Biochemical and Biophysical Research Communications 433 (2013) 513–518

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Effects of surface camouflaged islet transplantation on pathophysiological progression in a *db*/*db* type 2 diabetic mouse model

Jee-Heon Jeong [a], Simmyung Yook [a], Haeshin Lee [b,c], Cheol-Hee Ahn [d], Dong Yun Lee [e], Youngro Byun [a,f,*]

[a] Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea
[b] Department of Chemistry, College of Natural Science, Korea Advanced Institute of Science and Technology, Daejeon 305-701, South Korea
[c] Graduate School Nanoscience and Technology, College of Natural Science, Korea Advanced Institute of Science and Technology, Daejeon 305-701, South Korea
[d] Research Institute of Advanced Materials, Department of Materials Science and Engineering, Seoul National University, Seoul 151-742, South Korea
[e] Department of Bioengineering, College of Engineering, and Institute for Bioengineering and Biopharmaceutical Research, Hanyang University, Seoul 133-791, South Korea
[f] Department of Molecular Medicine and Biopharmaceutical Sciences, Graduated School of Convergence Science and Technology, Seoul National University, Seoul 151-742, South Korea

ARTICLE INFO

*Article history:*
Received 18 February 2013
Available online 21 March 2013

*Keywords:*
Islet transplantation
Type 2 diabetes
Surface modification
6-Arm-PEG–catechol
Anti-CD154 monoclonal antibody
Tacrolimus

ABSTRACT

To investigate the inhibition effects of pancreatic islet transplantation on the progression of obese type 2 diabetes, we analyzed the effects of surface camouflaged islet transplantation on delaying the disease progression in a *db*/*db* diabetic mouse model. Surface camouflaged islets using 6-arm-PEG–catechol were transplanted in *db*/*db* diabetic mice. The fat accumulation and toxicity in the liver, the expansion of islets in the pancreas, and the size change of abdominal adipocyte were analyzed. In addition, the blood glucose control, insulin levels and immunohistochemical staining of recovered tissues were analyzed after transplantation. Then co-administration of anti-CD154 monoclonal antibody and Tacrolimus (IT group) deterred the pathophysiological progression of obese type 2 diabetes. At day 3 of transplantation, the serum insulin concentration of IT group was increased compared to the *db*/*db* diabetic mice group. The immunohistochemical studies demonstrated that the mass of 6-arm-PEG–catechol grafted islet was preserved in the transplantation site for 14 days. Surface modification using 6-arm-PEG–catechol effectively inhibited the immune cell infiltration and activation of host immune cells when immunosuppressive drug was given to the *db*/*db* type 2 diabetes mice. Therefore, 6-arm-PEG–catechol grafted islets effectively restored the insulin secretion in islet recipients and prevented the disease progression in type 2 diabetes.



## 1. Introduction

Type 2 diabetes is characterized by a decrease in insulin secretion and an increase in insulin resistance [1–4]. Most obese type 2 diabetes show an insulin resistance associated with hyperinsulinemia in the early stage of the disease [5]. As the disease progresses, the functions of pancreatic beta cells are gradually deteriorated, finally developing severe insulin deficiency [6]. The morphological damage of the pancreatic islets in obese type 2 diabetes is induced by compensatory overexpression of insulin and the resultant exhaustion of beta cells in response to the increased insulin demand.

In these respects, we expected that additional exogenous insulin administration could compensate for excessive demand for insulin secretion from the beta cells, and furthermore, deter the progression of type 2 diabetes. The insulin therapy in early stage of obese type 2 diabetes had been found to improve both insulin action and secretion, thereby overcoming insulin resistance [7]. Thus, basal–bolus insulin therapy is one of the choices for regulating the blood glucose level of type 2 diabetic patients, offering a way to closely stimulate natural insulin delivery in the clinic [8,9]. However, the insulin therapy has shown many concerns with respect to hypoglycemia, patient willingness, and incompliance. Therefore, islet transplantation has been shown to be another alternative by which to properly deliver insulin to diabetic patients.

Several studies have used islet transplantation for the treatment of the type 2 diabetes in a mouse model [10–16]. Gates et al. have ameliorated the abnormalities of obese-hypoglycaemic by allogeneic implantation of islets [10,11]. They used a Millipore bag as a device for preventing graft rejection, but it had limitations in sensing glucose concentration and in maintaining islet viability [17]. Furthermore, it was reported that although MIN-6 cell transplantation decreased hyperglycemia in *db*/*db* mice over 100 days after transplantation, the transplanted MIN-6 cells had became the cancerous tissue [18]. Andersson et al. showed that intrasplenic

* Corresponding author at: Department of Molecular Medicine and Biopharmaceutical Sciences, Graduated School of Convergence Science and Technology, Seoul National University, Seoul 151-742, South Korea. Fax: +82 2 872 7864.
*E-mail address:* yrbyun@snu.ac.kr (Y. Byun).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.03.015

islet transplantation was failed to cure obese-hyperglycemic mice [13]. Barker et al. claimed that islet transplantation was not suitable as a treatment for *db*/*db* mice [15]. Katsuragi et al. examined that transplanted islet through the portal vein prevented the diabetic progression in Otsuka Long Evans Tokushima Fatty (OLEFT) rats. They reported on the possibility of transplanted islets to prevent the pancreas and mesangial matrix in the renal glomeruli from undergoing morphological changes [19]. Collectively, there were many studies reporting about the advantage of islet transplantation for deterring pathophysiological progression of type 2 diabetes.

However, it was hard to effectively deliver the insulin secreted from transplanted islets to type 2 diabetic patients because the grafted islets were easily rejected by host immune reaction. If transplanted islets would be survived for long-term periods without the immune rejection, transplanted islets would effectively delivery insulin and deter the progression of type 2 diabetes related with insulin resistance. To ameliorate these limitations in the treatment regime and to minimize the immune reaction after transplantation, and based on the previous studies, we introduced an islet surface modification technology combined with immunosuppressive medication as a treatment for type 2 diabetes [20,21]. In the previous study, surface modification of islet effectively inhibited the immune cell recognition and infiltration [22]. Particularly, the surface camouflage of pancreatic islet using 6-arm-PEG–catechol and the administration of Tacrolimus (FK506) and anti-CD154 mAb (MR-1) were very effective in preventing immune reactions against transplanted islets [20].

In this study, 6-arm-PEG–catechol grafted islets were transplanted into *db*/*db* mice with co-administration of MR-1 and FK506 to investigate the curing effect of type 2 diabetes. Furthermore, inhibition effects of this protocol on pathophysiological damage progression in type 2 diabetes were evaluated in a *db*/*db* type 2 diabetic mice model.

## 2. Materials and methods

### 2.1. Synthesis of 6-arm-PEG–catechol

Synthesis scheme was followed by previous study [20]. Briefly, six-arm-PEG–amine (1 g, MW 15 kDa) was dissolved in N-methylpyrrolidone (NMP, 10 ml) at 60 °C for 10 min. 3,4-Dihydroxyhydrocinnamic acid (DHCA, 0.8 mmol), bezotriazole-1-yl-oxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP, 0.8 mmol), 1-hydroxybenzotriazole (HOBt, 0.8 mmol) and N,N-diisopropylethylamine (DIPEA, 0.8 mmol) were dissolbed in NMP (5 ml) in separate vials. PEG and DHCA solutions were reacted at room temperature for 6 h and followed by reacting with BOP, HOBt and DIPEA until ninhydrin assay showed a negative result. The reacted materials were dialyzed (MWCO; 8 kDa) in distilled water in acidic condition (pH = 1–2) to prevent oxidation of catechol moieties, followed by lyophilization.

### 2.2. Surface camouflage of isolated pancreatic islet using 6-arm-PEG–catechol

Pancreatic islets were isolated from Sprague–Dawley (SD) rats (male, 8-weeks old, Orient Bio Inc., Seongnam, South Korea). Isolated islets were cultured in RPMI-1640 (Sigma) medium with 10% fetal bovine serum (FBS). The 6-arm-PEG–catechol molecules were chemically immobilized onto the surfaces [20]. After 2 days culture, HBSS (pH 8.0) containing 0.25% (w/v) 6-arm-PEG–catechol were added to the islets to chemically immobilize 6-arm-PEG–catechol onto the islet surfaces (Fig. 1A). To verify of 6-arm-PEG–catechol coverage, the fluorescein isothiocyanate (FITC) labeled 6-arm-PEG–catechol was immobilized on the surface of islets, and then, the coverage was evaluated using a laser scanning confocal microscope (LSM510, Carl Zeiss, Germany). All of the animal experiments were carried out according to the guidelines of the Institutional Animal Care & Use Committee, Seoul National University (IACUC No. SNU-070822-5).

Cell viability of islets was analyzed by Lived/Dead Viability/Cytotoxicy assay kit (Molecular Probes, Eugen, OR), Cell Counting Kit-8 (CCK-8) assay and measuring the rate of oxygen consumption (OCR) after surface modification [20].

### 2.3. Transplantation of 6-arm-PEG–catechol grafted islets

Six-week-old male *db*/*db* C57BL/KsJ mice (*db*/*db* group) and their lean non-diabetic heterozygous littermates, *db*/*m* mice (*db*/*m* group), were purchased from Japan SLC Inc. (Hamatsu, Japan). Spontaneously induced type 2 diabetic *db*/*db* mice were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylzine (16 mg/kg) after being housed for 2 weeks. The left kidney was exposed through a lumbar incision and capsulotomy was carried out at the cadual layer of the left kidney, followed by the transplantation of 500 IEQ 6-arm-PEG–catechol grafted islets (IT group). Thus, there were three following groups: *db*/*db*, *db*/*m* and IT group. Also, MR-1 (0.2 mg/mouse) was injected at 0, 2, 4, and 6 days of post-transplantation and FK506 (0.2 mg/kg) was daily injected into the recipient intrapertoneally.

### 2.4. Analysis of blood samples and intraperitoneal glucose tolerance test (IPGTT)

The non-fasting blood glucose (NBG) levels were measured by drawing blood from the tail veins using a portable glucometer (Super glucocoard II, Arkray, Kyoto, Japan) before and after of transplantation (at day 0, and 3 post transplantation). Insulin concentrations in serum were determined using the ELISA (rat/mouse insulin ELISA kit, Millipore, Billerica, MA). In addition, liver toxicity (aspartate aminotransferase, AST; alanine aminotransferase, ALT) was measured using FUJI DRI-CHEM 3500 (FUJIFILM, Tokyo, Japan).

In addition, IPGTT was performed to measure the glucose responsiveness at day 5 after islet transplantation. Each group was fasted for 6 h before intraperitoneal injection of 200 mg/ml glucose (200 μl/mouse) solution. Then, the blood glucose level was measured from the tail vein using portable glucometer (Super glucocard II, Arkray, Kyoto, Japan) at 0, 15, 30, 60, and 90 min after glucose injection.

### 2.5. Immunohistochemistry

Six-arm-PEG–catechol grafted islets were transplanted in the kidney capsule of *db*/*db* mice. At day 14 of islet transplantation, the kidney was retrieved and fixed in neutral 4% paraformaldehyde–phosphate-buffered saline, and embedded in paraffin. The tissue slides were stained with hematoxylin and eosin (H&E), anti-insulin (Abcam, Cambridge, MA), anti-$CD4^+$ (Abcam Inc., Cambridge, MA), anti-$CD8^+$ (BioLegend, San Diego, CA), and anti-$CD20^+$ (Santa Cruz Biotechnology Inc., Santa Cruz, CA) antibodies, respectively. At day 14 of islets transplantation, the pancreas, kidney, liver, and abdominal adipose tissue were stained with H&E and their morphological changes were evaluated. The sizes of adipocyte and pancreatic islet were also measured using the ACT-2U imaging software (Nikon, Tokyo, Japan). Three slides of each group were analyzed.

### 2.6. Statistical analysis

All the data were expressed as mean ± SEM. Statistically analysis was carried out using unpaired *t*-test or ANOVA one-way test. A

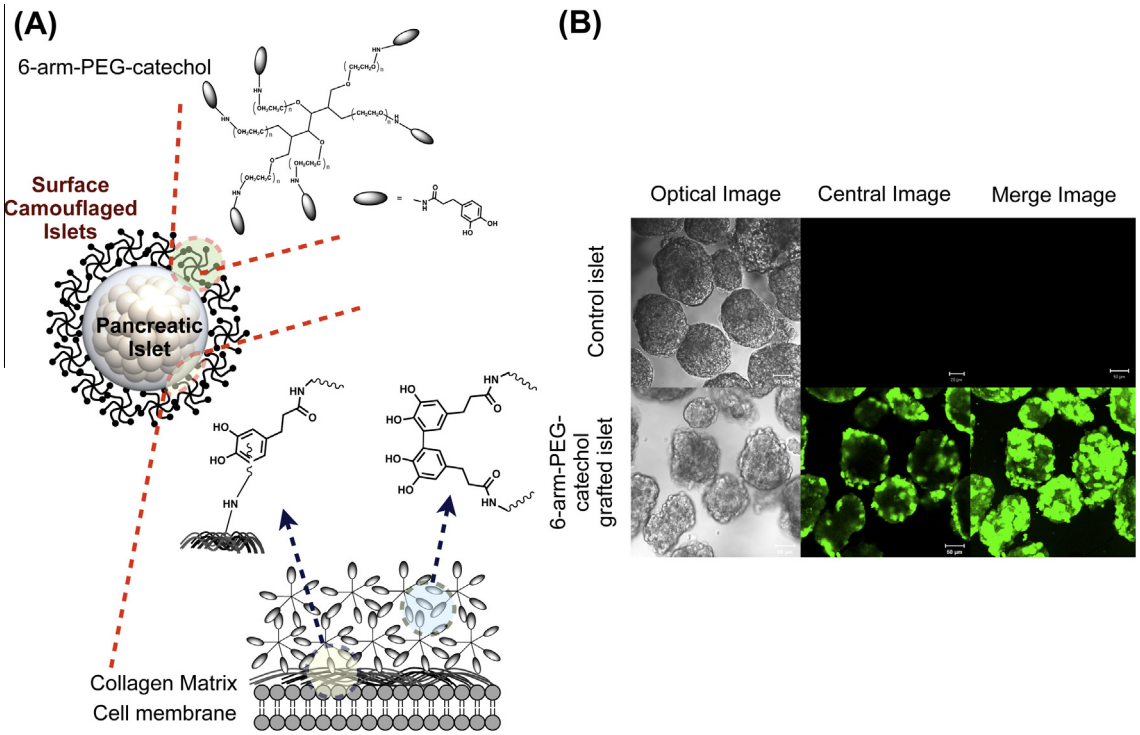


**Fig. 1.** (A) Illustration of 6-arm-PEG–catechol grafting onto the islet surface. (B) Confocal laser scanning microscopy images of FITC labeled 6-arm-PEG–catechol modified islets.

*P* value of less than 0.05 was considered to be statistically significant.

## 3. Results

### 3.1. Surface coverage of 6-arm-PEG–catechol on islets

Catechol conjugation to 6-arm-PEG–amine was monitored by ninhydrin test. This experiment indicated that all amine moieties of 6-arm-PEG reacted with 3,4-dihydroxyhydrocinnamic acid and approximately 20% of amine groups were conjugated to FITC molecules. The condition of 6-arm-PEG–catechol grafting to surface of islets was followed as indicated in the previous study [20]. Optimal 6-arm-PEG–catechol concentration was 0.25% (v/v) and this concentration did not show any cytotoxic effect when islets were incubated with the polymer for 1 h [20]. FITC-labeled 6-arm-PEG–catechol was evenly grafted to the surface of islets, which was evaluated using confocal laser scanning microscopy (CLSM). On the other hand, unmodified islets (control islets) did not emit any fluorescence (Fig. 1B).

### 3.2. Blood glucose control and IPGTT

The NBG level of *db*/*db* mice, which received 6-arm-PEG–catechol grafted islets, was decreased (220 ± 39.9 mg/dl) after transplantation with the co-administration of MR-1 and FK506 compared to that before transplantation (430.6 ± 33.6 mg/dl) (Fig. 2A). In addition, the insulin level of IT group was significantly increased at day 3 of transplantation; that is, the serum insulin level of IT group at day 3 (8.4 ± 2.1 ng/ml) was increased by 3.3-fold, compared to that in *db*/*db* group (2.6 ± 0.3 ng/ml) (Fig. 2B). Collectively, 6-arm-PEG–catechol grafted islets effectively expressed insulin, which compensated the insulin resistance and regulated the blood glucose level in the recipients. To confirm glucose responsiveness of transplanted 6-arm-PEG–catechol grafted islets, IPGTTs were carried out at day 5 of transplantation (Fig. 2C). The blood glucose level of *db*/*db* group rapidly increased to 600 mg/dl within 20 min. On the other hand, the blood glucose level of IT group reached the highest concentration (~400 mg/dl) after 30 min of glucose injection and then gradually decreased afterwards, showing that the insulin secreted from transplanted 6-arm-PEG–catechol grafted islet prevented a hasty increase of blood glucose level upon administration of high exogenous glucose solution.

### 3.3. Immunohistochemistry

To verify insulin secretion and immune cell recruitment around the transplanted 6-arm-PEG–catechol grafted islets at day 14 of post-transplantation, the left kidney containing transplanted islets was harvested. At day 14, insulin positive cells were detected around the transplantation site; however, CD4, CD8a and CD20 positive cells were rarely detected around the islet transplanted site (Fig. 2D). This result indicated that transplanted 6-arm-PEG–catechol grafted islets secreted insulin; in other words, islet functions were preserved even after the islet transplantation.

### 3.4. Pathophysiological analysis after 6-arm-PEG–catechol grafted islet transplantation

Photomicrographs of the pancreatic islets in *db*/*db*, *db*/*m*, and IT groups at day 14 of their transplantation were illustrated in Fig. 3A. The pancreatic islets of *db*/*db* group appeared much larger than those of *db*/*m* group and became multinodular due to the intrainsular proliferation of fibrous connective tissue. On the other hand, the pancreatic islets of IT group appeared as the normal although the intensity of insulin immunostaining was less than that of *db*/*m* group. The area quantification of insulin positive cells in *db*/*db* group was 17,920 ± 7372 $\mu m^2$. Contrastively, the area of IT group was 6379 ± 1661 $\mu m^2$, which was 2.8-fold smaller than that of *db*/*db* group (Fig. 3B). This result indicated that islet transplanta-

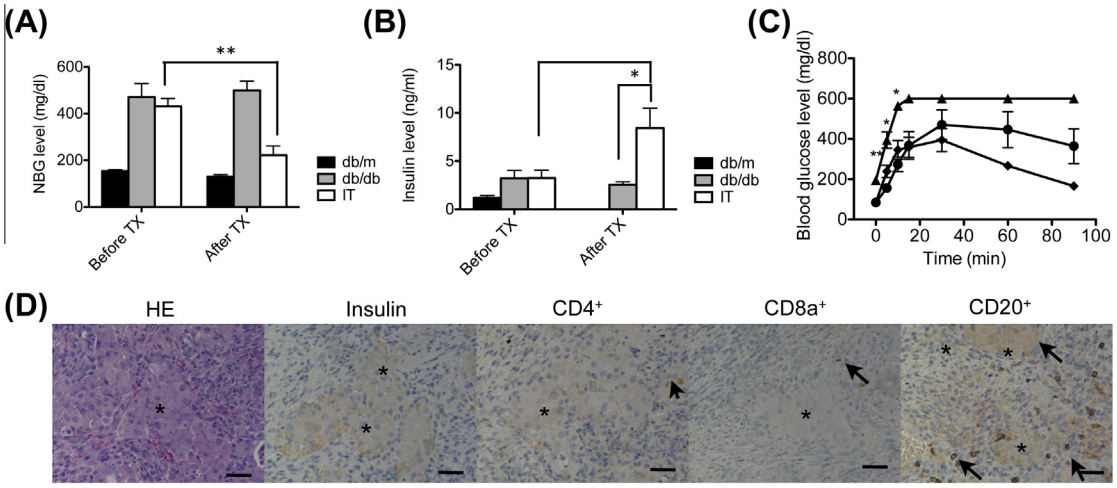


**Fig. 2.** (A) Fasting blood glucose and (B) fasting insulin serum levels at day 0 and 3 after 6-arm-PEG–catechol grafted islet transplantation. Data were expressed as mean ± SEM (*db*/*m*; $n = 6$, *db*/*db*; $n = 6$, IT; $n = 8$), ($^{*}P < 0.05$, $^{**}P < 0.01$). (C) The intraperitoneal glucose tolerance test (IPGTT) of *db*/*m* (black diamond), *db*/*db* (black triangle), and IT (black circle) groups at day 5 of transplantation. Data were expressed as mean ± SEM ($n = 4$), ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. IT group). (D) Immunohistochemical analysis of transplanted 6-arm-PEG–catechol grafted islets after 14 days of transplantation. Asterisk: transplanted islets, Scale bar = 25 μm.

tion could inhibit the enlargement of pancreatic islets and proliferation of fibrous connective tissue, which are usually induced in type 2 diabetic mice in order to compensate for hypoglycemia. Thus, the islet transplantation further delayed the destruction of pancreatic islets by reducing the size expansion of islet in *db*/*db* mice.

The color of the liver surface in *db*/*db* group was white-yellowish as a result of accumulating fat. On the other hand, the color of the liver surface in IT group was red as the normal, which was similar with those of *db*/*m* group (Fig. 3C). The hepatocytes in *db*/*db* group contained empty vacuoles in hematoxylin–eosin-stained sections. The fat was infiltrated into vacuole within the cytosol of hepatocytes in *db*/*db* group; however, the fat infiltration into the liver was not observed in IT group. To confirm the effect of 6-arm-PEG–catechol grafted islet transplantation on alleviating the liver toxicity in *db*/*db* mice, both ALT and AST levels in the serum of *db*/*db*, *db*/*m* and IT group were analyzed, respectively, using FUJI DRI-CHEM 3500 (FUJIFILM, Tokyo, Japan) at day 14 after 6-arm-PEG–catechol grafted islet transplantation (Fig. 3D). ALT and AST levels in *db*/*db* group were 302 ± 45 U/l and 262 ± 12 U/l, respectively. On the contrary, ALT and AST levels in IT group were statistically decreased to 151 ± 22 U/l and 176 ± 22 U/l, respectively. These results demonstrated that 6-arm-PEG–catechol grafted islet transplantation in a *db*/*db* diabetic mouse model partly recovered the liver from high fat accumulation and damage.

Adipocytes play an important role in regulating energy expenditure, food intake, and glucose metabolism. Therefore, the adipocyte is an important indicator for metabolic alterations related to the insulin resistance [18]. As shown in Fig. 3E and F, the size of an adipocyte, which was measured at the same time point of IT group in *db*/*m* and *db*/*db* groups, were 1082 ± 116, and 3259 ± 376.4 $\mu m^2$, respectively. On the other hand, the adipocyte size of IT group was 1675 ± 221 $\mu m^2$, which was statistically decreased compared to that of *db*/*db* group. These findings indicated that transplanted 6-arm-PEG–catechol grafted islets supplied a sufficient amount of insulin in place of host beta cells so that there was no need to compensate for hyperglycemia by enlarging the size of islet and the size of adipocytes.

## 4. Discussion

In this study, we investigated the therapeutic effects of surface camouflaged islets transplantation on the type 2 diabetes and its related pathophysiological phenomena in a *db*/*db* mouse model. In the previous study, we evaluated that islet surface modification was effective in inhibiting the immune cell activation and infiltration *in vitro* and *in vivo* [22,23]. PEG conjugation to islet did not elicit any activation of co-cultured immune cells such as lymphocytes, macrophages and splenocytes. In addition, the synergistic effect of islet PEGylation and administration of immunosuppressive drug into the diabetic recipient was observed as well [20]. PEG immobilization prevented the infiltration of immune cells into the transplanted islets and immunosuppressive drugs alleviated the immune cell and cytokine activities [22,24]. Thus, the PEG conjugation onto the surface of islets helped provide effective therapy over one year when accompanied with immunosuppressive drugs. In addition, a few rare T and B cells were observed around the PEG conjugated islets after the first one year of post-transplantation [25]. The similar immunoprotection protocol optimized for prolonging the graft survival time was applied to type 2 diabetic mice to evaluate its effects in inhibiting the pathophysiological disease progression. When 6-arm-PEG–catechol grafted islets were transplanted and both MR-1 and FK506 were administered to the mice, the blood glucose level of islet recipients was effectively decreased down to 200 mg/dl after transplantation. When the serum insulin level was high, a normal blood glucose level was observed, indicating that the insulin secreted from the transplanted islets was responsible for the regulation of blood glucose level. In addition, transplanted islets had insulin responsiveness to glucose and their clearance ability was somewhat increase in IT group. Following the injection of high exogenous glucose solution, a rapid increase of blood glucose level was prevented in the recipients transplanted with islets. On the other hand, a greater amount of insulin was necessary for *db*/*db* group to regulate hyperglycemia because of insulin resistance. The amount of secreted insulin from *db*/*db* mice was not enough to regulate the blood glucose level to maintain normal glycaemia. Moreover, the insulin positive cells were detected the transplanted site after 14 days of transplantation when 6-arm-PEG–catechol grafted islet transplanted with administering immunosuppressive drugs.

When morphological expansion and exhaustion of islets were observed in the type 2 diabetic mice, and the expansion of the islet size was prominently due to their need to compensate for hyperglycemia and the resistance against insulin. However, the transplantation of 6-arm-PEG–catechol grafted islets prevented the size expansion of islet in the pancreas. The size of adipocytes is

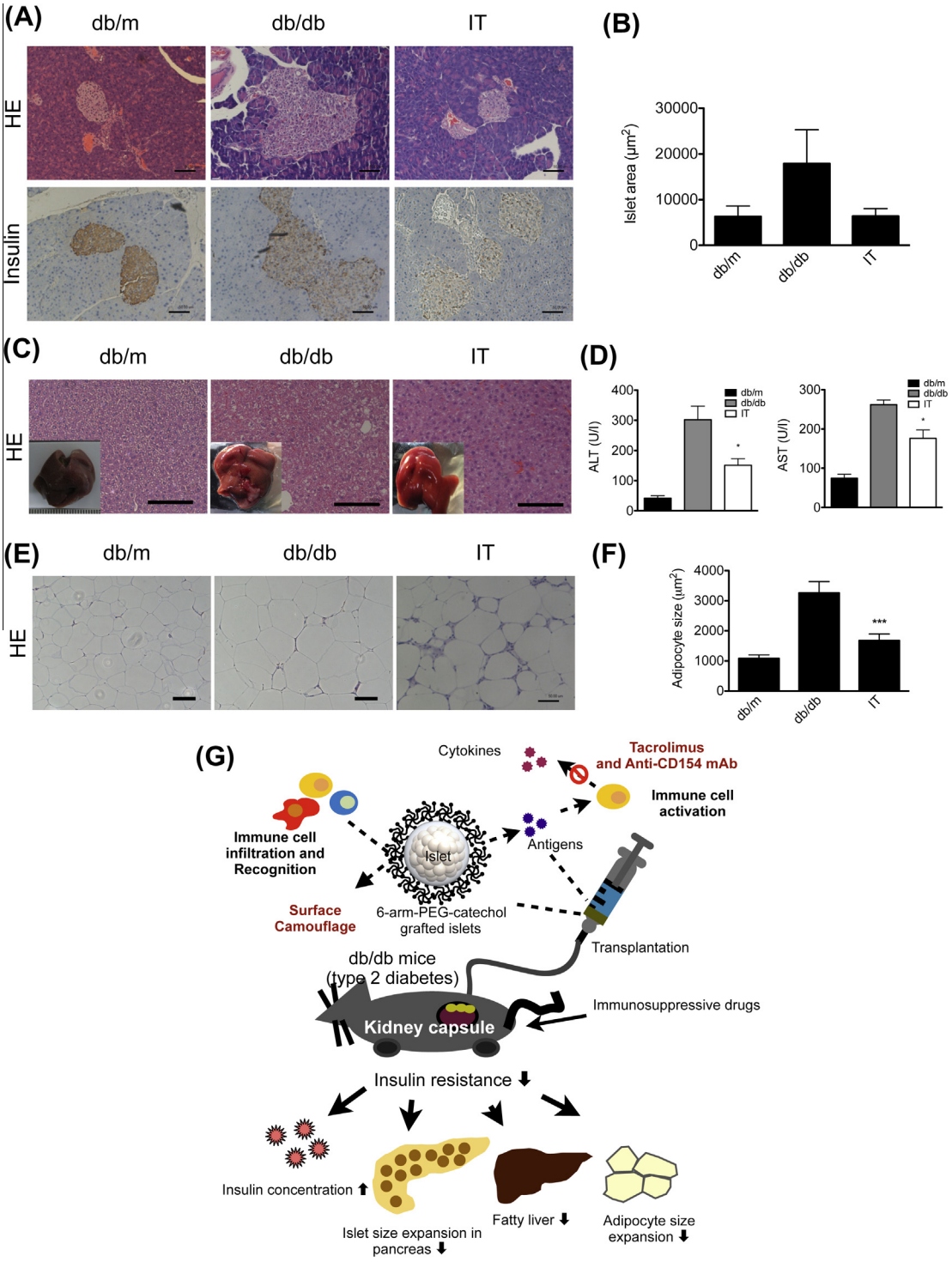


**Fig. 3.** (A) Morphology and size changes of islets in the pancreas at day 14 of 6-arm-PEG–catechol grafted islet transplantation in *db*/*m*, *db*/*db* and IT groups Hematoxylin–eosin and insulin staining of pancreatic islets, Scale bar = 50 μl. (B) The area of insulin-positive cells in the pancreas. Data were expressed as mean ± SEM. (C) The gross appearance and histological findings of the liver in the *db*/*m*, *db*/*db*, and IT groups at day 14 of 6-arm-PEG–catechol grafted islet transplantation. Scale bar = 100 μl. (D) Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level in the serum of *db*/*m*, *db*/*db*, and IT groups. Data were expressed as mean ± SEM (*db*/*m*; $n = 3$, *db*/*db*; $n = 3$, IT; $n = 5$). (E) Adipocyte size and morphology changes at day 14 of 6-arm-PEG–catechol grafted islet transplantation. Histology of abdominal adipose tissue in *db*/*m*, db/db, and IT groups. Scale bar = 50 μm. (F) Size of adipocyte in the *db*/*m*, *db*/*db*, and IT groups. Data were expressed as mean ± SEM (*db*/*m*; $n = 3$, *db*/*db*; $n = 3$, IT; $n = 5$; For each slide, 21 adipocytes were measured at random with an image analysis system); ($^{***}P < 0.001$ vs. *db*/*db* group). (G) Illustration of surface camouflaged islets transplantation for treatment of type 2 diabetes.

an indirect factor indicating the insulin resistance and glucose tolerance in the type 2 diabetes. Based on the severity of obesity in *db*/*db* mice, the size of adipocyte was also increased; however, the expansion of adipocyte was also significantly inhibited following the 6-arm-PEG–catechol grafted islet transplantation. At day 14, the size of adipocyte in IT group was much smaller than that of *db*/*db* group. At this point, insulin delivered from the transplanted 6-arm-PEG–catechol grafted islets might have affected the

diminution of insulin resistance and enhanced lipolysis in *db*/*db* mice. Thus, the enlargement of adipocytes in the 6-arm-PEG–catechol grafted islet transplanted *db*/*db* mice were effectively inhibited.

The AST and ALT values are sensitive indicators of hepatocellular injury, and these values are associated with insulin resistance, obesity and type 2 diabetes [26,27]. The AST and ALT values are also strongly associated with fat accumulation in the liver and diminution of these values indicates that the liver had somewhat recovered from its injured condition. The degree of fat accumulation, AST and ALT values of IT group were significantly decreased, compared to those of *db*/*db* group, indicating that the transplanted islets could reduce the degree of liver injury. Insulin secreted from transplanted 6-arm-PEG–catechol grafted islets effectively delivered glucose inside the cell, thereby decreasing the insulin resistance in the liver. Therefore, the excessive accumulation of fat in the liver was effectively diminished. Also inflammation was observed to be significantly decreased in the liver of IT group, but a strong in *db*/*db* group.

The ultimate goal of this study was to demonstrate the feasibility of islet camouflage technique used in combination with immunosuppressive drugs for the treatment of type 2 diabetes. We have verified that surface camouflage islets using 6-arm-PEG–catechol effectively inhibited pathophysiological deterioration of type 2 diabetes during that time by way of reducing islet size expansion in the pancreas, preventing adipocyte size expansion and fat accumulation in the liver (Fig. 3G).

Therefore, the present islet transplantation would help to successful inhibit progression of type 2 diabetes in various therapeutic approaches, thwarting complications related type 2 diabetes progression.

## Acknowledgments

This study was supported by a grant from the World Class University (WCU) program (Grant No. R31-2008-000-10103), the Converging Research Center Program (Grant No. 2012K001398) and the Basic Science Research Program (Grant No. 2010-0027955) of the National Research Foundation of Korea (NRF), which is by the Ministry of Education, Science and Technology, Republic of Korea.

## References

[1] D. Porte Jr., Clinical importance of insulin secretion and its interaction with insulin resistance in the treatment of type 2 diabetes mellitus and its complications, Diabetes Metab. Res. Rev. 17 (2001) 181–188.
[2] M. Prentki, E. Joly, W. El-Assaad, R. Roduit, Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes, Diabetes 51 (Suppl. 3) (2002) S405–S413.
[3] J.L. Leahy, Pathogenesis of type 2 diabetes mellitus, Arch. Med. Res. 36 (2005) 197–209.
[4] V. Poitout, R.P. Robertson, Minireview: secondary beta-cell failure in type 2 diabetes – a convergence of glucotoxicity and lipotoxicity, Endocrinology 143 (2002) 339–342.
[5] R.J. Mahler, Hyperinsulinemia and non-insulin-dependent diabetes mellitus, N. Engl. J. Med. 316 (1987) 110–111.
[6] D.A. Nugent, D.M. Smith, H.B. Jones, A review of islet of Langerhans degeneration in rodent models of type 2 diabetes, Toxicol. Pathol. 36 (2008) 529–551.
[7] W.J. Andrews, B. Vasquez, M. Nagulesparan, I. Klimes, J. Foley, R. Unger, G.M. Reaven, Insulin therapy in obese, non-insulin-dependent diabetes induces improvements in insulin action and secretion that are maintained for two weeks after insulin withdrawal, Diabetes 33 (1984) 634–642.
[8] I.B. Hirsch, R.M. Bergenstal, C.G. Parkin, E. Wright, J.B. Buse, A real-world approach to insulin therapy in primary care practice, Clin. Diabetes 23 (2005) 78–86.
[9] I. Lingvay, J.L. Legendre, P.F. Kaloyanova, S. Zhang, B. Adams-Huet, P. Raskin, Insulin-based versus triple oral therapy for newly diagnosed type 2 diabetes: which is better?, Diabetes Care 32 (2009) 1789–1795
[10] R.J. Gates, M.I. Hunt, N.R. Lazarus, Further studies on the amelioration of the characteristics of New Zealand Obese (NZO) mice following implantation of islets of Langerhans, Diabetologia 10 (1974) 401–406.
[11] R.J. Gates, M.I. Hunt, R. Smith, N.R. Lazarus, Studies on implanted islets of Langerhans: normalization of blood glucose concentration, blood insulin concentration and weight gain in New Zealand obese mice, Biochem. J. 130 (1972) 26P–27P.
[12] R.J. Gates, M.I. Hunt, R. Smith, N.R. Lazarus, Return to normal of blood–glucose, plasma–insulin, and weight gain in New Zealand obese mice after implantation of islets of Langerhans, Lancet 2 (1972) 567–570.
[13] A. Andersson, U. Eriksson, B. Petersson, L. Reibring, I. Swenne, Failure of successful intrasplenic transplantation of islets from lean mice to cure obese-hyperglycaemic mice, despite islet growth, Diabetologia 20 (1981) 237–241.
[14] I. Katsuragi, T. Okeda, H. Yoshimatsu, N. Utsunomiya, K. Ina, T. Sakata, Transplantation of normal islets into the portal vein of Otsuka, Exp. Biol. Med. (Maywood) (2001) 681–685.
[15] C.F. Barker, L.G. Frangipane, W.K. Silvers, Islet transplantation in genetically determined diabetes, Ann. Surg. (1977) 401–410.
[16] S. Inada, S. Kaneko, K. Suzuki, J. Miyazaki, H. Asakura, M. Fujiwara, Rectification of diabetic state in C57BL/KsJ-db/db mice by the implantation of pancreatic beta cell line MIN6, Diabetes Res. Clin. Pract. (1996) 125–133.
[17] J.F. Garvey, P.R. Millard, P.J. Morris, Experimental transplantation of fetal pancreas and isolated islets in the rat: studies of donor pretreatment and recipient immunosuppression, Transplant. Proc. 12 (1980) 186–189.
[18] C. Weyer, J.E. Foley, C. Bogardus, P.A. Tataranni, R.E. Pratley, Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance, Diabetologia 43 (2000) 1498–1506.
[19] I. Katsuragi, T. Okeda, H. Yoshimatsu, N. Utsunomiya, K. Ina, T. Sakata, Transplantation of normal islets into the portal vein of Otsuka Long Evans Tokushima Fatty rats prevents diabetic progression, Exp. Biol. Med. (Maywood) 226 (2001) 681–685.
[20] J.H. Jeong, S.W. Hong, S. Hong, S. Yook, Y. Jung, J.B. Park, C.D. Khue, B.H. Im, J. Seo, H. Lee, C.H. Ahn, D.Y. Lee, Y. Byun, Surface camouflage of pancreatic islets using 6-arm-PEG–catechol in combined therapy with tacrolimus and anti-CD154 monoclonal antibody for xenotransplantation, Biomaterials 32 (2011) 7961–7970.
[21] Y.S. Jung, J.H. Jeong, S. Yook, B.H. Im, J. Seo, S.W. Hong, J.B. Park, V.C. Yang, D.Y. Lee, Y. Byun, Surface modification of pancreatic islets using heparin-DOPA conjugate and anti-CD154 mAb for the prolonged survival of intrahepatic transplanted islets in a xenograft model, Biomaterials 33 (2012) 295–303.
[22] D.Y. Lee, J.H. Nam, Y. Byun, Effect of polyethylene glycol grafted onto islet capsules on prevention of splenocyte and cytokine attacks, J. Biomater. Sci. Polym. Ed. 15 (2004) 753–766.
[23] D.Y. Lee, S.J. Park, J.H. Nam, Y. Byun, A combination therapy of PEGylation and immunosuppressive agent for successful islet transplantation, J. Control. Release 110 (2006) 290–295.
[24] J.Y. Jang, D.Y. Lee, S.J. Park, Y. Byun, Immune reactions of lymphocytes and macrophages against PEG-grafted pancreatic islets, Biomaterials 25 (2004) 3663–3669.
[25] D. Yun Lee, J. Hee Nam, Y. Byun, Functional and histological evaluation of transplanted pancreatic islets immunoprotected by PEGylation and cyclosporine for 1 year, Biomaterials 28 (2007) 1957–1966.
[26] R.K. Schindhelm, M. Diamant, J.M. Dekker, M.E. Tushuizen, T. Teerlink, R.J. Heine, Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease, Diabetes Metab. Res. Rev. 22 (2006) 437–443.
[27] R.K. Schindhelm, M. Diamant, S.J. Bakker, R.A. van Dijk, P.G. Scheffer, T. Teerlink, P.J. Kostense, R.J. Heine, Liver alanine aminotransferase, insulin resistance and endothelial dysfunction in normotriglyceridaemic subjects with type 2 diabetes mellitus, Eur. J. Clin. Invest. 35 (2005) 369–374.