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Research Paper

Polymeric Gene Transfection on Insulin-Secreting Cells: Sulfonylurea Receptor-Mediation and Transfection Medium Effect

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Purpose. *In vitro* transfection of secreting cells is regarded as one strategy for improved cell engineering/transplantation. Insulin-secreting insulinoma cell lines or pancreatic β-cells could be genetically engineered using designed polymeric vectors which are safer than viral vectors. This study investigates the effects of the constituents in transfection media on polymeric transfection.

Methods. Polyplexes conjugated with sulfonylurea (SU) were evaluated under different transfection conditions for gene transfection and their effects on cytotoxicity and insulin secretion. Several components in transfection media specifically associated with the insulin secretion pathway were amino acids, vitamins, Ca^{2+} and K^+. The interactions of the polyplexes with insulin were monitored by surface charge and particle size to monitor how insulin as a protein influences transfection.

Results. For an insulin-secreting cell line (RINm5F), polyplexes in Ca^{2+}-containing KRH medium (Ca^{2+}(+)KRH) enhanced transfection and did not cause damage to biological functions. When adding amino acids, vitamins, or K^+ or depleting Ca^{2+} from Ca^{2+}(+)KRH, poly(L-lysine)/DNA complexes showed a greater reduction in transfection than SU receptor (SUR)-targeting polyplexes (SU-polyplex). Positively charged polyplexes interacted with insulin, developing a negative surface charge, and these interactions may cause a decrease in transfection.

Conclusion. The findings suggest that *in vitro* and *ex vivo* polymeric transfection of insulin-secreting cells can be modulated and enhanced by adjusting the transfection conditions.

KEY WORDS: insulin-secreting cells; polymeric gene carrier; receptor-mediated transfection; sulfonylurea; transfection medium.

INTRODUCTION

Cell transplantation is a potential solution to repair and replace impaired organs and to enhance specific biological functions within the body. In particular, transplantable secretory cells which produce bioactive molecules such as insulin and dopamine can cure diseases related to disorders of endocrine secretion and CNS function, such as Type 1 Diabetes and Parkinson's Disease. However, transplanted allogeneic or xenogeneic cells are often attacked by the host immune system, causing graft failure (1,2). In addition, cellular grafts encounter conditions harsher than the natural physiological environment such as hypoxia, resulting in apoptosis/necrosis. Thus, the *in vivo* systemic delivery of immunomodulatory genes and/or *in vitro* or *ex vivo* genetic manipulation of secretory cells for anti-apoptosis (3) and/or angiogenesis (4,5) prior to transplantation have been proposed. While most *in vivo* gene therapies target cells that have no particular physiological function, genetically engineering secreting cells *in vitro* or *ex vivo* is a promising approach for increasing cell therapy performance and survival post transplantation.

Currently, *in vitro* or *ex vivo* gene transfer to secreting cells (i.e., insulinoma cell lines, pancreatic β-cells and neuroendocrinal cells) has mostly utilized viral gene vectors (3,5,6). However, some viral vectors raised safety concerns because of unfavorable reactions such as immunogenicity and cytotoxicity (3). A few polymeric vectors have been investigated as a potential alternative to viral vectors. As an approach for effective polymeric gene transfection, polymeric vectors have been designed to target specific receptors expressed on secretory cells (i.e., sulfonylurea receptors (SUR) on insulin-secreting cells (7), high-affinity neurotensin receptors on neuronal cells (8), and nerve growth factor receptors on dopamine-secreting cells (9)).

In addition, *in vitro* transfection studies have shown that transfection is strongly influenced by the transfection medium. Specifically, serum proteins such as albumin inhibit non-viral transfection by electrostatically interacting with positively charged non-viral gene carriers (10). Thus, *in vitro* non-viral transfection frequently excludes serum from the transfection medium. Also, secretion functions or processes are often linked to environmental stimuli and chemicals. It is hypothesized that the transfection environment is linked to cellular secretion mechanisms, which in turn influence non-viral transfection processes. A few studies have considered the

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effects of medium components on transfection of secretory cells. For instance, Florea *et al.* suggested that secreting mucus composed of negatively charged glycosaminoglycans could reduce polymeric transfection of differentiated Calu-3 cells (11). Also, liposomal delivery of oligonucleotides to dopamine-secreting cells showed that the transfection medium could affect cellular uptakes although the reason was not known (12). However, when proteins, peptides, and chemicals secreted from cells cause negative effects on gene transfection, it is difficult to remove these secreted molecules from the transfection environment unlike serum. One method to modulate the amount of secreted molecules in the transfection environment is to control medium components such as amino acids (13–16), vitamins (17–19), and ions (20) which affect exocytosis of bioactive molecules (i.e., insulin and neurotransmitters) from secreting cells. However, the influence of secreting molecules on polymeric transfection is not well established yet (21). Thus, improving the efficiency of gene transfection by manipulating medium components *in vitro* or *ex vivo* is regarded to be highly significant in cell engineering with non-viral vectors.

In this study, we investigated the effects of transfection media components on the transfection process of insulin-secreting cells (RINm5F cells) using polymeric vectors. Amino acids (AA), vitamins (V), and ions were the medium components examined in conjunction with the effects of sulfonylurea (SU) in SU-*g*-poly(L-lysine) (PLL-SU) gene carriers. In our previous study, it was noted that PLL-SU/DNA polyplexes utilize receptor-mediated internalization and temporally stimulate insulin secretion (7).

MATERIALS AND METHODS

Materials

PLL·HBr (M_w(viscosity) = 27,400 Da, M_w (MALLS) = 30,200 Da), Fluorescein isothiocyanate (FITC), Dulbecco's phosphate buffered saline (DPBS), Dulbecco's modified Eagle's medium (DMEM), RPMI1640 medium, glibenclamide, bovine serum albumin (BSA), and 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemicals Co. (St. Louis, MO). Branched poly(ethyleneimine) (PEI; M_w = 25 kDa), glutaraldehyde, and dimethylsulfoxide (DMSO) were bought from Aldrich Chemicals Co. (Milwaukee, WI). 4-(2-Hydroxy-ethyl)-1-piperazine ethanesulfonic acid (HEPES), fetal bovine serum (FBS), and horse serum were obtained from GIBCO BRL (Grand Island, NY). All components for the transfection media as listed in Table I were purchased from Sigma–Aldrich Company. Recombinant human insulin was bought from Serologicals Corporation (Norcross, GA). BCA™ protein assay kit and luciferase assay kit were purchased from Pierce Biotechnology Inc. (Rockford, IL) and Promega Co. (Madison, WI), respectively. ^{125}I-Insulin radioimmunoassay (RIA) kit was purchased from MP Biomedicals, Inc (Irvine, CA).

Cell Culture

RINm5F cells (rat insulinoma cell line) were selected as the insulin-secreting cell line and PC12 cells (rat pheochromocytoma cell line) as a reference secreting cell. HepG2 cells (hepatoma cell line) and HEK293 cells (human embryonic kidney cell line) were used as controls (non-secreting cells). RINm5F cells and PC12 cells were cultured using RPMI1640 medium as a standard culture medium. DMEM medium was used for HEK293 and HepG2 cells. Glucose was added into the culture media as a supplement (11.1 mM for secreting cells and 25.2 mM for non-secreting cells). The cells were incubated in humidified air containing 5% CO_2 at 37°C.

Preparation of Polyplexes

Polyplexes were prepared using a model plasmid DNA (pCMV-Luc) and polymers. PLL, PLL-grafted sulfonylurea (PLL-SU), and PEI were selected as representative polymers. PLL-SU was synthesized as previously reported (7) and PLL-SU5% (a feed mole ratio of SU to L-lysine) was selected as a model receptor-targeting polymer. For cellular uptake studies, FITC-labeled polyplexes were utilized. A low amount of FITC (1 mol% of lysine units) label was used so as not to affect particle size and surface charge of the polyplexes at the considered complexation ratio, because these physico-chemical properties of polyplexes influence cellular uptake. Ten microliters of DNA solution (0.1 mg/ml; 1 μg of DNA) and an equal volume of polymer solution were mixed together to form polyplexes (20 μl). After 30 min incubation at room temperature, the polyplexes were utilized in further experiments. The complexation ratio between polymer and DNA was two by weight for PLL/DNA complexes and PLL-SU5%/DNA complexes and five (N/P ratio) for PEI/DNA complexes. The weight ratio of PLL/DNA complexes and PLL-SU5%/DNA complexes was selected because they had similar particle sizes and surface charges in order to prevent the influence of these polyplexes' physico-chemical properties in further studies, as previously reported (7).

Preparation of Transfection Media

For *in vitro* transfection studies, standard culture media and Krebs-Ringer-HEPES (KRH) medium were selected as transfection media. The standard culture medium used for transfection was RPMI1640 medium (RPMI) for RINm5F cells and PC12 cells and DMEM for HEK293 cells and HepG2 cells. Glucose was added into the transfection media for all cell types (5.6 mM for RINm5F cells, 11.1 mM for PC12 cells, and 25.2 mM for HEK293 cells and HepG2 cells). Ca^{2+}-containing KRH medium (Ca^{2+}(+)KRH) was supplemented with L-glutamine (Gln), L-arginine (Arg), a mixture of all the amino acids (AA) used in RPMI, or a mixture of all the vitamins (V) used in RPMI. Ca^{2+}-free KRH medium (Ca^{2+}(−)KRH) and Ca^{2+}(+)KRH with K^+ (40 mM) (Ca^{2+}(+)KRH/$K^+_{40\ mM}$) were also used. In order to compare the component effects of standard culture media utilized for transfection, KRH media prepared with equivalent concentrations of glucose were used for transfection. The major components of the transfection media employed in this study are summarized in Table I.

In vitro Transfection Study

In vitro transfection studies were performed in six-well plates and cells were seeded at a density of 5×10^5 cells/well. Seeded cells were cultured for 24 h prior to adding polyplexes.

Table I. Components Used in Transfection Media

Transfection medium	Components
Ca^{2+}(+)KRH	KCl 4.74 mM, KH_2PO_4 1.19 mM, $MgCl_26H_2O$ 1.19 mM, NaCl 119 mM, $CaCl_2$ 2.54 mM, $NaHCO_3$ 25 mM, HEPES 10 mM
Ca^{2+}(+)KRH/Gln	Ca^{2+}(+)KRH + L-glutamine (Gln; 2.05 mM)
Ca^{2+}(+)KRH/Arg	Ca^{2+}(+)KRH + L-arginine (Arg; 1.15 mM).
Ca^{2+}(+)KRH/GlnArg	Ca^{2+}(+)KRH + Gln (2.05 mM) + Arg (1.15 mM)
Ca^{2+}(+)KRH/AA	Ca^{2+}(+)KRH + a mixture of amino acids in RPMI1640 medium[a] (AA)
Ca^{2+}(+)KRH/V	Ca^{2+}(+)KRH + a mixture of vitamins in RPMI1640 medium[b] (V)
Ca^{2+}(+)KRH /AAV	Ca^{2+}(+)KRH + AA + V
Ca^{2+}(−)KRH	KCl 4.74 mM, KH_2PO_4 1.19 mM, $MgCl_2 \cdot 6H_2O$ 1.19 mM, NaCl 135 mM, $NaHCO_3$ 25 mM, HEPES 10 mM
Ca^{2+}(+)KRH/$K^+_{40\ mM}$	KCl 38.81 mM, KH_2PO_4 1.19 mM, $MgCl_2 \cdot 6H_2O$ 1.19 mM, NaCl 119 mM, $CaCl_2$ 2.54 mM, $NaHCO_3$ 25 mM, HEPES 10 mM

[a] A mixture of amino acids in RPMI1640 medium: L-arginine (0.2 g/l), L-asparagine (0.05 g/l), L-aspartic acid (0.02 g/l), L-cystine·2HCl (0.0652 g/l), L-glutamic acid (0.02 g/l), L-glutamine (0.3 g/l), glycine (0.01 g/l), L-histidine (0.015 g/l), hydroxy-L-proline (0.02 g/l), L-isoleucine (0.05 g/l), L-leucine (0.05 g/l), L-lysine·HCl (0.04 g/l), L-methionine (0.015 g/l), L-phenylalanine (0.015 g/l), L-proline (0.02 g/l), L-serine (0.03 g/l), L-threonine (0.02 g/l), L-tryptophan (0.005 g/l), L-tyrosine·2Na·$2H_2O$ (0.02883 g/l), and L-valine (0.02 g/l).

[b] A mixture of vitamins in RPMI1640 medium: D-biotin (0.0002 g/l), choline chloride (0.003 g/l), folic acid (0.001 g/l), myo-inositol (0.035 g/l) niacinamide (0.001 g/l), *p*-amino benzoic acid (0.001 g/l), D-pantothenic acid·1/2Ca (0.00025 g/l), pyridoxine·HCl (0.001 g/l), riboflavin (0.0002 g/l), thiamine·HCl (0.001 g/l), and vitamin B-12 (0.000005 g/l).

One hour before transfection, the culture medium with 10% FBS was replaced with serum-free transfection medium. Polyplexes (20 μl; 1 μg of DNA) were prepared 30 min before transfection. After adding the polyplexes, cells were incubated for 4 h, then rinsed twice with Ca^{2+}-free and Mg^{2+}-free DPBS solution ($Ca^{2+}Mg^{2+}$(−)DPBS) and incubated for 2 d with normal culture medium containing 10% FBS. After the transfection experiment, cells were rinsed twice with $Ca^{2+}Mg^{2+}$(−)DPBS and lysed using a reporter lysis buffer (200 μl/well). Relative luminescent unit (RLU) and protein content in the cells were evaluated using conventional protocols.

Cell Viability

The transfection medium-dependent cytotoxicity of polyplexes was examined by MTT assay. Cells (RINm5F cells) were seeded in a 12-well plate at a density of 2.5×10^5 cells/well. The experimental procedure was the same as described above for the *in vitro* transfection study except for the amount of loaded polyplexes added (10 μl, 0.5 μg of DNA). After incubation for 2 d, 100 μl of 5 mg/ml MTT solution was added. The cells were incubated for an additional 4 h. After removing the MTT-containing medium, formazan crystals formed by the living cells were dissolved in 0.5 ml of DMSO and quantified using a SpectraMax® M2 microplate reader by reading the absorbance at 570 nm (Molecular devices; Sunnyvale, CA).

In vitro Transfection Study with Competitive Inhibition by Free SU

A transfection medium-dependent competition study with free SU (glibenclamide) using RINm5F cells was performed as described above for the *in vitro* transfection study. SU was dissolved in DMSO at different concentrations. The SU solution (10 μl) was added to the cells before adding polyplexes. To minimize DMSO-induced toxicity, the loading volume of DMSO was limited to 0.1% of the total volume.

Insulin Secretion Study

After incubating the cells for 2 d for transfection, RPMI1640 culture medium was replaced by Ca^{2+}(+)KRH containing 16.7 mM glucose and 0.5% (w/v) BSA. After 1 h insulin stimulation, the medium was sampled and analyzed by RIA. Insulin secretion was normalized to the amount of protein evaluated by BCA™ protein assay.

Influence of Medium Components on Particle Size and Surface Charge of Polyplexes

The effects of medium components on the size and surface charge of PLL/DNA complexes were evaluated in various transfection media as listed in Table I. Ca^{2+}(+)KRH was used as a control medium and amino acids, vitamins and ions were either added or depleted from Ca^{2+}(+)KRH. PLL/DNA complexes were added to the transfection media to adjust the concentration of DNA to 1.5 μg/ml. Surface charge and particle size of the polyplexes were measured using a Zetasizer 3000HS (Malvern Instrument, Inc., Worcestershire, UK) at a wavelength of 677 nm with a constant angle of 90° at 37°C.

Interaction Between Insulin and Polyplexes

Interactions between insulin and polyplexes (PLL/DNA complexes and PLL-SU5%/DNA complexes) were evaluated in HEPES buffer (20 mM, pH 7.4) at 37°C. HEPES buffer was used as a low salt buffer to avoid physico-chemical influences induced by high concentrations of salt. Polyplexes were added to the buffer so that the concentration of DNA was 1.5 μg/ml. After adding insulin to a polyplex-containing solution, surface charge of the polyplexes was monitored for 30 min. Particle size was also measured after mixing insulin with the polyplex.

Cellular Uptake of Polyplexes

Cellular uptake of FITC-labeled polyplexes (FITC-PLL/DNA complexes and FITC-PLL-SU5%/DNA complexes) was performed using RINm5F cells. Transfection media used were RPMI, Ca^{2+}(+)KRH, Ca^{2+}(−)KRH, and Ca^{2+}(+)KRH/V. The preparation of cells was the same as in the *in vitro* transfection study. Polyplexes were added to cells in a serum-free transfection medium. After 1 or 4 h incubation, cells were rinsed with $Ca^{2+}Mg^{2+}$(−)DPBS, detached with 0.25% Trypsin-EDTA solution and fixed using 2.5% glutaraldehyde. Cells were evaluated using flow cytometry (FACScan Analyzer, Becton–Dickinson) with a primary argon laser (488 nm) and a fluorescence detector (530 ± 15 nm) for FITC. The instrument was calibrated with a control (non-treated cells) to identify viable cells and then FITC-uptake in cells was analyzed from a gated viable population of 10,000 cells.

Statistical Analysis and Reproducibility

Results are expressed as mean ± standard error of the mean (SEM) and were analyzed by Student's *t*-test for unpaired data or ANOVA test with $p < 0.05$ significance. The reproducibility of all data was confirmed at least twice.

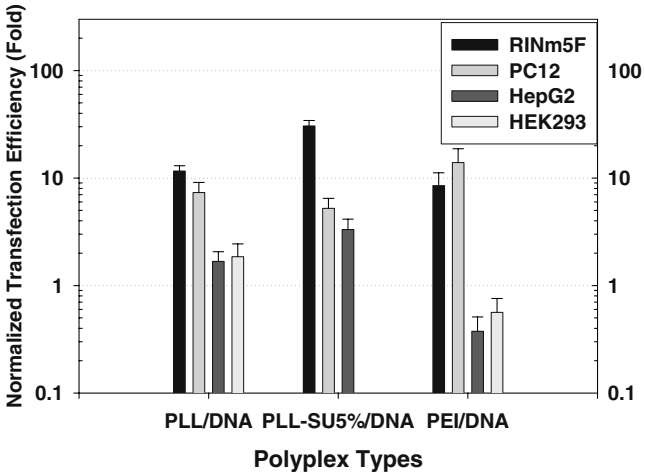


Fig. 1. The effect of Ca^{2+}-containing KRH medium (2.54 mM Ca^{2+}) on polymeric transfection. Two SUR^+ cell lines (RINm5F and PC12) and two SUR^- cell lines (HepG2 and HEK293) were tested. Normalized transfection efficiency was calculated for comparison and the transfection efficiency (RLU/mg Protein) of each polyplex in standard culture media (RPMI1640 or DMEM) was set as unity. Complexation ratios applied were two (weight ratio of Polymer/DNA) for PLL/DNA complexes and PLL-SU5%/DNA complexes and five (N/P ratio) for PEI/DNA complexes. ($n = 4$, Mean ± SEM).

RESULTS AND DISCUSSION

Effect of Transfection Media on Biological Functions

The expression levels of a reporter gene complexed using three different polymeric carriers (PLL, PLL-SU5%, and PEI) in two different transfection media (standard culture medium and Ca^{2+}(+)KRH) were assessed. To compare how polymeric transfection efficiencies were influenced by these media, normalized transfection efficiency was used and the transfection efficiency of each polyplex in standard culture medium was set as unity. The effects of transfection media on transfection efficiency were first evaluated using RINm5F cells. As shown in Fig. 1, polymeric transfection in Ca^{2+}(+)KRH medium induced at least eight times higher gene expression than in RPMI (standard culture medium for RINm5F cells) regardless of the type of polyplex. In secretory RINm5F cells, Ca^{2+}(+) KRH enhanced polymeric transfection 11.6-fold ($p < 0.01$) for PLL/DNA complexes, 30.5-fold ($p < 0.01$) for PLL-SU5%/DNA complexes (SU-polyplex), and 8.5-fold ($p < 0.05$) for PEI/DNA complexes compared to RPMI. In order to analyze whether the effect of transfection media was cell-specific, three more cell lines (PC12, HepG2, and HEK293 cells) were tested. PC12 is a secreting cell line, while HepG2 and HEK293 are non-secreting cell lines.

For PC12 cells, Ca^{2+}(+)KRH medium positively influenced polymeric transfection compared to RPMI as noted with RINm5F cells. As seen in Fig. 1, Ca^{2+}(+)KRH induced better gene expression: 7.3-fold ($p < 0.02$) for PLL/DNA complexes, 5.2-fold ($p < 0.02$) for SU-polyplexes, and 13.9-fold ($p < 0.05$) for PEI/DNA complexes compared to RPMI. However, non-secreting cells either showed no significant effects or negative effects in transfection. For PLL/DNA complexes, HepG2 and HEK293 cells showed 1.7-fold ($p = 0.1$) and 1.9-fold ($p = 0.25$) better efficiency, respectively, when transfected in Ca^{2+}(+)KRH compared to DMEM (standard culture medium for HepG2 and HEK293 cells). However, HepG2 and HEK293 cells transfected by PEI/DNA complexes in Ca^{2+}(+)KRH showed lower gene expression compared to those in DMEM (0.38-fold and 0.56-fold, respectively). For HepG2 cells transfected with SU-polyplexes, Ca^{2+}(+)KRH showed slight enhancement of polymeric transfection (3.3-fold ($p < 0.05$)).

These results suggest that the media affect polymeric transfection differently depending upon the cell type and the carriers. For example, Ca^{2+}(+)KRH enhanced PLL-based and PEI-based transfection in RINm5F and PC12 cells (secreting cells), whereas polymeric transfection of non-secretory HepG2 and HEK293 cells was not positively affected by the media.

Assuming that this cell type-dependent medium effect is relevant to secretory functions, further investigation focused on the insulin-secreting RINm5F cells. The evaluation of biological functions (such as cell viability and insulin secretion) after transfection in two different media (Ca^{2+}(+)KRH and RPMI) was assessed because gene vectors and their transfection process presumably affect not only the viability but also secretory functions of the cells (4,7). The viability of transfected cells was not noticeably different when transfection studies were conducted in the two media. As shown in Fig. 2, when the viability of the cells cultured in RPMI (no transfection) was set as 100%, cells incubated in Ca^{2+}(+)KRH showed 99.2 ± 5.4% viability with no statistical significance ($p = 0.9$; unpaired Student's *t*-test). When treated with either PLL/DNA complexes or PLL-SU5%/DNA complexes, the transfected cells in the two media showed almost 100% cell viabilities. Thus, viability of the transfected cells was not damaged by the presence of polyplexes or the transfection media.

The insulin secretion study was intended to assess how the secretion function is influenced by the particular vector

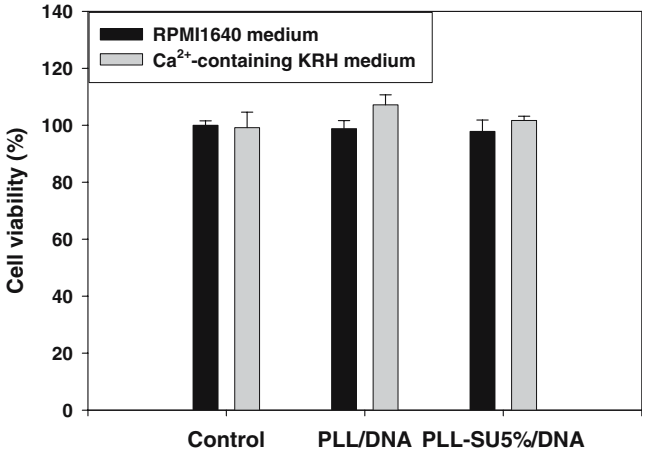


Fig. 2. Effect of transfection medium on cell viability (RINm5F cells). MTT solution was applied for 4 h after finishing the transfection procedure (2 d). Cell viability for cells cultured in RPMI1640 medium only was set as 100%. Polyplexes were prepared using a weight ratio of two (Polymer/DNA). (n = 4, Mean ± SEM).

and transfection conditions employed in this study despite the fact that viability is sustained using relatively less toxic vectors. This assessment can be achieved by using a reporter gene, rather than introducing any other gene that could influence insulin secretion, which allows the use of an easy assay to assess transfection efficiency. Thus, glucose-dependent insulin secretion function was examined after transfection experiments. Insulin secretion after transfection by PLL/DNA or SU-polyplex in two different media was evaluated for 1 h (Fig. 3). For comparison, insulin secreted from non-transfected cells (control) in RPMI was set as 100%. In order to judge whether gene carriers and transfection media affected insulin secretion, a two-way ANOVA test was performed. The gene carrier and transfection medium independently and positively affected insulin secretion with a high statistical significance ($p < 0.0002$ and $p = 0.000002$, respectively). In addition, the combination of the two showed

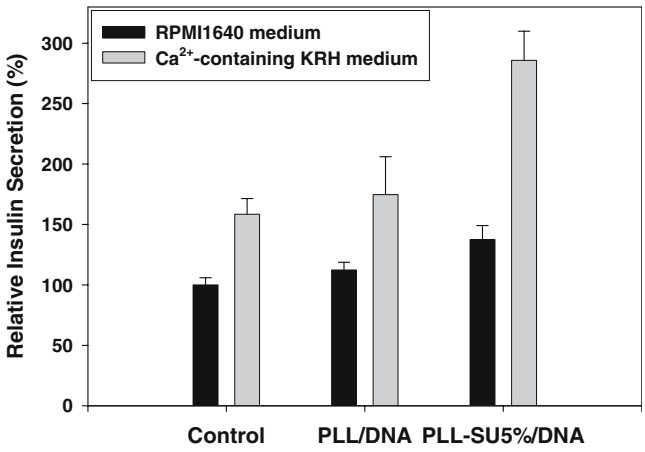


Fig. 3. Effects of transfection media and PLL-based polyplexes on the insulin secretion of RINm5F cells. Insulin secreted after 1 hr stimulation in Ca^{2+}-containing KRH medium (2.54 mM Ca^{2+}) with 16.7 mM glucose was monitored after finishing the transfection procedure (2 d). Insulin secretion from control cells cultured in RPMI1640 medium was set as 100%. Polyplexes were prepared with a weight ratio of 2 (Polymer/DNA) (n = 5, Mean ± SEM).

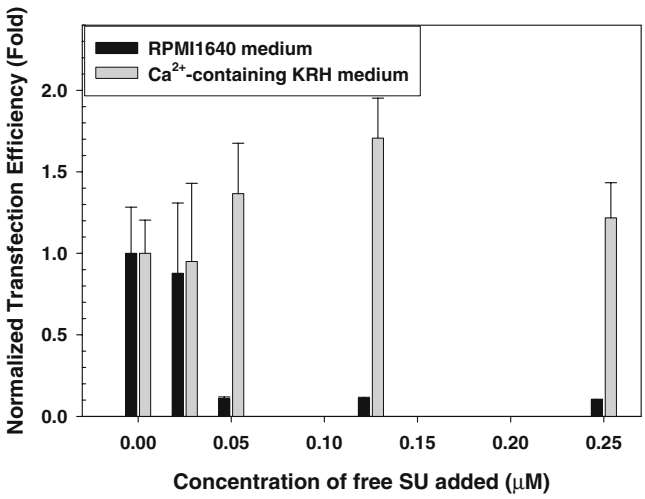


Fig. 4. Competitive transfection studies of PLL-SU5%/DNA complex with free sulfonylurea in two different transfection media (RPMI1640 medium and Ca^{2+}-containing KRH medium (2.54 mM Ca^{2+})). Normalized transfection efficiency was applied and the transfection efficiency (RLU/mg Protein) of PLL-SU5%/DNA complex in each transfection medium was set as unity. RINm5F cells were used and polyplexes were prepared by with weight ratio of two (Polymer/DNA). (n = 4, Mean ± SEM).

a significant difference in insulin secretion with $p = 0.025$. For transfected cells in Ca^{2+}(+)KRH, treatment with SU-polyplexes resulted in about 2.1 times more insulin secretion ($p = 0.0005$) than the control (non-transfected cell cultured in RPMI). In Ca^{2+}(+)KRH medium, both untreated cells and cells transfected by PLL/DNA complexes secreted approximately 1.6 ($p < 0.1$) times more insulin when compared with insulin secreted from untreated cells in RPMI. However, when cells were in the same transfection medium, insulin secretion from both treated and untreated cells did not show any significant difference. As previously reported (7), it was observed that SU-polyplexes stimulated cells to secrete insulin in both media. Also, cells transfected with SU-polyplexes in Ca^{2+}(+)KRH (286% of the control) showed higher insulin-secreting activity than those in RPMI (137% of the control). These results were attributed to insulin stimulation from the SU moiety, which is a known insulinotropic agent. Although insulin stimulation effects of

Table II. Effect of Transfection Medium on Particle Size and ζ-Potential of PLL/DNA Complexes

Transfection medium	Particle size[a] (nm)	ζ-potential[b] (mV)
Ca^{2+}(+)KRH	290 ± 54	30.5 ± 3.2
Ca^{2+}(+)KRH/Gln	295 ± 36	29.1 ± 2.4
Ca^{2+}(+)KRH/Arg	320 ± 53	29.3 ± 2.9
Ca^{2+}(+)KRH/GlnArg	329 ± 51	30.4 ± 2.4
Ca^{2+}(+)KRH/AA	309 ± 58	27.6 ± 2.8
Ca^{2+}(+)KRH/V	314 ± 67	28.7 ± 4.1
Ca^{2+}(+)KRH /AAV	297 ± 38	26.4 ± 1.0
Ca^{2+}(−)KRH	284 ± 55	29.2 ± 1.9
Ca^{2+}(+)KRH/$K^{+}_{40\ mM}$	317 ± 24	28.9 ± 3.6

The values are expressed as Mean ± SD.
[a] Particle size was obtained from its distribution.
[b] ζ-potential was obtained by n = 3.

SU-polyplexes in two different media of Ca^{2+}(+)KRH and RPMI differ each other which are not elucidated, the effects may pertain to changing amounts of SUR present on the plasma membrane, altered by the different transfection environments.

An *in vitro* competitive inhibition study on transfection with free SU (glibenclamide) was carried out using polyplexes (PLL/DNA and SU-polyplexes) and RINm5F cells. Transfection of PLL/DNA in either media was not noticeably affected by the presence of free SU (Data not shown). In contrast, when SU-polyplexes were used, transfection was influenced differently by the concentration of free SU depending on the medium (Fig. 4). In RPMI, co-treatment of SU-polyplexes and free SU showed a decrease in transfection efficiency (nine-fold lower; $p < 0.05$; unpaired Student's *t*-test) for concentrations of free SU higher than 50 nM compared to single treatment of SU-polyplexes without free SU. However, in Ca^{2+}(+)KRH the combined dose of SU-polyplexes and free SU did not induce any reduction in transfection within the same concentration range of free SU. Interestingly, with 125 nM free SU, cells treated with SU-polyplexes showed about 1.7 times higher transfection efficiency than those treated with SU-polyplexes alone, although the value is not significant ($p = 0.07$). These results suggest that in RPMI,

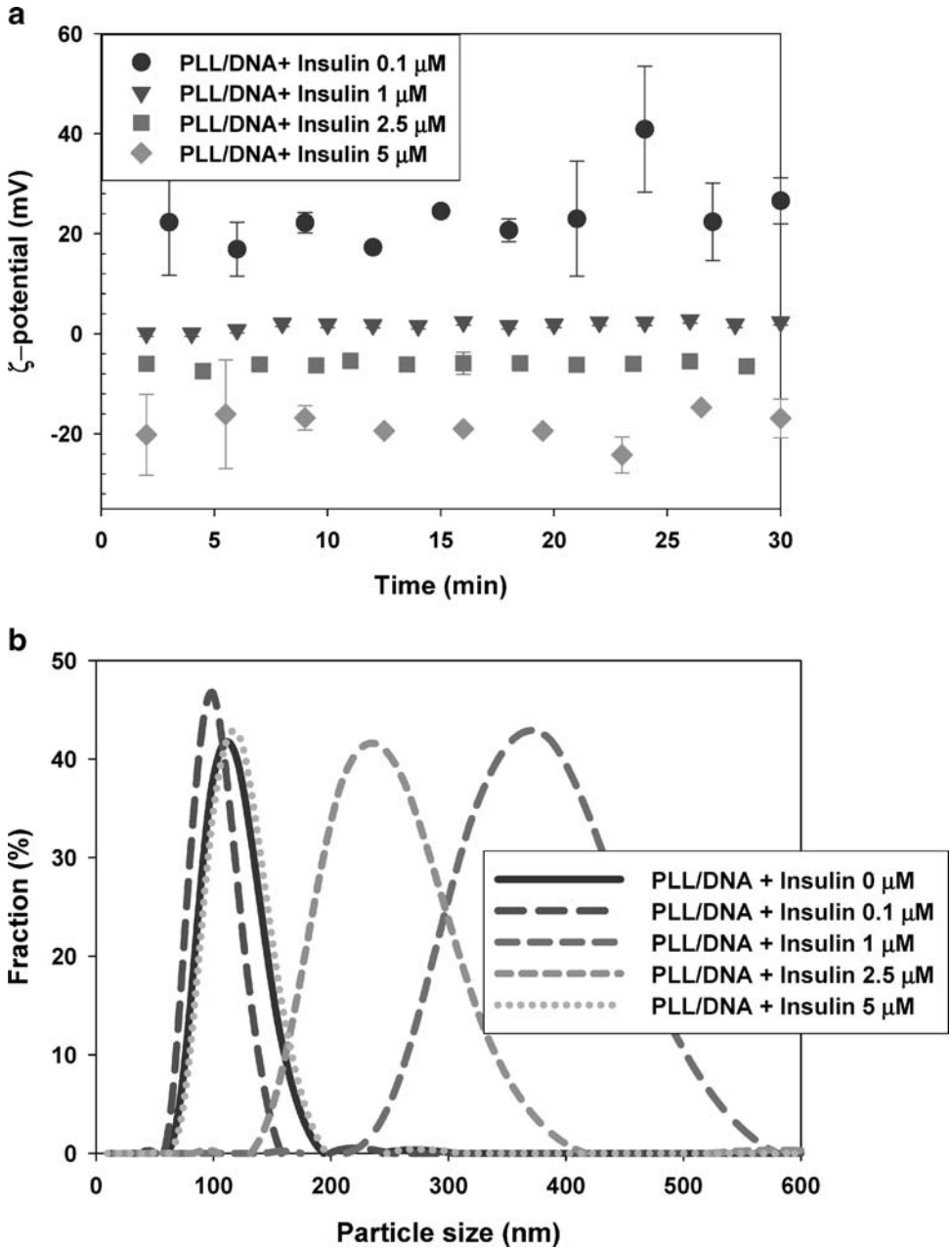
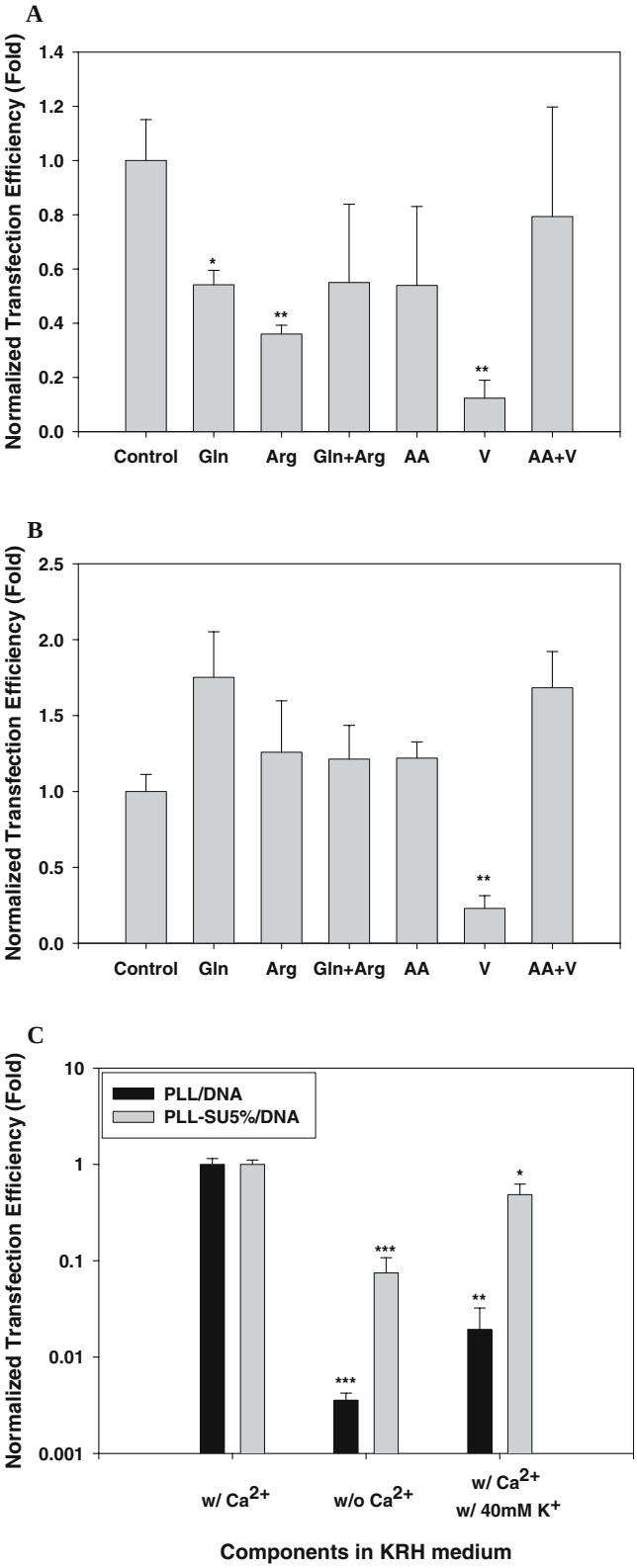


Fig. 5. Interactions between insulin and PLL/DNA complexes. A Surface charges and B Particle sizes. Polyplexes were prepared with a weight ratio of two (Polymer/DNA). Representative trends of surface charges and particle sizes were determined after checking reproducibility. *Error bar* means the distribution of zeta-potentials at each point.

free SU significantly influences the binding frequency of SU-polyplexes to SUR, resulting in a reduction in translocation. However, the inhibition of SUR-binding by free SU was not effective in Ca^{2+}(+)KRH. These results may be related with the amount of SUR on plasma membrane.



Influence of Medium Components on Particle Size and Surface Charge of Polyplexes

Particle size and surface charge of polyplexes affect cellular uptake, thereby influencing gene transfection. Medium components pertaining to insulin-secreting processes may directly affect such properties of polyplexes. Thus, the effects of amino acids, vitamins, and ions on particle size and surface charge of polyplexes were examined. The particle size and surface charge of PLL/DNA complexes in Ca^{2+}(+)KRH was 290 ± 54 nm and 30.5 ± 3.2 mV, respectively. The particle size of PLL/DNA complexes in Ca^{2+}(+)KRH was larger (~100 nm) than that in HEPES buffer (20 mM) because high salt concentrations can induce loose polymer/gene condensation (10). However, surface charges in Ca^{2+}(+)KRH and HEPES buffer (20 mM) were not significantly different. In addition, the particle size and surface charge of PLL/DNA complexes showed about 300 ± 50 nm and about 27–30 mV without significant difference depending on transfection media, as shown in Table II. These results suggest that the influences of amino acids, vitamins, and ions in Ca^{2+}(+)KRH on the physico-chemical properties of polyplexes tested in this study are not significant.

Interaction Between Insulin and Polyplexes

The interactions between cationic polyplexes and insulin are expected, which may negatively affect polymeric transfection as albumin does (10,22). As shown Fig. 5, the presence of insulin at pH 7.4 and 37°C altered surface charges and particle sizes. With 1 μM of insulin, PLL/DNA complexes became almost neutral (1.7 mV as mean), while the control (PLL/DNA complexes at 0 μM of insulin) was about 32 mV. The PLL/DNA surface became more negatively charged with increasing amounts of insulin. The particle sizes of PLL/DNA were also affected by the amount of insulin present. At neutral charges, the polyplex formed bigger particles, probably resulting from aggregation. Particle size increased with increasing insulin concentration and formed the biggest particles at neutral charge. Increasing insulin concentration induces negative surface charges and interestingly, the polyplex size returned back towards 100 nm in diameter. It seems that the surface charge of the polyplexes strongly affects the particle size. That is, strong positive or negative charges prevent the particles from aggregating due to charge–charge repulsion, but a neutral surface causes interparticle interaction and aggregation. SU-polyplexes showed an interaction pattern with insulin similar to the case of PLL/DNA complexes (Data not shown). However, a higher amount of insulin was required to ap-

Fig. 6. Effects of medium components on polymeric transfection. Effects of amino acids and/or vitamins on RINm5F cell lines were tested using two polyplexes (A) PLL/DNA and (B) PLL-SU5%/DNA in Ca^{2+}-containing KRH medium (2.54 mM Ca^{2+}). (C) Effects of Ca^{2+} (2.54 mM or 0 mM) and K^+ (40 mM) on polymeric transfection in KRH medium were studied using both polyplexes. Normalized transfection efficiency was applied and the transfection efficiency (RLU/mg Protein) of each polyplex in Ca^{2+}-containing KRH medium (2.54 mM Ca^{2+}) was set as unity. Amino acids, L-glutamine, L-arginine, and vitamins are designated as AA, Gln, Arg, and V, respectively. (n = 4, Mean ± SEM). (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

proach neural surface charges compared to the case of PLL/DNA complexes. At neutral surface charges, SU-polyplex formed bigger particles than PLL/DNA. Regardless of the polyplex type, the results indicate that negatively charged insulin covers the cationic polyplexes, leading to changes in particle size.

Effect of Medium Components on Polymeric Transfection

To understand the influence of secreted molecules on transfection efficiency is important because secreted molecules are continuously present in the media. Due to interactions of insulin with cationic polyplexes, the effects of medium components which influence insulin secretion such as amino acids, vitamins, and cations were investigated in transfection processes. In addition to those medium components, the effect of SU in polymeric carriers on transfection efficiency was also examined.

Amino acid-induced insulin secretion normally utilizes K_{ATP} channels and/or voltage-dependent Ca^{2+} channels to promote insulin exocytosis (15), but the extent of insulin secretion induced by amino acids was different depending on the cell type and the concentration of amino acids. For example, L-lysine and L-arginine stimulate insulin secretion (13,14), whereas L-valine inhibits it (13). Furthermore, the effect of Gln on insulin secretion is controversial (15,16). Thus, in these studies, Gln and Arg were selected as model amino acids for the *in vitro* transfection studies because of

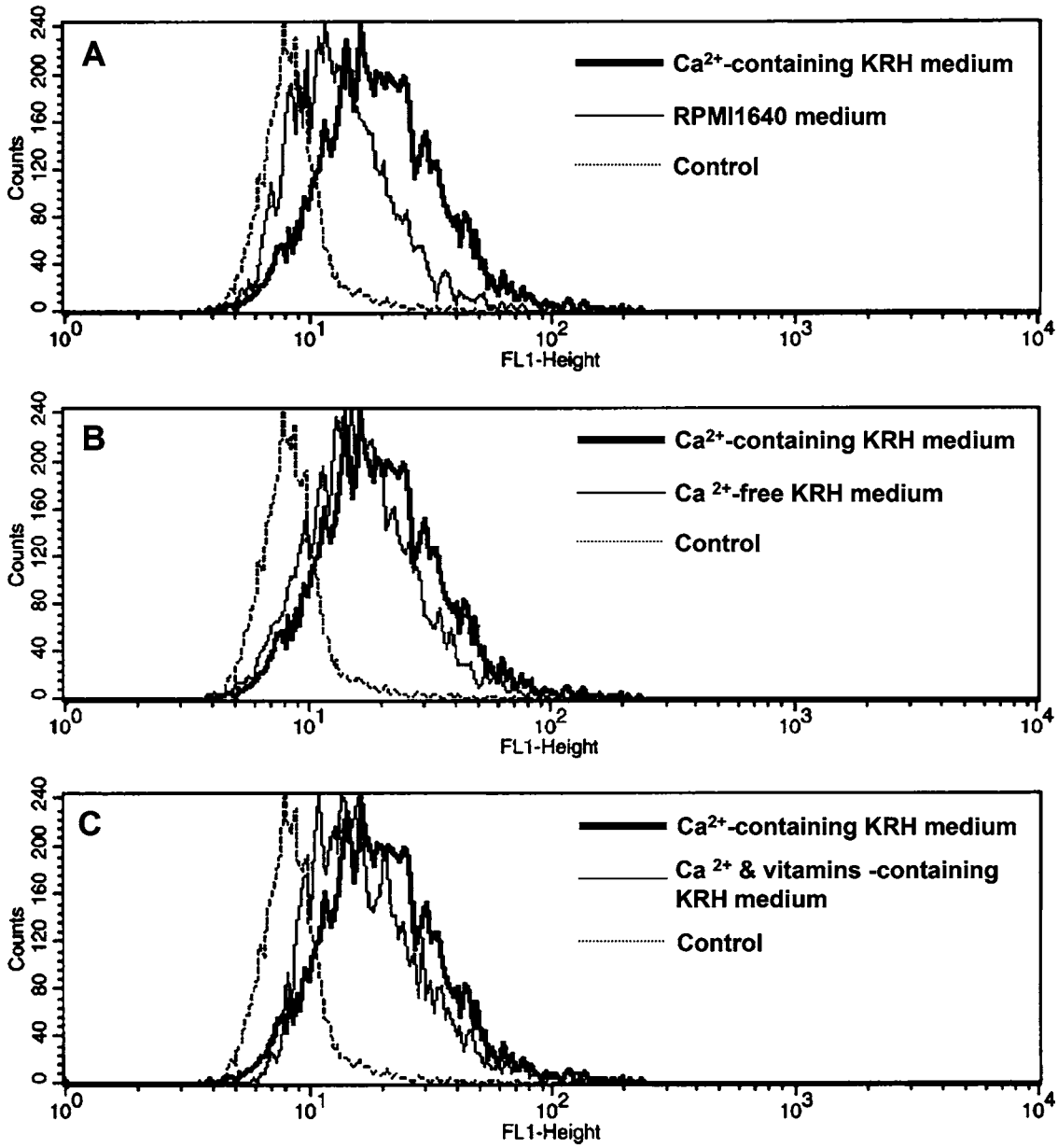


Fig. 7. Cytograms which show transfection medium-dependent cellular uptake of FITC-labeled polyplexes in RINm5F cells after 4 h incubation. A–C FITC-PLL/DNA complexes and D–F FITC-PLL-SU5%/DNA complexes. FITC-polymers were designed to prevent the alteration of the polyplexes' particle sizes and surface charges. The target label amount of FITC was 1 mol% of lysine unit. Polyplexes were prepared with a weight ratio (Polymer/DNA) of two. Controls in the cytograms were obtained by treating cells with pH 7.4 buffer.

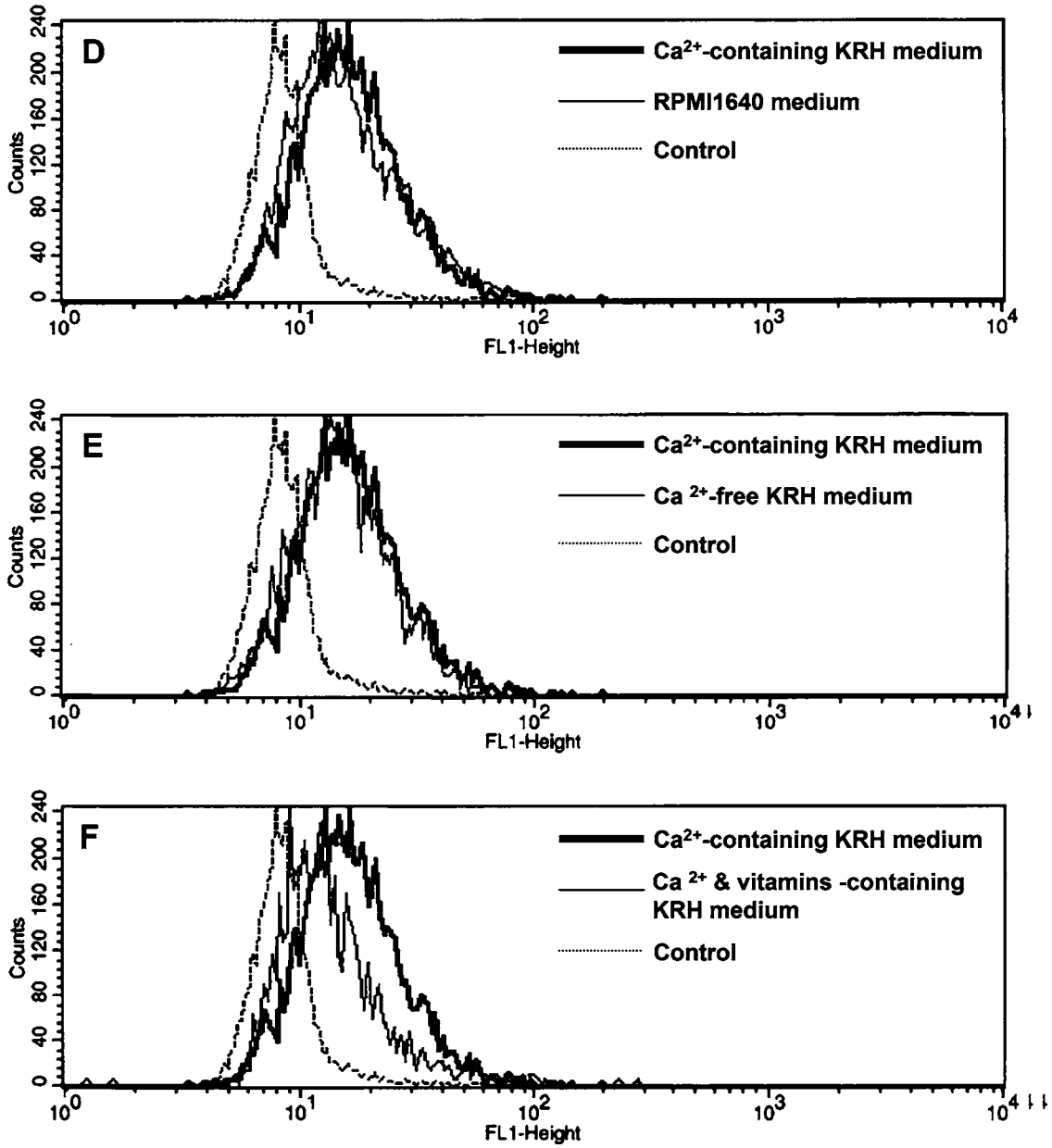


Figure 7 (continued)

their high concentrations in RPMI (Gln: 2.05 mM; Arg: 1.15 mM; other amino acids: <0.4 mM). All amino acids in the formulation of RPMI (see the components listed in Table I) were also taken into consideration. The effects of amino acids on transfection efficiency in amino acids-containing Ca^{2+}(+)KRH (i.e., Ca^{2+}(+)KRH/GlnArg, Ca^{2+}(+)KRH/Gln, and Ca^{2+}(+)KRH/Arg) were evaluated by comparing the transfection efficiencies of PLL/DNA and SU-polyplex in Ca^{2+}(+)KRH (without amino acids and vitamins), which were set as unity in Fig. 6A and B, respectively. Gln or Arg in Ca^{2+}(+)KRH caused a reduction in transfection with PLL/DNA: (0.54 ± 0.05; p = 0.03) for Gln and (0.36 ± 0.03; p = 0.006) for Arg relative to the control (Ca^{2+}(+)KRH). When transfecting in Ca^{2+}(+)KRH/GlnArg or Ca^{2+}(+)KRH/AA, PLL/DNA also showed decreased transfection efficiency: (0.55 ± 0.29; p = 0.2) and (0.54 ± 0.29; p = 0.2), respectively. However, unlike PLL/DNA complexes, SU-polyplexes in Ca^{2+}(+)KRH/Gln and Ca^{2+}(+)KRH/Arg increased normalized transfection efficiency but without statistical significance: (1.75 ± 0.30; p = 0.06) for Gln and (1.26 ± 0.34; p = 0.5) for Arg. A mixture of Gln and Arg and AA also showed no statistically significant effect on transfection: (1.21 ± 0.22; p = 0.4) and (1.22 ± 0.11; p = 0.2), respectively. These results may be primarily affected by facilitated endocytosis of SU-polyplexes (a positive effect in transfection) compared to the insulin stimulation of SU moiety (a negative effect in transfection).

Besides amino acids, the composition of vitamins is different between the two transfection media. RPMI contains a variety of water soluble vitamins such as biotin (0.82 μM), choline chloride (21.5 μM), folic acid (2.27 μM), *myo*-inositol (0.19 mM), niacinamide (8.19 μM), and thiamine (2.96 μM).

These vitamins can directly or indirectly affect insulin secretion (17–19). In the present study, transfection efficiencies of PLL/DNA and SU-polyplexes were significantly decreased in Ca^{2+}(+)KRH/V ((0.12 ± 0.07; p = 0.005) and (0.23 ± 0.08; p = 0.004), respectively) as shown in Fig. 6A and B. In addition, when AA and V were added together, the normalized transfection efficiencies of PLL/DNA and SU-polyplex were (0.79 ± 0.40; p = 0.65) and (1.68 ± 0.24; p = 0.04), respectively. These results suggest that vitamins in Ca^{2+}(+)KRH/V can induce a greater reduction in polymeric transfection and/or more insulin stimulation than amino acids in Ca^{2+}(+)KRH/AA. However, a combined dose of vitamins and amino acids showed less reduction in transfection efficiency than a single treatment of vitamins. It is likely that certain amino acids such as valine in Ca^{2+}(+)KRH/AAV could inhibit insulin stimulation caused by vitamins and other amino acids.

The effects of ions on transfection were considered because insulin secretion is closely linked to Ca^{2+} and K^+. In particular, glucose, which is one of the major insulin stimulants, induces insulin secretion primarily after activating Ca^{2+}-dependent depolarization of the plasma membrane (23). High K^+ concentrations (>30 mM) induce rapid exocytosis of insulin granules like glucose-induced insulin stimulation (20). Thus, in order to evaluate the role of cations in polymeric transfection, Ca^{2+}(+)KRH, Ca^{2+}(−)KRH, and Ca^{2+}(+)KRH/$K^+_{40\ mM}$ were tested and the results are shown in Fig. 6C. When compared to the transfection efficiencies of PLL/DNA and SU-polyplex in Ca^{2+}(+)KRH which were set as unity, transfection efficiencies in Ca^{2+}(−)KRH showed significant reduction, as low as (0.0036 ± 0.0007; p = 0.0006) and (0.075 ± 0.033; p = 0.0002), respectively. Ca^{2+}(−)KRH caused relatively lower transfection levels than other conditions. This observation can be explained by Ca^{2+}-independent insulin secretion (24) as well as Ca^{2+}-dependent biochemical functions such as gene expression (25). That is, a low level of Ca^{2+} in intracellular environments might limit the protein production from the delivered genes. In the calcium-free condition, SU-polyplex still induced better transfection than PLL/DNA.

In Ca^{2+}(+)KRH/$K^+_{40\ mM}$, the normalized transfection efficiencies of PLL/DNA and SU-polyplex were lower than those in Ca^{2+}(+)KRH as expected ((0.019 ± 0.013; p = 0.003) and (0.49 ± 0.14; p = 0.03), respectively). This result also seems relevant to insulin granule exocytosis stimulated by $K^+_{40\ mM}$. As before, SU-polyplex represented more transfection than PLL/DNA.

Effect of Transfection Media on Cellular Uptake

The cellular uptake of FITC-labeled polyplexes was performed to evaluate how the transfection media affects cellular uptake rate. Laser scanning confocal microscopy (Data not shown) and flow cytometry (Fig. 7) were applied to perform a cellular uptake study of polyplexes in RPMI and Ca^{2+}(+)KRH. In confocal images of cells 1 and 4 h after transfection with FITC-labeled PLL/DNA, cells in Ca^{2+}(+)KRH showed higher fluorescence intensity than those in RPMI regardless of the transfection time period. Ca^{2+}(−)KRH and Ca^{2+}(+)KRH/V were selected as model transfection media for the flow cytometry studies because

Table III. Summary of Normalized Transfection Efficiencies (Mean ± SEM)

(A) Cell-dependent and polymer-dependent transfection in standard culture medium (RPMI or DMEM) and Ca^{2+}(+)KRH

	Normalized transfection efficiency[a]							
	Secretory cells				Non-secretory cells			
	RINm5F		PC12		HepG2		HEK293	
Polyplex	RPMI	Ca^{2+}(+)KRH	RPMI	Ca^{2+}(+)KRH	DMEM	Ca^{2+}(+)KRH	DMEM	Ca^{2+}(+)KRH
PLL/DNA	1.00 ± 0.26	11.64 ± 1.37	1.00 ± 0.31	7.33 ± 1.78	1.00 ± 0.16	1.68 ± 0.39	1.00 ± 0.34	1.85 ± 0.58
PLL-SU5%/DNA	1.00 ± 0.11	30.49 ± 3.81	1.00 ± 0.29	5.22 ± 1.25	1.00 ± 0.33	3.31 ± 0.84	N.T.	N.T.
PEI/DNA	1.00 ± 0.16	8.52 ± 2.66	1.00 ± 0.25	13.92 ± 4.82	1.00 ± 0.28	0.38 ± 0.14	1.00 ± 0.47	0.56 ± 0.20

(B) Transfection medium-dependent transfection of PLL/DNA complexes and PLL-SU5%/DNA complexes

	Normalized transfection efficiency[b]	
Transfection medium	PLL/DNA complexes	PLL-SU5%/DNA complexes
Ca^{2+}(+)KRH	1.00 ± 0.15	1.00 ± 0.11
Ca^{2+}(+)KRH/Gln	0.54 ± 0.05	1.75 ± 0.30
Ca^{2+}(+)KRH/Arg	0.36 ± 0.03	1.26 ± 0.34
Ca^{2+}(+)KRH/GlnArg	0.55 ± 0.29	1.21 ± 0.22
Ca^{2+}(+)KRH/AA	0.54 ± 0.29	1.21 ± 0.11
Ca^{2+}(+)KRH/V	0.12 ± 0.07	0.23 ± 0.08
Ca^{2+}(+)KRH /AAV	0.79 ± 0.40	1.68 ± 0.24
Ca^{2+}(−)KRH	0.0036 ± 0.0007	0.075 ± 0.033
Ca^{2+}(+)KRH/$K^+_{40\ mM}$	0.019 ± 0.013	0.49 ± 0.14

(A) [a] Each transfection efficiency of each polyplex in standard culture medium was set as the unity on each cell-type.
N.T. Not tested
(B) [b] Each transfection efficiency of each polyplex in Ca^{2+} (+)KRH was set as the unity.

these media caused a significant reduction in polymeric transfection compared to Ca^{2+}(+)KRH. FITC-PLL/DNA in Ca^{2+}(+)KRH showed about 1.9-fold (for 1 h incubation) and 2.4-fold (for 4 h incubation; Fig. 7A) more cellular uptake compared to RPMI. As more time elapsed, the difference in the amount of internalized polyplexes in Ca^{2+}(+)KRH and RPMI grew. This result may be because AA and V in RPMI stimulate more insulin release than Ca^{2+}(+)KRH and secreted insulin affects the properties of PLL/DNA complexes as seen by the results in Fig. 5. The change of their particle size and surface charge could inhibit or retard their cellular uptake. It seems that the difference in cellular uptake between Ca^{2+}(+)KRH and RPMI caused the remarkable difference in normalized transfection efficiency seen in Fig. 1.

For cellular uptake of FITC-PLL/DNA complexes in Ca^{2+}(−)KRH and Ca^{2+}(+)KRH/V, a 1 h incubation did not induce significant differences between Ca^{2+}(−)KRH or Ca^{2+}(+)KRH/V and Ca^{2+}(+)KRH (data not shown). However, a 4 h incubation revealed more differentiated cellular uptake. Ca^{2+}(−)KRH and Ca^{2+}(+)KRH/V showed about 30% less and 20% less cellular uptake compared to Ca^{2+}(+)KRH (Fig. 7B and C). These results indicate that both calcium-free conditions and vitamins may induce insulin stimulation. However, the transfection results of PLL/DNA complexes in both media (Fig. 6A and C) showed more reduction compared to that in RPMI. Based on the *in vitro* transfection studies and cellular uptake studies, it is inferred that Ca^{2+}(−)KRH and Ca^{2+}(+)KRH/V can affect insulin secretion (17–19,24) as well as gene expression (19,25).

For the case of FITC-SU-polyplexes, 1 h incubation resulted in 25% less uptake in RPMI than Ca^{2+}(+)KRH (Data not shown). However, after 4 h incubation, cellular uptake of polyplexes in Ca^{2+}(+)KRH and in RPMI were not different as shown in Fig. 7D. RPMI can stimulate more insulin secretion than Ca^{2+}(+)KRH. However, SU moieties in SU-polyplexes are also an insulin production stimulant. This seems to relate to the cellular uptake rate of the polyplexes. SU-polyplexes in Ca^{2+}(+)KRH probably could endocytose rapidly compared to those in RPMI because of the different insulin secretion. However, 4 h could be enough time for complete cellular uptake of the polyplexes.

One and 4 h incubation (Fig. 7E) of FITC-SU-polyplexes in Ca^{2+}(−)KRH represented similar cellular uptakes to those in Ca^{2+}(+)KRH. Although cellular uptake of the polyplexes in Ca^{2+}(+)KRH/V for 1 h was close to that in Ca^{2+}(+)KRH (Data not shown), 4 h incubation in Ca^{2+}(+)KRH/V induced about 30% less uptake of polyplexes compared to in Ca^{2+}(+)KRH (Fig. 7F). As seen for PLL/DNA complexes, it seems that weak Ca^{2+}-independent insulin stimulation for Ca^{2+}(−)KRH and strong vitamin-mediated insulin stimulation for Ca^{2+}(+)KRH/V affect the cellular uptake of SU-polyplexes.

This study investigated the effect of transfection medium components on polymeric transfection of insulin-secreting cells. The findings show that insulin secretagogues can affect polymeric transfection (as summarized in Table III) even though the transfection medium does not contain insulin at beginning. However, SU-polyplexes showed a lower reduction in transfection than PLL/DNA complexes. These results are not clearly explained because the SU moiety is a strong insulin stimulant. One possibility is an increase of SUR on the plasma membrane during insulin granule exocytosis (20). It has been known that the fusion of insulin granules with the plasma membrane increases the number of SUR on the plasma membrane because SUR exists in the plasma membrane as well as in intracellular compartments, especially insulin granules (26). Increased SURs could mediate increased or faster cellular uptake of SU-polyplexes compared to PLL/DNA, leading to enhanced transfection. Furthermore, the supply of SUR could explain the different patterns for competitive transfection of SU-polyplexes with free SU. The competition of SU-polyplex with free SU against SUR on the plasma membrane caused reduced transfection efficiency in RPMI, whereas in Ca^{2+}(+)KRH, the competitive inhibition of free SU on transfection efficiency was not significant within the tested range of SU concentrations (up to 250 nM) as shown in Fig. 4. These results could be caused by an increase of available SUR on the cellular membrane. That is, SU-polyplexes could be binding more easily or in increased amounts with SUR in Ca^{2+}(+)KRH than in RPMI. Most likely, the range of free SU that is effective for binding inhibition could be shifted towards higher concentrations of free SU than the conditions tested.

Furthermore, it is also expected that membrane depolarization and granule mobilization (20) influences polymeric transfection. Membrane depolarization from −60 to −10 mV during insulin secretion could induce less interaction between plasma membrane and positively charged polyplexes. This may contribute to less transfection efficiency because non-targeting polyplexes generally follow fluid-phage and/or adsorptive endocytosis to enter cells and their cellular uptake is strongly affected by the negatively charged cellular membrane. In addition, the loss of insulin granules due to insulin secretion causes their biogenesis from the Golgi complex and the newly generated granules move closer to the plasma membrane by cytoskeleton microtubules (20,27). Thus, the mobilization of negatively charged insulin granules and their membrane characteristics may cause interactions between positively charged polyplexes and the granules, inhibiting nuclear import of delivered genes. However, this plausible explanation requires further investigation at sub-cellular levels.

CONCLUSION

In this study, the effects of transfection media on several biological functions of insulin-secreting cells were examined, including gene transfection, cell viability, and insulin secretion. Cellular mechanisms induced by the transfection conditions either inhibited or promoted polymeric transfection. Thus, for improved polymeric transfection *in vitro* and *ex vivo*, our findings show that understanding the cellular mechanisms activated by the transfection medium is important. It is expected that this study will help to improve the *ex vivo* polymeric transfection of secretory cells such as pancreatic islets and chromaffin cells for transplantation.

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REFERENCES

1. C. Ricordi and T. B. Strom. Clinical islet transplantation: advances and immunological challenges. *Nat. Rev. Immunol.* **4**:259–268 (2004).
2. M. K. Lee and Y. H. Bae. Cell transplantation for endocrine disorders. *Adv. Drug Deliv. Rev.* **42**:103–120 (2000).
3. R. Bottino, P. Lemarchand, M. Trucco, and N. Giannoukakis. Gene- and cell-based therapeutics for type I diabetes mellitus. *Gene Ther.* **10**:875–889 (2003).
4. A. S. Narang, K. Cheng, J. Henry, C. Zhang, O. Sabek, D. Fraga, M. Kotb, A. O. Gaber, and R. I. Mahato. Vascular endothelial growth factor gene delivery for revascularization in transplanted human islets. *Pharm. Res.* **21**:15–25 (2004).
5. D. Casper, S. J. Engstrom, G. R. Mirchandani, A. Pidel, D. Palencia, P. H. Cho, M. Brownlee, D. Edelstein, H. J. Federoff, and W. J. Sonstein. Enhanced vascularization and survival of neural transplants with *ex vivo* angiogenic gene transfer. *Cell Transplant* **11**:331–349 (2002).
6. G. Leibowitz, G. M. Beattie, T. Kafri, V. Cirulli, A. D. Lopez, A. Hayek, and F. Levine. Gene transfer to human pancreatic endocrine cells using viral vectors. *Diabetes* **48**:745–753 (1999).
7. H. C. Kang, S. Kim, M. Lee, and Y. H. Bae. Polymeric gene carrier for insulin secreting cells: Poly(L-lysine)-*g*-sulfonylurea for receptor mediated transfection. *J. Control. Release* **105**: 164–176 (2005).
8. D. Martinez-Fong, I. Navarro-Quiroga, I. Ochoa, I. Alvarez-Maya, M. A. Meraz, J. Luna, and J. A. Arias-Montano. Neurotensin-SPDP-poly-L-lysine conjugate: a nonviral vector for targeted gene delivery to neural cells. *Brain Res. Mol. Brain Res.* **69**:249–262 (1999).
9. J. Zeng and S. Wang. Enhanced gene delivery to PC12 cells by a cationic polypeptide. *Biomaterials* **26**:679–686 (2005).
10. H. C. Kang, M. Lee, and Y. H. Bae. Polymeric gene carriers. *Crit. Rev. Eukaryot. Gene Expr.* **15**:317–342 (2005).
11. B. I. Florea, C. Meaney, H. E. Junginger, and G. Borchard. Transfection efficiency and toxicity of polyethylenimine in differentiated Calu-3 and nondifferentiated COS-1 cell cultures. *AAPS PharmSci* **4**:E12, 2002 (2002).
12. R. Acosta, C. Montanez, P. Gomez, and B. Cisneros. Delivery of antisense oligonucleotides to PC12 cells. *Neurosci. Res.* **43**:81–86 (2002).
13. S. Charles, T. Tamagawa, and J. C. Henquin. A single mechanism for the stimulation of insulin release and 86Rb+ efflux from rat islets by cationic amino acids. *Biochem. J.* **208**:301–308 (1982).
14. A. Sener, F. Blachier, J. Rasschaert, A. Mourtada, F. Malaisse-Lagae, and W. J. Malaisse. Stimulus-secretion coupling of arginine-induced insulin release: comparison with lysine-induced insulin secretion. *Endocrinology* **124**:2558–2567 (1989).
15. N. H. McClenaghan, C. R. Barnett, F. P. O'Harte, and P. R. Flatt. Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic B-cell line. *J. Endocrinol.* **151**:349–357 (1996).
16. C. Li, C. Buettger, J. Kwagh, A. Matter, Y. Daikhin, I. B. Nissim, H. W. Collins, M. Yudkoff, C. A. Stanley, and F. M. Matschinsky. A signaling role of glutamine in insulin secretion. *J. Biol. Chem.* **279**:13393–13401 (2004).
17. H. Sone, M. Ito, K. Sugiyama, M. Ohneda, M. Maebashi, and Y. Furukawa. Biotin enhances glucose-stimulated insulin secretion in the isolated perfused pancreas of the rat. *J. Nutr. Biochem.* **10**:237–243 (1999).
18. P. Rathanaswami, A. Pourany, and R. Sundaresan. Effects of thiamine deficiency on the secretion of insulin and the metabolism of glucose in isolated rat pancreatic islets. *Biochem. Int.* **25**:577–583 (1991).
19. C. J. Barker, I. B. Leibiger, B. Leibiger, and P. O. Berggren. Phosphorylated inositol compounds in β-cell stimulus-response coupling. *Am. J. Physiol. Endocrinol. Metab.* **283**:E1113–E1122 (2002).
20. P. Rorsman and E. Renstrom. Insulin granule dynamics in pancreatic beta cells. *Diabetologia* **46**:1029–1045 (2003).
21. A. T. L. Young, R. B. Moore, A. G. Murray, J. C. Mullen, and J. R. T. Lakey. Assessment of different transfection parameters in efficiency optimization. *Cell Transplant* **13**:179–185 (2004).
22. M.Ogris, S. Brunner, S. Schuller, R. Kircheis, and E. Wagner. PEGylated DNA/transferrin-PEI complexes: reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery. *Gene Ther.* **6**:595–605 (1999).
23. D. Mears. Regulation of insulin secretion in islets of Langerhans by Ca(2+)channels. *J. Membr. Biol.* **200**:57–66 (2004).
24. M. Komatsu, T. Schermerhorn, T. Aizawa, and G. Sharp. Glucose stimulation of insulin release in the absence of extracellular Ca^{2+} and in the absence of any increase in intracellular Ca^{2+} in rat pancreatic islets. *Proc. Natl. Acad. Sci. USA* **92**:10728–10732 (1995).
25. I. Quesada, J. Rovira, F. Martin, E. Roche, A. Nadal, and B. Soria. Nuclear K_{ATP} channels trigger nuclear Ca^{2+} transients that modulate nuclear function. *Proc. Natl. Acad. Sci. USA* **99** 9544–9549 (2002).
26. B. J. Zunkler, M. Wos-Maganga, and U. Panten. Fluorescence microscopy studies with a fluorescent glibenclamide derivative, a high-affinity blocker of pancreatic beta-cell ATP-sensitive K^+ currents. *Biochem. Pharmacol.* **67**:1437–1444 (2004).
27. M. Molinete, J. C. Irminger, S. A. Tooze, and P. A. Halban. Trafficking/sorting and granule biogenesis in the beta-cell. *Semin. Cell Dev. Biol.* **11**:243–251 (2000).