Immuno-Isolation of Pancreatic Islet Allografts Using Pegylated Nanotherapy Leads to Long-Term Normoglycemia in Full MHC Mismatch Recipient Mice

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Abstract

Two major hurdles need to be surmounted for cell therapy for diabetes: (i) allo-immune rejection of grafted pancreatic islets, or stem/precursor cell-derived insulin-secreting cells; and (ii) continuing auto-immunity against the diabetogenic endogenous target antigen. Nanotherapeutics offer a novel approach to overcome these problems and here we ask if creation of “stealth” islets encapsulated within a thin cage of pegylated material of 100–200 nanometers thick provides a viable option for islet transplantation. The aims of this study were to test islet viability and functionality following encapsulation within the pegylated cage, and functional efficacy in vivo in terms of graft-derived control of normoglycemia in diabetic mice. We first demonstrated that pegylation of the islet surface, plus or minus nanoparticles, improved long-term islet viability in vitro compared to non-pegylated (naked) control islets. Moreover, pegylation of the islets with nanoparticles was compatible with glucose-stimulated insulin secretion and insulin biogenesis. We next looked for functionality of the created “stealth” DBA/2 (H-2d) islets in vivo by comparing glycemic profiles across 4 groups of streptozotocin-induced diabetic C57BL/6 (H-2b) recipients of (i) naked islets; (ii) pegylated islets; (iii) pegylated islets with nanoparticles (empty); and (iv) pegylated islets with nanoparticles loaded with a cargo of leukemia inhibitory factor (LIF), a factor both promotes adaptive immune tolerance and regulates pancreatic β cell mass. Without any other treatment, normoglycemia was lost after 17 d (+/−7.5 d) in control group. In striking contrast, recipients in groups (ii), (iii), and (iv) showed long-term (>100 d) normoglycemia involving 30%; 43%; and 57% of the recipients in each respective group. In conclusion, construction of “stealth” islets by pegylation-based nanotherapeutics not only supports islet structure and function, but also effectively isolates the islets from immune-mediated destruction. The added value of nanoparticles to deliver immune modulators plus growth factors such as LIF expands the potential of this novel therapeutic approach to cell therapy for diabetes.

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Introduction

Pancreatic β cell transplantation, either in the form of harvested pancreatic islets, or as cells derived from embryonic precursors or following trans-differentiation in vitro, has the potential to restore the recipients’ ability to respond to blood glucose levels and secrete insulin in a physiological manner [1]. However major problems in achieving this ideal include lack of donor islets available for transplantation; loss of the valuable resource of islets during the harvesting procedure; and loss of islets following transplantation due to immune mediated allo-rejection plus lack of trophic support [2]. Although future advances in regenerative medicine may alleviate the problem of availability, all these issues are compounded by continuing autoimmunity against the diabetogenic endogenous target antigen. Considering the immune aspects, while efforts have been centered on systematic modulation of host immune responses for transplantation tolerance, the converse of strategies focused on direct protection of the allograft itself has not been adequately explored. With the advent of new technologies and especially nano-scale devices and materials, the concept of creating physical barriers combined with therapeutic support of transplanted islets or cell populations becomes a realistic option.

Islet encapsulation using immune-isolation devices to facilitate the transplantation of islets so reducing the need for immunosuppression has been explored [3,4]. Macro-capsules (encapsulation of the whole islet graft) and micro-capsulation (encapsulation of
single islets) are the most common approaches for encapsulation [5,6]. However, use of agarose- or alginate-based macro- and micro-capsules is problematic on several counts including lack of clinical-grade biocompatible polymers; the physical thickness of the macro-capsules (mm level) that prevents efficient molecular exchange between the cells of the islet and their micro-environment; and the islet death due to hypoxia and subsequent fibrosis [for review, see [7]]. In the field of transfusion medicine, research has shown that surface modification of red blood cell membranes with non-immunogenic materials such as methoxy- polyethylene glycol] (mPEG) could yield antigenically silent (“stealth”) cells [8]. These “stealth” cells exhibit little or no antiserum-mediated agglutination or antibody binding, and show markedly decreased immunogenicity. Moreover, for lymphocytes mPEG modification prevented MHC class II-mediated T cell activation in the mixed leukocyte reaction [9] and the pegylation procedure itself has no negative effects on normal cell structure, function, or viability [10–12]. Following these findings, attempts to modify the surface of islets with bioreactive chemicals showed that blood-mediated inflammatory responses to the islets can be reduced [13]; furthermore, pegylated islets exhibit prolonged survival in allogeneic hosts without any immunosuppressive treatment [14], whilst a short course of cyclosporine A therapy synergized for even longer survival [15].

Ideally, islet encapsulation with biocompatible materials should exert both isolation and immunomodulation effects by physically isolating islets from inflammatory cytokines and host immune cells, whilst simultaneously delivering immune regulatory factors plus supportive growth factors to the islets. The latter point may allow for relatively low numbers of donor islets providing efficacious control, thereby addressing not only the problem of immune-mediated rejection but also the problems of limited islet supply. However, the PEG of the pegylated layer has insufficient rigidity for loading with a therapeutic cargo; therefore we have explored combining pegylation with nanotherapy.

Very recently biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticles have been designed to carry therapeutic agents plus surface targeting moieties able to decorate the surface of pegylated islets [16–19]. Compared to the traditional immunosolation and immunoregulation methods, such nanoparticles provide a biodegradable, biocompatible slow release vehicle for paracrine-type delivery of cargo to the targeted cell or islets. PLGA has been used for drug delivery and is approved by FDA based on its biodegradability, biocompatibility, adjustable biodegradation kinetics, mechanical properties, ease of processing, and safety [20,21]. PLGA undergoes hydrolysis of the ester linkages in the presences of water to produce the naturally occurring monomers lactic acid and glycolic acid. It has been shown that PLGA nanoparticles loaded with leukemia inhibitory factor (LIF) and targeted to CD4+ T lymphocytes reduce the inflammatory immune response in vivo by promoting regulatory T cells (Treg) [22]. In addition to promoting immune tolerance via Treg, LIF is also well known to promote islet cell survival and LIF regulates β cell mass [23–25]. Using a full mismatch mouse model, here we ask, (i) does construction of “stealth” islets by pegylation decorated with LIF-nano support islet structure and function? and (ii) are such islets able to maintain normoglycemia following transplantation?

Materials and Methods

Animals

Male C57BL/6 and DBA/2 mice at 6–8 weeks of age were purchased from the Jackson Laboratory (Bar harbor, ME). All procedures were carried out using animals less than 12 weeks old and protocols were approved by the IACUC committee at Medical University of South Carolina.

Islet isolation

DBA/2 mice were anesthetized by intraperitoneal injection of ketamine and xylazine. Each pancreas was perfused with collagenase (type V, 0.6 mg/mL, Sigma Aldrich, St. Louis, MO) through the pancreatic ducts. The dissected enzyme-containing pancreas was then incubated in 37°C water bath with constant shaking to release the islets which were isolated by density gradient separation using standard techniques as described [26]. Islet yield was assessed by the dithizone staining (DTZ, Sigma Aldrich, St. Louis, MO) and converted to a standard number of islet equivalents (IEQ) of islets where the diameter was standardized to 150 µm. Islets were cultured in vitro in Dulbecco’s Modified Eagles Medium (DMEM) containing 10% of fetal bovine serum at 37°C with 5% CO2 using normal or low attachment cell culture plates (Corning, Tewksbury, MA).

Pegylation and nanoparticle attachment to pegylated islets

Pegylation of freshly isolated mouse islets was carried out by incubation in serum-free DMEM containing the EZ-Link Amine-PEG11-Biotin (Thermo Scientific, Rockford, IL) at 20 mg/mL at room temperature for 30 min, followed by washing with PBS. Nanoparticle preparation has been described in detail elsewhere [22]. Briefly, avidin-coated PLGA nanoparticles were loaded with a cargo of either fluorescent dye (coumarin-6), or mouse recombinant LIF (Santa Cruz, CA), using a modified water/oil double emulsion technique. The diameter of PLGA nanoparticles generated was 100±20 nm (mean ± S.D.). For the LIF-nanoparticles the cumulative LIF release was 1000±50 picograms per milligram particles over a 7-day period [22]. Nanoparticle coating of the islets was performed using a two-step method: freshly isolated mouse islets were first pegylated as above; after washing with PBS, the islets were next incubated with the avidin-coated nanoparticles in complete DMEM medium at 37°C for another 30 min. The decorated islets were then washed in PBS to remove unbound nanoparticles.

Scanning electron microscopy

Islets were preserved with 1% gluteraldehyde and 0.01% osmium tetroxide followed by dehydration using a graded series (10–100%) of ethanol. Samples were placed on an aluminum stub using double stick tape and sputter coated with gold-platinum using a Denton Vacuum Desk II Sputter Unit prior to examination using a JEOL 5600LV SEM.

Cell viability analysis

Islets in 1 mL of PBS were stained with 100 µL of SytoGreen 13 (25 M, Invitrogen, Carlsbad, CA) and 100 µL ethidium bromide (EB, 50 M, Sigma Aldrich) at room temperature in the dark. Fluorescence vital staining based on membrane integrity was observed under a confocal microscope. Using this method, dead cells are stained red and live cells are green. Percentage of dead cells in total cells was calculated. At least 10 islets were included in each treatment group. Experiments were repeated for at least 3 times.

Detection of insulin expression using immunohistochemistry

Naked islets or islets coated with PEG plus empty nanoparticles were cultured in DMEM with high glucose in low attachment

[58x31]PLOS ONE | www.plosone.org 2 December 2012 | Volume 7 | Issue 12 | e50265
plates for 1, 7, 14 and 21 days. Islets were fixed in 4% paraformaldehyde and insulin expression was analyzed by staining with the guinea pig anti-insulin polyclonal antibody (Sigma-Aldrich). A phycoerythrin (PE)-labeled anti-guinea pig secondary antibody was used to detect expression of insulin in individual islets.

Glucose-stimulated insulin secretion (GSIS) assay
Islets that were either (i) naked, (ii) pegylated, or (iii) pegylated plus nano-empty were placed in 100 mm petri dishes overnight, using some 20 islets per dish. The islets were first treated with DMEM-low glucose (2.8 mM) for 1 hr, and then challenged with DMEM high glucose (28 mM) for a second hour. Cell culture medium was collected and the concentration of insulin released into the growth medium was measured using mouse insulin ELISA kit (ALPCO, Salem, NH). Insulin stimulation index (SI) was calculated as: SI = Insulin concentration after 28 mM glucose stimulation/Insulin concentration after 2.8 mM glucose stimulation.

Islet transplantation
C57BL/6 (H-2b) mice were rendered diabetic by one-time injection of streptozotocin (STZ) given intraperitoneally (i.p.) at 225 mg/kg. Five days after STZ administration, mice with two consecutive blood glucose levels exceeding 350 mg/dL were deemed diabetic and used as recipients. Encapsulated DBA/2 islets (500–600 IEQ) were transplanted under the kidney capsule of each recipient: four groups each of 6–7 recipients each received either (i) naked islets, (ii) pegylated islets; (iii) pegylated plus empty-nano islets; or (iv) pegylated plus LIF-nano islets. Islet function was monitored indirectly by measuring blood glucose levels twice per week. Mice with a blood glucose <200 mg/dL were considered normoglycemic. Grafts were deemed to have been rejected when two consecutive glucose levels were >300 mg/dL after a period of primary graft function evidenced by normoglycemia.

Statistical analyses
Kaplan-Meier survival curves were based on measurements of normoglycemia and performed by using the StatView software and the statistical differences were assessed by the Log-rank test. Values of p<0.05 were considered significant. Survival data are expressed as mean survival time ± standard deviation (MST ± SD). Differences between each treatment group were compared for statistical significance by the Student’s t test.

Results
1. Encapsulation improves long-term structural integrity of islets in vitro
We first asked, could islets pre-draped with PEG be further decorated with nanoparticles? Freshly isolated mouse islets were incubated with biotin-PEG and then with avidin-nanoparticles loaded with fluorescent dye coumarin-6 (Fig. 1). After washing, the islets were cultured in DMEM with high glucose for 24 h before being examined under fluorescent and confocal microscopes. Fig. 2 shows that these islets became completely covered with fluorescently labeled nanoparticles (Fig. 2A, a). This was confirmed by the Z-stack analysis of confocal microscopy to scan single layers of an islet (Fig. 2A, b), thus revealing penetration of the nanoparticles within the islet mass. The interaction of nanoparticles with islet was confirmed using SEM. Here naked islets showed a smooth surface contoured by bumps of individual cells within the islet (Fig. 2B, c). In contrast, islets that had been further incubated with coumarin-6-nanoparticles had a rough surface due to the surface bound nanoparticles (Fig. 2B, d). These observations demonstrate that avidin-nanoparticles bind to islets coated with biotin-PEG.

We next asked, does encapsulation preserve islet structure? Here we took either naked islets, or islets coated with PEG alone, or islets coated with both PEG plus coumarin-6-nano, and cultured them on normal (high attachment) cell culture plates for up to 21
days. The cultures were monitored daily using fluorescence phase
contrast microscopy. In naked islet group, islets lost their coherent
islet structure and there was migration of single cells that formed a
monolayer. This would be in accordance with loss of basement
membrane integrity during islet isolation leading to cell escape
from the islets in the absence of pegylation (Fig. 2C, e–g). In
striking contrast, islets encapsulated with PEG, with or without
coumarin-6-nanoparticles, retained an intact islet morphology, as
shown for the PEG-nano treated islets in Fig. 2C, h–j. Notably, in
those islets decorated with both PEG and coumarin-6-nano-
particles, the nanoparticulate coating persisted over the 3 week culture
period as indicated by the green fluorescence seen in Fig. 2C, h–j.
Although at 3 weeks the coumarin-6 dye may not reflect the
distribution of the nanoparticles themselves, but rather of diffused
drug derived from the nanoparticles, overall the data confirms that
nanoparticles do coat the pegylated islets and thereafter release of
cargo may continue over 3 weeks when cultured in vitro.

2. Islet functionality is not impaired by encapsulation
Having established that islets can be decorated with a
combination of PEG plus nanoparticles with preservation of islet
structure, we next determined functional integrity of the encap-

Figure 3. Encapsulation does not affect islet function. (A) Insulin secretion (ng/mL/h/islet) was measured in naked (light grey bars, CTR) and
nano-PEG-encapsulated (dark grey bars, Nano) islet cultures cultured overnight after encapsulation stimulated with 2.8 mM, or 28 mM glucose for
24 h. (B) Insulin stimulation index of the naked and nanoparticle-coated islets shown in (A). At least 20 islets were included in each group, and the
data represents 3 individual experiments.
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Figure 4. Prolonged viability of encapsulated islets in vitro. (A) Staining of viable (green) versus dead (red) cells in cultures of naked islets
(CTR), pegylated islets (PEG), or pegylated plus empty-nanoparticle islets (Nano) at 1 d, 7 d, 14 d, and 21 d. (B) Percentages of viable cells in the
different groups during culture. (C) Insulin staining in naked (CTR) and PEG-Nano-coated islets at 2 d and 14 d culture: more insulin positive cells were
observed in islets encapsulated with nanoparticles (lower panels) compared to naked islets (upper panels). At least 10 islets were included in each
group. Red represents insulin staining, blue staining (DAPI) represents nuclear staining in all cells. * p<0.05 and ** p<0.01.
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sulated islets in terms of ability to respond to glucose stimulation. Comparing naked islets with encapsulated islets cultured 1 h in low glucose (2.8 mM) then with 1 h high glucose (28 mM), after overnight in primary culture, we found similar glucose response profiles for insulin release levels (Fig. 3A), with correspondingly similar stimulation indices (Fig. 3B). We deduced that the encapsulation process did not impair β cell function (i) in sensing glucose change and (ii) in responding to this change with insulin release.

3. Prolonged viability of encapsulated islets in vitro

Islet viability in vitro is highly relevant to the potential use of harvested islets for clinical transplantation. We therefore compared naked versus encapsulated islets over a period of 21 d using low attachment conditions to mimic clinical harvest procedure. Three groups, naked islets, pegylated islets, and pegylated islets with empty nanoparticles, were cultured in DMEM on low attachment cell culture plates. Live and died cells were analyzed at 1, 7, 14 and 21 days after culture using Syto Green and EB staining. Fig. 4A and B shows that, although viability at 1 d and 7 d was comparable across the three groups at around 75%, there was an unexpected prolongation of long-term viability at both 14 d (~72%) and 21 d (~40%) specifically associated with the combined PEG plus nanoparticles. This beneficial effect was significantly greater than pegylation alone at 21 d. The pegylated islets without nanoparticles also showed marked benefits in terms of survival at 14 d (~60%) and 21 d (~27%) when compared to the naked islets 14 d (~33%) and 21 d (~11%).

Having demonstrated improved long-term viability of islets in vivo afforded by surface pegylation plus nanoparticles, we next confirmed relevance of the surviving islets in terms of their insulin activity. Immunohistochemistry revealed greater positivity in the pegylated plus nanoparticle coated islets compared to naked islets: notably, the difference was clear at 2 d and 14 d (Fig. 4, C). Thus, although viability scores were equivalent at these time points, a difference in the numbers of cells apparently responsive in terms of insulin expression was already present. This may reflect early differential vulnerability of β cells following the trauma of isolation, and/or preferential preservation of β cells following pegylation. Future studies will explore any further protection of β cells gained from attachment of LIF-nanoparticles, or of compound LIF/EGF-nanoparticles, to the pegylated drape, given that LIF is known to support β cells whilst LIF plus EGF synergise in pancreatic β cell differentiation [24].

4. Prolonged functionality of encapsulated islets in vivo

Given the improved viability with continued functionality of β cells in pancreatic islets draped with PEG plus nanoparticles in vitro, we next asked, do these pegylated “stealth” islets also show improved functionality in vivo? More specifically, could encapsulation protect transplanted islets from the hostile environment of a full MHC mismatched recipient? Using glycaemia as a surrogate indicator of graft function, four groups of six streptozotocin-induced diabetic C57BL/6 recipients were transplanted with islets as follows: (i) naked control islets; (ii) pegylated islets; (iii) pegylated islets with nanoparticles (empty); and (iv) pegylated islets with nanoparticles (LIF-Nano).

| GROUP                        | N   | N>100 d | N<100 d | <100 d Mean ± SD (days) | P value vs. control |
|------------------------------|-----|---------|---------|------------------------|---------------------|
| (i) Control                  | 6   | 0       | 6       | 17.0±7.5               | -                   |
| (ii) Pegylated               | 6   | 2       | 4       | 22.5±2.6               | 0.09                |
| (iii) Pegylated+Empty-Nano   | 7   | 2       | 5       | 21.5±8.8               | 0.03                |
| (iv) Pegylated+LIF-Nano      | 7   | 4       | 3       | 27.6±6.5               | 0.003               |

Grafts were placed under the kidney capsule. No immunosuppressive therapy was given.

*”>100 d” indicates number of recipients reaching >100 days normoglycemia.

*”<100 d” indicates number of recipients failing to reach long-term normoglycemia.

Table 1. Long-term normoglycemia derived from pegylated DBA/2 islet grafts in C57BL/6 recipients.
nanoparticles loaded with a cargo of leukemia inhibitory factor (LIF). LIF was chosen because LIF is known to promote adaptive immune tolerance in addition to LIF playing a key role in regulation of pancreatic β cell mass [24]. The islets were placed under the kidney capsule and no immunosuppressive therapy was given. In group (i) controls, normoglycemia was lost after 17.0 ± 7.5 d. In striking contrast, recipients in groups (ii), (iii) and (iv) showed long term (<100 d) normoglycemia involving some 30%, 43% and 57% of the recipients in each respective group. As detailed in Fig. 5 and Table 1, the incidence of long-term normoglycemia in the treated groups (ii)–(iv) was significantly different from the control group (i). This significant therapeutic gain from nanotherapeutic immune-isolation of the islets was interpreted to reflect prolonged islet survival and β cell functionality in vivo in the absence of immunosuppression and despite a full MHC mismatch donor/recipient pair combination.

Discussion

We have constructed “stealth” islets by pegylated nanotherapy wherein the encapsulating pegylated layer is physically linked to nanoparticles for targeted paracrine-type delivery of therapeutic cargo to the immediate microenvironment of the encapsulated islet. The specific aim of this study was firstly to test islet viability and functionality following encapsulation within a pegylated nanoparticle cage, and secondly to test functional efficacy in vivo in terms of allograft-derived control of normo-glycaemia in diabetic recipient mice. We demonstrate (i) in vitro, prolonged viability and functionality of the “stealth” islets and in particular β cell responsiveness to glucose challenge; and (ii) in vivo, prolonged functionality of the “stealth” islet allografts in maintaining normoglycemia in MHC-mismatched diabetic hosts.

Pegylation-based nanotherapeutics of the pancreatic islets significantly reduced the rate of β cell death [Fig. 1] – a highly significant point and, even though the in vitro model has its limitations in fully mimicking the cell destructive process after transplantation, the data clearly demonstrate that pegylated, nanoparticle decorated islets have superior survival advantages over naked islets. This will underpin new in vivo studies aimed at optimising nanotherapeutic cargo for further support of the intra-islet β cell population. Our findings also have immediate relevance to work of others aimed at deriving β cells from stem cells, precursor cells, or by trans-differentiation: the pegylated-nanotherapeutic coat may create cellular micro-environments not only promoting β cell neogenesis, but also thereafter for their “stealth” delivery. For example, we anticipate nanotherapeutic delivery of factors including LIF plus EGF, known to synergise in β cell transdifferentiation from pancreatic exocrine cells [24,25]. Exocrine pancreas as a source for β cell neogenesis might also be promoted by targeted delivery of inhibitors of the hedgehog signalling pathway based on the recent findings [27].

The ability to reduce the allo-immune response using nanotherapeutics integrated into the pegylated coat of the graft is also a major finding. The added value of targeting immune-modulatory growth factors such as LIF, able to bias allo-responsive T cells towards the Treg lineage [28], becomes especially significant when considering ongoing autoimmunity to endogenous diabetogenic antigen. Although our data is limited to islet allografts under the kidney capsule, and in hosts that are not primed against a diabetogen, the concept holds that shifting differentiation of islet-reactive T cells towards Treg will be beneficial. Importantly, since Treg release LIF upon stimulation by cognate antigen, a self-sustaining state of both immune tolerance plus support for the β cells (via LIF) may arise [29,30].

In conclusion, nanotherapeutic immune-isolation of grafts creates “stealth” pancreatic islets that show significantly prolonged viability and functionality in vitro and also in vivo. The long-term normoglycemia in fully mismatched diabetic hosts in the absence of all immunosuppression emphasizes the promise of the “stealth” approach, not only for islet but also for β cell transplantation including for cells generated from stem, precursor, or trans-differentiated, cell sources.

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Author Contributions

Conceived and designed the experiments: WG HW TMF SMM. Performed the experiments: HD HW WG SLM XD. Analyzed the data: WG HW TMF SMM. Contributed reagents/materials/analysis tools: TMF. Performed the experiments: HD HW WG SLM XD. Analyzed the data: Conceived and designed the experiments: WG HW TMF SMM. Wrote the paper: HW WG LI DBA.

References

1. Biancone L, Ricordi C (2002) Pancreatic islet transplantation: an update. Cell Transplant 11: 309–311.
2. Mercani S, Shapay AM (2006) Current status of pancreatic islet transplantation. Clin Sci (Lond) 110: 611–625.
3. Fort A, Fort N, Ricordi C, Stabler CL (2008) Biohybrid devices and encapsulation technologies for engineering a bioartificial pancreas. Cell Transplant 17: 997–1003.
4. Giraldo JA, Weaver JD, Stabler CL (2010) Tissue engineering approaches to enhancing clinical islet transplantation through tissue engineering strategies. J Diabetes Sci Technol 4: 1238–1247.
5. Kobayashi T, Aomatsu Y, Kanehiro H, Hisanaga M, Nakajima Y (2003) Protection of NOD islet isograft from autoimmune destruction by agarose microencapsulation. Transplant Proc 35: 404–405.
6. Wilson JT, Chaihof EL (2008) Challenges and emerging technologies in the immunomodulation of cells and tissues. Adv Drug Deliv Rev 60: 124–143.
7. Orive G, Hernandez RM, Gascon AR, Calafaro R, Chang TM, et al. (2003) Cell encapsulation: promise and progress. Nat Med 9: 104–107.
8. Sawhney AS, Pathak CP, Hubbard JL (1994) Modification of islet of langerhans surfaces with immunoprotective poly(ethylene glycol) coatings via interfacial polymerization. Biotechnol Bioeng 44: 383–386.
9. Murad KL, Greselin FJ, Eaton JW, Scott MD (1999) Stealth cells: prevention of major histocompatibility complex class II-mediated T-cell activation by cell surface modification. Blood 94: 2135–2141.
10. Murad KL, Mahany KL, Brugnara C, Kuypers FA, Eaton JW, et al. (1998) Structural and functional consequences of antigenic modulation of red blood cells with methoxy(polyethylene glycol). Blood 93: 2121–2127.
11. Scott MD, Murad KL (1998) Cellular camouflage: fooling the immune system with polymers. Curr Pharm Des 4: 423–430.
12. Scott MD, Murad KL, Koumpouras F, Talbot M, Eaton JW (1997) Chemical camouflage of antigenic determinants: stealth erythrocytes. Proc Natl Acad Sci U S A 94: 7566–7571.
13. Teramura Y, Ivata H (2008) Islets surface modification prevents blood-mediated inflammatory responses. Bioconjug Chem 19: 1309–1309.
14. Lee DY, Park SJ, Lee S, Nam JH, Byun Y (2007) Highly poly(ethylene glycolylated islets improve long-term islet allograft survival without immunosuppressive medication. Tissue Eng 13: 2133–2141.
15. Yun-Hee L, Hee Nam J, Byun Y (2007) Functional and histological evaluation of transplanted pancreatic islet immunoprotected by PLGA scaffold for 1 year. Biomaterials 28: 1957–1966.
16. Elcin YM, Elcin AE, Bretzel RG, Linn T (2003) Pancreatic islet culture and transplantation using chitosan and PLGA scaffolds. Advances in experimental medicine and biology 534: 255–264.
17. Giovagnoli S, Luca G, Casaburi I, Blasi P, Macchiarello G, et al. (2005) Long-term delivery of superoxide dismutase and catalase entrapped in poly(lactide-co-glycolide) microspheres: in vitro effects on isolated neonatal porcine pancreatic cells. Journal of controlled release: official journal of the Controlled Release Society 107: 65–77.
18. Mao GH, Chen GA, Bai HY, Song TR, Wang YX (2009) The reversal of hyperglycaemia in diabetic mice using PLGA scaffold seeded with islet-like cells derived from human embryonic stem cells. Biomaterials 30: 1706–1714.
19. Basarab A, Singh J (2009) Poly(lactide-co-glycolide)-polyethyleneate nanoparticles for intramuscular delivery of plasmid encoding interleukin-10 to prevent autoimmune diabetes in mice. Pharmaceutical research 26: 72–81.
20. Jain R, Shah NH, Malick AW, Rhodes CT (1998) Controlled drug delivery by biodegradable poly(ester) devices: different preparative approaches. Drug Dev Ind Pharm 24: 703–727.

21. Jain RA (2000) The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21: 2475–2490.

22. Park J, Gao W, Whiston R, Strom TB, Metcalfe S, et al. (2010) Modulation of CD4+ T lymphocyte lineage outcomes with targeted, nanoparticle-mediated cytokine delivery. Mol Pharm 8: 145–152.

23. Baeyens L, Bonne S, German MS, Ravassard P, Heinberg H, et al. (2006) Ngn3 expression during postnatal in vitro beta cell neogenesis induced by the JAK/STAT pathway. Cell death and differentiation 13: 1892–1899.

24. Baeyens L, De Breuck S, Lardon J, Mfopou JK, Roosman I, et al. (2005) In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. Diabetologia 48: 49–57.

25. De Breuck S, Baeyens L, Bouvens L (2006) Expression and function of leukemia inhibitory factor and its receptor in normal and regenerating rat pancreas. Diabetologia 49: 108–116.

26. Wang H, Lee SS, Gao W, Gzimadie E, McDaid J, et al. (2005) Donor treatment with carbon monoxide can yield islet allograft survival and tolerance. Diabetes 54: 1400–1406.

27. Mfopou JK, Baeyens L, Bouvens L (2012) Hedgehog signals inhibit postnatal beta cell neogenesis from adult rat exocrine pancreas in vitro. Diabetologia 55: 1024–1034.

28. Gao W, Thompson L, Zhou Q, Putheti P, Fahmy TM, et al. (2009) Treg versus Th17 lymphocyte lineages are cross-regulated by LIF versus IL-6. Cell Cycle 8: 1444–1450.

29. Metcalfe SM, Watson TJ, Shurey S, Adams E, Green CJ (2005) Leukemia inhibitory factor is linked to regulatory transplantation tolerance. Transplantation 79: 726–730.

30. Metcalfe SM (2011) LIF in the regulation of T-cell fate and as a potential therapeutic. Genes Immun 12: 157–168.