
**UNIVERSITI PUTRA MALAYSIA**

# *COMPARISON OF GIP AND GLP-1 PROMOTER-MEDIATED INSULIN EXPRESSION IN GUT K AND L-CELLS FOR THE POTENTIAL TREATMENT OF DIABETES MELLITUS*

**MINA RASOULI**

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**By**

**MINA RASOULI**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the degree of Doctor Philosophy**

**October 2012**

**DEDICATION**

I would like to dedicate this thesis to:

My parents, “Hassan Rasouli” & “Azar Bakhshandeh”

My sister, “Minoo Rasouli”

My brother, “Meysam Rasouli”

For their unconditional love and support in whole my life

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**COMPARISON OF GIP AND GLP-1 PROMOTER-MEDIATED INSULIN EXPRESSION IN GUT K AND L-CELLS FOR THE POTENTIAL TREATMENT OF DIABETES MELLITUS**

By

**MINA RASOULI**

**October 2012**

**Chair: Zalinah Ahmad, PhD**

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Diabetes mellitus is characterized by abnormally high blood glucose levels, which causes serious complications. It is hypothesized that gut K and L-cells could make potential candidates for diabetes gene therapy manipulation. The project was carried out to determine and compare the quality and quantity of the human insulin expressed in both mice K and L-cells *in vitro* and *in vivo* which have been transfected with human insulin gene construct with GIP and GLP-1 promoters. These cells do not naturally produce insulin but respond to glucose and other nutrients in the gut by secreting GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide-1) which then stimulate beta cells of pancreas to produce insulin. For this purpose, two plasmids containing neomycin with GIP or GLP-1 were constructed to isolate pure surrogate models for *in vitro* studies. The next two plasmids containing insulin with GIP or GLP-1 were constructed in order to study efficiency and efficacy of insulin expression

*in vitro* and *in vivo* and the last two plasmids containing GFP with GIP or GLP-1 were constructed to study delivery efficiency of gene vehicle *in vivo*. We also studied different nutrients stimulus effects and time expression of the insulin secretion in both the K and L cells. Finally, the insulin expression efficacy of the reconstructed plasmids with GIP and GLP-1 promoters in lowering the glucose levels was compared in diabetes animal model. To study these hypotheses, neomycin, insulin and GFP genes were inserted downstream of the promoters in 6 different recombinant constructs. QRT- PCR was used to authenticate the purity of the isolated K and L-cell lines. Isolated K and L-cell lines were then transfected by recombinant insulin constructs. RT-PCR, immunocytostaining and Western blotting were used to analyze qualification of mature insulin while QRT- PCR and ELISA were employed to quantify the secreted insulin from both cell-lines. Glucose and meat hydrolysate (MH) were used to study different stimulant effects on insulin expression in both cell lines. The secretion of insulin was investigated for 180 min in 6 different time points. For *in vivo* studies, chitosan was employed to transfer the recombinant insulin constructs into the target cells orally. The ability of chitosan to deliver the constructs was investigated using immunohistostaining on the intestine of the mice. The effect of secreted insulin on lowering the glucose levels in STZ-induced diabetic mice was investigated by blood glucose testing in two weeks duration. QRT-PCR results proved that the isolated cells were pure K and L-cell. The Western blotting and immunocytostaining results of insulin secretion studies revealed that both cells were able to produce mature and active insulin. Statistical analysis revealed that the difference between insulin expressions from K and L-cells in relation to the glucose and MH stimulations were not significant. The

immunohistostaining results showed that chitosan was an efficient gene vehicle to transfect the intestinal cells. In addition, RT-PCT and ELISA confirmed that pure human insulin was expressed *in vivo*. Blood glucose testing results showed that the treatment with the recombinant insulin gene significantly reduced glucose levels in diabetic mice. As a conclusion, recombinant neomycin constructs successfully produced pure K and L-cells. Recombinant insulin constructs comparably efficiently produced mature insulin protein in K and L-cells *in vitro* and *in vivo*. The response of K and L-cells to induction of both stimulators was mostly comparable. However, meat hydrolysate is found to be a more potent insulin secretion agonist for both cells than glucose. In addition, our *in vivo* results provided successful preliminary data that proved the analogous ability of both GIP and GLP-1 insulin constructs in lowering blood glucose levels of the diabetic mice.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PERBANDINGAN GIP DAN GLP-1 PROMOTER PENGHANTARAN TEKANAN INSULIN DALAM GUT K DAN SEL-L UNTUK POTENSI RAWATAN DIABETES MELLITUS**

Oleh

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Diabetes mellitus adalah disebabkan oleh paras glukosa yang tinggi di dalam darah dan ini seterusnya boleh menyebabkan komplikasi yang serius. Hipotesis menyatakan bahawa sel K dan L di usus adalah antara sel yang berpotensi untuk digunakan di dalam manipulasi terapi gen bagi merawat diabetes. Projek ini telah dijalankan untuk menentukan dan membandingkan kualiti dan kuantiti insulin yang dihasilkan oleh kedua-dua sel K dan L secara *in vitro* dan *in vivo*. Bagi tujuan ini, dua plasmid mengandungi neomycin dengan GIP atau GLP-1 telah dihasilkan untuk digunakan bagi pengasingan model gantian yang tulen untuk digunakan di dalam kajian secara *in vitro*. Seterusnya, dua plasmid yang mengandungi insulin dengan GIP atau GLP-1 telah dihasilkan untuk mengkaji kecekapan dan keberkesanan insulin yang dihasilkan dan ini akan dijalankan secara *in vitro* dan *in vivo*, dan dua plasmid terakhir mengandungi GFP dengan GIP atau GLP-1 telah dicipta bagi mengkaji kecekapan kaedah penghantaran gen secara *in vivo*. Kami juga mengkaji

kesan perbezaan rangsangan nutrient yang berbeza dan juga masa yang diambil untuk proses rembesan insulin oleh kedua-dua sel K dan L. Akhir sekali, keberkesanan rembesan insulin yang dihasilkan oleh plasmid yang dicipta dengan penganjuran GIP atau GLP-1 dalam menurunkan paras glukosa dibandingkan pada model haiwan yang berpenyakit diabetes. Untuk mengkaji hipotesis ini, gen-gen neomycin, insulin dan GFP dimasukkan ke hilir penganjur dalam 6 konstruk rekombinan yang berbeza. QRT-PCR telah digunakan untuk mengesahkan ketulenan talian sel K dan L yang telah diasingkan. Talian sel K dan L yang diasingkan telah ditransfekkan dengan konstruk rekombinan insulin yang berbeza. RT-PCR, pelumuran antibody secara histologi dan blot Western telah digunakan untuk menganalisa kualiti penghasilan insulin matang manakala QRT-PCR dan ELISA telah digunakan untuk mengukur kuantiti insulin yang dirembeskan dari kedua-dua sel. Glukosa dan hidrolisat daging (MH) telah digunakan untuk mengkaji perbezaan kesan rangsangan keduanya terhadap rembesan insulin dalam kedua-dua talian sel. Rembesan insulin telah diperhatikan selama 180 minit dalam 6 titik masa yang berbeza. Dalam kajian *in vivo*, kitosan telah digunakan untuk menghantar konstruk rekombinan insulin ke sel sasaran melalui pengambilan melalui mulut. Keupayaan kitosan untuk manghantar konstruk disiasat menggunakan pelumuran antibodi secara histologi pada usus tikus. Kesan rembesan insulin dalam menurunkan paras glukosa disiasat pada tikus diabetik di bawah pengaruh STZ melalui ujian penentuan tahap glukosa darah selama dua minggu. Keputusan QRT-PCR membuktikan bahawa sel-sel yang diasingkan adalah sel K dan L yang tulen. Keputusan blot Western dan pelumuran antibodi secara histologi terhadap rembesan insulin menunjukkan bahawa kedua-dua sel mampu untuk menghasilkan insulin yang matang dan aktif. Analisis statistik mendedahkan keupayaan rembesan insulin oleh sel K dan L hasil

rangsangan glukosa dan MH tidak begitu ketara perbezaannya. Keputusan pelumuran antibodi secara histologi menunjukkan bahawa kitosan adalah kenderaan penghantaran gen yang cekap kepada sel-sel usus. Di samping itu, RT-PCT dan ELISA telah mengesahkan bahawa insulin manusia yang tulen telah Berjaya dirembeskan secara *in vivo*. Keputusan ujian glukosa darah menunjukkan bahawa rawatan dengan konstruk rekombinan insulin dari kedua dua penganjur mampu menurunkan tahap glukosa yang ketara pada tikus diabetes. Kesimpulannya, konstruk neomycin rekombinan bejaya menghasilkan sel K dan L yang tulen. Konstruk rekombinan insulin dengan cekapnya Berjaya menghasilkan protein insulin yang matang dalam sel K dan L secara *in vitro* dan *in vivo*. Keupayaan sel K dan L merembeskan insulin secara *in vitro* adalah setanding. Hidrolisat daging merupakan agonis nutrient yang lebih kuat berbanding glukosa dalm merangsang perembesan insulin. Di samping itu, keputusan yang kami perolehi secara *in vivo* berjaya memberikan data awal yang membuktikan kebolehan kedua-dua konstruk rekombinan insulin dengan penganjur GIP dan GLP-1 dalam menurunkan paras glukosa darah tikus berpenyakit diabetis.

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I certify that a Thesis Examination Committee has met on **25 October 2012** to conduct the final examination of MINA RASOULI on her thesis entitled **"Comparison of GIP and GLP-1 promoter mediated insulin expression in gut K and L-cells for the potential treatment of diabetes mellitus"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the **degree of Doctor of Philosophy**.

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## DECLARATION

I certify that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not currently submitted for any of other degree at Universiti Putra Malaysia or at any other institutions.

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**MINA RASOULI**

Date: 25 October 2012

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| | |
|---|---|
| ADA gene | adenosine deaminase gene |
| APS | Ammonium persulfate |
| ATP | Adenosine triphosphate |
| bp | Base pair |
| BCIP | 5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt |
| BSA | bovine serum albumin |
| $CaCl_2$ | Calcium chloride |
| CCK | cholecystokinin |
| cDNA | complementary deoxyribonucleic acid |
| CMV | Cytomegalovirus |
| DAPI | 4, 6 Diamidino-2 phenylindole |
| DKA | diabetic ketoacidosis |
| DM | Diabetes mellitus |
| DMEM | Dulbecco's modified eagle medium |
| DMSO | Dimethyl sulphoxide |
| DNA | Deoxyribonucleic acid |
| DNase | Deoxyribonuclease |
| dNTP | Deoxyribonucleotide phosphate |
| EDTA | disodium ethylenediaminetetraacetate |
| EF-1-α | elongation factor 1 alpha |
| ELISA | Enzyme-linked immunosorbent assay |
| ER | endoplasmic reticulum |
| FBS | fetal bovine serum |

| | |
|---|---|
| G | Gram |
| G418 | Geneticin antibiotic |
| G6Pase | glucose 6-phosphatase |
| GFP | green fluorescent protein |
| GIP | glucose-dependent insulinotropic polypeptide |
| GLP-1 | glucagon-like peptide-1 |
| GLUTs | glucose transporters |
| HbA1c | Haemoglobin A1c |
| HONK | hyperglycaemic nonketotic coma |
| Hr | Hour |
| IDDM | Insulin-dependent diabetes mellitus |
| KATP | ATP-sensitive potassium |
| kb | kilo base |
| KCl | Potassium chloride |
| KDa | Kilo dalton |
| lad | DNA ladder |
| LB | Lysogeny broth |
| L-PK | liver-pyruvate kinase |
| mEpo gene | murine erythropoietin gene |
| mg | Miligram |
| mg/dL | milligrams/decilitre |
| mg/L | milligram/liter |
| $MgCl_2$ | Magnesium chloride |
| MH | meat hydrolysis |
| min | Minute |

| | |
|---|---|
| ml | Millilitre |
| mM | Millimolar |
| mRNA | Messenger ribonucleic acid |
| MTT | 3-(4,5-Dimethylthiazol-2-yl) |
| NaOH | Sodium hydroxide |
| NBT | nitro-blue tetrazolium chloride |
| ng | Nanogram |
| NHMS | National health and morbidity survey |
| NIDDM | Non-insulin-dependent diabetes mellitus |
| OD | Optical density |
| P mole | Pico mole |
| PAGE | Polyacrylamide gel electrophoresis |
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| pH | Hydrogen ion concentration |
| Q-PCR | Quantitative PCR |
| QRT-PCR | Quantitative Reverse Transcription PCR |
| rAAV | recombinant adeno-associated virus |
| rIGFBP-1 | rat insulin-like growth factor binding protein-1 |
| RNA | Ribonucleic acid |
| RT-PCR | Reverse transcription polymerase chain reaction |
| rpm | rotation per minute |
| SDS | sodium dodecyl sulfate |
| SDS-PAGE | sodium dodecyl sulfate- Polyacrylamide Gel Electrophoresis |

| | |
|---|---|
| sec | Second |
| STZ | streptozotocin |
| TAE | Tris-Acetate- EDTA buffer |
| Taq | Thermus aquaticus |
| TEM | transmission electron microscopy |
| TEMED | Tetramethylethylenediamine |
| Tm | melting temperature |
| UV | Ultraviolet |
| V | Volt |
| WHO | World Health Organization |
| α | Alpha |
| β | Beta |
| µ | Micron |
| µl | Microliter |
| µ mol / L | micromole per litre |
| $^{o}$C | Celsius |

# CHAPTER 1

# INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by abnormally high blood glucose levels. This abnormality is mainly the consequence of disorder in insulin production and/or insulin function. Unsuccessful regulation of glucose level in diabetic patients causes acute and chronic complications. Acute complication in diabetes mellitus comprises hypoglycemia, diabetic ketoacidosis (DKA), hyperosmolar hyperglycaemic nonketotic coma (HONK) that could lead to coma and death whereas examples of chronic complication of diabetes are cardiovascular disease, renal failure and blindness (Aiken et al., 1994). Thus, the survival and patients' quality of lives are completely dependent on the ability of the patients to control their blood glucose levels. Treatment of diabetes and complication are crucial matters for the patients, patients' families, society and the government because diabetes is lifelong disease that has a major financial physical and emotional impact.

Diabetes mellitus is subdivided into two main pathogenic categories: 1) Insulin-dependent diabetes mellitus, IDDM (more commonly referred to as type I diabetes), which mainly is the result of autoimmune destruction of the pancreatic β-cells causing insufficient or absolute deficiency of insulin production. It usually develops during childhood, leading to serious complications. 2) Non-insulin-

dependent diabetes mellitus, NIDDM (more commonly referred to as type II diabetes), which result from interaction of environmental /life style with genetic defects that could cause insulin resistance and/or insulin deficiency or both. It typically presents in adult patients and being recognized with increasing frequency in the obese population. Type II diabetes is the most prevalent form of this disease (Crofford, 1995).

Diabetes is one of the most significant diseases in the developed world as much as developing countries such as Malaysia. The prevalence of diabetes has increased significantly worldwide. The World Health Organization (WHO) reported that in year 2000, 120 million people worldwide had suffered from diabetes mellitus and the number of affected people will reach 350 million by 2030 worldwide. 10 - 15% of diabetic cases are classified as type I, while 85 - 90% of diabetics suffer from type II diabetes. Based on WHO records, more than 23 million people (7.8% of the total population) are diabetic in the United States alone. In some regions such China and India the frequency of diabetes is low, (1 case per 100,000 individuals per year), but in other countries such as Finland, the reported frequency is 50 cases per 100,000 persons (Pablos-Velasco et al., 2001; Wild et al., 2004).

The World Health Organization (WHO) estimated that in the year 2030, the prevalence of diabetes in Malaysia would be a total of 2.48 million people. In Malaysia, the First National Health and Morbidity Survey (NHMS I) conducted in

1986 reported a diabetes prevalence of 6.3%, while in the Second National Health and Morbidity Survey (NHMS II) in 1996, this data had risen to 8.3%. The third National Health and Morbidity Survey (NHMS III), which was conducted between April to July 2006, reported that the rate of diabetes has reached 11.6%. The overall prevalence of diabetes among adults above 30 years increased by 80% over a decade, representing an average in increment of 8% per year. Based on ethnicity, Indians have the highest prevalence of diabetes (19.9%), followed by Malays (11.9%) and Chinese (11.4%). The diabetes prevalence rate in Malaysia has increased dramatically, almost doubling over the last decade. These data reveal that diabetes is a challenging disorder requiring more significant attention to find convenient and efficient treatments (Ismail et al., 2002; Letchuman et al., 2010).

The major goal for therapeutic approaches in diabetes is to reduce circulating blood glucose levels. Insulin replacement is the only way to reduce hyperglycemia in type I diabetes. Although, the main pathophysiology of type II diabetes is not because the patients are unable to produce insulin, insulin usually is needed when the disease is progressing towards its final stage. The current standard treatment of diabetes type I and poor expression of type II includes subcutaneous insulin injections (Guthrie & Guthrie, 2004). Several types of insulin have been developed over the years. Insulin was initially prepared through isolation from animal pancreatic tissues. Insulin therapy with the animal insulin reduced the complications resulting from diabetes, but this insulin could not replace natural human insulin. This is because the animal insulin is not

completely homologous to human insulin. Therefore, the human peripheral cells did not response ideally as effectively to that of animal insulin. Thus, animal insulin is not able to efficiently control glucose levels in the diabetics. In addition, animal insulin causes immunogenicity problems in some patients (Allan, 1972).

Since 1982, human insulin is prepared through recombinant DNA techniques. Recombinant insulin has eliminated the immunogenicity issues of the animal insulin. However, ideal glucose homeostasis is still not achieved with recombinant insulin. This is because the injected insulin in only being distributed in the system by passing the liver. The blood glucose fluctuations result in chronic complications associated with the disease, such as renal failure, cardiovascular disease and neuropathy. In addition, other problems such as multiple daily injections, frequent glucose monitoring, dietary restrictions and tallying the correct dosage of injected insulin at different lifestyles and dietary condition are heavy burden on diabetic patients (many of whom are very young) (Ahrens et al., 1986; cited by Yechoor & Chan, 2005).

Another promising treatment for patients with diabetes is pancreas transplantation to replace damaged and non-functional insulin-producing cells with functional pancreatic β-cells. It is because complications of diabetes can be prevented if blood glucose is constantly maintained at normal levels. In most cases, this method provides successful glycemic control and delays the development of severe diabetic complications. However, the pancreatic transplantation has several

major challenges of its ones, such as the small numbers of organs available for transplantation, complications after severe transplantation surgery (which is especially critical in diabetic patients), graft rejection and requirement for long-term immunosuppressive therapy (Larsen & Stratta, 1996).

It has been reported that islet transplants is a safer alternative treatment to whole pancreas transplantation. In this approach, the Langerhans islets are isolated from donor and transferred into the recipient. The islet transplantation has shown the potential to correct glucose homeostasis. In addition, it is a simpler technique in comparison to severe surgical procedures of the whole pancreas transplantation approach. It poses less risk of surgery-related complications. However, it is also limited by the immunosuppressive requirement and the availability of donor islet cells (Shapiro et al., 2000).

In order to avoid the harmful and unpleasant side effects of commonly use insulin therapy, immunosuppressive drugs and surgeries from current treatments; gene therapy has been developed as an alternative treatment of diabetes. In this approach, non β-cells are engineered for the production, processing and secretion of insulin. The target cells for insulin gene therapy must be able to accurately detect and appropriately respond to changes in glucose levels. Gene therapy can be a successful approach if insulin can be produced through a glucose-regulated pathway and if this insulin can elicit responses to glucose fluctuation levels that are most similar to those induced by natural insulin secretion (Yoon & Jun, 2002).

Gene therapy approaches offer remarkable advantages in comparison with the available treatment options for diabetes. The insulin that is produced endogenously by engineered cells control glucose levels more efficiently than injected insulin. This method is more convenient and safer than frequent insulin injections. In addition, since the insulin gene therapy can be developed in such way that therapeutic plasmids are taken orally; it is more feasible therapy of choice than whole pancreas or islet transplants. There are no risks for the transmission of infectious agents after the surgery and there are no side effects due to the toxicity of immunosuppressive drugs (Flotte, 2007).

Several cell types have been genetically modified to replace β-cells in producing functional insulin, such as hepatocytes (Kolodka et al., 1995), pituitary gland (Moore et al., 1983), skin (Falqui et al., 1999) and muscle cells (Bartlett et al., 1997). These surrogate β-cells were synthesized and aimed to secrete the active insulin protein. Unfortunately, these insulin secretions approaches were not glucose regulated. Therefore, the engineered cells could not produce satisfactory insulin substitutes. This is because these cell types do not possess all the essential properties that would mimic the natural physiological regulation of insulin secretion (Ramshur et al., 2002).

Recently, several evidences have supported the hypothesis that the enteroendocrine cells which are located in the gut lumen are potential candidates for diabetic gene therapy (Cheung et al., 2000). The enteroendocrine cells secrete

incretin hormones, such as glucagon-like peptide-1 (GLP-1, from L-cells) and glucose-dependent insulinotropic polypeptide (GIP, from K-cells). The specific factors that regulate GLP-1 and GIP secretion by K and L-cells are very similar to those that regulate insulin secretion by β-cells. There are some advantages of using these cells for gene therapy. Firstly, K and L-cells express prohormone convertases 2 and 3 that are necessary to process proinsulin to mature insulin (Seidah et al., 1994). Secondly, the presence of some hormones, such as glucokinase and glucose transporter II, in K and L-cells lead to the activation of glucose-sensitive systems similar to that of pancreatic β-cells (Lynch et al., 1987). By this, activation of insulin secretion is upon the detection of glucose levels in the blood. Thirdly, K and L-cells have cell-specific promoters like GIP and GLP-1. These two promoters are only recognized by gut K and L-cells, respectively (Drucker et al., 1986). Finally, K and L-cells are easily-accessible cells. This is a crucial requirement for target cells in gene therapy approaches.

The animal body is a complex system with unknown components. Many factors are involved in gene expression pathways that are absent in cell culture studies. Consequently, *in vitro* systems cannot show the actual aspects of insulin gene expression in intestinal cells. Several factors from different tissues and cells in the body may influence insulin expression from intestinal K and L-cells. Therefore, this project covered both *in vitro* and *in vivo* studies to determine the efficacy of this approach in both situations. To develop a potential method for gene therapy, the selection of suitable target cells is an important step but successful gene therapy also needs an efficient gene transfer vehicle as well, especially if the

treatment modality being aimed is orally. There are several systems that are used to transfer foreign recombinant plasmids into the target cells *in vivo* (Somia & Verma, 2000). The ideal gene delivery vehicle must protect the plasmid DNA until it reaches the target tissue. The DNA plasmid needs to escape the processes that affect the decomposition of molecular structure. In addition, the gene delivery vehicle must be small enough to allow internalization into cells before releasing the plasmid DNA into the cytoplasm.

In terms of intestinal gene therapy, chitosan nanoparticle is the most appropriate candidate for gene delivery proposes (Cryan & O'Driscoll, 2003). Chitosan is a non-toxic biodegradable polycationic polymer that has low immunogenicity. The positively-charged chitosan interacts with the negatively-charged DNA. Therefore, chitosan can effectively bind to the plasmid DNA and protect it from nuclease degradation. It has been shown that chitosan has mucoadhesive feature as well. These properties of chitosan increase the transcellular and paracellular transport of macromolecules across the intestinal epithelial monolayers. In addition, chitosan is stable in the acidic pH of the stomach, facilitating oral gene delivery of the therapeutic plasmids (Tong et al., 2009). The oral delivery system is a convenient and non-injurious method of transferring recombinant plasmids to the diabetic patients. Thus, pose the friendliest modality of treatment.

Based on the background detailed above, we hypothesized that K and L-cells along with their special promoters are potential candidates for diabetic gene

therapy due to their ability to produce sustainable, mature and regulated insulin. The general objective of the present study is to evaluate and compare the ability and efficiency of engineered K and L-cells in expressing insulin *in vitro* and *in vivo* in order to recommend the best intestinal candidate target cells for treatment of diabetes. Thus, the study was carried out to meet the following specific objectives.

1. To construct six recombinant plasmids containing GIP or GLP-1 promoters for the purpose of isolating pure cell lines, studying insulin expression and confirming gene delivery efficiency.

2. To isolate pure K and L-cell lines as basic intestinal models using pGIP/Neo/pBlu and pGLP-1/Neo/pBlu plasmids for the preparation of intestinal insulin producing cell lines.

3. To establish intestinal insulin producing K and L-cell lines using pGIP/Ins/pBud and pGLP-1/Ins/pBud plasmids for insulin expression study.

4. To determine and compare the quantity and quality of insulin being expressed with GIP and GLP-1 promoter *in vitro.*

5. To develop chitosan nanoparticles of the pGIP/GFP/pBlu and pGLP-1/GFP/pBlu plasmids for gene delivery efficiency conformation.

6. To determine the quantity and quality of insulin being expressed with GIP and GLP-1 promoter *in vivo* and investigate the efficiency and efficacy of the constructed pGIP/Ins/pBud and pGLP-1/Ins/pBud nanoparticles in secreting insulin and controlling blood glucose levels of type I diabetes mouse model.

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