Transfection of Rat Pancreatic Islet Tissue by Polymeric Gene Vectors

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Abstract

Background: *In vitro* genetic modification has been regarded as one option to improve the viability and functionality of pancreatic islets when used for transplantation in patients with diabetes, either as naked islets or in a type of bioartificial pancreas. In this approach, vector safety and poor transfection efficiency are major concerns.

Methods: In this study, the influence of *in vitro* transfection conditions on polyplexes constructed of polyethyleneimine (PEI) and plasmid DNA (pDNA) on the transfection efficiency was investigated by varying the transfection medium, the pDNA dose, and the amines of polycation/phosphates of pDNA (N/P) ratio.

Results: Ca^{2+} -containing Krebs-Ringer-HEPES medium was more effective than RPMI 1640 medium by increasing transfection efficiency (2.5-fold). An increase in pDNA dose slightly reduced the transfection efficiency but had minimal influence on islet loss. However, the N/P ratio had a large effect on islet viability and transfection efficiency. For example, the PEI/pDNA ratio at N/P = 10 caused greater islet loss (56% vs. 28%) and 30-fold less transfection efficiency than at N/P = 5. Even under a set of best conditions selected from this study, mostly a fraction of cells located in the peripheral regions of an islet were transfected, and the viability and insulin secretion from the treated islets were not altered. However, it was found that the extent of apoptosis was noticeably higher (~16%) than in untreated islets (~2%).

Conclusions: These results suggest that the gene delivery efficacy to isolated islets can be improved by manipulating the transfection conditions. Polymeric vectors will broaden the options for islet transfection, which is currently limited to viral vectors.

Introduction

UPON TRANSPLANTATION, pancreatic islets face a detrimental environment containing an undeveloped vascular network, hypoxia, and autoimmunity, which induce apoptosis and seriously hamper islet viability and insulin secretion ability. This prompted investigators to develop long-acting islets through various means. As one strategy, genetic modification of islet cells following isolation from a pancreas has been attempted with various genes for immunomodulation (e.g., *CTLA-4lg*¹), β -cell proliferation (e.g., *hepatocyte growth factor*²), anti-hypoxia (e.g., *myoglobin*³), neovascularization (e.g., *vascular endothelial growth factor*^{4,5}), anti-apoptosis (e.g., *bcl-2*^{6,7}), and cytoprotection (e.g., *interleukin-4*⁸). It was hypothesized that such genetic modification would improve the viability and functionality of transplanted islets.^{9–13}

Islet transfection has mostly been conducted using viral or liposomal gene carriers. The use of viral vectors raises several safety concerns (e.g., toxicity, mutagenesis, carcinogenesis, and antiviral responses)^{14–17} and presents serious technical

challenges (e.g., limited loading capacity, limited gene size, and difficulties in production/purification).^{17–19} Liposomal vectors have shown poor gene expression,^{5,20–22} toxicity to islets at high liposome/plasmid DNA (pDNA) ratios,^{21,22} and impaired insulin secretion.²² The drawbacks of using viral or liposomal vectors have spurred interest in testing polymeric vectors.

Polymeric vectors have favorable safety profiles (e.g., low immunogenicity and no oncogenesis) and good technical features (e.g., mass productivity, easy quality control, and unlimited pDNA loading capacity).^{17,23} Polyethyleneimine (PEI)²¹ and Superfect™ (Qiagen, Chatsworth, CA) (polyamidoamine)⁵ have been tested using islets and had poor outcomes. One possible reason for such a negative outcome could have been that the experimental conditions used in the published reports^{5,21} had been adapted from the protocols for non-pancreatic cells (e.g., 3T3, COS7, HepG2, Jurkat, etc.).

This study attempted to investigate how the transfection conditions of polyplexes made from branched PEI (25 kDa)/pDNA influenced the viability of rat islets and insulin

secretion function. Three parameters of transfection medium, pDNA dose, and amines of polycation/phosphates of pDNA (N/P) ratio were examined.

Materials and Methods

Islet isolation and culture

Islets of Langerhans were isolated from male Sprague-Dawley rats (weighing 200–300 g; Charles River Laboratories, Wilmington, MA) by using standard collagenase digestion and discontinuous Ficoll density gradient methods.²⁴ Islet yield was about 350 islet equivalents (IEQ) per pancreas at 2 days post-isolation. The islets were cultured in RPMI 1640 medium supplemented with 11.1 mmol/L glucose and 10% heat-inactivated fetal bovine serum under humidified air containing 5% CO₂ at 37°C.

Preparation of PEI/pDNA polyplex

Polyplexes were prepared containing pDNA encoding either firefly luciferase (*gWiz-Luc*; Aldevron, Fargo, ND) or green fluorescent protein (GFP) reporter gene (*gWiz-GFP*; Aldevron) and branched PEI (25 kDa; Aldrich Chemical Co., Milwaukee, WI) in HEPES buffer (20 mM, pH 7.3) supplemented with 5% glucose. After pDNA and PEI were mixed under predetermined complexation conditions, the polyplexes (0.02 mL/ μ g of pDNA) were incubated for 30 min at room temperature.

Particle size and surface charge of PEI/pDNA complexes

The polyplexes (0.2 mL; 10 μ g of pDNA) were added to HEPES buffer (20 mM, pH 7.3) supplemented with 5% glucose or transfection medium (1.8 mL). The concentration of pDNA in the polyplex solution was 5 μ g/mL. Surface charge and particle size of the polyplexes were measured using a Zetasizer 3000HS (Malvern Instrument, Inc., Malvern, Worcestershire, UK) at a wavelength of 677 nm with a constant angle of 90° at room temperature.

In vitro islet transfection

Polymeric transfection of pancreatic islets was optimized using PEI and a luciferase reporter gene. The parameters tested were transfection medium (RPMI 1640 medium at a fixed [Ca²⁺] of 0.424 mmol/L and Ca²⁺-containing Krebs-Ringer-HEPES medium [Ca²⁺ (+)KRH] with varying [Ca²⁺] in a range of 0.127–2.54 mmol/L),²⁵ pDNA dose (3–10 μ g of pDNA/100 IEQ), and PEI/pDNA N/P ratio (N/P = 3–10). At 2 days post-isolation, the isolated islets were transferred to a Petri dish (10 cm in diameter) and were cultured in serum-free transfection medium (2 mL) such as RPMI 1640 medium with glucose (5.6 mmol/L) or Ca²⁺(+)KRH (4.74 mmol/L KCl, 1.19 mmol/L KH₂PO₄, 1.19 mmol/L MgCl₂ 6H₂O, 119 mmol/L NaCl, 0.127–2.54 mmol/L CaCl₂, 25 mmol/L NaHCO₃, 10 mmol/L HEPES, and 5.6 mmol/L glucose). After a 1-h incubation, the islets (200 IEQ) were transfected with PEI/pDNA complexes. After an additional 4-h incubation, culture medium (RPMI 1640 medium with 11.1 mmol/L glucose and 10% heat-inactivated fetal bovine serum; 20 mL) was added to the transfected islets. At 12 h post-transfection, the islets were transferred into fresh culture medium.

To evaluate the extent of luciferase expression, the transfected islets were examined at 1 day post-transfection. Islets were first washed with Ca²⁺/Mg²⁺-free Dulbecco's phosphate-buffered saline. After addition of lysis buffer (60 μ L), the islets were gently vortex-mixed and centrifuged at 13,400 g for 2 min. Supernatants were collected to analyze luciferase expression (20 μ L) and protein levels (20 μ L). Luciferase activity in the transfected islets was quantified using a luciferase assay kit (Promega Co., Madison, WI). The protein amount was evaluated using a BCATM protein assay kit (Pierce Biotechnology Inc., Rockford, IL) to normalize luciferase expression.

In vitro islet toxicity

PEI/pDNA-mediated islet toxicity was examined in terms of remaining islets (%), apoptosis (per IEQ), and cell viability (per IEQ). At 1 day post-transfection, remaining transfected islets were counted, and the change in islet number before and after transfection was evaluated as follows:

Remaining islets (%) =

$$\frac{\text{Number of remaining islets after transfection (IEQ)}}{\text{Number of initial islets before transfection (IEQ)}} \times 100$$

For cell viability (per IEQ), the transfected islets were transferred to a 96-well plate at a density of 10 IEQ per well and were cultured at their final volume (0.1 mL per well). At 1 day post-transfection, CellTiter-Blue[®] reagent (Promega) (20 μ L per well) was added to the islets. After a 4-h incubation at 37°C, fluorescence was measured at 560 nm (excitation) and 590 nm (emission). Cell viability of transfected islets was expressed in relative fluorescent units (RFU) per IEQ and was compared to the cell viability of untransfected islets. After the cell viability assay was performed, Apo-One[®] caspase-3/7 reagent (Promega) (0.1 mL per well) was added to the islets. After a 2-h incubation, fluorescence was measured at 499 nm (excitation) and 521 nm (emission) to assess apoptosis. Transfection-induced apoptosis was also expressed as percentage apoptosis per IEQ, which was converted from RFU/IEQ by comparing results to fluorescence levels obtained from dispersed, dexamethasone (100 nmol/L)-treated islet cells.²⁶

Insulin secretion of transfection islets

Glucose-stimulated insulin secretion was evaluated following islet transfection. At 12 h post-transfection, the transfected islets were transferred to a 24-well plate at a density of 20 IEQ per well and were cultured at their final volume (1 mL). At 1 day post-transfection, the islets were rinsed twice with RPMI 1640 medium, which was supplemented with 2.8 mmol/L glucose, for 20 min to remove secreted insulin. Then, the islets were incubated in fresh RPMI 1640 medium supplemented with 2.8 mmol/L glucose, and after 30 min of starvation, the medium was collected. The glucose-starved islets were then exposed to 16.7 mmol/L glucose in RPMI 1640 medium to stimulate insulin secretion, and samples were collected after 30 min. The medium samples from these conditions were stored at –20°C until analysis. The medium used contained 10% fetal bovine serum regardless of glucose concentration. Insulin secreted from the transfected islets in RPMI 1640 medium supplemented with 2.8 mmol/L glucose or 16.7 mmol/L

glucose was evaluated by ^{125}I -insulin radioimmunoassay (MP Biomedicals, Solon, OH). Insulin stimulation index values were calculated as follows:

Insulin stimulation index =

$$\frac{\text{Insulin secreted at 16.7 mmol/L glucose}}{\text{Insulin secreted at 2.8 mmol/L glucose}}$$

GFP expression

To monitor the location of transfected cells within an islet, pDNA encoding a GFP reporter gene was introduced using optimal transfection conditions. Transfection of the GFP gene was monitored as described in the *in vitro* islet transfection study. Following transfection, GFP expression from the transfected islets was evaluated using a laser scanning confocal microscope (model FV1000, Olympus, Center Valley, PA) with Olympus Fluoview™ installed at an excitation wavelength of 488 nm. Confocal images were sectioned every 15 μm of thickness, and images were to reconstruct images of whole cells.

Statistical analysis

All tests were performed with at least $n = 3$, and these results are expressed as mean $\pm$ standard error of the mean (SEM) except for particle size and surface charge of polyplexes, which are expressed as mean $\pm$ standard deviation (SD). Statistical analysis by Student's *t* test or one-way analysis of variance (ANOVA) with Tukey's HSD (Honestly Significantly Different) test was evaluated with $P < 0.05$ significance.

Results and Discussion

Various chemicals, biological agents, and ions can directly or indirectly influence biological functions. For pancreatic islets, glucose, amino acids, vitamins, and Ca^{2+} are known to effect viability and insulin secretion. β -Cells in an islet are tuned to produce and secrete insulin in response to glucose concentration in the blood and culture environment. During culture, glucose levels are linked to islet apoptosis; islets exposed to a glucose concentration of ≤ 2 mmol/L or ≥ 24.4 mmol/L for 48 h presented more apoptotic events than islets exposed to a glucose concentration of 5.5–11.1 mmol/L.²⁷

Normal cell growth medium (e.g., RPMI 1640 medium) supplemented with amino acids and vitamins has frequently been used for cell culture and gene transfection. However, amino acids promote insulin exocytosis via K_{ATP} channels or voltage-dependent Ca^{2+} channels,²⁸ and vitamins can directly or indirectly influence insulin secretion.^{29–31} We reported that RPMI 1640 medium stimulated higher insulin release from an insulinoma RINm5F cell line than $\text{Ca}^{2+}(+)\text{KRH}$ [$[\text{Ca}^{2+}] = 2.54$ mmol/L is $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$].³² The negatively charged insulin interacted with the cationic polyplexes, leading to reduced cellular uptake of the polyplexes and decreased polymeric transfection.²⁵

Thus, RPMI 1640 medium and $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$ were selected for comparison because RPMI 1640 medium contains several amino acids and vitamins, whereas $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$ does not. A glucose concentration of 5.6 mmol/L was used to minimize glucose-induced insulin secretion and apoptosis. As a starting point, PEI/pDNA complexes containing 5 μg of pDNA were prepared at

$\text{N/P} = 5$. Rat islets (100 IEQ) were treated with polyplexes in either $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$ or RPMI 1640 medium ($[\text{Ca}^{2+}] = 0.424$ mmol/L). PEI/pDNA complexes ($\text{N/P} = 5$) in $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$ had larger particle sizes than the complexes in RPMI 1640 medium, although both particles had similar surface charges (Table 1). Nevertheless, islet transfection performed in $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$ resulted in an approximately 2.5-fold ($P = 0.02$ by unpaired Student's *t* test) higher luciferase expression than in RPMI 1640 medium (Fig. 1, columns). This result was consistent with our previous report in which insulin-secreting RINm5F cells were treated with different polyplexes.²⁵

The transfection media we compared did not show any significant difference in the number of remaining islets after transfection (as a percentage) ($P = 0.30$ by unpaired Student's *t* test); the number of intact islets was approximately 76% and 78% of the initial number of islets in RPMI 1640 medium and $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$, respectively (Fig. 1, solid circles). These results are concurrent with our previous cell viability data using RINm5F cells transfected in both transfection media.²⁵

Ca^{2+} influx mediated by insulin stimulants (e.g., glucose and sulfonylurea) increases intracellular $[\text{Ca}^{2+}]$, which modulates insulin exocytosis as well as β -cell apoptosis.³³ The effects of extracellular $[\text{Ca}^{2+}]$ on PEI/pDNA-mediated islet transfection in the presence of cationic PEI were investigated in $\text{Ca}^{2+}(+)\text{KRH}$ having different $[\text{Ca}^{2+}]$ (i.e., 0.127–2.540 mmol/L). Particle size and surface charge of PEI/pDNA complexes ($\text{N/P} = 5$) presented similar values in $\text{Ca}^{2+}(+)\text{KRH}$ regardless of $[\text{Ca}^{2+}]$ (Table 1). Also, exposing islets to different extracellular $[\text{Ca}^{2+}]$ for 4 h did not result in any statistically significant differences in transfection efficiency ($P = 0.53$ by one-way ANOVA) (Fig. 1, columns). As shown in Figure 1 (solid circles), the remaining islets (as a percentage) after transfection were approximately 71–80% with no statistical significance ($P = 0.29$ by one-way ANOVA), although the values were somewhat decreased in lower extracellular

TABLE 1. PARTICLE SIZE AND SURFACE CHARGE OF PEI/pDNA COMPLEXES IN VARIOUS BUFFERS

N/P ratio	Buffer	Particle size (nm)	Surface charge (mV)
3	HBG	345 $\pm$ 65	0.8 $\pm$ 2.0
	$\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$	429 $\pm$ 68	−14.5 $\pm$ 2.7
5	HBG	134 $\pm$ 13	11.5 $\pm$ 2.7
	RPMI 1640	335 $\pm$ 29	11.9 $\pm$ 2.2
	$\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$	412 $\pm$ 55	13.4 $\pm$ 4.4
	$\text{Ca}^{2+}_{1.905 \text{ mmol/L}}(+)\text{KRH}$	404 $\pm$ 48	12.0 $\pm$ 4.1
	$\text{Ca}^{2+}_{1.270 \text{ mmol/L}}(+)\text{KRH}$	417 $\pm$ 50	11.1 $\pm$ 4.0
	$\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$	370 $\pm$ 57	12.0 $\pm$ 4.8
7	$\text{Ca}^{2+}_{0.127 \text{ mmol/L}}(+)\text{KRH}$	416 $\pm$ 59	11.5 $\pm$ 5.2
	HBG	141 $\pm$ 10	18.1 $\pm$ 7.6
10	$\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$	422 $\pm$ 57	20.7 $\pm$ 3.4
	HBG	133 $\pm$ 11	17.2 $\pm$ 3.0
	$\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$	409 $\pm$ 69	25.9 $\pm$ 4.8

Data are mean $\pm$ SD values ($n = 3$). $\text{Ca}_x^{2+}(+)\text{KRH}$, Ca^{2+} -containing Krebs-Ringer-HEPES medium with the subscript indicating the Ca^{2+} concentration; HBG, HEPES buffer (20 mM, pH 7.3) supplemented with 5% glucose.

FIG. 1. Effects of transfection medium on transfection efficiency (columns) and remaining islet number (solid circles) in islets transfected with PEI/pDNA polyplex at 1 day post-transfection. PEI/pDNA polyplexes were prepared at a pDNA dose of 5 μ g of pDNA per 100 IEQ and an N/P ratio of 5. RLU, relative luminescent units. (* $P < 0.05$ by unpaired Student's t test when compared with $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$.)

[Ca^{2+}]: e.g., $71 \pm 2\%$ in $\text{Ca}^{2+}_{2.54 \text{ mmol/L}}(+)\text{KRH}$, $76 \pm 2\%$ in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$, and $80 \pm 3\%$ in $\text{Ca}^{2+}_{1.27 \text{ mmol/L}}(+)\text{KRH}$. Transfection medium containing a low glucose concentration (5.6 mmol/L) may not stimulate Ca^{2+} -mediated islet death because PEI is probably not an insulin stimulant. Nevertheless, the depletion of [Ca^{2+}] from the transfection medium caused poor transfection²⁵ because [Ca^{2+}] is essential for gene expression.³⁴ Thus, for further optimization, a condition of [Ca^{2+}] = 0.635 mmol/L in $\text{Ca}^{2+}(+)\text{KRH}$ ($\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$) was used to reduce the risk of high [Ca^{2+}]-mediated damages to islet and low/depleted [Ca^{2+}]-mediated loss on gene expression.

Islet transfection using PEI/pDNA polyplexes was reported to be ineffective when a dose of 1.5 μ g of pDNA was given for every 100 IEQ.²¹ Thus, pDNA dose dependency for PEI/pDNA (N/P = 5)-transfected islets in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$ was investigated in the range of 3–10 μ g of pDNA per 100 IEQ. From the doses used, transfection levels were the highest at a dose of 5 μ g of pDNA per 100 IEQ. This level was similar to that at 3 μ g of pDNA per 100 IEQ but was approximately 2.9-fold ($P = 0.07$ by one-way ANOVA with Tukey's HSD test) and 2.3-fold higher than for 7 and 10 μ g of pDNA doses per 100 IEQ, respectively (Fig. 2, columns). IEQ losses were approximately 20–30% at all doses tested ($P = 0.73$ by one-way ANOVA); the remaining islets (as a percentage) were $77 \pm 4\%$ at 3 μ g of pDNA, $76 \pm 2\%$ at 5 μ g of pDNA, $78 \pm 4\%$ at 7 μ g of pDNA, and $73 \pm 3\%$ at 10 μ g of pDNA in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$ (Fig. 2, solid circles). The lower transfection levels at higher pDNA doses (7 and 10 μ g of pDNA per 100 IEQ) is most likely related to the reduced viability of individual cell types, such as α -, β -, δ -, and PP-cells, in an islet rather than the loss of whole islets, which was visually observed before and after treatments.

After selection of a pDNA dose (5 μ g of pDNA per 100 IEQ) and a transfection medium [$\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$], the PEI/pDNA N/P ratio was changed from 3 to 10. N/P ratios higher than N/P = 10 were excluded because of the potential toxicity of PEI/pDNA polyplexes to cells and animals. The transfection efficiency of PEI/pDNA polyplexes was highest at N/P = 5; this was 15-, eight-, and 30-fold higher than transfection levels using N/P = 3, N/P = 7, and N/P = 10, re-

FIG. 2. Effects of pDNA doses on transfection efficiency (columns) and remaining islet number (solid circles) in islets transfected with PEI/pDNA polyplex at 1 day post-transfection. PEI/pDNA polyplexes were prepared in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$ and an N/P ratio of 5. RLU, relative luminescent units.

spectively ($P < 0.002$ by one-way ANOVA with Tukey's HSD test) (Fig. 3, columns). This optimum complexation ratio is consistent with results from liposomal islet transfection.^{21,22} The poor transfection of PEI/pDNA complexes at N/P = 3 could be caused by the negative surface charge of these particles in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$ (Table 1) because negative surface charges cause less cellular uptake of polyplexes than positive surface charges. In addition, the low transfection levels of PEI/pDNA complexes at higher N/P ratios (N/P = 7 and 10) could be due to uncomplexed PEI-mediated islet loss because uncomplexed PEI induces greater cytotoxicity than PEI complexed with genes.³⁵ These transfection results may explain why islet loss was not significant at N/P = 3 and why islet loss increased with higher N/P ratios ($P < 0.001$ by one-way ANOVA). Approximately $79 \pm 3\%$ of islets maintained their integrity at N/P = 3, $76 \pm 2\%$ at N/P = 5, $60 \pm 2\%$ at N/P = 7, and $44 \pm 1\%$ at N/P = 10 ($P < 0.01$ compared with N/P = 3 and N/P = 5 by one-way ANOVA with Tukey's HSD test) (Fig. 3, solid circles).

From the above screening tests, the best transfection efficiency of PEI/pDNA-transfected islets was attained when PEI/pDNA polyplexes were prepared at an N/P ratio of 5 and at a ratio of 5 μ g of pDNA per 100 IEQ and transfected into rat

FIG. 3. Effects of N/P ratios on transfection efficiency (columns) and remaining islet number (solid circles) in islets transfected with PEI/pDNA polyplex at 1 day post-transfection. PEI/pDNA polyplexes were prepared in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}}(+)\text{KRH}$ at a pDNA dose of 5 μ g of pDNA per 100 IEQ. RLU, relative luminescent units.

islets in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}(+) \text{KRH}}$. Under these conditions, the location of PEI/pDNA-mediated transfected cells in a single islet was monitored using a GFP gene because a single islet is composed of approximately 1,000 cells (α -, β -, δ -, and PP-cells). Transfected islets showed higher fluorescence intensity

compared to untransfected islets (Fig. 4a). Also, their sectioned images indicated that PEI-mediated islet transfection mostly occurred in the peripheral region of the islet (Fig. 4b). This result could be caused by the size of PEI/pDNA polyplexes ($\sim 400 \text{ nm}$ in a diameter) in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}(+) \text{KRH}}$,

FIG. 4. GFP expression of PEI/pDNA-transfected islets under optimal transfection conditions: (a) whole islet images and (b) sectioned images. The polyplexes containing $5 \mu\text{g}$ of pDNA prepared at $\text{N/P} = 5$ per transfected 100 IEQ of rat islets in $\text{Ca}^{2+}_{0.635 \text{ mmol/L}(+) \text{KRH}}$. Transfected and untransfected islets were compared and analyzed by laser scanning confocal microscopy. Color images available online at www.liebertonline.com/dia.

FIG. 5. Cell viability (per IEQ), apoptosis (per IEQ), and insulin secretion of transfected islets under optimal transfection conditions: $\text{Ca}^{2+}_{0.635 \text{ mmol/L}}$ (+)KRH, $5 \mu\text{g}$ of pDNA per 100 IEQ, and N/P = 5. Transfected and untransfected islets were compared and analyzed by unpaired Student's *t* test. The *P* values were 0.08 for cell viability (per IEQ), 0.000004 for apoptosis (per IEQ), and 0.98 for insulin stimulation index.

which might be too big for homogeneous islet transfection. Also, this peripheral transfection of islets indicates that PEI-mediated transfection might occur in non- β -cells because these cells reside in the peripheral regions of an islet, unlike β -cells, which are located in the core of islets. This result was consistent with the results from other studies.²¹

The number of viable cells in a single islet is another critical factor for islet transfection. In particular, the viability of β -cells is directly linked to the success of islet grafts. Thus, under the optimum transfection conditions defined above, cell viability (per IEQ) and apoptosis (per IEQ) were evaluated. Transfected and untreated control islets had similar cell viabilities (per IEQ), whereas the extent of apoptosis (per IEQ) of transfected islets ($\sim 16\%$) was about eightfold higher than that of the control islets ($\sim 2\%$) (Fig. 5).

In addition, the insulin-secreting ability of transfected islets was evaluated to determine whether transfection affects the cellular function of β -cells. In this study, the insulin stimulation index was considered instead of secreted insulin (e.g., pM or μIU) because insulin secretion of islets is strongly dependent on islet size^{36,37} and β -cell proportion (e.g., 600–800 cells in an islet).³⁸ Glucose-stimulated insulin secretion index values of PEI-transfected islets were similar in value to that of untransfected islets. These insulin stimulation indices suggest that insulin secretion of transfected islets, especially β -cells, was not impaired. This result was supported by the distribution of GFP-transfected cells in an islet.

Peripheral transfection may be useful for delivering therapeutic genes of proteins to be secreted, such as vascular endothelial growth factor for angiogenesis and CTLA-4Ig for immunomodulation because these genes do not require homogeneous transfection of the whole islet. However, further development of custom-designed polymeric systems, which can control size, surface charge, and functional modification of polyplexes, may enable more homogeneous transfection of islets. For therapeutic genes that translate into non-secreted proteins, such as bcl-2 for anti-apoptosis, homogeneous transfection should result in improved viability and functionality of transplanted islets during islet grafts.

The use of polymeric vectors rather than viral vectors may allow therapeutic genes to be delivered in a safer manner. Additionally, unlike viral vectors, polymeric vectors are particularly well suited for the simultaneous delivery of multiple therapeutic genes, because of their unlimited gene loading capacity.

Conclusions

This study demonstrated that the optimization process is important in improving polymeric islet transfection. The optimum conditions of PEI-mediated islet transfection investigated in this study are an N/P ratio of 5, a ratio of $5 \mu\text{g}$ of pDNA per 100 IEQ, and $\text{Ca}^{2+}_{0.635 \text{ mmol/L}}$ (+)KRH as the transfection medium. The transfection conditions identified in this study may be useful for PEI-mediated transfection of islets with therapeutic genes such as vascular endothelial growth factor and CTLA-4Ig. Although this study used branched PEI, a similar optimization process can be applied to various functionalized polymeric vectors to achieve more efficient gene transfection in pancreatic islets.

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Author Disclosure Statement

No competing financial interests exist.

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