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Efficient GLP-1 gene delivery using two-step transcription amplification plasmid system with a secretion signal peptide and arginine-grafted bio-reducible polymer

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Abstract

Glucagon-like peptide (GLP-1) encoding dual plasmid (pDNA) system (TSTA (SP-GLP-1)) which is composed of p β -Gal4-p65 and pUAS-SP-GLP-1 was constructed to improve the production and secretion of expressed GLP-1 by combining the advantages of signal peptide (SP) and two-step transcription amplification (TSTA) system. Its potential for GLP-1 gene delivery system was investigated with employment of arginine-grafted bio-reducible polymer (ABP) as a gene carrier. Their polyplexes have about 140 nm-sizes and 20 mV Zeta-potential values. ABP showed no cytotoxicity contrary to PEI25k. It was found in RT-PCR experiments that TSTA-SP pDNA systems showed increased GLP-1 gene transcription level in comparison with mono pDNA system (p β -GLP-1). It was also observed in GLP-1 ELISA that GLP-1 secretion level of TSTA (SP-GLP-1) pDNA system was 2.7–3.4 times higher than those of p β -GLP-1 and 1.5–1.7 times than TSTA (GLP-1). Additionally, 2.5–3.5 folds increased level of GLP-1 secretion was found in ABP gene carrier system in comparison with PEI25k. When transfection medium containing secreted GLP-1 was transferred to NIT-1 insulinoma cells, the highest secretion level of insulin was induced in ABP/TSTA (SP-GLP-1) polyplex medium-treated cells. Therefore, this novel system could be utilized as a safe and efficient GLP-1 gene delivery system for type 2 diabetes therapy.

Keywords

Gene delivery; Glucagon-like peptide-1; two-step transcription amplification; secretion signal peptide; arginine-grafted bio-reducible polymer

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1. Introduction

Glucagon-like peptide-1 (GLP-1) is one of incretins, intestinal hormones that augment insulin secretion in elevated glucose level [1–5]. GLP-1 is secreted by intestinal L-cells, which can stimulate insulin secretion from islet β -cells. It was reported that GLP-1 can stimulate all steps of insulin biosynthesis, upregulate genes involved in insulin secretion, provide new β -cells and inhibit glucagon secretion and gastric emptying [6–13]. Most of all, GLP-1 can enhance insulin secretion only when blood glucose level is higher than 250 mg/dL so it doesn't cause hypoglycemia during treatment, not like insulin or other chemical drugs such as sulphonylurea [14,15]. Therefore, treatment of GLP-1 was proposed as a new potential therapy for type 2 diabetes arising from insulin resistance, combined with relative insulin deficiency [16]. Another incretin, glucose-dependent insulintropic polypeptide (GIP) tends to lose its biological activity in type 2 diabetes [17]. However, GLP-1 has a major drawback, very short half-life (< 5 min) in the plasma, which is due to rapid degradation by dipeptidyl peptidase IV (DPPIV) [18,19]. Two active forms of GLP-1 [20], GLP-1 (7–36) and GLP-1 (7–37) lose their two N-terminal amino acid residues and biological activity by action of DPPIV quickly in the body.

So, GLP-1 gene delivery systems were designed as alternative strategies of conventional GLP-1 treatments because gene therapy can overcome the above limitation of GLP-1 by secreting GLP-1 with enough concentration in the body during prolonged period. Among them, non-viral GLP-1 pDNA delivery systems should be comprised of pDNAs enable to stably express GLP-1 in cells and gene carriers possessing high biocompatibility and gene delivery potency.

In the part of pDNAs, various GLP-1 pDNA systems have been constructed by using a chicken β -actin promoter (p β -GLP-1) [21] or a SV40 promoter/enhancer with nuclear factor κ B binding sites (pSI-GLP-1-NF κ B) [22,23]. Administration of polyplexes (PEI25k/p β -GLP-1 or pSI-GLP-1-NF κ B) showed increased insulin secretion and decreased blood glucose levels longer than 2 weeks in mouse model after a single injection. However, blood glucose level was not normalized to 250 mg/dL, which suggests GLP-1 encoding pDNA should be upgraded. Recently, another GLP-1 pDNA system (p β -Gal4-p65 + pUAS-GLP-1) was developed using two-step transcription amplification (TSTA) system [24]. In this system, Gal4-DNA binding domain (DBD)/p65-transactivation domain (TAD) fusion protein expressed from p β -Gal4-p65 binds to the upstream activating sequence (UAS) in the second pDNA, pUAS-GLP-1 and then stimulates the transcription of GLP-1. TSTA (GLP-1) system was proven to be an efficient GLP-1 expression system by showing much higher GLP-1 expression level in 293 cells than one-step expression system, p β -GLP-1. Here, more improved TSTA system encoding GLP-1 (TSTA (SP-GLP-1)) was constructed by attaching secretion signal peptide (SP) to GLP-1 in order to facilitate its secretion in non-endocrine cells or tissues because small increment of GLP-1 secretion may improve its therapeutic effect. Signal peptide can target expressed GLP-1 to the constitutive secretory pathway of cells and allow post-translational processing of the fusion peptide by signal peptidase [25]. In the part of gene carriers, PEI25k [21,22] and reducible poly(amido ethylenimine) [23] have been used for GLP-1 gene delivery systems. Chitosan was also used for GLP-1 gene delivery in type 2 diabetes animal model [26]. Here, we employed arginine-grafted bioreducible polymer (ABP). ABP was reported to show much improved transfection efficiency in comparison with PEI25k or its backbone polymer, poly(CBA-DAH) due to the cell penetrating functionality of grafted arginine residues [27]. Arginine is thought to function importantly in cell penetrating peptides which can translocate non-permeable molecules into cells efficiently [28]. In addition, ABP possesses minimal cytotoxicity due to its reducing environment-sensitive biodegradation because internal disulfide bonds of ABP

could be cleaved by high concentration of intracellular glutathione. Therefore, ABP is also expected to be a promising gene carrier for GLP-1 gene delivery.

In this work, TSTA (SP-GLP-1) pDNA system is newly constructed and used with ABP for novel GLP-1 gene delivery systems. The physiochemical properties of polyplexes are characterized and GLP-1 mRNA transcription level, GLP-1 secretion level, and finally insulin secretion-inducing ability of this system are investigated.

2. Materials and methods

2.1. Materials

ABP was synthesized according to the method of the previous report [27]. Hyperbranched poly(ethylenimine) (bPEI, 25 kDa) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). SYBR safe DNA gel stain (10,000X in DMSO), fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (DPBS), Dulbecco's modified Eagle's medium (DMEM), and Dulbecco's modified Eagle's medium: Nutrient Mixture F-12 (DMEM/F12) were purchased from Invitrogen (Carlsbad, CA). All other chemicals were purchased and used without any further purification.

2.2. Construction of plasmids

p β -GLP-1, pUAS-GLP-1, and p β -Gal4-p65 were previously constructed [21,24]. The DNA fragment encoding the GLP-1 secretion signal peptide was synthesized chemically and inserted into pUAS-GLP-1 at the Kpn I site between the sequences of Gal4 UAS/E1b TATA and GLP-1 to construct pUAS-SP-GLP-1. Restriction enzyme studies and direct sequencing confirmed the construction of the plasmids. The plasmids were introduced into *E. coli* strain DH5 α , and purified by Qiagen plasmids Maxi Kits (Qiagen, Valencia, CA). The purities of plasmids were certified by OD₂₆₀/OD₂₈₀ ratio. The plasmids were stored at -20 °C until use.

2.3. Agarose gel electrophoresis

Agarose gel electrophoresis assay was performed to examine pDNA condensation profile of ABP. PEI25k was used as a control reagent. Agarose gel (0.7 %, w/v) containing 0.1 μ L/mL SYBR Safe DNA gel stain was prepared in Tris-Acetate-EDTA (TAE) buffer. The prepared pDNAs and polyplexes (0.5 μ g pDNA) were prepared in Hepes buffered saline (10 mM Hepes, 1 mM NaCl, pH 7.4). After 30 minutes of incubation at room temperature, the electrophoresis was run for 15 min at 120 V. The locations of pDNA bands were visualized by UV illuminator (Gel Documentation Systems, Bio-Rad, Hercules, CA).

2.4. Average particle size and Zeta-potential measurements

Average particle size and Zeta-potential values of polyplexes were examined by using Zetasizer 3000 (Malvern Instruments, USA) with a He-Ne Laser beam (633 nm, fixed scattering angle of 90°) at 25 °C. 0.5 mL of polyplex solutions (10 μ g pDNA) were prepared in Hepes buffered saline. After 30 min of incubation, polyplex solutions were diluted to final volume of 4 mL before measurement. Measured average sizes and Zeta-potential values were presented as the average values of 5 runs.

2.5. Cell culture

NIH3T3 mouse embryonic fibroblast cells were maintained in DMEM supplemented with 10% FBS in a 5% CO₂ incubator. NIT-1 insulinoma cells established from NOD/Lt mice [29] were purchased from ATCC (Manassas, VA) and maintained in DMEM/F12 (1:1)

supplemented with 10% FBS in a 5% CO₂ incubator. After every 2 or 3 days of incubation, old growth medium for NIT-1 cells was exchanged with fresh medium.

2.6. Cytotoxicity

MTT assays were performed to examine the cytotoxicity of polymeric gene carrier, ABP. NIH3T3 cells in 90 μ L DMEM (10% FBS) were seeded in a 96-well plate at 1×10^4 cells/well. Cells achieving 70–80 % confluence were exposed to 10 μ L polymer solutions for 4 h at different concentration in serum-free media. The final polymer concentrations per well ranged from 1 to 100 μ g/mL. PEI25k was used as a control. Subsequently, the media were changed with fresh culture media. After 24 h, the cells were treated with 25 μ L of MTT stock solution (2 mg/mL in DPBS) and incubated for 2 h at 37°C. After removing each medium carefully, 150 μ L of DMSO was added to each well to dissolve the formazan crystal formed by proliferating cells. The absorbance was measured at 570 nm using a UV/VIS photometry microplate reader (Model 680, Bio-Rad Lab, Hercules, CA). Results were presented as relative cell viabilities (RCV, percentage values relative to value of untreated control cells). All experiments were performed in quadruplicate.

2.7. Transfection experiments in vitro

NIH3T3 cells were seeded at a density of 4×10^5 cells/well in a 6-well plate in DMEM medium containing 10% FBS and grown to reach 70~80 % confluence prior to transfection. Before transfection, medium of each well was exchanged with fresh serum-free medium. The cells were treated with polyplex solutions (total 4 μ g pDNA) at different weight ratios for 4 h at 37 °C. After exchange with fresh medium containing 10 % FBS, cells were further incubated for 2 days before assay.

2.8. Reverse transcription-polymerase chain reaction (RT-PCR)

The transfected NIH3T3 cells were washed with DPBS. The total RNA of cell was prepared by Purelink RNA Mini kit (Invitrogen, Carlsbad, CA) and quantified by OD₂₆₀ measurement using the NanoDrop ND-1000 (Nanodrop Technologies, Wilmington, DE). A part of GLP-1 cDNA was obtained and amplified from one microgram of the total RNA via reverse transcription and consecutive polymerase chain reaction by using OneStep RT-PCR kit (Qiagen, Valencia, CA). The sequences of the oligonucleotide primers for GLP-1 are as followings: forward primer, 5'-ATGCGTCAACGTCGTCATGC-3'; backward primer, 5'-GCCTTTCACCAAGCAA-3'. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) housekeeping gene was used as a internal control. The RT-PCR reaction consisted of reverse transcription (50 °C for 30 min), initial PCR activation (95 °C for 15 min), and 30 cycles of PCR (94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min), followed by an extension of 10 min at 72 °C. The sizes of the expected products were 105 bp. Analysis was performed by using Quantity One software (Bio-Rad, Hercules, CA).

2.9. GLP-1 level measurements by enzyme-linked immunosorbent assay (ELISA)

100 μ L of growth medium was collected from the transfected NIH3T3 cells and the secreted GLP-1 concentration of the growth medium was determined by ELISA (Linco Research, St. Charles, MO) according to the manufacturer's manual, which measure biological active GLP-1 forms, GLP-1 (7–36) amide and GLP-1 (7–37).

2.10. Insulin level measurements by ELISA

NIT-1 insulinoma cells were seeded at a density of 8×10^5 cells/well in a 6-well plate in DMEM/F12 medium (1:1) containing 10% FBS and grown to reach 80~90 % confluence. Growth medium was removed and cells were washed with DPBS. Media from transfected NIH3T3 cells were then transferred to NIT-1 cells in order to study the biological activity of

GLP-1 secreted from NIH3T3 cells for insulin secretion. After 4 h of incubation, each medium was collected and diluted 20 times with fresh medium for assay. 10 μ L of diluted medium was used for the determination of insulin concentration by Rat/Mouse Insulin ELISA (Millipore, Billerica, MA) according to the manufacturer's manual.

3. Results and Discussion

3.1. Construction of plasmids

Control pDNAs expressing GLP-1, p β -GLP-1 and TSTA (GLP-1) which is composed of pUAS-GLP-1 and p β -Gal4-p65 were previously constructed [21,24]. TSTA (GLP-1) system showed higher GLP-1 expression level than conventional mono plasmid, p β -GLP-1 [24]. This enhancement will be beneficial for the GLP-1 gene therapy, since the delivery of p β -GLP-1 expressed therapeutic level of GLP-1 as proved in the previous study and even small increment of the GLP-1 expression can enhance its therapeutic effect. In addition, secretion signal peptide (SP) which is attached to GLP-1 would benefit the secretion of GLP-1 from non-endocrine tissues in systemic administration of non-targeted GLP-1 gene delivery systems. Therefore, in order to combine the advantages of TSTA (GLP-1) pDNA system and SP, pUAS-SP-GLP-1 pDNA was constructed, of which transcription would be amplified by p β -Gal4-p65 in TSTA (SP-GLP-1) system as shown in scheme 1.

The DNA fragment encoding the secretion signal peptide was synthesized by a DNA synthesizer and inserted into pUAS-GLP-1 at the Kpn I site between the sequences of Gal4 UAS/E1b TATA and GLP-1 to construct pUAS-SP-GLP-1. However, introduction of SP at the N-terminus of GLP-1 might reduce the activity of GLP-1. Therefore, a furin recognition sequence was inserted between SP and GLP-1, which would be cleaved by furin after the translated GLP-1 was transferred to Golgi apparatus for secretion [21]. In this way, SP can increase the secretion efficiency of GLP-1 without any interference in the activity of GLP-1. The construction of pUAS-SP-GLP-1 was confirmed by restriction enzyme studies and direct sequencing. Finally, prepared pDNAs from transformed *Escherichia coli* DH5 α were electrophoresed and observed at the expected locations (p β -Gal4-p65 : 5.414 kb, pUAS-GLP-1 : 3.483 kb, pUAS-SP-GLP-1 : 3.671 kb) in Figure 1.

3.2. Agarose gel electrophoresis

Agarose gel electrophoresis was performed to confirm the pDNA condensation profiles of cationic polymers for dual pDNA systems. pDNA solutions were prepared by mixing p β -Gal4-p65, and pUAS-GLP-1 or pUAS-SP-GLP-1 at 1 : 2 ratio (w/w) to form TSTA (GLP-1) or TSTA (SP-GLP-1) respectively. The mixing ratio was determined in accordance with the previous study, which showed that TSTA (GLP-1) system (p β -Gal4-p65 : pUAS-GLP-1 = 1 : 2, w/w) exhibited the highest transfection efficiency among various mixing ratios [24]. All the mixing ratios of pDNAs were fixed as above in this study. Polyplexes were prepared by adding pDNA solutions to PEI25k or ABP solutions at weight ratios (polymer/pDNA) of 1 or 50, respectively. After electrophoresis of polyplexes, no migration of pDNAs was observed at lane #5 to #8 in Figure 1, which means all the polymers showed good pDNA condensation ability regardless of dual pDNA systems at used weight ratios.

3.3. Average particle size and Zeta-potential measurements

Average particle size and Zeta-potential of polyplexes were measured by Zeta-sizer. It was reported that the proper size (< 200 nm) of polyplexes would be suitable for efficient cellular uptake of polyplex [30] and the positive charges of polyplexes can improve their adhesion onto cellular membrane, increasing the chance of cellular uptake [31], finally leading to enhanced transfection. TSTA pDNA systems (p β -Gal4-p65 = 3.33 μ g, pUAS-GLP-1 or

pUAS-SP-GLP-1 = 6.67 μ g) were prepared to adjust the used pDNA amounts of total 10 μ g for the measurements.

As shown in Table 1, all the polyplexes have 126~145 nm sizes and 18~21 mV Zeta-potential values, which confirms that PEI25k or ABP can form positively charged and nm-sized (< 200 nm) polyplex particles with TSTA dual pDNA systems, satisfying the requirements mentioned above. Although considerable differences among measured values were not observed, Zeta-potential values of ABP polyplexes have about 2 mV higher values than PEI25k polyplexes, which may be due to strong positive charges of arginine residues of ABP.

3.4. Cytotoxicity

Cytotoxicity of the polymers was investigated by MTT assay in NIH3T3 cells. RCV of PEI25k-treated cells was abruptly decreased with the concentration in Figure 2, showing significant cytotoxicity of PEI25k. In contrast with PEI25k, ABP-treated cells displayed high RCV (> 100 %), irrespective of increasing polymer concentration. ABP showed no cytotoxicity due to its biodegradation in reducing intracellular environment as expected from the previous study reporting ABP also has little cytotoxicity in C2C12 cells [27].

3.5. GLP-1 mRNA transcription

Level of GLP-1 mRNA transcription was evaluated by RT-PCR in order to study the effect of SP or gene delivery carriers to transcription level. Total 4 μ g of pDNA was used for each well in transfection experiments. After 2 days of transfection, cells were washed with DPBS and lysed for preparation of total RNA. GLP-1 cDNA was obtained from the RNA after reverse-transcription and amplified by consecutive polymerase chain reaction.

Figure 3 displays that cDNA products from GLP-1 mRNA of all transfected cells were observed at expected location in agarose gel, which means all transcription processes were conducted in cells well after transfection except non-treated cells. Each band density of GLP-1 was normalized by its corresponding GAPDH cDNA control as follows (lane #2: 76.4 %, #3: 88.7 %, #4: 90.1 %, #5: 77.6 %, #6: 92.3 %, #7: 93.7 % in Figure 3). p β -GLP-1 systems showed less GLP-1 mRNA level (84~86 %) in comparison with TSTA (GLP-1) systems, elucidating that TSTA system enhanced the transcription level of GLP-1 mRNA as expected. It is interesting that almost similar transcription levels of GLP-1 mRNA were observed in TSTA (SP-GLP-1) systems and TSTA (GLP-1) systems, which was supported by the report that SP can improve secretion of the expressed peptide by targeting to secretory pathway, not enhance the transcription level itself.

From the viewpoint of gene delivery carriers, only a little higher transcription levels of GLP-1 mRNA (3.6 %) were found in ABP polyplexes-treated cells than PEI25k polyplexes-treated cells. Distinct differences between them were not observed contrary to the expectation based on the previous report about strong pDNA nuclear localization ability of guanidine group in arginine residues and resultant higher gene transcription level [32]. One of possible explanation is that small improvement of transcription levels can be much more amplified in protein translation levels by cell machinery.

3.6. GLP-1 expression and secretion

Secreted level of GLP-1 was examined by GLP-1 ELISA. After 2 days of transfection, the growth medium was collected for the assay. Regardless of gene delivery carriers, the highest level of secreted GLP-1 was observed in TSTA (SP-GLP-1) system, which was about 1.5~1.7 times higher than that of TSTA (GLP-1) system and about 2.7~3.4 times higher than that of p β -GLP-1 (Figure 4). Therefore, it was elucidated that SP could improve the

secretion of expressed GLP-1 due to the targeting ability of GLP-1 peptide to secretory pathways despite just a little higher transcription level. Therefore, it was confirmed that TSTA (SP-GLP-1) dual pDNA system could increase GLP-1 production and secretion efficiently in comparison with TSTA (GLP-1) pDNA system. In addition, ABP polyplexes-transfected cells showed about 2.5~3.5 times higher GLP-1 expression level than PEI25k polyplexes-transfected cells, meaning transfection efficiency of ABP is superior to that of PEI25k as expected above and as already shown in luciferase reporter gene transfection in the previous study [27]. Therefore, the highest level of GLP-1 secretion was observed ABP/TSTA (SP-GLP-1) polyplex system, which proposes its potential for efficient GLP-1 gene delivery systems.

3.7. Insulin secretion

The biological activity inducing insulin secretion of secreted GLP-1 was examined by insulin ELISA. Each growth media containing secreted GLP-1 from transfected NIH3T3 cells was transferred to NIT-1 insulinoma cells because secreted GLP-1 from non-endocrine cells should circulate in blood stream and is expected to stimulate insulin production and secretion from insulin-secreting cells such as β -cells in pancreas. After 4 h of incubation, a part of medium was collected and insulin level was determined by insulin ELISA (Figure 5). Background insulin concentration was measured to about 170 ng/mL in non-treated cells. Insulin secretion level was lowest in β -GLP-1 medium-treated cells among secreted GLP-1 containing medium-treated cells and it showed a tendency to increase according to the following order (β -GLP-1 < TSTA (GLP-1) < TSTA (SP-GLP-1)). However, when PEI25k was used as a gene carriers, insulin concentrations were almost similar between TSTA (GLP-1) and TSTA (SP-GLP-1) medium-treated cells. ABP polyplex-treated cells displayed 1.1–1.3 folds higher secretion levels of insulin than PEI25k polyplex-treated cells. It is interesting that insulin secretion results didn't show significant differences among cells unlike GLP-1 secretion measurements, which may be due to the low susceptibility of used NIT-1 cells to GLP-1. The highest level of insulin secretion was obtained in ABP/TSTA(SP-GLP-1) polyplexes-treated cells as expected, which suggests a potential for GLP-1 gene delivery systems inducing insulin secretion in body.

4. Conclusion

Novel GLP-1 encoding dual pDNA system (TSTA (SP-GLP-1)) which is composed of p β -Gal4-p65 and pUAS-SP-GLP-1 was constructed to improve the secretion of expressed GLP-1. It could form nano-sized and positively charged nanoparticles with arginine-grafted bioreducible polymer (ABP). TSTA (SP-GLP-1) pDNA systems showed much improved GLP-1 secretion in transfected cells in comparison with TSTA (GLP-1), which means attached SP sequence can play an important role for expressed peptide secretion. Moreover, it was confirmed that ABP could slightly increase the GLP-1 gene transcription and finally lead to the large enhancement of GLP-1 production and secretion in comparison with PEI25k, displaying its high capability as an efficient and nontoxic gene delivery carrier. By combining the advantages of dual pDNA system containing SP sequence and ABP, ABP/TSTA (SP-GLP-1) polyplexes could exhibit the highest GLP-1 secretion level, which consecutively led to the highest induction of insulin secretion in insulinoma cells. Therefore, this novel system could be utilized as a safe and efficient GLP-1 gene delivery system for type 2 diabetes therapy.

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Figure 1.

Agarose gel electrophoresis of pDNAs and polyplexes. Left texts indicate the sizes of ladder DNA marker. Lane #1: DNA marker, #2: p β -Gal4-p65, #3: pUAS-GLP-1, #4: pUAS-SP-GLP-1, #5: PEI25k/(#2 + #3), #6: PEI25k/(#2 + #4), #7: ABP/(#2 + #3), #8: ABP/(#2 + #4).

Figure 2. MTT assay results in NIH 3T3 cells. RCV (relative cell viability) is presented as relative value (%) to non-treated cells.

Figure 3. RT-PCR results in NIH 3T3 cells. (A) GAPDH results, (B) GLP-1 results. M: 100 bp DNA marker, lane #1: non-treated cells, #2-#4: PEI25k/(p β GLP-1, TSTA(GLP-1), TSTA(SP-GLP-1)), #5-#7: ABP/(p β GLP-1, TSTA(GLP-1), TSTA(SP-GLP-1)).

Figure 4.
GLP-1 ELISA results in NIH3T3 cells.

Figure 5.
Insulin ELISA results in NIT-1 cells.

Scheme 1.

The structures and transcription mechanisms of pDNAs. Gal4-p65 transcription factor from first pDNA, pβ-Gal4-p65 can bind Gal4 UAS region of second pDNA, pUAS-GLP-1, stimulating GLP-1 gene transcription. For the case of pUAS-SP-GLP-1, signal peptide (SP) attached to GLP-1 can improve the secretion of GLP-1 by targeting GLP-1 to secretory pathway, leading to higher GLP-1 level than pUAS-GLP-1.

Table 1

Average sizes and Zeta-potential value measurement results.

	PEI25k		ABP	
	TSTA (GLP-1)	TSTA (SP-GLP-1)	TSTA (GLP-1)	TSTA (SP-GLP-1)
Size (nm)	126.48±3.1	144.48±8.3	143.92±3.2	138.34±3.7
Zeta-potential (mV)	18.13±0.4	18.57±1.0	20.13±1.0	20.35±0.3