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Allogeneic Bone Marrow Co-Cultured with Human Islets Significantly Improves Islet Survival and Function in vivo

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Abstract

Background—A significant barrier to islet transplantation is the rapid loss of human islet function in vivo. The present study evaluates whether bone marrow (BM) could be used to support human islet survival and function in vivo.

Methods—We co-cultured human islets and BM for three weeks prior to transplantation into the left subrenal capsule of diabetic SCID mice.

Results—The co-cultured human islets prior to transplantation demonstrated improved viability, increased size and migration capacity in vitro. After 4 months, animals transplanted with pre-cultured BM /human islets exhibited euglycemia and detectable human insulin levels (157 μ U/ml), while no human insulin was detected in the islet only transplantation group. Furthermore, the

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removal of the transplants on day 126 resulted in hyperglycemia indicating that the reduction of blood glucose was dependent on the transplants. Diabetic mice transplanted with BM/islets demonstrated the longest survival period (130 days vs 40 days for those with islet only transplants). The transplanted BM/islets showed signs of vascularization and migration from the renal capsule into medulla.

Conclusions—Our results suggest that BM pre-cultured with human islets may enhance the survival and function of transplanted islets, thus significantly improving the therapeutic efficacy of islet transplantation for type 1 diabetes.

Keywords

Allogeneic Bone Marrow; Human Islet; Diabetes

Introduction

The occurrence of diabetes mellitus has increased steadily worldwide (1, 2). Improvements in immunosuppressive regimens have made islet transplantation a feasible clinical choice with which to treat type 1 diabetes (3). However, efforts toward routine islet cell transplantation have been hindered not only by scarce islet availability but also by low rates of post-transplantation islet survival and function (4, 5). Islets generally required to achieve insulin independence is 12,000 islet equivalents per kg of recipient body weight, and this number is usually obtained by transplanting more than one set of islet preparation per patient (6). Early graft loss resulting from repeated transplantation of islets (7) is a major component of islet dysfunction, which occurs in syngeneic islet transplantation (8) as well as T-cell activation (9, 10). After transplantation, only small proportions are successfully engrafted while large numbers of islets are destroyed. In experimental models of syngeneic islet transplantation, up to 60% of islet cell mass underwent apoptosis with half of these losses occurring within the first 3 days of transplantation (11). Functional islet mass is reduced even in successfully transplanted recipients compared to healthy individuals. Poor islet viability may be attributed to the loss of a suitable microenvironment.

Numerous efforts have been made to improve islet cytoprotection and the success rate of transplantation (12). The early application of perfluorocarbons into transplantable tissue (two-layer method) allowed for the increased availability of oxygen to the tissue and permitted increased adenosine triphosphate (ATP) content in the organ (13, 14). Use of additives in the culture media (antioxidants, hormones, etc.) resulted in reduced islet cell death, improved islet recovery after isolation, and better function, representing a minimally invasive strategy for the optimization of islet engraftment (15, 16). Molecular biology approaches to achieve islet cytoprotection have used various vectors (including viruses) to transfer genes that may inhibit apoptosis, increase growth factors (17) or even reprogram cells (18). Delivery of cytoprotective proteins by protein transduction allows delivery of proteins/peptides fused into small cationic cell-penetrating peptides to cells or tissues in order to prevent islet apoptosis (19).

We have previously reported that allogeneic bone marrow is capable of supporting human islet survival and function for over six months (20). Bone marrow reduced the release of IL-1 β in islets, thus inhibiting the apoptotic process in cultured islets (21, 22). BM subtype MSCs were also demonstrated to be able to secrete paracrine factors such as HGF, IL-6, and TGF- β resulting in protection against hypoxia and a reduction of apoptosis (23–25).

We propose two potential ways in which allogeneic BM derived mesenchymal stem cells (MSCs) and endothelial progenitor cell (EPCs) are capable of creating a suitable islet microenvironment. One possibility is EPCs initiating angiogenesis for the revascularization

of islets, which repair destroyed microvessels in the islet, thus supporting islet repair and function. This includes the action of MSCs and EPCs in initiating vascularization within human islets (26) to support α , β , and other endocrine cells. This strategy can be used in the recipient's bone marrow cells with allogeneic human islets, which could alleviate the immune response. In this specific study, the interaction of human islets and bone marrow in the in vitro culture system has been explored and analyzed.

Results

Interaction between human BM and islets in vitro

Towards the beginning of co-culture (7 hours to 96 hours), labeled human BM cells gradually migrated towards islets to form an integrated tissue (Figure 1). Initially, the two types of cells merged together (Figure 1A, 1B). After 24 hours co-culture, cells which indicate the merger between bone marrow cells (red) and insulin-producing cells (green) appeared (yellow) (Figure 1C, 1D). To investigate the long-term interaction between human islet and bone marrow, in vitro co-culture samples were kept for further observation. Without the support of bone marrow, human islets gradually lose structure and became monolayer at day 60 (Figure 2D, E, F) while co-cultured human islets recruited bone marrow cells (Figure 2A,B,C). Human islet growth was observed throughout long-term culture (Figure 3G,H). Grids at the bottom of culture slides were used to navigate cell location and migration of human islets. Islet size on day 71 was significantly larger than that on day 54 ($p < 0.01$). The size changes in co-cultured human islets and bone marrow was observed and indicated by the arrows. Images of two co-cultured human islets were taken starting on day 2 of culture and continuously monitored. Consecutive images (every 4 days) showed migration (Figure 3. A,B,C,D) to form a single islet after 22 days of culture (Figure 3.E, F). This phenomenon only existed in the BM/islets co-culture system (Figure 3).

Human islet insulin release in vitro and in vivo

Prior to transplantation, tissues were maintained in culture for three weeks and insulin concentrations from each group were measured in order to ensure the quality of the transplanted human islets. Figure 4D showed approximately equal levels of insulin release (over 2000 $\mu\text{U/ml}$) between the human islet only and the human islet plus bone marrow co-culture transplantation group. Human insulin was detected in animal blood in the bone marrow and human islet co-culture transplantation group with a concentration of 130 $\mu\text{U/ml}$ (Table 1). Human insulin concentrations in the other groups were below the detectable range (less than 1 $\mu\text{U/ml}$).

Lifespan of diabetic mice post transplantation

The lifespan of the diabetic mice was used as another indication of the therapeutic efficacy of different transplantation tissue. In figure 4D, diabetic mice transplanted with bone marrow and human islets survived the longest with an average of over 130 days. Diabetic mice with human islet transplantation alone survived for approximately 40 days. The bone marrow alone group showed to have a lifespan of 30 days. The diabetic mice control group had a lifespan of only 20 days.

Hyperglycemia control via human islet transplantation

BM transplantation alone was unable to reduce glucose levels (Figure 4B.). When fresh islets (500 IEQ) were transplanted alone, glucose levels were reduced on day 5 through day 40. However, this effect was not permanent as glucose levels rose again shortly thereafter (Figure 4B). Co-cultured islets (pre-cultured for 3 weeks) transplanted into the renal capsule of diabetic SCID mice reduced hyperglycemia in concordance with detectable levels of

human insulin in animal blood for 4 months. Hyperglycemia returned when transplanted islets were removed, illustrating that the reduction in glucose levels was a direct result of BM co-cultured islets. Animals transplanted with the islet only cultures showed no human insulin in blood and high blood glucose levels, suggesting that human islets without BM (also pre-cultured for 3 weeks) did not offer significant long term benefits for the reduction of blood glucose levels in hyperglycemic animals (Figure 4C).

BM co-cultured human islets have the capability to migrate and induce angiogenesis

Transplanted animal kidneys were harvested and evaluated using CD 31 antibodies for endothelia proliferation and human insulin antibody for islet β -cells. A significant portion of co-cultured human islets (with positive human insulin staining) was localized in vascular areas of the kidney with an increased endothelia cell presence (CD 31+) versus no significant insulin staining and few CD31+ endothelia cells were observed in islet only groups (Figure 5.B). The location of the CD31+ cells and insulin producing cells after transplantation was focused in the canal area of the kidney versus the original transplantation site on the outer layers of the renal capsule (Figure 5.A). Figure 5E showed insulin producing cells located close to the blood vessels around the kidney medulla and can be clearly observed by the staining of CD 31+ cells. In Figure 5F and 5G, image analysis of the CD31+ expressed cells and insulin positive cells showed that kidney tissue sections collected from BM/islets transplanted diabetic mice contain more CD31+ and insulin positive cells.

Discussion

Our previous study reported in vitro human islets co-cultured with bone marrow sustained function and structure of human islets for over 6 months versus the loss of integrity of human islets when cultured alone. In the current study, the interaction between bone marrow cells and human islets in long term co-culture was further examined. The addition of bone marrow cells marked with PKH26 (red) to human islets showed immediate bone marrow migration towards human islets to form an integrated tissue. The newly formed tissue further demonstrated growth and migration.

Quality of native human islets after isolation from the host tissue deteriorates rapidly. Up to 60% of pancreatic islet tissue undergoes apoptosis in vitro (27–29). The incorporation of bone marrow promoted cell proliferation resulted in an increase in human islet size (Figure 3). The specific mechanism behind this effect was unveiled partially by our previous studies showing bone marrow-initiated human islet vascularization and growth (30).

However, we believe that human islet function was maintained not only through a reduction of apoptosis but also through regeneration by bone marrow cells. The phenomenon of bone marrow migration towards human islets in vitro is intriguing. There is strong evidence for the phenomenon of BM migration into areas of tissue damage to promote vascular infrastructure and potentially regenerating into insulin positive cells (31–35). More specifically, MSCs have shown to ability to secrete matrix metalloproteinases to degrade extracellular matrix in order to allow the migration of endothelial cells (12, 36). The promotion of endothelial cell migration was also observed with Adipose-derived stem cells (37). In vivo studies suggest (38–40) that BM contributes to pancreatic islet regeneration. MSCs are seen as the subtype attracted by pancreatic islets via the MSC cell surface markers. Despite a wide expression of MSC surface markers, CXCL12 and CX3CL1 are believed to be involved in the migration of MSCs to pancreatic islets (41).

The significance of this phenomenon in vivo was discovered when we analyzed the transplanted kidney capsules. The two groups of cells fused into one cluster, indicating a

possible mechanism behind the effect of bone cells on human islet growth and development. This process is crucial to the growth and development of human islets as it allows human islets to migrate into nutrient rich areas to maintain survival and activity after transplantation.

Diabetic mice transplanted with co-cultured bone marrow and human islets survived longer than every other group (the control group, human islet transplantation group and the bone marrow transplantation group – Figure 4D). However, Islet transplantation alone was able to prolong lifespan due to glycemic control. Interestingly, bone marrow alone was also able to prolong lifespan, which may be attributed to mechanisms related to tissue repair or anti-inflammation.

Transplanted human islets reduced blood glucose levels for a short duration as seen in Figure 4B. However the function gradually diminished after transplantation as indicated by the hyperglycemia shown in Figure 4C. This is consistent with previous reports that human islets are very sensitive to the culture environment and the function of fresh islet transplants deteriorate rapidly after isolation (27). The longevity of human islet transplantation is still a significant challenge for the effective application of cell transplantation to treat diabetes. Bone marrow transplantation has been initially used to achieve donor-specific transplantation tolerance from an immunological perspective. Through modulating the host's immune response, bone marrow transplantation could enhance human islet survival rates (42). On the other hand, the effects of BM on islets does not seem to be thwarted by immune-related factors. In limited studies, we observed minimal T-cell loads and there was no correlation between matched versus mismatched HLA and islet/BM co-culture function (preliminary studies not shown). This is consistent with studies which have reported the inhibition of T-cells when bone marrow was cultured in vitro (43).

Several studies reported the differentiation capacity of bone marrow mesenchymal stem cells into insulin-producing cells (44, 45). The role of bone marrow on human islets is still a topic of debate. Negative results have been reported on whether bone marrow cells are capable of differentiation (46, 47). In our study, we found that bone marrow transplantation alone cannot reverse hyperglycemia in diabetic mice (Figure 4B). On the other hand, transplantation of human islets cultured with bone marrow significantly improved human islet function in terms of reducing hyperglycemia (Figure 4C). Bone marrow cells migrated towards human islets and gradually integrated within human islet tissue (Figure 1). We believe that a direct interaction between BM and islets through co-culturing prior to transplantation is necessary to achieve islet survival and function. Prior studies show an increase in the number of insulin positive cells as a result of BM differentiation (48). This could also be coupled with β -cell regeneration due to a supportive microenvironment. The failure of bone marrow transplantation alone to correct hyperglycemia could be attributed to insufficient interaction between stem cells and islets in vivo due to the location of transplants in the kidney capsule. Bone marrow may require stimuli via released factors and or direct contact with islets in order to support islet function.

In our previous studies, we observed a vessel-like structure within human islets (30). This finding indicates revascularization after two months of culture versus a loss of structure and function after the same period of culture for the islet only group. In the present study, the signs of angiogenesis were confirmed by the immunohistochemical staining images shown in Fig 5. Bone marrow derived cell initiated angiogenesis in human islets have been confirmed in both in vitro and in vivo studies (12, 31, 33). Many CD31+ positive cells were observed around the insulin positive cells (Fig. 5) and most CD 31+ and insulin positive cells were localized around the kidney medulla (Fig 5E). The migration from the renal cortex to the medulla observed in vivo is consistent with the in vitro observations (Fig 3).

The quantification of CD 31+ cells within two types of explants (human islet only versus human islet and bone marrow transplantation) showed more CD 31+ and insulin positive cells existed in BM/islets transplanted kidney tissue sections (Fig. 5F, G), indicating that bone marrow is very important for initiating vascularization.

Based on these results, the positive effects of human islets co-cultured with bone marrow for islet transplantation can be summarized into three main aspects: 1) improved human islet function; 2) angiogenesis within human islets; 3) human islet proliferation and regeneration in vivo.

Materials and Methods

Human islet and bone marrow (BM) cell culture

Human pancreatic islets—Human islets, from healthy donors, were obtained from Integrated Islet Distribution Program (IIDP) in the IIDP Basic Science Islet Distribution Program, Human Islet Laboratory, University of Pennsylvania (Philadelphia, PA), Massachusetts General Hospital (Boston, MA), City of Hope National Medical Center (Duarte, CA) and University of Miami (Miami FL). The use of these cells is approved by the Institutional Review Board (IRB) at Roger Williams Hospital and the IIDP Committees.

Harvested Human BM—Human BM from normal donors was obtained under a separate Roger Williams Hospital IRB approved protocol. Bone-marrow erythrocytes were isolated by Ficoll-Paque™ Plus (Amersham Biosciences; Amersham, UK) per manufacturer directions. Cells were then washed twice with 10% fetal calf serum (FCS) in phosphate buffered saline (PBS), resuspended in culture medium (see below). Trypan blue staining was used (manufacturer) to assess for cell viability.

Allogeneic BM co-culture with human islets—Human islets were received from Islet Cell Resource Centers (ICRs) within 48 hours after harvest from cadaveric donors. Purity of islets in total isolated tissue was 75 ~ 90% as assessed by dithizone staining and viability was > 95% as determined by trypan blue dye exclusion. Islets were maintained in RPMI 1640 (GIBCO) supplemented with 10% heated inactivated Fetal Bovine Serum (HiFBS, GIBCO), 5.5 mM glucose, 10 mM HEPES, and 1% PS. Whole BM progenitors were used in co-culture at 1×10^6 allogeneic BM cells/mL. The ratio of islet equivalent to BM cells was $1:10^4$. Co-cultured cells were in the same culture conditions as islet-only groups.

Labelling BM cells with PKH26—BM cells labeling with PKH26, a fluorescent membrane dye, was performed according to the manufacturer's instructions (Sigma, St Louis, Mo.). Specifically, bone marrow cells (1×10^6 allogeneic BM cells/ml) in culture medium were incubated with 0.2 μ M PKH26 for 2 min at room temperature. Cell images were taken under fluorescent microscopy.

Human insulin assay—Insulin concentrations in specimens (cell culture media or animal blood) were measured using a Human Insulin ELISA Kit (Linco Research, St. Charles, MO) according to the manufacturer's instructions. Briefly, insulin standards and diluted (1:50–1:500) samples were added to an insulin antibody-coated 96-well microplate and incubated for 2 hours at 4 °C. After 5 washings, anti-human insulin enzyme conjugate was added to each well and incubated for 30 minutes at room temperature. After 7 more washings, enzyme substrate solution was added and then incubated for 45 minutes at room temperature in the dark. The reaction was halted by adding 1N sulfuric acid. Absorbance at 450 nm was measured with a μ Quant™ microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) and

insulin concentrations calculated using KC Junior[®] microplate reader software (Bio-Tek Instruments, Inc.).

Transplantation of cultured human islets into SCID mice

After seven days post arrival, mice (8 weeks old male Balb/c SCID mice, Charles River, MA) were injected with streptozotocin (STZ, St. Louis, Sigma) 200 mg/kg body weight intraperitoneal (i.p) to induce diabetes (blood glucose levels over 400 mg/dL). After three days of consistent hyperglycemia, animals were transplanted. Islets (500 IEQ) co-cultured with BM for three weeks (average insulin level 2000 μ U/ml) served as the experimental group and an equal amount of islet only cultures served as control. Under anesthesia, cultured islets with or without BM were implanted into the left subrenal capsule of the diabetic SCID mice. Blood glucose levels in these animals were monitored daily in the first week post operation and once a week after a significant reduction in glucose levels. Glucose levels were checked using Accu-Check test strips with blood drawn (one drop) from the tail vein. After blood glucose levels decreased to under 200 mg/dL for two months, the implanted tissues were removed from animals and animals were sacrificed after two days. The protocol for animal studies was approved by the Institute Animal Care and Use Committee (IACUC) at Roger Williams Hospital.

Immunohistochemistry

Kidney tissues were frozen with liquid nitrogen, cut to 5 μ m in thickness, and fixed with 4% paraformaldehyde on glass slides followed by exposure to 10% normal goat serum. The slides were blotted and primary antibodies were applied. The slides were then incubated in a moist chamber at 4 °C overnight. The slides were washed 3 times, followed by exposure to the secondary antibody for 45 minutes at room temperature. After washing, diluted secondary antibody was applied and the slides were incubated for 15 minutes. The slides were subsequently washed with PBS and the above process repeated with a second fluorescent dye and a third antigen-detecting antibody. Slides were cover slipped using a fluorescent mount medium and evaluated using fluorescent microscopy(48). Primary antibodies used in this study include: mouse mAb to proinsulin (1: 100 dilution Abcam), rabbit pAb to CD31(Abcam) (1:100 dilution). Secondary antibodies used include: Texas Red anti rabbit IgG (1:1000; Vector) and Fluorescein anti mouse IgG (1:1000 dilution; Vector).

Image analysis

To evaluate islet size and vascularization, 20 images from each group were captured and images were analyzed through examining size via pixels using the NIH Image J software according to previous publications (48).

Statistical Analysis

The correlation of islet size to islet vascularization was analyzed by two ways ANOVA. Data is presented as mean $\pm$ standard error (std). In the text and figures, all data were presented as means $\pm$ STD. Data were analyzed by ANOVA statistics program using a two factor analysis of variance of repeated measures. Post hoc comparisons among individual means were made by Turkey's Test.

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References

1. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*. 2006; 355:1318. [PubMed: 17005949]
2. Rother KI, Harlan DM. Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. *J Clin Invest*. 2004; 114 (7):877. [PubMed: 15467822]
3. Azzi J, Geara AS, El-Sayegh S, Abdi R. Immunological aspects of pancreatic islet cell transplantation. *Expert Rev Clin Immunol*. 6(1):111. [PubMed: 20383895]
4. Cravedi P, van der Meer IM, Cattaneo S, Ruggerenti P, Remuzzi G. Successes and disappointments with clinical islet transplantation. *Adv Exp Med Biol*. 654:749. [PubMed: 20217523]
5. Vaithilingam V, Tuch BE. Islet transplantation and encapsulation: an update on recent developments. *Rev Diabet Stud*. 8(1):51. [PubMed: 21720673]
6. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. 2000; 343 (4):230. [PubMed: 10911004]
7. Davalli AM, Scaglia L, Zangen DH, Hollister J, Bonner-Weir S, Weir GC. Vulnerability of islets in the immediate posttransplantation period. Dynamic changes in structure and function. *Diabetes*. 1996; 45 (9):1161. [PubMed: 8772716]
8. Nagata M, Mullen Y, Matsuo S, Herrera M, Clare-Salzler M. Destruction of islet isografts by severe nonspecific inflammation. *Transplant Proc*. 1990; 22 (2):855. [PubMed: 2109420]
9. Chahine AA, Stoeckert C, Lau HT. Local immunomodulation to promote co-stimulatory blockade. *Clin Transplant*. 1995; 9 (3 Pt 2):215. [PubMed: 7670166]
10. Kaufman DB, Platt JL, Rabe FL, Dunn DL, Bach FH, Sutherland DE. Differential roles of Mac-1+ cells, and CD4+ and CD8+ T lymphocytes in primary nonfunction and classic rejection of islet allografts. *J Exp Med*. 1990; 172 (1):291. [PubMed: 2113565]
11. Barshes NR, Wyllie S, Goss JA. Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts. *J Leukoc Biol*. 2005; 77 (5):587. [PubMed: 15728243]
12. Johansson U, Rasmusson I, Niclou SP, et al. Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization. *Diabetes*. 2008; 57 (9):2393. [PubMed: 18519803]
13. Noguchi H, Naziruddin B, Onaca N, et al. Comparison of modified Celsior solution and M-kyoto solution for pancreas preservation in human islet isolation. *Cell Transplant*. 19(6):751. [PubMed: 20955657]
14. Miyamoto M, Morimoto Y, Balamurugan AN, et al. Improvement of modified two-layer preservation method (PFC/Kyoto solution) in islet isolation from breeder pigs. *Transplant Proc*. 2000; 32 (7):1660. [PubMed: 11119879]
15. Sandler S, Andersson A, Korsgren O, et al. Tissue culture of human fetal pancreas. Effects of nicotinamide on insulin production and formation of isletlike cell clusters. *Diabetes*. 1989; 38 (Suppl 1):168. [PubMed: 2521329]
16. Eizirik DL, Sandler S, Welsh N, Bendtzen K, Hellerstrom C. Nicotinamide decreases nitric oxide production and partially protects human pancreatic islets against the suppressive effects of combinations of cytokines. *Autoimmunity*. 1994; 19 (3):193. [PubMed: 7605871]
17. Wang H, Ferran C, Attanasio C, Calise F, Otterbein LE. Induction of protective genes leads to islet survival and function. *J Transplant*. 2011:141898. [PubMed: 22220267]
18. Giannoukakis N, Rudert WA, Ghivizzani SC, et al. Adenoviral gene transfer of the interleukin-1 receptor antagonist protein to human islets prevents IL-1beta-induced beta-cell impairment and activation of islet cell apoptosis in vitro. *Diabetes*. 1999; 48 (9):1730. [PubMed: 10480601]
19. Embury J, Klein D, Pileggi A, et al. Proteins linked to a protein transduction domain efficiently transduce pancreatic islets. *Diabetes*. 2001; 50 (8):1706. [PubMed: 11473028]
20. Luo L, Badiavas E, Luo JZ, Maizel A. Allogeneic bone marrow supports human islet beta cell survival and function over six months. *Biochem Biophys Res Commun*. 2007; 361 (4):859. [PubMed: 17686461]

21. Maedler K, Sergeev P, Ris F, et al. Glucose-induced beta cell production of IL-1 β contributes to glucotoxicity in human pancreatic islets. *J Clin Invest*. 2002; 110 (6):851. [PubMed: 12235117]
22. Welsh N, Cnop M, Kharroubi I, et al. Is there a role for locally produced interleukin-1 in the deleterious effects of high glucose or the type 2 diabetes milieu to human pancreatic islets? *Diabetes*. 2005; 54 (11):3238. [PubMed: 16249450]
23. Lu Y, Jin X, Chen Y, et al. Mesenchymal stem cells protect islets from hypoxia/reoxygenation-induced injury. *Cell Biochem Funct*. 28(8):637. [PubMed: 21061411]
24. Park KS, Kim YS, Kim JH, et al. Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after transplantation. *Transplantation*. 89(5):509. [PubMed: 20125064]
25. Karaoz E, Genc ZS, Demircan PC, Aksoy A, Duruksu G. Protection of rat pancreatic islet function and viability by coculture with rat bone marrow-derived mesenchymal stem cells. *Cell Death Dis*. 1:e36. [PubMed: 21364643]
26. Brissova M, Powers AC. Revascularization of transplanted islets: can it be improved? *Diabetes*. 2008; 57 (9):2269. [PubMed: 18753672]
27. Noguchi H, Naziruddin B, Shimoda M, et al. Comparison of fresh and cultured islets from human and porcine pancreata. *Transplant Proc*. 42(6):2084. [PubMed: 20692414]
28. Schroppel B, Zhang N, Chen P, Chen D, Bromberg JS, Murphy B. Role of donor-derived monocyte chemoattractant protein-1 in murine islet transplantation. *J Am Soc Nephrol*. 2005; 16 (2):444. [PubMed: 15601743]
29. Amrani A, Verdaguer J, Thiessen S, Bou S, Santamaria P. IL-1 α , IL-1 β , and IFN- γ mark beta cells for Fas-dependent destruction by diabetogenic CD4(+) T lymphocytes. *J Clin Invest*. 2000; 105 (4):459. [PubMed: 10683375]
30. Luo JZ, Xiong F, Al-Homsi AS, Roy T, Luo LG. Human BM stem cells initiate angiogenesis in human islets in vitro. *Bone Marrow Transplant*. 46(8):1128. [PubMed: 21132023]
31. Sakata N, Chan NK, Chrisler J, Obenaus A, Hathout E. Bone marrow cell cotransplantation with islets improves their vascularization and function. *Transplantation*. 89(6):686. [PubMed: 20101199]
32. Ito T, Itakura S, Todorov I, et al. Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. *Transplantation*. 89(12):1438. [PubMed: 20568673]
33. Figliuzzi M, Cornolti R, Perico N, et al. Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats. *Transplant Proc*. 2009; 41 (5):1797. [PubMed: 19545731]
34. Wu XH, Liu CP, Xu KF, et al. Reversal of hyperglycemia in diabetic rats by portal vein transplantation of islet-like cells generated from bone marrow mesenchymal stem cells. *World J Gastroenterol*. 2007; 13 (24):3342. [PubMed: 17659673]
35. Oh SH, Muzzonigro TM, Bae SH, LaPlante JM, Hatch HM, Petersen BE. Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes. *Lab Invest*. 2004; 84 (5):607. [PubMed: 15034596]
36. Ding Y, Xu D, Feng G, Bushell A, Muschel RJ, Wood KJ. Mesenchymal stem cells prevent the rejection of fully allogeneic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9. *Diabetes*. 2009; 58 (8):1797. [PubMed: 19509016]
37. Traktuev DO, Merfeld-Clauss S, Li J, et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ Res*. 2008; 102 (1):77. [PubMed: 17967785]
38. Zorina TD, Subbotin VM, Bertera S, et al. Recovery of the endogenous beta cell function in the NOD model of autoimmune diabetes. *Stem Cells*. 2003; 21 (4):377. [PubMed: 12832692]
39. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest*. 2003; 111 (6): 843. [PubMed: 12639990]
40. Hess D, Li L, Martin M, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol*. 2003; 21 (7):763. [PubMed: 12819790]
41. Sordi V, Malosio ML, Marchesi F, et al. Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood*. 2005; 106 (2):419. [PubMed: 15784733]

42. Exner BG, Fowler K, Ildstad ST. Tolerance induction for islet transplantation. *Ann Transplant*. 1997; 2 (3):77. [PubMed: 9869869]
43. Sajic M, Hunt DP, Lee W, et al. Mesenchymal stem cells lack efficacy in the treatment of experimental autoimmune neuritis despite in vitro inhibition of T-cell proliferation. *PLoS One*. 7(2):e30708. [PubMed: 22359549]
44. Neshati Z, Matin MM, Bahrami AR, Moghimi A. Differentiation of mesenchymal stem cells to insulin-producing cells and their impact on type 1 diabetic rats. *J Physiol Biochem*. 66(2):181. [PubMed: 20422338]
45. Iskovich S, Goldenberg-Cohen N, Stein J, Yaniv I, Farkas DL, Askenasy N. beta-Cell neogenesis: experimental considerations in adult stem cell differentiation. *Stem Cells Dev*. 20(4):569. [PubMed: 20973695]
46. Choi JB, Uchino H, Azuma K, et al. Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells. *Diabetologia*. 2003; 46 (10):1366. [PubMed: 12898006]
47. Lechner A, Yang YG, Blacken RA, Wang L, Nolan AL, Habener JF. No evidence for significant transdifferentiation of bone marrow into pancreatic beta-cells in vivo. *Diabetes*. 2004; 53 (3):616. [PubMed: 14988245]
48. Luo L, Luo JZ, Xiong F, Abedi M, Greer D. Cytokines inducing bone marrow SCA+ cells migration into pancreatic islet and conversion into insulin-positive cells in vivo. *PLoS One*. 2009; 4 (2):e4504. [PubMed: 19225560]

Figure 1.

Florescent images of in vitro human islet and bone marrow co-culture: 7 hours (A), 24 hours (B), 48 hours (C) and 96 hours (D). Bone marrow cells were labeled with PKH26 (Red), whereas human islet β cells were labeled with pre-insulin antibody (Green), cell nuclei were stained with DAPI (blue). Magnification: 40x for ABCD, 67x for enlarged images in the corner of C and D.

Figure 2.

Bone marrow cells maintained the integrity and original structure of human islets. Cell images observed under light microscopy and fluorescent microscopy. Human islets co-cultured with Bone marrow cells (labeled with PKH26 in red color) in vitro on day 1 (A), day 21 (B), day 60 (C). Human islets only cultures on day 1 (A), day 21 (B), and day 60 (C). Magnification: 40x.

Figure 3.

Two human islets co-cultured with bone marrow migrated towards each other and finally integrated into a single islet after 18 days of culture. Images were taken on day 2 (A), day 6 (B), day 10 (C), day 14 (D), Day 18 (E), day 22 (F). The arrows indicate the movement of the cells. Magnification: 20X. Continuous growth of human islets observed under light microscopy (G,H). Islet growth was traced with a grid under the culture slide on day 54, day 60, day 67, and day 71. (G). NIH Image J software measured islet size by calculating pixel numbers covered by islet surface area. Islet size on day 71 is significantly larger than that on day 54. * $p < 0.01$ (H). Magnification: 20x.

Figure 4.

Schematic diagram of in vivo transplantation: co-cultured bone marrow cells and human islets were directly injected into the kidney capsules of diabetic SCID mice. Black arrow refers to the initial implantation site in the renal capsule (A). Monitoring in vivo glucose levels after transplantation with fresh BM, fresh islets, or saline (B). Monitoring glucose levels after transplantation with 3 week pre-cultured islet only cultures, and 3 week co-cultured human islet and bone marrow groups. Islet only mice were euthanized on day 106 due do significantly deteriorating conditions (C) (n=12). Lifespan and human insulin concentrations measured from extracted animal blood for animals without transplant (control), islet (islet only), co-culture (Islet + bone marrow), and bone marrow (bone marrow only) (D)

Figure 5.

Immunohistochemical fluorescent images of kidney explants after two months of transplantation. Transplanted tissue migrated towards vasculature near the renal medulla (A). CD+31 positive cells were stained with Texas red (red). Insulin was stained with FITC (green) and cell nuclei were stained with DAPI (blue). Human islet only transplants (B,C); Human islet and bone marrow transplants (D,E). (Magnification 20x). Quantification of CD 31+ cells within the kidney after transplantation (F); Quantification of human insulin positive cells within the kidney after transplantation (G). (n=3) The CD31+ and human insulin positive cells were counted by capturing the fluorescent images, then followed by measuring the pixels using the NIH Image J software.