effect of FGF-1 released from our redesigned alginate microcapsules on the function of the graft.

Methods—Isolated rat islets were encapsulated in an inner core made with 1.5% low-viscosity high-mannuronic acid (LVM) alginate followed by an external layer made with 1.25% low-viscosity high-guluronic acid (LVG) alginate with or without FGF-1, in microcapsules measuring 300 – 400 µm in diameter. The two alginate layers were separated by a perm-selective membrane made with 0.1 % Poly-L-Ornithine (PLO), and the inner LVM core was partially chelated using 55 mM sodium citrate for 2 min.

Results—A marginal mass of encapsulated islet allografts (~2000 islets/kg) in Streptozotocin-diabetic Lewis rats caused significant reduction in blood glucose levels similar to the effect observed with encapsulated islet isografts. Transplantation of allo-islets co-encapsulated with FGF-1 did not result in better glycemic control, but induced greater body weight maintenance in transplant recipients compared to those that received only allo-islets. Histological examination of the retrieved tissue demonstrated morphologically and functionally intact islets in the microcapsules, with no signs of fibrosis.

Conclusion—We conclude that the omentum is a viable site for encapsulated islet transplantation.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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Keywords

alginate; microencapsulation; islets; immunoisolation; allotransplantation; omentum

1. Introduction

Diabetes is an epidemic which places an overwhelming burden on the patient's quality of life and the health-care system. The DCCT study showed that while intensive insulin treatment may result in good glycemic control in type 1 diabetes, it can only delay but does not completely prevent diabetic complications [1]. Better alternative therapeutic strategies to traditional insulin therapy are therefore required for the management of this disease [2].

A primary therapeutic target for diabetes is stable glycemic control resulting in prevention of secondary complications, and islet transplantation has been shown to be a feasible treatment option for type 1 diabetic patients [3]. However, critical donor organ shortage (requirement for multiple donors), gradual loss of graft function over time (low islet survival rates over the long term), and most importantly, the need for chronic immunosuppression, which causes undesirable side-effects, limit the application of this therapy to a small group of patients [4]. Cell encapsulation provides an attractive means to transplant cells without the need for immunosuppression. The cells are immunoisolated by enclosing them in polymeric devices with semipermeable membrane that allows selective permeation of nutrients and therapeutics while isolating the cells from the immune system of the host's body [5]. Furthermore, this strategy allows for the potential utilization of tissue from allo- and xeno-sources, which could increase the organ donor pool. Although the technique of microencapsulation of islets was introduced more than three decades ago [6], its full clinical potential has not yet been realized, hence some investigators have continued to explore different strategies to improve the technique [7–11]. Other investigators have also been exploring different strategies to enhance the function of encapsulated islets [12–15].

A major issue which has not been adequately addressed is the optimal site for encapsulated islet transplantation. Based on technical ease of procedure and space requirement to accommodate a large transplant volume, encapsulated islet transplantation has been routinely performed in the peritoneal cavity, an avascular site that has limited oxygen and nutrient diffusion [16]. This hinders transplant success as the graft needs to rapidly establish a blood supply because islets have an intensive demand of oxygen for survival [17–21]. An alternative anatomical site for islet or encapsulated islet transplantation, which offers maximum engraftment, efficacious utilization of secreted insulin, and maximum patient safety is urgently needed [22–24]. Thus, our aim in this work was to evaluate the viability of encapsulated islets in an omentum pouch, a well vascularized site, which would also accommodate a large transplant volume in large animals and humans. In addition, this site permits subsequent graft retrieval for post-transplant evaluation and also offers drainage of secreted insulin directly into the portal vein which ensures the most efficient use in the liver [25–30]. Furthermore, in order to assess the effect of FGF-1 induced neovascularization, we examined the effect of co-encapsulation of islets with FGF-1 on the function of a marginal mass of encapsulated islets transplanted in immune-competent diabetic rats.

2. Materials and Methods

2.1 Solutions

High-mannuronic acid-low viscosity alginate (LVM, 20–200 mPas; Nova-Matrix, Sandvika, Norway) was dissolved at 1.5% in Eagle's minimum essential medium (MEM) (M0518 w/o Ca^{2+} ; Sigma-Aldrich, St. Louis, MO,) overnight under sterile conditions. Poly-L-Ornithine

(PLO; mol wt 30–70 kDa, Sigma-Aldrich), sodium citrate (Sigma-Aldrich) and high-guluronic acid-low viscosity alginate (LVG, 20–200 mPas; Nova-Matrix) were also prepared sterile in MEM media at 0.1%, 55 mM, and 1.25%, respectively. LVM and LVG alginates were reported by the manufacturer to have molecular weights 75–200 kDa and G/M ratios of 1 and 1.5, respectively. Alginate microbead cross-linking (CaCl_2 ; 100 mM; Acros) and wash (NaCl ; 0.9 %) solutions were prepared in deionized water. All solutions were pH balanced at 7.4 and stored at 4°C until use. Dithizone was obtained from Sigma-Aldrich, and prepared in solution by dissolving 50 mg in 5 mL of DMSO, mixing for 15 minutes, and then adding 15 mL Hank's balanced salt solution (HBSS, Sigma-Aldrich), and mixing for an additional 15 minutes prior to filtering through a 0.45 μm filter syringe. Human fibroblast growth factor-1 (FGF-1) was purchased from Peprotech (Rocky Hill, NJ).

3. Animals

Male Wistar-Furth (WF) and Lewis rats were purchased from Harlan (Dublin, VA) and housed 2 rats/cage in a temperature-controlled room with a 12-hr light-dark cycle where the animals had unlimited access to food and water. All animal protocols were approved by the Wake Forest University Institutional Animal Care and Use Committee (IACUC).

3.1 Islet isolation and purification

Male WF and Lewis rats (250–300 g) were used as the source of islets for allograft and isograft experiments, respectively. Islets were isolated from the pancreata of the rats using the procedure of collagenase digestion of pancreatic tissue [31] with modifications [32]. Briefly, following euthanasia by CO_2 asphyxiation, shaving, cleaning, and laparotomy of the animals the pancreatic ducts were cannulated for the infusion of 12 ml of 1 mg/ml freshly prepared Collagenase P solution (Roche (Indianapolis, IN) through the bile duct in order to distend the pancreases. The distended pancreases were collected in ice-cold glass tubes pre-filled with 3 ml of same collagenase solution and digested for 20 min at 37°C with uniform shaking in a water bath. The digestion mixture was filtered through a nylon mesh (500 μm) and washed twice with wash buffer comprising HBSS, 10% FBS and 10 mM HEPES (Sigma-Aldrich), and pelleted by centrifugation for 1 min at 200 g and 4°C.

Islets were purified and separated from the exocrine tissue with Optiprep (Sigma-Aldrich) density gradients. Two density solutions were prepared at 1.125 g/ml and 1.09 g/ml in University of Wisconsin solution (UWS; Perfadex, Xvivo Perfusion, Englewood, CO). The digested tissue was pelleted and dispersed in 10 ml of the 1.125 density solution to bring the resulting density to 1.1 g/ml. It was topped with 10 ml of 1.09 and 10 ml of UWS solution (1.045 g/ml) without mixing in a 50 ml centrifuge tube. This column was carefully centrifuged for 5 min at 375 g at 4°C. The first interface between the top two density layers (1.045 and 1.09) was carefully pipette out and washed with wash buffer. The purified islets were cultured overnight in RPMI-1640 (Sigma-Aldrich) media supplemented with 10% FBS and 10 mM HEPES.

3.2 Microencapsulation procedure

Microencapsulation of islets was performed using a modification [33] of a previously described procedure [8]. After overnight culture the islets were pelleted for 1 min at 200 g and 4°C, and were then dispersed in 1.5% LVM at 2000 islets/ml. The islet suspension was pumped at a constant flow rate of 1.4 ml/min and microspheres were formed using a high-throughput flow focusing microfluidic device at 1.4 psi pressure [34]. These microspheres were collected in 100 mM calcium chloride solution where they were allowed to crosslink for 15 min. The microbeads were subsequently transferred to a 50 ml conical tube and washed twice with the wash solution prior to incubation in 0.1% PLO for 20 min at 4°C.

with gentle rotational mixing to apply a uniform semi-permeable coating. The PLO-coated microcapsules were partially liquefied by incubating in 55 mM sodium citrate solution for 2 min at 4°C. After washing with wash solution, a final outer coating of LVG was applied with 1.25 % LVG solution in the absence or presence of FGF-1 (1.79 µg/100 microcapsules) and heparin (5 U/ml) for 5 min at 4° C with uniform mixing and the addition of 22 mM solution of CaCl₂ to crosslink the outer layer. Two additional washes were performed with 2 mM CaCl₂ and 0.9% NaCl solution to remove unbound alginate. The microcapsules were finally rinsed with normal saline solution and dried prior to transplantation. These microcapsules which have an alginate-PLO-alginate make up were termed APA microcapsules. The islet containing APA microcapsules were cultured overnight in RPMI 1640 medium and transplanted the next day.

3.3 *In vitro* Studies

3.3.1 Live/Dead Staining—To assess viability, samples of both naked islets and islets encapsulated in the APA microcapsules were stained with Dithizone and live-dead dyes (Live/Dead reduced biohazard cell viability kit, Invitrogen). For Dithizone staining, islets were stained with 2 mg/mL Dithizone solution for 2 minutes prior to imaging. For the live/dead cell assessments, naked and encapsulated islets were hand-picked and suspended in PBS solution of CFDA/PI live green fluorescent nucleic acid and dead red (Ethidium Homodimer-2) nucleic acid stains, according to the protocol guidelines provided in the kit and the stained islets were imaged using confocal microscopy.

3.4 *In Vivo* Procedures

3.4.1 Induction of diabetes in rats—Lewis rats were obtained at 350–400 g and made diabetic after a week of arrival. Briefly, a Streptozotocin (STZ) solution was prepared in 0.1 M sodium citrate pH 4.5. Each rat was injected intraperitoneally with 65 mg of STZ/kg of body weight. The rats were monitored for any sign of sickness or behavioral change. Immediately after receiving the STZ injection the animals were put on wet chow. The blood glucose was closely monitored using a glucometer and the rats were judged to be diabetic when blood glucose levels were higher than 400 mg/dL for two consecutive days [35]. After diagnosis of diabetes, insulin pellets (Linshin, Canada) were subcutaneously (s.c.) inserted in the back of the neck to maintain the health of the animals for at least one week before they were used for transplant studies.

3.4.2 Transplantation of Microcapsules—For each group (n=5), rats were anaesthetized with Isoflurane and a small incision was made on the left side below the stomach. The omental tissue was exposed and spread over a wet gauze and an omental pouch was sewed with a 4.0 vicryl suture. Microcapsules were placed carefully in the pouch and it was closed and carefully positioned back in the peritoneal cavity. In order to examine the effect of FGF-1 on the function of the encapsulated islets, a marginal mass of 800 islets was transplanted, (~ 2000 islets/kg) in each recipient in both isografts (Lewis islets to Lewis rats) and allografts (WF islets to Lewis rats) transplants experiments. Samples of all encapsulated islet preparations were tested for viability and only islets shown to be viable by histological and functional tests were used for transplant studies. A control group of animals received an equivalent number of empty microcapsules containing neither islets nor FGF-1. All animals in the studies were given morphine and painkillers following surgery. After 5 days of microcapsule transplantation, animals were placed on normal *ad libitum* chow diet. According to our approved protocol guidelines, whenever two consecutive blood glucose measurements in any transplant recipient were >400 mg/dL, insulin was administered subcutaneously at the dose of 10 U/kg.

3.5 Post-transplant assessments

3.5.1 Blood collection and Blood Glucose and Body Weight monitoring—Blood samples were collected once in two weeks after the surgery in order to assess the endocrine functions of encapsulated islets *in vivo*. Plasma were separated and stored in -20°C until they were used to measure C-peptide. Food intake, body weight, random-fasting blood glucose levels were routinely measured. Blood glucose was measured from the tail vein using the lancet technique with a glucometer and body weight was measured with an electronic scale.

3.5.2 Plasma C-Peptide—C-Peptide levels were measured with a rat C-Peptide ELISA kit (Mercodia) according to the protocol guidelines provided with the kit.

3.5.3 Hematoxylin and Eosin (H&E) Stain—At the end of 90 days transplant follow up, all transplant recipients were euthanized and omentum pouches and pancreases were harvested and fixed in 10% neutral buffered formalin overnight. The tissues were washed with deionized water and transferred to 60% ethanol prior to being processed with a tissue processor (Leica ASP300 S) and embedded in paraffin blocks (Leica EG1160). The microscope slides were accordingly labeled with an automated inkjet printer (Leica IP S). Thin sections of 5 μm thicknesses were cut with a fully automated rotary microtome (Leica RM2255). These sections were stained with an autostainer (Leica Autostainer XL ST5010 staining system) for H&E.

3.5.4 Immunohistochemistry—Five μm thick tissue sections were also stained for insulin and CD-31 markers. The paraffin sections were first de-paraffinized and hydrated. Antigen retrieval was performed with a microwave. After blocking the slides for non-specific binding with Dako serum free protein block, the primary antibodies dilutions for insulin (1:2000; mouse monoclonal anti-insulin antibody, Sigma-Aldrich) and CD-31 (1:25; PECAM-1, goat polyclonal IgG, Santa Cruz, CA) were prepared in Dako antibody diluent. These primary antibodies were detected with goat anti-mouse IgG (Alexa Fluor 488, Invitrogen) and rabbit anti-goat IgG (Alexa Fluor 594, Invitrogen) at dilutions of 1:200.

Both H&E and immunohistological sections were imaged at 20x with an upright microscope (Leica DM4000 B, Leica Microsystems), using the Imagepro software. Cell viability was quantitatively assessed in the live-dead assay in which the CFDA/PI technique stains the living cell with green dye, while if the cell membrane is compromised, the Ethidium Homodimer binds to the nuclei of the damaged cells and stains it red. Cells in both naked islets and islets encapsulated in the microcapsules were imaged with a confocal microscope utilizing z-stack imaging. The z-stacks were compressed to a single image to get a composite image consisting of information from the whole z-stacks and representing the 3D morphology. Quantitation of the live/dead islet cells was performed by counting green and red pixels from the areas of interest (islets only) and viability was calculated based on the pixel count using the NIH Image J software.

4 Data analyses

Statistical evaluation of multiple groups of data was performed using a one-way analysis of variance (ANOVA) with the Tukey-Kramer post-tests (GraphPad Prism software). For comparison of two groups of data, the *student's* t-test was used. In all cases, a value of p 0.05 was considered as significant.

5. Results

Figure 1 shows images of samples of Dithizone-stained islets used to assess viability at different stages during our studies. Figure 1a shows dispersed islets immediately after digestion of the pancreas; at this stage there is lot of exocrine tissue in the tissue digest. Figure 1b shows an islet after the Optiprep purification step indicating significant improvement in the purity. Following the Optiprep purification, we further purified the islets by hand-picking using a stereo-microscope under a cell culture hood (Biosafety level II) thus leaving very little exocrine tissue in the preparation before encapsulation (Figure 1c). Figure 1d shows an islet within an APA microcapsule ready for transplantation.

Prior to transplantation, the islets were first characterized for viability and functionality. Figure 2 shows a sample of pictures of live/dead stains of naked and encapsulated islets used for quantitative viability analyses. While the mean $\pm$ SD viability was similar for the fresh islets before and after encapsulation ($75\pm3\%$ and $77\pm2.7\%$ for naked and encapsulated, respectively), the encapsulated islets retained their viability ($75\pm3.4\%$) in contrast to naked islets whose viability dropped to $60\pm4.2\%$, ($p = 0.001$, $n = 5$) after overnight culture in RPMI. This suggests that the 3D environment created by the microcapsules helps to preserve the structural integrity of the islets thus preventing them from losing cell mass in culture.

We found no difference in daily food intake among the transplant groups throughout the study. Two weeks after transplantation insulin pellets were carefully removed from all control (empty) microcapsule and islet transplant recipients, and the control animals were then maintained on daily insulin injections throughout the study. The mean non-fasting blood glucose levels measured in the isografts, allografts, and control (empty microcapsule) recipient animals are shown in Figure 3. The marginal mass of transplanted islets reduced the blood glucose of the recipients to a range of 300 – 400 mg/dL compared to consistently greater than 500 mg/dL for control animals. The mean blood glucose measured in the allografts was reduced by 22% compared to control ($p<0.001$), and this level of reduction in blood glucose levels was similar to that observed with isografts, showing that our microencapsulation procedure effectively protected the allografts from the host body's immune system in the absence of any immunosuppressant. These observations on blood glucose levels were in agreement with the level of insulin secretion in the three groups of animals based on C-peptide levels measured in the animals. Once the diabetic model was established the animals did not produce any detectable C-peptide, but following transplantation with encapsulated islets, C-peptide levels increased significantly and remained stable through the duration of study (Figure 4). The plasma C-peptide levels measured after transplantation of the marginal mass of islets was lower than the level measured in normal non-diabetic rats, as shown in Figure 4; however, the post-transplant insulin secretion was sufficient to maintain the pre-transplant body weights of the islet transplant recipients in contrast to the control group whose mean body weight fell precipitously after transplantation of empty microcapsules and removal of the insulin pellets. Despite receiving daily insulin injections, the body weights of the control group remained lower than the body weights of the islet transplant recipients ($p<0.05$) throughout the study, as shown in Figure 5.

We did not observe any additional effect of co-encapsulation of FGF-1 with islets on blood glucose levels (Figure 6), albeit, the plasma C-peptide levels (Figure 4) were significantly lower ($p<0.05$) in the alloislets + FGF-1 microcapsule recipients compared to the recipients of encapsulated islet allografts alone. Interestingly, despite lower level of insulin secretion as determined by the C-peptide levels, the rats that received FGF-1 with islet transplants gained significantly more weight ($p<0.05$) than those that received islet transplants alone

(Figure 7), suggesting that FGF-1 might have a trophic effect on body weight independent of insulin.

At the end of the 90 days of islet transplantation the animals were sacrificed and the pancreata and omentum pouches were retrieved and characterized (See Figures, Supplemental Digital Content 1 and 2). In summary, Supplementary figure 1(a) shows a retrieved omentum pouch after 90 days transplantation in a diabetic rat. Microcapsules can be clearly seen with the blood vessels spread throughout the pouch indicating good integration of the blood supply to the microcapsules. Supplementary figure 1(b) shows the H&E section for a normal rat pancreas. The islets can be seen distributed throughout the tissue with blood vessels in the section. In contrast, a diabetic rat pancreas which contains no live islets is shown in Supplementary figure 1 (c). Supplementary figure 1 (d), (e) and (f) show the H&E sections of microcapsules in an omentum pouch retrieved after 90 days from animals which received empty microcapsules, encapsulated islet allografts, and encapsulated islet isografts, respectively. Both encapsulated allogeneic and isogeneic islets can be observed in the microcapsules with surrounding blood vessels.

Supplementary fig. 2a and 2b shows the sections of pancreases from a normal healthy rat and our diabetic model, respectively. We can clearly detect insulin in the normal rat section while no insulin was visible in the diabetic rat. Supplementary fig 2(c), (d) and (e) are the sections of the omentum pouch where the microcapsules were transplanted. Insulin is clearly visible in both 2c and 2d, which received allografts and isografts, respectively. This suggests that islets were still active and functional after 90 days of microencapsulated islet transplantation. No necrotic centers in the islets were detected. These data are also supported by blood glucose, c-peptide and animal weight results. Fig 2(e) is a section from blank microcapsules transplanted in omentum tissue of control animals and hence has no insulin. No immune cells were detected inside the microcapsules and no morphological damage to the microcapsules was detected, this indicates that the APA microcapsule protected the islets from the host body immune cells

6. Discussion

Microencapsulated islets have been routinely transplanted in the peritoneal cavity, a site from which graft retrieval is difficult and where islets are prone to extended periods of hypoxia [9]. Numerous studies have provided strong evidence indicating that prolonged hypoxia is deleterious to islet survival and function [19–21, 36]. To address these issues, we recently described a modified microencapsulation scheme suitable for islet immunoisolation and angiogenic protein delivery for enhanced graft vascularization in a retrievable omentum pouch [12]. In that report we showed that the sustained delivery of FGF-1 from this system can increase vascular density around the microcapsule graft. In the present study, we have observed that islets encapsulated in the re-designed microcapsules and transplanted in the omentum pouch have sustained graft function in immune-competent STZ-diabetic rats indicating the effectiveness and durability of the microcapsules as immunoisolating devices for islet transplantation.

The alginate microcapsules used contained a perm-selective membrane made with poly-L-ornithine (PLO), which has been shown to be superior to poly-L-lysine (PLL), as it invokes less immunogenic reaction and produces mechanically stronger capsules [8,37]. However, one study had suggested that a higher immune response was induced by PLO and poly-D-lysine coating compared to PLL [38] in contrast to observations in the more recent study by Tam SK et al [37]. The reason for the discrepancies reported in immune responses to the polycationic coating membranes in these previous studies is unclear, but our data in the present study are consistent with the observations from the study by Tam SK et al. We

would like to point out that in the modified encapsulation procedure used in our study the PLO membrane is covered with a cross-linked alginate layer, which would preclude the exposure of the PLO membrane attributable to any degradation of the external alginate coat, thereby preventing possible reactions to the membrane that could occur. It has been recently shown that degradation of the uncross-linked outer alginate layer leaves the underlying PLO membrane exposed, which ultimately leads to reduced biocompatibility *in vivo* [39]. Our modified microencapsulation system contains a thick and crosslinked outer alginate layer which may explain why the microcapsules remained intact without any apparent fibrotic reactions in the retrieved omentum tissues after 90 days of transplantation. It is noteworthy that alginate microcapsules with perm-selective PLO membrane for encapsulated pig islets are currently being used in clinical trials by Living Cell Technologies, Auckland, New Zealand [40]

In a previous study, we had observed that sustained delivery of FGF-1 from the outer alginate layer of the multilayer APA microcapsule resulted in enhanced vascularization of the microcapsule grafts in the omentum pouch [12]. In the present study, the transplantation of APA microcapsules with islets encapsulated in the inner alginate core and FGF-1 in the outer alginate layer did not result in improved graft function as judged by the assessment of glycemic control and C-peptide secretion, perhaps because the endogenous vascularity of the omentum [41] was sufficient for adequate supply of oxygen and nutrients to the graft. A recent report by Veriter S, et al [42] showed that although co-encapsulation of islets with mesenchymal stromal cells (MSCs) improved implant oxygenation and neoangiogenesis it had only slight improvement in the function of encapsulated islets transplanted subcutaneously, a site that is even less vascularized than the omentum. Surprisingly, in our studies with islets co-encapsulated with FGF-1, we found that although insulin secretion after transplantation of these islets in the omentum pouch was lower than obtained in the transplantation of encapsulated islets alone, the diabetic rats transplanted with islets and FGF-1 gained significantly more weight than those that received encapsulated islets without FGF-1. This observation suggests a potential trophic effect of FGF-1 hitherto unreported.

The stable plasma C-peptide levels that were measured for 90 days after transplantation of the islets encapsulated in multi-layer alginate microcapsules in the omentum pouch clearly demonstrate long-term viability of the encapsulated islet transplants at this site. A previous study with islets encapsulated in agarose and transplanted in omentum pouches created in NOD mice had also shown long-term survival of these encapsulated islets at this transplant site [25]. However, agarose is a neutral polymer [7] that is difficult to use for immunoisolation of cells for transplantation, in contrast to alginate, the most widely used biopolymer for perm-selective coating of microcapsules [7, 43], which we have used in the present study.

7. Conclusions

In this study we have shown that our multilayer APA microcapsules can be effectively used for islet graft immunoisolation in immune-competent STZ-diabetic rats. Furthermore our data indicate that the retrievable omentum pouch is a viable site of encapsulated islet transplantation. We therefore propose that the omentum should be considered as an alternative site to the avascular peritoneal cavity where encapsulated islet transplants are subjected to a long period of hypoxia and subsequent cell death. Our data also suggest that the induction of new microvessels by FGF-1 delivered from the outer layer of the microcapsules [12] offers no additional benefit to encapsulated islet grafts in the omentum. It is well known that the omentum is a highly vascularized tissue with endogenous angiogenic factors [41], and it appears that the ambient vascularity of the omentum provides adequate supply of nutrients and oxygen to the islet transplants. The lack of effect of

enhanced neovascularization on islet graft function observed in this study is consistent with recent reports which indicate that islet vascularization is necessary in developing but not adult pancreatic islets in which the primary role of blood vessels has been suggested to be facilitating rapid and adequate delivery of insulin [44–46]. It is noteworthy that there is abundant omental tissue to accommodate high transplant volumes of encapsulated islets in large animal and human studies. Finally, our novel observation of a possible FGF-1 mediated promotion of body weight gain in diabetic rats that received transplantation of islets co-encapsulated with FGF-1 warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Immediately following digestion and washing of the pancreatic tissue digest, islets were stained red with Dithizone amidst a lot of acinar tissue (a). After purification with the Optiprep gradient, the purity of the preparation improved with little exocrine tissue (b), prior to handpicking for enhanced purity before encapsulation (c). After encapsulation and Dithizone staining, an islet enclosed in the APA microcapsule is shown in (d).

Figure 2.

For quantitative viability assessments live and dead cells islets were stained with the CFDA/PI dyes and the live and dead cells were imaged and assessed immediately after isolation (a); 24 hours after isolation (b); immediately following microencapsulation (c); and 24 hours after encapsulation (d).

Figure 3.

Microencapsulated islets, isolated from either normal Lewis rats (isografts) or Wistar-Furth rats (allografts) were transplanted in omentum pouches created in STZ-diabetic Lewis rats. Control empty microcapsules were also transplanted in a group of diabetic rats and non-fasting blood glucose levels were measured daily for 90 days. Data represent the mean blood glucose levels in each group without error bars to avoid distortion of group means.

Figure 4.

Islets isolated from either normal Lewis rats (isografts) or Wistar-Furth rats (allografts) were microencapsulated with or without FGF-1 prior to transplantation in omentum pouches created in diabetic Lewis rats. Control empty microcapsules were also transplanted in a group of diabetic rats and plasma C-peptide levels were measured biweekly in all for 90 days using the Mercodia rat C-Peptide ELISA kit. The mean plasma C-peptide levels measured in normal Lewis rats are provided for reference, while no C-peptide was detected in the control microcapsule recipients, which received daily insulin injections.

Figure 5. Microencapsulated islets isolated from either normal Lewis rats or Wistar-Furth rats (allografts) were transplanted in omentum pouches created in diabetic Lewis rats. Control empty microcapsules were also transplanted in a group of diabetic rats and body weights were measured daily for 90 days.

Figure 6.

Islets isolated from Wistar-Furth rat donors were either microencapsulated alone (islet microcapsules) or coencapsulated with FGF-1 (islet+FGF-1 microcapsules) prior to transplantation in omentum pouches created in diabetic Lewis rats, and blood glucose levels were measured for 90 days. Data represent the mean blood glucose levels in each group without error bars to avoid distortion of group means.

Figure 7.

Islets isolated from Wistar-Furth rat donors were either microencapsulated alone (islet microcapsules) or coencapsulated with FGF-1 (islet+FGF-1 microcapsules) prior to transplantation in omentum pouches created in diabetic Lewis rats, and body weights were measured for 90 days. The data represent mean body weight in each group, $n = 5/\text{group}$ without the error bars to avoid distortion of group data. The mean $\pm$ SD weight gained by the islet+FGF-1 group was 61.7 ± 12.6 g compared to 33.2 ± 18.6 g gained by the islet alone transplant group ($p < 0.05$).