Diabetes gene therapy by means of nanoparticles

Terapia gênica do diabetes por meio de nanopartículas

Maria Rita Campante Moreira Prina*[1]; Janira Maria Alves[2], José Lamartine Soares Sobrinho[2], Antonio José Ribeiro[1]

[1]*College of Pharmacy, University of Coimbra, Coimbra, Portugal.*
[2]*Universidade Federal de Pernambuco, Programa de Pós Graduação em Inovação Terapeutica, Recife, Pernambuco Brasil.*

* Correspondencia:
E-mail: aribeiro@ff.uc.pt

RESUMO

O desenvolvimento tecnológico anda de mãos dadas com uma necessidade crescente de melhores soluções terapêuticas. Quando aplicado a diabetes mellitus (DM), terapia gênica oferece uma alternativa mais promissora para o que diz respeito ao controlo dos sintomas, ou mesmo a cura desta doença metabólica. Têm sido feitos esforços no sentido de encontrar a melhor maneira de aplicar a terapia genética para DM. Ele pode ser aplicado para melhorar os resultados de transplante de ilhéus, a administração do gene da insulina em um nível gastrointestinal ou mesmo através de silenciamento de genes como maneira de manter os níveis sanguíneos de glicose perfeitos. Apesar do potencial terapêutico da terapia gênica, para a sua aplicação vários fatores devem ser considerados. Sistemas de distribuição constituem um desses fatores sendo decisivo para a eficácia da terapia genética. Vetores não virais têm grande importância neste domínio devido à sua essencial características *in vivo*, tais como baixa imunogenicidade e conseqüente segurança. Nanopartículas à base de quitosano têm sido utilizados como vetores não virais e estudados para aplicação em terapia gênica no diabetes tipo 2 de tipo I.

Palavras-chave: diabetes *mellitus*; gene terapia; vetores não viral; nanoparticula

ABSTRACT

The technological development walks hand on hand with an increasing need of better therapeutic solutions. When applied to diabetes *mellitus* (DM), gene therapy offers a more promising alternative to what concerns the symptom control or even the cure of this metabolic disease. Efforts have been made towards finding the Best way to apply gene therapy to DM. It can be applied to improve the outcomes of islet transplantation, insulin gene administration in a gastrointestinal level or even through gene silencing as way to keep perfect glucose blood levels. Despite the therapeutic potential of gene therapy for its application various factors need to be considered. Delivery systems constitute one of those factors being decisive in gene therapy's efficacy. Non-viral vectors have great importance in this Field due to their essential *in vivo* characteristics such as low immunogenicity and consequent safety. Chitosan-based nanoparticles have been used as non-viral vectors and studied for gene therapy application both in type I diabetes and type 2 diabetes.

Keywords:diabetes *mellitus*; gene therapy; non-viral vectors; nanoparticle

INTRODUCTION

Progress on the treatment of diabetes *mellitus* (DM) has been made during the years, but no therapy has been effective in eliminating or reversing the disease's progression. A rising need to discover alternative therapeutics for the treatment of this disease has made gene therapy gain importance.

As a consequence of the diabetes disease, an autoimmune destruction of the beta cells occurs in type I diabetes (TID), while in type II diabetes (T2D), these cells are unable to keep up with the body's insulin requirements. This leads to a loss in the concentration of glucose in the blood, and consequently causes hyperglycemia.

It is, therefore, imperative to develop an alternative approach to correct the relative or absolute insulin deficiency in DM patients, so that tight control of blood glucose can be achieved to prevent the end-organ damage caused by prolonged hyperglycemia. Diabetes gene therapy offers a novel strategy for this purpose since the insulin gene was first cloned and expressed in cultured cells in the late 1970s (XUet al., 2003).

The principle underpinning gene therapy is theoretically straightforward, but difficult to achieve in practice. The principle entails the introduction of a gene into the genetic complement of a cell, such that subsequent expression of the gene achieves a therapeutic goal (GARY, 2007; WALSH, 2007). This goal can be islet transplantation improvement or related with generating surrogate insulin-producing cells to replace beta cells.

However, a key limitation at present is the availability of efficient and reliable methods for delivery and sustained expression of the transferred deoxyribonucleic acid (DNA). Non-viral vectors present important advantages in relation to viral vectors, such as their low/non-immunogenicity andnon of integration of the therapeutic gene into host chromosome (this eliminates the potential to disrupt essential host genes or to activate host oncogenes).

The present review is intended to summarize the current efforts made regarding gene therapy applied to DM, focused on non-viral delivery systems. Although important research is still needed to continue with the achievement of important goals, important studies involving these delivery systems are described as well as their principle conclusions and progress towards successful gene therapy.

Diabetes *mellitus* (DM): Type I and Type 2

Diabetes *mellitus* caused by insulin deficiency or a resistance to insulin, resulting in elevated blood glucose levels (hyperglycemia). The consequences of the long-term hyperglycemia can lead to end stage micro-and macrovascular damage, leading to organ failure such as neuropathy, nephropathy, retinopathy, peripheral vascular disease, morbidity and mortality. Furthermore, lifestyle changes over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide (XUet al., 2003) and therefore an increased risk of complications of this devastating disease.

Insulin is secreted by the beta cells in the islets of Langerhans in the pancreas. It is the primary hormone responsible for controlling the simulation of intracellular use and storage of glucose and the main factor controlling the synthesis and secretion of insulin is the blood glucose concentration. As a result, in DM this relationship is altered. Insulin plays a central role in regulating blood glucose levels, generally keeping it within narrow defined limits.

Diabetes is caused by an absolute (type I) or relative (type 2) insulin deficiency. Although hyperglycemia is an indicator for both TID and T2D, the clinical features and pathophysiology between the two disorders is vastly different (GLASER, 2007). In TID, there is almost universally an autoimmune destruction of the beta cells of the pancreas, while in T2D the beta cells are unable to keep up with the body's insulin requirements, which are increased due to insulin resistance.

Even though there have been important advances in the understanding of both the pathogenesis and management of both forms of diabetes, treatment options, which include injectable insulin and oral agents, are neither the ideal or definitive treatments. While insulin replacement has drastically improved the lifespan of diabetics, its administration fails to mimic its physiologic regulation and that of glucoregulation in the body (D'ANNEOet al., 2006) and has been associated with patient noncompliance resulting in suboptimal control and ensuing diabetic complications. In addition, frequent hypoglycemia, due to the lack of physiological regulated insulin delivery, leads to significant morbidity and even mortality (YECHOOR & CHAN, 2005).

Gene Therapy Progress and Prospects

In order to improve the clinical status of patients with diabetes, gene therapy can generally be defined as any therapeutic modality that uses gene transfers technology. More specifically, gene therapy, a promising therapeutic approach, is the

delivery of genetic material to target cells with the purpose of either restoring a function which has been lost, or blocking an undesirable effect (HARRISON, 2008).

DM has long been targeted, as yet unsuccessfully, as being curable with gene therapy. The main hurdles not only been vector-related toxicity but also the lack physiological regulation of the expressed insulin (YECHOOR & CHAN,2005). The first gene therapy trials were initiated in 1990. However, the lack of a stable and strong gene expression, a target-specific vector and appropriate regulation system to control the transgene expression, the problems associated with immune and inflammatory responses as well as the limited knowledge regarding the genetic components of the disease have been blocking its clinical application (XUet al., 2003).

However, gene therapy has rapidly developed, having occupied for the last 30 years, a very important part of scientific research, due to an enormous progress in molecular biology (XUet al., 2003). Diseases that occur from DNA mutation have grown substantially; gene therapy has shown to be promising in the treatment, cure, and prevention of these diseases. The first studies regarding genetic engineering gene therapy were performed with the aim of curing cancer. Nowadays, gene therapy is used in other fields, as neurologic and metabolic diseases, such as diabetes (YECHOOR & CHAN,2005). Diabetes gene therapy should ideally lead to euglycemia, the normal glucose level in the blood, and elimination of the necessity for frequent injections or tissue transplantation demanding immunosuppression (XUet al., 2003).

The use of lifelong injections of insulin, and islet transplantation, which faces the problems of donor shortage and rejection, makes gene therapy an auspicious approach, even though specific obstacles still remain unresolved (Xu et al., 2003). For instance, in TID, islets are the target for autoreactive T cell destruction. Insulin deficiencies and resultant hyperglycemia occur with the lack of islets. Gene therapy is a useful technique to treat TID; either the insulin gene can be replaced in a host or the autoreactive T cell can be suppressed (WONG, HAWTHORNE & MANOLIOS,2010). Besides this, both TID and T2D, and their associated complications, appear to be amenable to gene therapy technology.

Nanoparticles

Nanotechnology, along with related concepts such as nanoparticles, nanomaterials and nanostructures has become a priority area for scientific research and technological development. One of the most important nanotechnology applications developed over the past decade have been nanovehicles, nanoscale compounds used as a therapeutic tool and designed to specifically accumulate in the sites of the body where they are needed in order to improve pharmacotherapeutic outcomes (BOULAIZet al., 2011).

Effectively, the ability of nanoparticles to manipulate the molecules and their structures has revolutionized the conventional drug delivery system, in increasing therapeutic effectiveness while obtaining lower toxicity rates (BOULAIZet al., 2011). Chitosan nanoparticles area good example of this reality: because of their biodegradability, biocompatibility, better stability, low toxicity, simple and mild preparation methods, they offer a valuable tool to novel drug delivery systems (NAGPAL, SINGH & MISHRA, 2010).

Gene transfer methods: delivery systems

Main somatic gene transfer approaches employ either viral or non-viral vectors such as calcium phosphate co-precipitation, lipofection, direct microinjection, electroporation andbiolistics (TROS DE LLARDUYA, SUN & DUEZGUENES, 2010).

The choice of these approaches depends on various factors. Viral vectors show high gene transfers efficiency, but on the other hand they are deficient in several areas, such as the induction of a host inflammatory and unexpected immune response. Therefore, non-viral vehicles, such as cationic liposomes or polymers, can be employed, overcoming some of these problems. The complexes formed with polymers and DNA are defined as "lipoplexes" whereas the complexes formed with polymers and DNA are called "polyplexes, constituting the most promising alternative to the use of viral vectors for gene therapy. Non-viral vectors have benefits in terms of their simplicity of use, their easiness in large-scale production and their absence of specific immune response. Therefore, non-viral vectors have received rising attention to achieve an elevated high level of cellular transfection in an effective and harmless way (TROS DE LLARDUYA, SUN & DUEZGUENES, 2010).

The gene transfer approaches can be categorized into two general groups: naked DNA delivery, by a physical method, such as electroporation and gene gun, and delivery, mediated by a carrier such as a cationic polymer and lipid.

Gene therapy may involve one of the three strategies described below:

In vivo gene therapy comprises systemic or directed injection of the vector for expression of the gene(s) of interest. This approach requires the development of vectors capable of recognizing and binding only to specific, predefined cell types. Through appropriate biospecific interactions they would only delivery their nucleic acid payload to the specific target cells (SAMSON &CHAN, 2006).

In situ gene therapy involves the direct injection of the vector contiguous to the body target cells (SAMSON & CHAN, 2006).

The *ex vivo* gene therapy requires the removal tissue, for subsequent culture and transduction *in vivo* with gene therapy vectors. After the cells express the protein or achieve the desired phenotype, they are implanted in the hope of curing the recipient. In practical terms, this would require removal of a small amount of tissue from the recipient, such as with a biopsy, for *in vitro* culture, transduction and re-implantation. Because *ex vivo* transduction would avoid systemic infection, there is less potential for vector toxicity and transduction of non-target tissues. Moreover, it might allow more quality control, while only successfully differentiated cells would be selected for implantation (SAMSON & CHAN, 2006).

Non-viral methods

Calcium phosphate co-precipitation is a simple and non-expensive method for genetically modifying pancreatic cells. A precipitate is formed as the DNA of interest and calcium chloride is added to a buffered saline/phosphate solution. This DNA-containing precipitate can either be endocytosed or phagocytized by cells.

Also used as high efficiency transfection agents of cells both *in vivo* and *in vitro* are DNA-containing liposomes, contrary to calcium phosphate co-precipitation, which is conducted *in vitro.* *In vivo* lipofection is considered advantageous, as liposomes may be injected into the bloodstream, being less invasive than other treatments, such as transplantation. These carriers have minimal positive charges which increase their interaction with target cells and the subsequent transfection efficiency (TORCHILIN, 2006).

Microinjection consists on directly injecting DNA into cells and is an effective technique for transfecting cells. Nevertheless, this is a labor intensive method, as each cell needs to be targeted individually, and is not appropriate for the targeting of large cell numbers (TROS DE LLARDUYA, SUN & DUEZGUENES, 2010).

Electroporation that creates permeable membranes for gene transfer by applying high voltages to cells causing cell death in many cases. In order to permit proficient gene transfer to surviving beta cells the islets need to be dissociated from the tightly clustered sacs of cells into single cells suspensions. The dissociated islets may be non-functional, if there is not conservation of their morphology. Electroporation cannot efficiently integrate DNA into the host genome, even though it is possible for gene transfer into the cell (TROS DE LLARDUYA, SUN & DUEZGUENES, 2010).

Biolistics is another non-viral method which uses of a "gene gun" to transfect cells with a transgene. The "gene gun" quickly discharges DNA-microprojectiles into cells (WONG et al.,2010). This method produces higher transfection efficiencies than calcium phosphate co-precipitation or lipofection.

The use of cationic lipids and cationic polymers for gene transfers was introduced by FELGNER et al., (1987) and WU and WU (1987), respectively. Their use has progressed rapidly from transfection of cell cultures to clinical gene therapy applications (TROS DE LLARDUYA, SUN & DUEZGUENES, 2010). Over the past decade significant progress has been made in the understanding of the cellular pathways and mechanisms involved in lipoplex- and polyplex-mediated gene transfection. The general mechanisms of delivery comprise the following steps (ELOUAHABI & RUYSSCHAERT,2005): nano-sized liloplex or polypex particles are prepared from the combination of cationic liposomes or polymers with nucleic acids in a buffered aqueous solution. Between the positively charged complexes and the negatively charged cell surface, the particles bind to the cell surface by non-specific, electrostatic interactions, entering cells by endocytosis (TROS DE LLARDUYA, SUN & DUEZGUENES, 2010).

Whereas the cationic lipids, known by lipoplex, can be synthesized by the interaction of a large variety of liposomes and DNA, cationic polymers comprise natural DNA – binding proteins (for example histones, synthetic polypeptides, cationic detriments, or carbohydrate-based polymers are synthetic compounds. The size of the particles size is essential for gene transfer- the smaller the particle, the higher the transfection efficacy, particularly *in vivo*. In this sense, cationic polymers are advantageous as they can be combined with DNA to develop a particulate complex, and can condense DNA molecules to a relatively small size, compared to cationic liposomes (TROS DE LLARDUYA, SUN & DUEZGUENES, 2010).

The stability of these vehicles is related to

their structure charge and formulation, affecting consequently their efficiency of gene transfection. Wile lipids that facilitate transformation of lipoplexes to non-bilayer phases mediate high transfection activity *in vitro*, lipids, like cholesterol, that ensure stability in serum, are more suitable for gene delivery *in vivo* (TROS DE LLARDUYA, SUN & DUEZGUENES, 2010).

Viral Vectors

A non-viral vector is of low immunity and low cost, and is handy for preparation, but the main barrier for its wide usage results from its poor delivery efficiency and transient gene expression. In contrast, viral vectors have been proved to have the highest efficiency in gene delivery to date since many of them involved specific machinery for delivering DNA to cells. These vectors are packaged as viral particles containing only the necessary regulated viral sequences and from which all viral genes have been removed. If prepared appropriately, these viruses are so defective that after infecting the target cells, no further replication or infection is theoretically possible, the viral DNA integrates into the host cell genome, thus conferring the potential for stable therapeutic gene expression (XU et al.,2003).

Table 1. Comparison between the cost, immunity and transfection efficiency of non-viral and viral vectors

PROPERTIES	NON-VIRAL VECTORS	VIRAL VECTORS
Cost	+++	+
Immunity	+++	+
Transfectionefficiency	+	+++

Therapeutic in type I diabetes melitus: a world of approaches involved with gene therapy

Over the past century, the Discovery of insulin and development of insulin analogues have been life-saving for treatment of hyperglycemia in DM. Insulin, however, is not cure and even intense therapy does not perfectly simulate the strict control of blood glucose that is provided by beta cell (SAMSON AND CHAN, 2006). So we need to look forward to find better solutions among which gene therapy.

A promising therapeutic approach to improve TID is islet or pancreas transplantation that may physiologically release insulin in DM patients.

However, these strategies have several problems, such as surgical risks, organ shortage, high cost, and immune rejection, which hinder the wide spread of the therapeutic measures. Furthermore, transplant patients have to receive long-term-probably lifetime-immunosuppression therapy with all is associated side-effects (D'ANNEO et al., 2006).

In fact, these limitations can be potentially circumvented with cell/gene therapy. Indeed advances in cell and gene therapy have rekindle the possibly of not only ameliorating but also potentially curing diabetes (YECHOOR & CHAN, 2005).

Gene therapy can be applied from many different angles: such as the suppression of autoreactive T cells to prevent islet destruction (prophylactic) or the of replacement of the insulin gene (post-disease). The need for a better method for providing euglycemia arose from insufficient numbers of cadaver islet for transplantation and the immunosuppression required post-transplant (WONG et al., 2010). Therefore, as TID results from autoimmunity against pancreatic islet beta cells, causing the loss of these cells, therapies have to both block autoimmunity and restore the beta-cell mas (PRUD'HOMME, DRAGHIA-AKLI & WANG, 2007).

Moreover, owing to the heterogeneity and complexity of DM it is possible to outline several different gene transfer strategies that might prevent or cure the disease. These strategies could be divided into Five major categories, namely; prevention of beta cell destruction in TID; simulation of beta cell differentiation and regeneration; ectopic production of insulin by substitute cells; cell therapy using genetically manipulated beta cells and *ex vivo* gene transfer to donor pancreatic islets destined for transplantation in patients with TID (BARBU & WELSH, 2007).

The ability to engineer pancreatic beta cells is prerequisite for a successful application of many gene therapy approaches in TID. Since pancreatic islet are terminally differentiated

Cell clusters, gene transfer into islet cells poses significant technical hurdles. To date, several gene therapy vectors have demonstrated their utility in genetic modification of islet cells (BARBU & WELSH, 2007).

While viral vectors show the most promising gene transfer efficiency into islet cells, it is likely that non-viral vector systems will more easily satisfy biosafety concerns in clinical trials (BARBU &WELSH, 2007).

Table 2. Comparison between the cost, immunity and transfection efficiency of non-viral and viral vectors

		ADVANTAGES	DISADVANTAGES	REFERENCES
Non-viral vectors	Naked DNA DNA Complexes	High Clinical safety Easyna inexpensive to produce Nonimmunogenic	Low transfection efficiency Transient gene expression	LAKEY et al., 2001.
Adenovirus		High transduction efficiency High viral titre Infects nondividing cells	Transient gene expression Immunogenic Clinical safety issues	NOGUCHI & MATSUMOTO, 2006; BARBU et al., 2006.
Adeno-asssociatedvírus		Potencial site-specific integration Infects nondividing cells No immune response High clinical safety	Very difficult to generate	REHMAN et al., 2005.
Lentivirus		High transduction efficienty Lonh-term expression. Easy to generate	Possibility of insertional mutagenesis with clinical effects	KOBINGER et al., 2004
Herpes simplex virus		High transductionefficiency	Inflammatory and toxic reactions in patients	EPSTEIN et al., 2005

Reinventing the islet

Islets considered being a potentially effective therapy for insulin deficient diabetes. Islet transplants, however, are limited by the availability of donors and the majority of transplanted islet experience damage and apoptosis during the isolation process, a blood –mediated inflammatory microenvironment in the portal veins upon islet infusion, hypoxia induced by the low oxygenated milieu, and poor revascularization-mediated lack of nutrients, and impaired hormone modulation in the local transplanted site (D'ANNEO et al., 2006).

These hurdles can be overcome by strategies using genetic modification methods trough over expression or silencing of those proteins involved in promoting new formation of blood vessels or inhibition of apoptosis and consequently improve islet engraftment outcomes (WANG et al. 2011).

The consequence of RNA interference in islet transplantation

Ribonucleic acid interference (RNAi) is a novel strategy to selectively degrade target messenger RNA (mRNA). The use or RNAi Technologies to down regulate the expression of harmful genes has the potential to improve the outcome os islet transplantation (LI & MAHATO,2011).

Insulin-producing betacells in the islets of Langerhans are the target of autoimmune aggression in TID, and non betacells and the exocrine pancreas is unaffected. The islet constitutes only a tiny fraction (1%) of the whole pancreas and it is functionally unnecessary to transplant the whole pancreas when only its endocrine tissue is required. Islet transplantation provides a less invasive alternative approach for the treatment of TID with reduced antigen load, relative simplicity, and low morbidity.

However, since the initiation of clinical islet transplantation from early 1970s, most of these trials failed, until the breakthrough at the University of Alberta in Edmonton, Canada. The "Edmonton protocol" was published in 2000, reporting that seven patients with TID became insulin independent after receiving islet transplantation with a prednisone-free protocol. The Edmonton protocol hás been replicated and further modified worldwide with many successes. Despite these successes, only less than 10% of the recipients remain insulin independent for up 5 years, and most recipients return insulin because the islet function decreased over time (RYAN et al. 2005).

Ex vivo genetic modification of islets before transplantation has the potential to overcome several problems associated with islet transplantation (PANAKANTI & MAHATO, 2009b; PANAKANTI & MAHATO, 2009a). Growth factor gene expression has the potential to promote islet revascularization while anti-apoptotic gene expression can reduce inflammation and immune rejection and prevent islet cell apoptosis. This strategy can help to reduce the number of islet needed for each recipient and prolong the time that a recipient can maintain insulin independence transplantation. The genetic modification of islet

could be the over-expression of protective genes or inhibition of harmful gene expression (MAHATO, 2009). Antisense oligonucleotides (ODNs), ribozymes, and RNAi are major approaches which are currently being used to sequence specifically reduce or inhibit gene expression. Among these approaches, RNAi is relatively new and evolutionally conserved biologic process that regulates gene expression using small interfering RNA (siRNA) mediated sequence specific, post-transcriptional gene silencing. Since the Discovery of RNAi by fire and milo in 1998, it has been widely used as a tool for basic research as well as a potential therapeutics (CHEN,CHENG & MAHATO 2008).

Delivery strategies for enhanced gene silencing

Since the Discovery of the RNAi technique, good progress has been made. However, due to the numerous biological barriers for siRNA delivery, which result in the degradation of siRNA. Systemic delivery of siRNA is required to block the host against islet graft immune rejection (JULIANO et al., 2009). Also, the lack of targeting ability of systemic siRNA delivery is another concern, as this technique involves the use of targeted delivery system to circumvent gene silencing on other tissues or organs. In addition, the systemic administration will possibly induce the immune reaction not only by the siRNA but also by the delivery system, especially, when a viral vector is used. Alternatively, *ex vivo* delivery is na idyllic method for genetic modification of islet prior to transplantation. The transfer of siRNA into islet prior to transplantation can be done, since after isolation, islet are commonly conserved in storage solutions before transplantation (LI & MAHATO, 2011).

Ever since lipofection was introduced in the late 1980s, various cationic liposomes have been tested for delivery of plasmids, and recently for the delivery of siRNA, various commercial cationic lipid formulations for siRNA delivery are used, including lipofectamine 2000, oligofectamine, lipofectamine, RNAifect, and Fugene HD.

Lipofectamine has been tested by Li and Mahato (2011) to transfect a plasmid encoding an enhanced green fluorescent protein (EGFP) gene into intact human Islets. Nevertheless, low transfection efficiency was obtained, since human islets are a cluster of around 1000 non-dividing cells (MAHATO et al., 2003). Another study by Li and Mahato (2011), used lipofectamine 2000 to transfect fluorescein-labeled siRNA. An increase in transfection efficiency was observed with an increase in the siRNA concentration, with maximum transfection efficiency of 95,9% and 28,3% on rat beta cell line and intact human islets, respectively (LI & MAHATO, 2008). This confirmed the viability of *in vitro* genetically modifying human islets with siRNA/lipid complex (LI & MAHATO, 2008).

Besides cationic lipids, caionic polymers and also used for siRNA delivery. Although decent *in vitro* transfection efficiencies could be achieved by optimizing transfection conditions, the cellular toxicity of cationic carriers is a major concern for its therapeutic applications. Besides this, the poor intracellular dissociation of siRNA/polycation complex may reduce the intracellular bioavailability of siRNA (LI & MAHATO, 2011).

The bioconjugation of siRNA with lipids, polymers and others molecules will also bring advantages to the systemic stability, cellular uptake, and targeted delivery to specific cells. The properties of the siRNA conjugate are based on molecules used for conjugation with siRNA. Attachment of lipids usually increases the hydrophobicity of siRNA, which are highly hydrophilic macromolecules. Lipophilic molecules such as cholesterol, bile acid and long chain fatty acid and vitamin E can also conjugated with siRNA. Depending on the degree of hydrophobicity, after systemic injection, siRNA conjugate can bind with lipoproteins.

Currently, there are more than ten ongoing and completed clinical trials as recent progresses have already demonstrated its great potential. However, the improvement in the outcome of islet transplantation is still na inexpert area. The development of safe and efficient delivery systems is the key for efficacious gene silencing (LI & MAHATO, 2011).

The importance of vascular endothelial growth factor in islet transplantation

Vascular endothelial growth factor (VEGF) is an angiogenic growth factor, extensively used in vascular diseases, ischemic injuries, and in other transplanted areas. It can be useful when applied to one of the significant obstacles to efficacious pancreatic islet transplantation: inadequate graft vascularization during the first days after transplantation, which can cause dysfunction and death of the transplanted islet tissue (KIM, 2004; ROBERTSON, 2004). Moreover, an optimistic *in vitro* result was obtained by transplanting VEGF gene adenovirus-vector transfected islet cells in diabetic mice: VEGF gene therapy was reported to increase islet graft revascularization in a mouse model. However, the gene delivery system used

was a viral vector, which showed disadvantages such as affecting cellular immunogenicity and/or inducing the production of several chemokines and their receptors, and thus may limit its use in terms of clinical application. Furthermore, current developments in gene delivery carriers offer greater levels of safety, as for example the use of non-viral vectors for human cell therapy (CHAE et al., 2005).

Hypoxic damage is one of the major causes of islet graft failure and VEGF is kwon to play a fundamental role in revascularization. Chae and co-authors (2005) evaluated whether a non-viral cationic lipid reagent can be used as a reagent for gene delivery in non-dividing islet cells, and whether the VEGF transgene in islet grafts can increase islet revascularization, and therefore, increase transplanted islet survival rates to attain effective glycemic control in diabetic mouse models (CHAE et al., 2005).

Successful islet transplantation is dependent on adequate islet mass, stable engraftment, and on the prevention of rejection. Unlike allogenic islet transplantation, where islets are transplanted from an unrelated individual to a member of the same species, and which requires immunosuppressive treatment, autologous islet recipients transplanted with >250,000 islets can achieve a stable euglycemic state (70-80%). This is considered to be an ideal model type for studying islet engraftment. However, it is likely that no more than 30% of the transplanted islet cells mass become engrafted in TID recipients, though they ultimately compose 50% of the beta cell mass present in a normal individual. Moreover, hypoxic damage and cytokine-mediated nonspecific injure to islets may play important roles in inadequate islets engraftment (CARLSSON & MATTSSON 2002).

Islets pre-exposed to VEGF protein *in vitro* before transplantation showed no survival gain, thus indicating that VEGF was directly involved and that it must be secreted continuously during islet engraftment.

An effectene lipid reagent was used in a transfection experiment using mouse islets to address the effectiveness of a cationic lipid reagent as a VEGF gene carrier, and the beneficial effect of VEGF-transfected islets on glycemic control (CHAE et al. 2005).

Modified gold nanoparticle vectors

The complex architecture of the pancreatic islets leads to challenging problems towards the delivery of molecular therapeutic cargos into islets. Islets are constituted of a cluster of approximately 1,000-2,000 cells, ranging in size from 50 to 400 mm in diameter and are richly vascularized, innervated, and delimited by connective tissue. Usually, only the periphery of an islet is transfected or transduced efficiently, penetrating approximately 2-3 cells layers, with most cells within the islets staying unaffected (ZHANG et al., 2005, BARBU et al., 2006). Furthermore, in addition to traditional delivery systems increasing immunogenicity, they also compromise islet function and cause potential oncogenic risks (JAIN, 2007; WILLNER & WILLNER, 2007; STEWART et al., 2008, CHEN et al., 2007).

Modified gold nanoparticles (AuNPs) represent a potential alternative to viral vectors, due to their inherent capability to enter many cell types with high efficacy, biocompatibility, unique binding properties, and simple conjugation chemistry (Massich et al. 2009). These AuNP conjugates are composed primarily of aAuNP core with a densely packed layer of oligonucleotides, which reduces enzymatic degradation by endonucleases. At the time of the incubation of the oligonucleotide-modified AuNPs with cells, they have the distinctive aptitude to initiate endocytosis by adhering to signaling proteins in the extracellular matrix without the use of transfection agents and demonstrate minimum or no toxicity (MASSICH et al., 2009, SEFEROS et al., 2009).

The siRNA-AuNP conjugates can achieve efficient *in vitro* gene silencing abilities, resistant against endonuclease degradation while preserving serum stability (GILJOHANN et al., 2009, ELBASHIR et al., 2001). In the cell, the conjugated siRNA escapes from de AuNPs surface and deactivates the targeted genes. The amount of gene silencing attained with the siRNA-AuNP complex was significantly improved in comparison with other siRNA techniques (VEGA et al., 2010).

Human insulin gene chitosan nanoparticles for use in gastrointestinal administration in diabetic rats and NHI3T3 cells

Insulin gene therapy, including any approach involving the introduction of a foreign gene into any cell type in the body, can produce insulin (D'ANNEO et al., 2006). The gene(s) introduced could be the insulin gene itself, perhaps under control of a tissue specific promoter, allowing for expression in a selected non betacell type, or in a gene encoding for a factor that activates the insulin gene, thereby allowing for ectopic insulin production. In insulin gene therapy, one of the key issues is the development of

efficient delivery systems (NIU et al., 2008a). Despite the advances in gene transfer technology, including viral and non-viral vectors, no ideal vector system is available at present (GIANNOUKAKIS & TRUCCO, 2005). Although viral vectors can introduce exogenous genes into cells precisely and effectively, they can easily cause immune reactions because of the existence of the antiviral immune system. Due to the growing concerns over the toxicity and immunogenicity of viral DNA delivery systems, DNA delivery *via* improving viral routes has become more desirable and advantageous (ZAIA, 2007). More and more researchers are interested in non-viral vectors.

Chitosan nanoparticles, in addition to have good biocompatibility and no toxicity are also economically available (DOUGLAS, PIECIRILLO & TABRIZIAN,2006). The transfection efficiency of chitosan can be regulated by changing its molecular weight, plasmid concentration, and the chitosan/plasmid ratio. After the plasmid is embedded in chitosan, it can resist the degradation of nucleases. It also exhibits an antibacterial activity by inhibiting the bacterial metabolism (NIU et al., 2008a).

There is a considerable amount of endocrinal cells in gastrointestinal tracts, wich are considered ideal target cells in the gene therapy of diabetes (REN et al., 2007, FODOR et al., 2007). Considering these facts, Niu and co-workers (2008b) attempted to transfer the exogenous human insulin gene by gastrointestinal tracts. The transfer of the human insulin gene to diabetic rats by gastrointestinal tracts is very safe, painless, and can effectively avoid infections. The authors constructed an expression plasmid (pCMV.Ins) expressing the human insulin gene. They then wrapped pCMV. Ins with chitosan nanoparticles, which was transfected to NIH3T3 cells and diabetic rats to evaluate the efficiency of chitosan nanoparticles as a vector and novel route for the transfection of the human insulin gene in diabetic rats. Their findings demonstrated that the human insulin gene wrapped by chitosan nanoparticles can be expressed efficiently in NIH3T3 cells and the gastrointestinal tracts of diabetic rats, indicating chitosan nanoparticles are a promising non-viral vector with potential for gene expression (NIU et al., 2008b).

In conclusion, the human insulin gene can be transfected successfully by chitosan nanoparticles *in vitro* and *in vivo*, being expressed efficiently in NIH3T3 cells and the gastrointestinal tracts of diabetic rats, indicating that chitosan is a promising safe and effective non-viral vector for gene expression. The benefits of using chitosan are its safety and painlessness. If it can be applied on clinics, it will be willingly accepted by patients. Although much work remains to be done, the rapid progress in insulin gene therapy provides an optimistic Outlook for clinical applications for TID (NIU et al., 2008b).

Gene therapy in type 2 diabetes

Considering the worldwide obesity epidemic predictions, the increasing diabetes prevalence from 2.8% in 2000 to 4.4% in 2030 in every age group is not surprising. Since not every obese person develops T2D, some interaction between genetic susceptibility and environmental factors is clearly involved in the continuing diabetes epidemic (REIMANN et al., 2009).

Due to GLP-I's insulinotropic action, it has been proposed that this insulinotrophic hormone can be used in the treatment of T2D. GLP-I's gene delivery has been considered an important delivery system, since, although GLP-I presents several advantages, it also suffers the drawback of an extremely short half-life as a result of its degradation by the dipeptidyl peptidase IV protease (JEAN et al., 2011).

Gene therapy approaches have shown to lower blood glucose levels effectively in murine models of severe T2D (JEAN et al., 2011). Another important feature of gene delivery systems is the ability to condense/release nucleic acid sequences and protect them from the extracellular matrix nuclease rich environment during nanoparticle residence time at the cell surface (JEAN et al., 2012).

Jean and co-authors (Jean et al. 2012) developed a study where the glucosamine-based polymer chitosan was used as a cationic polymer-based *in vitro*delivery system for GLP-I, DPP-IV (Dipedtidyl peptidase-4) resistant GLP-I analogues and siRNA targeting DPP-IV mRNA. The nanoparticle formulations efficiently deliver GLP-I or siRNA-DPP-IV, in order to achieve an ideal intermediate stability profile, and at the same time offer nuclease protection and intracellular disassembly for efficient endosomal escape with high transfection efficiencies. Indeed, these formulations protected and delivered nucleic acids, suggesting promising combined delivery of both GLP-I and siRNA-DPP-IV to enhance glycemic control in T2D. In addition, DPP-IV gene silencing, using the described chitosan formulation, reached levels comparable to those of the commercially available lipoplex (DharmaFECT®), reduced toxicity as an important advantage. The versatility of these specific formulations to deliver plasmid DNA and siRNA render their promising use as a combined *in vivo* therapy for the control of T2D.

CONCLUSIONS

Diabetes and its associated complication represent evermore a problem of public health. Thus, and with the emergence of new technologies, gene therapy has assumed the most relevant position in this field for offering a preventive perspective by delaying the onset of diabetes or even a possible cure for this glucose homeostasis disorder.

The feasibility of gene therapy depends of the development of an adequate gene delivery system. Viral vectors were extensively studied because of their high transduction efficiency, nevertheless it is known today that their use has weakened due to safety issues an also due to the disadvantageous time consuming process construction.

Non-viral vectors have become a promising approach because in addition to being economically viable, they gather the most relevant advantages for possible use in human trials: security combined with non-immunogenicity. Regarding the therapies applied to DM, these are a possible key to solve the delivery system problems. Several approaches have been made since they offer the possibility of reducing the disease complications or even enable an individualized therapy. An example of application is the improvement of islet transplantation through gene silencing using cationic lipids and cationic polymers, and recently modified gold nanoparticles although some obstacles like low transferring efficiency demand further studies.

Non-viral vectors like chitosan containing the human insulin gene also seem an interesting hypothesis in gastrointestinal administration.

It is still necessary to understand in more detail how to apply these vectors, which genes to silence, which cells to target. Moreover, despite the advances in the field, the delivery of nucleic acids to target cells and tissues is still challenging and lots of efforts remain to be made.

REFERENCES

BARBU, A. R. B.; BONDIN, M.; WELSH, L. J.; JANSSON; WELSH, N. A perfusion protocol for highly efficient transduction of intact pancreatic islets of Langerhans. **Diabetologia,** v.49, p. 2388-2391, 2006;

BARBU, A. R.; WESH, N. Diabetes *Melllitus*: Gene therapy. **Encyclopedia of lyfe sciences**, 2007.

BARON, R.; WILLNER B.; WILLNER I. Biomolecule-nanoparticles hybrids as functional units for nanobiotechnology. **Chemical Communications**, p. 323-332, 2007;

BOULAIZ, H.; ALVAREZ, P. J.; RAMIREZ, A.; MARCHAL, J. A.; PRADOS, J.; RODRIGUEZ-SERRANO, F.; PERAN, M.; MELGUIZO, C.; ARANEGA, A. Nanomedicine: Application Areas and Development Prospects. **International Journal of Molecular Sciences**, v. 12, p. 3303-3321, 2011;

CARLSSON, P. O.; MATTSSON, G. Oxigen tension and blood flow in relation to revascularization in transplanted adult and fetal rat pancreatic islets. **Cell Transplantation**, v. 11, p. 813-820, 2002;

CHAE, H. Y.; LEE, B. W.; OH, S. H.; AHN Y. R.; CHUNG, J. H.; MIN, Y. K.; LEE, M. S.; LEE, M. K. KIM, K. W. Effective glycemic control achieved by transplanting non-viral cationic liposome-mediated VEGF-transfected islets in streptozotocin-induced diabetic mice. **Experimental and Molecular Medicine**, v. 37, p. 513-523, 2005;

CHEN, Y.; CHENG, G.; MAHATO, R. I. RNAi for treating hepatitis B viral infection. **Pharmaceutical Reseach**, n. 25, p. 72-86, 2008;

D'ANNEO, A.; ROOD, P.; BOTTINO, R.; BALAMURUGAN, A. N.; HE, J.; GIANNOUKAKIS, N. Gene therapy for type I diabetes – is it ready for the clinic? **Immunologic Research**, v. 36, p. 83-89, 2006;

DOUGLAS, K. L.; PIECIRILLO, C. A.; TABRIZIAN, M. Effects of alginate inclusion on the vector properties of chitosan-based nanoparticules. **Journal of Controlled Release**, v.115, p. 354-361, 2006;

ELBASHIR, S. M.; HARBORTH, J.; LENDECKEL, W.; YALCIN, A.; WEBWE, K.; TUSCHL, T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. **Nature,** v. 411, p. 494-498, 2005;

ELOUAHABI, A.; RUYSSCHAERT, J. M. Formation and intracellular trafficking of lipoplexesanpolyplexes. **Molecular therapy**, v. 11, p. 336-347, 2005;

EPSTEIN, A. L.; MARCONI, L. P.; ARGNANI, R.; MANSERVIGI, R. HSV-I-derived recombinant and amplicon vectors for gene transfer and gene therapy. **Current Gene Therapy**, v. 5, p. 445-458, 2005;

FEDOR, A.; HAREL, C.; FODOR, L.; ARMONI, M.; SALMON, P.; TRONO, D.; KARNIELI, E. Adult rat liver cells transdifferentiated with lentiviral IPFI vectors reverse diabetes in mice: an *ex vivo* gene therapy approach. **Diabetologia**, v. 50, p. 121-130, 2007;

GARY, W. **Pharmaceutical Biotechnology. University of Limerick**, Republic of Ireland. 2007;

GIANNOUKAKIS, N.; TRUCCO, M. Gene therapy for type I diabetes.**American Journal of Therapeutics.** v. 12, p. 512-528, 2005;

GILJOHANN, D. A.; SEFEROS, D. S.; PRIGODICH, A. E.; PATEL, P. C.; MIRKIN, C. A. Gene regulation with polyvalent sirRNA-nanoparticles conjugates. **Journal of the American Chemical Society**, v. 131, p. 2072-+, 2009;

GLASER, B. Type 2 diabetes: Hypoinsulinism, hyperinsulinism, or both? **Plos Medicine**, v. 4, p. 619-620, 2007;

HARRISON, P. T.; Application of gene therapy in diabetes care.**Infectious disorders drug targets**, v. 8, p. 129-133, 2008;

JAIN, K. K. Use of nanoparticles for drug delivery in glioblastoma multiforme. **Expert review of neurotherapeutics,** v. 7, p. 363-372, 2007;

JEAN, M.; ALAMEH, M.; BUSCHMANN, D.; MERZOUKI, A.Effective and safe gene-based delivery of GLP-I using chitosan/plasmid-DNA therapeutic nanocomplexes in an animal model of types 2 diabetes. **Gene therapy**, v. 18, p. 807-816, 2011;

JEAN, M.; ALAMEH, M.; DE JESUS, D.; THIBAULT M.; LAVERTU, M.; DARRAS, V.; NELEA, M.; BUSCHMANN, M. D.; MERZOUKI, A. Chitossan-based therapeutic nanoparticles for combination gene therapy and gene silencing of in vitro cells lines relevant to type 2 diabetes. **European Journal of Pharmacology**, v. 45, p. 138-149, 2012;

JULIANO, R.; BAUMAN, J.; KANG, H.; MING, X. Biological barriers to therapy with antisense and siRNA oligonucleotides. **Molecular pharmaceutics**, v. 6, p. 689-695, 2009;

KIM, K. W. Islet transplantation: a realistic alternative for the treatment of insulin deficient diabestes mellitus. **Diabetes research and clinical practice**, v. 66, p. S11-S17, 2004;

KOBINGER, G. P.; DENG, S. P.; LOUBOUTIN, J. P.; VATAMANIUK, M.; MATSCHINSKY, F.; MARKMANN, J. F.; RAPPER, S. E.; WILSON, J. M. Transduction of human islets with pseudotypedlentiviral vectors. **Human gene therapy**, v. 15, p. 211-219, 2004;

LAKEY, J. R. T., YOUNG, A. T. L.; PARDUE, D.; CALVIN, S.; ALBERTSON, T. E.; JACOBSON, L.; CAVANAGH, T. J. Nonviral transfection of intact pancreatic islets. **Cell transplantation**, v.10, p. 697-708, 2001;

LI, F.; MAHATO, R. I. iNOS gene silencing prevents inflammatory cytoline-induced beta-cell apoptosis. **Molecular Pharmaceutics**, v. 5.p. 407-417, 2008;

LI, F.; MAHATO, R. I. RNA interference for improvingthe outcome of islet transplantation. **Advanced drug delivery review**, v. 63, p. 47-68, 2011;

MAHATO, R. I. Gene expression and silencing for improved islet transplantation. **Journal of controlled release**, v. 140, p. 262-267, 2009;

MASSING, M. D.; GILJOHANN, D. A.; SEFEROS, D. S.; LUDLOW, L. E.; HORVATH, C. M.; MIRLIN, C. A. Regulating immune response using polyvalent nucleic acid-gold nanoparticles conjugates. **Molecular pharmaceutics**, v. 6, p. 1934-1940, 2009.

NAGPAL, K.; SINGH, S. K.; MISHRA, D. N. Chitosan nanoparticles: a promising system in novel drug delivery. **Chemical & pharmaceutical bulletin**, v. 58, p. 1423-1430, 2010;

NIU, L.; XU, Y. C.; DAI, Z.; TANG, H . Q. Gene therapy for type I diabetes *mellitus* in rats by gastrointestinal administration of chitosan nanoparticles containing human insulin gene. **World journal of gastroenterology**, v. 14, p.

4209-4215, 2008a;

NIU, L.; XU, Y. C.; DAI, Z.; TANG, H . Q. Expression of human insulin gene wrapped with chitosan nanoparticles in NIH3T3 cells and diabetic rats. **Acta Pharmacological sinica**, v. 19, p. 1342-1349, 2008b;

NOGUCHI, H.; MATSUMOTO,S. Protein transduction technology offers a novel therapeutic approach for diabetes. **Journal of hepato-biliary-pancreatic surgery**, v. 13, p. 306- 316, 2006;

PANAKANTI, R.; MAHATO, R. I. Bipartite adenoviral vector encoding hHGF and hIL-I Ra fr improved human islet transplantation. **Pharmaceutical research**, v. 26, p. 587-596, 2009a;

PRUD'HOMME, G. J.; DRAGHIA-AKLI, R.; WANG, Q. Plasmid-based gene therapy of diabetes mellitus. **Gene therapy**, v. 14, p. 553-564, 2007;

REHMAN, K. K.; WANG, Z.; BOTTINO, R.;Ç BALAMURUGAN, A. N.; TRUCCO, M.; LI, J.; XIAO, X. ROBBINS, P. D. Efficient gene delivery to human and rodent islets with double-stranded (ds) AAV-based vectors. Gene therapy, v. 12.p. 1313-1323, 2005;

REIMANN, M.; BONIFACIO, E.; SOLIMENA, M.; SCHWARZ, P. E. H.; LUDWIG, B.; HANEFELD, M.; BORNSTEIN, S. R. An update on preventive and regenerative therapies in diabetes *mellitus*. **Pharmacology & therapeutics**, v. 121, p. 317-331, 2009;

REN, B.; O'BRIEN, B. A.; SWAN, M. A.; KOINA, M. E.; NASSIF, N.; WEI, M. Q.; SIMPSON, A. M. Long-term correction of diabetes in rats after lentiviral hepatic insulin gene therapy. **Diabetologia**, v. 50, p. 1910-1920, 2007;

ROBERTSON, R. P.; Medical progress: Islet transplantation as a treatment for diabetes – a work in progress. **New England journal of medicine**, v. 350, p. 694-705, 2004;

RYAN, E. A.; PATY, B. W.; SENIOR, P. A.; BIGAM, D.; ALFADHLI, E.; KNETEMAN, N. M.; LAKEY, J. R. T.; SHAPIR, A. M. J. Five-year follow-up after clinical islet transplantation. **Diabetes**, v. 54, p. 2060-2069, 2005;

SAMSON, S. L.; CHAN, L. Gene therapy for diabetes: reinventing the islet. **Trends in endocrinology and metabolism**, v. 17, p. 92-100, 2006;

SEFEROS, D. S.; PRIGODICH, E. A.; GILJOHANN, D. A.; PATEL, P. C.; MIRKIN, C. A. Polyvalent DNA nanoparticles conjugates stabilize nucleic acids. **Nano letters**, v. 9, p. 308-311, 2009;

SHAPIRO, A. M. J.; RICORDI, C.; HERING, B. J.; AUCHINCLOSS, H.; LINDBLAD, R.; ROBERTSON, P.; SECCHI, A.; BRENDEL, M. D.; BERNEY, T.; BRENNAN, D. C.; CAGLIERO, E.; ALEJANDRO, R.; RYAN, E. A.; DIMERCURIO, B.; MOREL, P.; POLONKKY, K. S.; REEMS, J. A.; BRETZEL, R. G.; BERTUZZI, F.; FROUD, T.; KANDASWAMY, R.; SUTHERLAND, D. E. R.; EINSENBARTH, G.; SEGAL, M.; PREIKSAITIS, J.; KORBUTT, G. S.; BARTON, F. B.; VIVIANO, L.; SEYFERT-MARGOLIS, G.; BLUESTONE, J.; LAKEY, J. R. T. International trial of the Edmonton protocol for islet transplantation. **New England journal of medicine**, v. 355, p. 1318-1330, 2006;

STEWART, M. E.; ANDERTON, C. R.; THOMPSON, L. B.; MARIA, J.; GRAY, S. K.; ROGERS, J. A.; NUZZO, R. G. Nanostructured plasmatic sensors. **Chemical reviews**, v. 108, p. 494-521, 2008;

TORCHILIN, V. P. Recent approaches to intracellular delivery of drugs and dna and organelle targeting. **Annual review of biomedical engineering**, v. 8, p. 343-375, 2008;

TROS DE LLARDUYA, C.; SUN, C. Y.; DUEZGUENES, N. Gene delivery by lipoplexes and polyplexes.**European Journal of Pharmaceutical Science**, v. 40, p. 159-170, 2010;

VEGA, R. A.Ç; WANG, Y.; HARVAT, T.; WANG, S.; QI, M.; ADEWOLA, F. A.; LEE, D.; BENEDETTI, E.; OBERHOLZER, J. Modified gold nanoparticles vectors: A biocompatible intracellular delivery system for pancreatic islet cell transplantation. **Surgery**, v. 148, p. 858-865, 2010;

WALSH, G. **Pharmaceutical Biotechnology**. University of Limerick, Republic of Ireland.

WANG, X. MELOCHE, M. VERCHERE, C. B.; OU, D.; MUI, A.; WARNOCK, G. L. Improving islet engraftment by gene therapy. **Journal of transplantation**, v. 2011, 2011;

WONG, M. S. HAWTHORNE, W. J.; MANOLIOS, N. Gene therapy in diabetes. **Self Nonself**, v. 1, p. 165-175, 2010;

XU, R., LI, H.; TSE, L. Y.; KUNG, H. F.; LU, H.; LAM, K. S. Diabetes gene therapy: potential and challenges. **Current gene therapy**, v. 3, p. 65-82, 2003;

YECHOOR, V.; CHAN, L. Gene therapy progress and prospects: gene therapy for diabetes mellitus. **Gene therapy**, v. 12, p. 101-107, 2005;

ZAIA, J. A. The status of gene vectors for the treatment of diabetes. **Cell biochemistry and biophysics**, v. 48, p. 183-190, 2007;

Zhang, N.; CLEMENT, N.; CHEN, D. M.; FU, S.; ZHANG, H. J.; REBOLLO, P.; LINDEN, R. M. BROMBERG, J. S. Transduction of pancreatic islets with pseudotypedadeno-associated virus: Effect of viral capsid and genoma conversion. **Transplantation**, v. 80, p. 683-690, 2005.