

Gene-based therapy for Type 1 diabetes mellitus: viral and nonviral vectors



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Practice points

- Type 1 diabetes mellitus (T1DM) is a chronic disease caused by autoimmune destruction of the pancreatic β cells, leading to insulin deficiency.
- Gene therapy is a viable alternative to life-long insulin injection, for the treatment and/or prevention of T1DM.
- Gene delivery can be performed via viral or nonviral vectors. Each delivery system has its own pros and cons, which should be taken into consideration during their selection process.
- Gene therapy strategies for T1DM uses different approaches that can be used for treatment and prevention of T1DM, such as maintenance of euglycemia, promoting of islets survival and proliferation, insulin secretion from surrogate cells and immunomodulation.

SUMMARY Type 1 diabetes mellitus (T1DM) is a global epidemic with an alarming number of patients suffering from the disease worldwide. The main goal of the therapy directed towards diabetes mellitus DM is achieving normal blood glucose levels. Gene therapy is one of the many approaches utilized to accomplish this in a safe, specific and efficient manner. Since the first cloning of insulin gene, the gene therapy approach for the treatment of T1DM has been widely investigated. Gene therapy for T1DM encompasses a broad area, from targeting noninsulin producing cells (e.g., hepatocytes, fibroblasts and myocytes) for stimulating the production and storage of insulin, and to genetically manipulate β cells to avoid autoimmune destruction. For this purpose, different types of viral and nonviral gene delivery vectors have been studied. This review aims mainly to discuss the most commonly gene delivery systems used for the treatment or prevention of T1DM. Several recent examples of viral and nonviral gene delivery vectors are provided, while pointing out the most relevant references found in the literature.

KEYWORDS

- diabetes • gene therapy
- insulin • viral vectors
- nonviral vectors

Diabetes mellitus (DM) is one of the most widespread chronic diseases of modern time [1]. According to the International Diabetes Federation, 382 million people had diabetes worldwide in 2013 and by 2035 this number is expected to increase to approximately 592

million [2]. In the USA alone, a total estimated cost of diagnosed diabetes in 2012 was of US\$245 billion, which included US\$176 billion in direct medical costs and US\$69 billion in reduced productivity, making DM one of the largest socioeconomic burden [3–6].

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DM is a group of chronic metabolic diseases characterized by high blood glucose levels (hyperglycemia), which is either due to relative or absolute insufficiency in insulin production and/or decreased insulin sensitivity. DM is associated with many major complications such as renal failure, retinopathy, amputation, and major risk of myocardial infarction and stroke [7]. DM can be classified into three main types: Type 1 DM (T1DM), Type 2 DM (T2DM) and gestational diabetes. T1DM, also known as insulin-dependent diabetes mellitus or juvenile-onset diabetes, accounts for 5–10% of the total DM cases. T1DM is associated with progressive and specific destruction of β cells, caused by cellular-mediated autoimmune destruction, which is responsible for producing insulin leading to absolute insulin deficiency. T2DM, also known as noninsulin-dependent diabetes mellitus or adult-onset diabetes, is a complex disease characterized by insulin resistance and relative insulin deficiency. T2DM accounts for 90–95% of the cases, in which sedentary lifestyle and obesity are often identified as the major causes. Gestational diabetes is the least common type of DM, which is associated with pregnancy, without previous diagnosis of DM, which may evolve to T2DM postpregnancy [8–10].

Current treatment approaches for T1DM include lifelong subcutaneous injections of insulin. However, these therapies are often associated with life-threatening hypoglycemic adverse events. This rises the need of close monitoring of blood glucose levels during insulin therapy and, moreover, parenteral route for administration of insulin severely reduces the patient compliance [11]. Thus, it is of utmost importance to develop an alternative therapeutic approach to correct the insulin deficiency in T1DM patients in order to prevent the organ damage caused by prolonged hyperglycemia and/or occurrence of hypoglycemic events [10]. Several therapeutic approaches from micro- and nano-particles for oral insulin [12,13], glucose nanosensors [14,15] to islet transplantation [16,17] have been explored to improve diabetes therapy. Among these, islet or full pancreas transplantation are currently the only way to treat T1DM with tight regulation of blood glucose levels [18].

Gene therapy is one of the alternative therapeutic approaches used for T1DM treatment, and has been explored tremendously in the past. Gene therapy for DM embraces a broad therapeutic area ranging from prevention or delay of

onset of DM, correction of insulin deficiency, promotion of β -cell proliferation and survival, modulation of immune response and inflammation and insulin secretion from surrogate cells [19,20]. The gene delivery approach for DM has also been discussed before in many reviews [10,21–23]. Gene delivery can be attained by two main approaches: *in vivo* and *ex vivo* as shown in **Figure 1** [19]. The *in vivo* approach involves the delivery of selected genes directly to the site of action by gene delivery systems. On the other hand, *ex vivo* approach includes removal of specific target cells from the host which, after transfection with the gene, is implanted back into the patient [24].

This article discusses mainly the most commonly gene delivery systems used for the treatment or prevention of T1DM. Several recent examples of viral and nonviral gene delivery vectors are provided, while pointing out to the most relevant references found in the literature.

Gene delivery systems

Gene delivery technology for the treatment and prevention of T1DM can be achieved efficiently by delivering the gene to the cells with high specificity and safety. The gene delivery system, route of administration and the targeting system are the major factors that influence the effective transgene expression at the targeted organ or cells. Efficient gene delivery with sustained long-term effect of the expressed gene along with the immunity and targeting system are some of the major concerns for the use of gene therapy for the treatment and prevention of diabetes. In case of gene delivery for diabetes, appropriate post-translational processing is required, since the exogenous insulin expression is tightly regulated based on changes in blood glucose levels [25]. For β cells, a number of viral and nonviral vectors have been studied along with other physical and chemical methods. This section of the review highlights and discusses the different viral and nonviral vector-based gene delivery systems for the T1DM gene therapy.

• Viral vectors for therapy of diabetes

Viral vectors have evolved as one of the most efficient gene delivery systems to the cells. They are constructed in such a way that the pathogenicity of the virus is eliminated while keeping the gene transfer capability intact [25]. Viral vectors eliminate all the viral genes that are responsible for viral replication and, thereby only containing

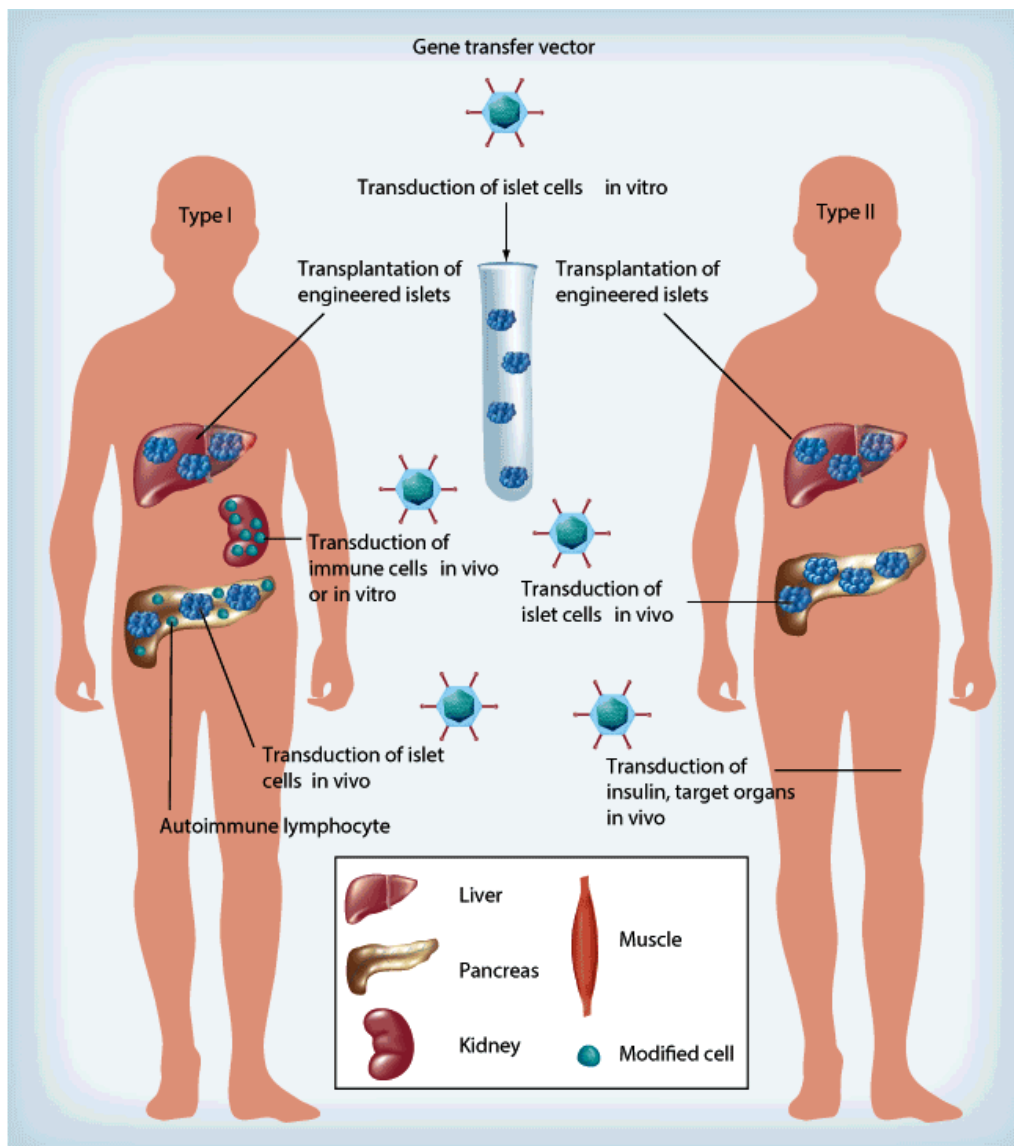


Figure 1. Gene delivery approaches for Type 1 diabetes mellitus and Type 2 diabetes mellitus.

Either the islet cells or islet cell replacements can be genetically modified *ex vivo* or viral vectors can be administered to the diabetes mellitus patient *in vivo* in order to modify his islet cells.

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the necessary viral sequence for gene delivery [10]. In comparison with the nonviral vectors, viral vectors are more efficient gene delivery systems. However, considerations regarding the cytotoxicity, inflammation and immunogenicity are crucial when developing a viral vector system [24].

The main mechanism of viral vectors uptake by the cells in the absence of specific and selective targeting moieties is endocytosis [24,26]. The first step of gene delivery includes binding of the viral vector complex with the cell membrane, followed by the internalization into the cytoplasm where it must escape the endosome and delivery

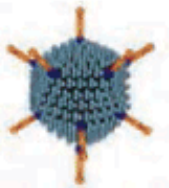

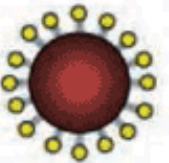
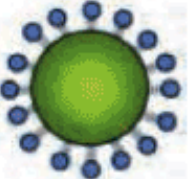
the payload intracellularly. After this, the complex can be either directly internalized into the nucleus where it unpacks releasing the plasmid, or the complex first dissociates in the cytoplasm releasing the plasmid, which then enters the nucleus, for integration and gene expression [26]. There are different viral vectors that are used as gene delivery systems and each system has its pros and cons, and depending on the therapeutic applications, viral vectors should be selected with further optimization [27]. A summary of the characteristics of the most used viral vectors is presented in **Table 1** [28].

Adenovirus

Adenovirus (Ad), a member of the Adenoviridae family, is medium-sized (90–100 nm), nonenveloped virus that contains a dsDNA [29,30]. Among the different serotypes of Ad available, serotypes 5 and 2 are the most used ones and the only ones used in humans to date [28]. Ad vectors are associated with many advantages such as high transduction efficiency, ability to transduce both dividing and nondividing cells and relatively large transgene capability, among others [10]. Owing to these advantageous properties, Ad has been one of the most common viral vector, used in 23.3% of the clinical trials in 2012 [31]. However, the Ad vector has limited duration of expression with a decrease in the level of the expressed proteins after a couple of weeks, followed by unsuccessful readministration due to intensive humoral response. Moreover, tendency

to provoke strong immune and inflammatory responses are also major drawbacks of this delivery system [10,30]. The immune response evoked by the Ad virus could be coped with the coadministration of immunosuppressors or having transgene expression that leads to immunosuppression [32]. Moreover, the pre-existing immunity against Ad due to previous exposure to wild-type virus, but with the same serotype, is also one of the major drawbacks. To overcome this, another serotype Ad virus can be used to avoid the immune response against the first serotype [33]. The recombinant Ad (rAd) vectors are also associated with safety problems, poor gene capacity and transient transgene expression. Thus, new rAd's were further developed such as the gutless vector, also designated as 'high capacity' Ad (HC-Ad) or 'helper dependent' Ad (HD-Ad). These HD-Ad vectors have no viral

Table 1. Characteristics of different viral vectors.

Vector type	Maximum expression cassette capacity (kb)	Transfers gene to nondividing cells	Antivector immunogenicity	Chromosomal integration	Expression	Other characteristics
Adenovirus 	7–8	Yes	High	No	Transient	Typically mediates expression for 1–3 weeks
Adeno-associated virus 	4.5	Yes	Low	No	Persistent in nondividing cells	Expression initiates in 1–3 weeks
Retrovirus 	8	No	None	Yes	Persistent	Risk of insertional mutagenesis and difficult to produce in high titer
Lentivirus 	8	Yes	None	Yes	Persistent	Safety concerns regarding HIV components, risk of insertional mutagenesis and difficult to produce high titer

Data taken from [28].

coding sequence and it can incorporate up to 36 kb of transgenic sequence into its genome. However, these HD-Ad vectors require helper virus, which can provide genes for virus assembly. These helper viruses require physical purification methods, which is labor intensive. These HD-Ad vectors also allow long-term transgenic expression without chronic toxicity [10,25,34].

Adeno-associated virus

Adeno-associated viruses (AAVs) belong to the genus *Dependovirus* and *Parvoviridae* family. The small virion is approximately 25 nm in diameter containing ssDNA molecules (4.7 kb). AAV has been classified as dependovirus because it requires a coinfection with other viruses, such as Ad and Herpes simplex virus, for effective infection [10,35–36]. The AAV virus expresses *rep* and *cap* genes, responsible for coding of replication and capsid protein. However, in the vectors, the *rep* and *cap* genes are removed and replaced by expression cassette [28]. The genome containing the ssDNA is converted to dsDNA once inside the cell, which is then stabilized mainly as nonintegrated episomal form [36,37]. This could explain the slow rise in gene expression after the gene transfer. The use of AAV as gene delivery vector has been widely explored owing to its available safety profile with no known disease associated with AAV infection, long-term transgene expression, many available serotypes of AAV and ability to transduce both dividing and nondividing cells [10,38–39].

One drawback associated with AAV is the limited size of the expression cassette that can be packed, thereby limiting its use. AAV is also associated with complex and small-scale production process with low titer, which raises the concerns for the need of high amounts for human dosing. Moreover, AAV is also associated with host-mediated humoral immune response against the capsid protein, although not as strong as for Ad virus, which could compromise the readministration of the vector of the same serotype. Also, contamination with helper virus and replication competent wild-type AAV can also restrict its use [10,28].

Retrovirus & lentivirus

Retroviruses (RV) are another common type viral vectors used as gene delivery systems. RV belongs to the family of *Retroviridae* and was originally derived from murine viruses. Moloney murine leukemia virus (Mo-MLV) was the first

of its kind to be widely used in gene therapy. The genome consists of ssRNA with limited insertion capacity of 8 kb. After entering the target cells, the RNA is retrotranscribed into linear dsDNA, which is then integrated into the cell chromatin [28,40–41]. The integration into the chromatin is an effective way to maintain the genetic material in the self-renewing tissues; however, it does not guarantee stable expression of the transduced genes. Moreover, good transduction is highly dependent on the mitosis of the target cells after virus entry, therefore limiting its application to only selected targets *ex vivo* [42].

RV is associated with many advantages such as long-term and stable integration, large cloning capacity and no viral gene transfer. However, insertional mutagenesis is a great risk with the use of RV, and to avoid such adverse effect, self-inactivating RVs have been developed. Moreover, RV can only transduce dividing cells, with a decrease in the expression with time [43–45].

One type of retroviral vector, lentivirus (LV), has an additional advantage of transducing nondividing cells or terminally differentiated cells, when compared with other retroviruses. The vectors are derived from HIV-1 and the genome of the lentivirus constitutes of ssRNA and other components of HIV-1 [28]. These lentiviral vectors have efficient *in vivo* delivery and integration along with stable expression of transgenes. Although lentiviral vectors are attractive and interesting vectors for other clinical studies, important ethical and biosafety questions, such as emergence of replication competent virus have risen, which might limit their application in humans in the future [46]. However, hybrid lentivirus from nonhuman lentivirus, such as feline and equine, among others, have been explored to make lentiviral gene delivery systems more acceptable to be used [47,48]. Moreover, limited capacity available for the transgene cassette also limits the use of lentivirus [25].

• Nonviral based gene delivery for therapy of diabetes

Over the past decade, many nonviral methods have been investigated for gene therapy, gaining particular interest due to their advantages of safety, nontoxicity and nonantigenicity when compared with viral vectors. These nonviral vectors can be divided in two major groups: physical and chemical methods. Physical methods facilitate the transfer of genes from the extracellular environment to cells by creating transient

openings in cellular membranes using physical forces such as mechanical (e.g., injection and gene gun), electrical, ultrasonic, hydrodynamic and laser energy.

Among the nonviral vectors, the chemical vectors are the preferred ones with most of the studies based on cationic lipids and cationic polymers. Due to their positive charge, cationic lipids and polymers spontaneously form condensed complexes with the negatively charged genetic material through electrostatic interactions. Encapsulation within and/or adsorption on top of these biomaterials are also alternative ways to pack the genetic material [49]. Compared with other nonviral delivery systems, the complexes formed with the cationic lipids and polymers tend to mediate a higher level of transfection in numerous cell lines since they can absorb more effectively to the anionic plasma membrane of mammalian cells via electrostatic interactions, which facilitates the cellular uptake and intracellular delivery [50,51]. These complexes are internalized into the cell through a vesicular pathway, which is followed by the release of the genetic material within the cell cytoplasm, as shown in Figure 2 [52]. Once inside the cells, if the genetic

material is DNA it can migrate to the nuclei and in case the case of RNAi, it will stay in the cytoplasm [53]. Furthermore, the complexes formed are also able to protect and stabilize the genetic material until it reaches the target site of action. They also have the most advantage of having no limitation in the size of the amount of genetic material that can be incorporated [54].

Cationic lipids are the most studied and used nonviral vectors in common genetic therapy, with dozens of lipids developed and tested for this purpose [52]. The lipids share a common structure of positively charged hydrophilic head connected via a linker to a hydrophobic tail [50]. The complex formed between the lipid and the genetic material is commonly called lipoplex. Although a simple method, lipoplex formation can be tricky with many factors to be considered that influence their final features. Due to their positive charge, cationic liposomes may interact unspecifically with negative cellular components, which may lead to defects in the cellular membrane integrity, resulting in the hemolysis of the cells. Moreover, the reagents normally used to prepare the lipoplexes may be injurious to cells [52,55-56]. The main issues and

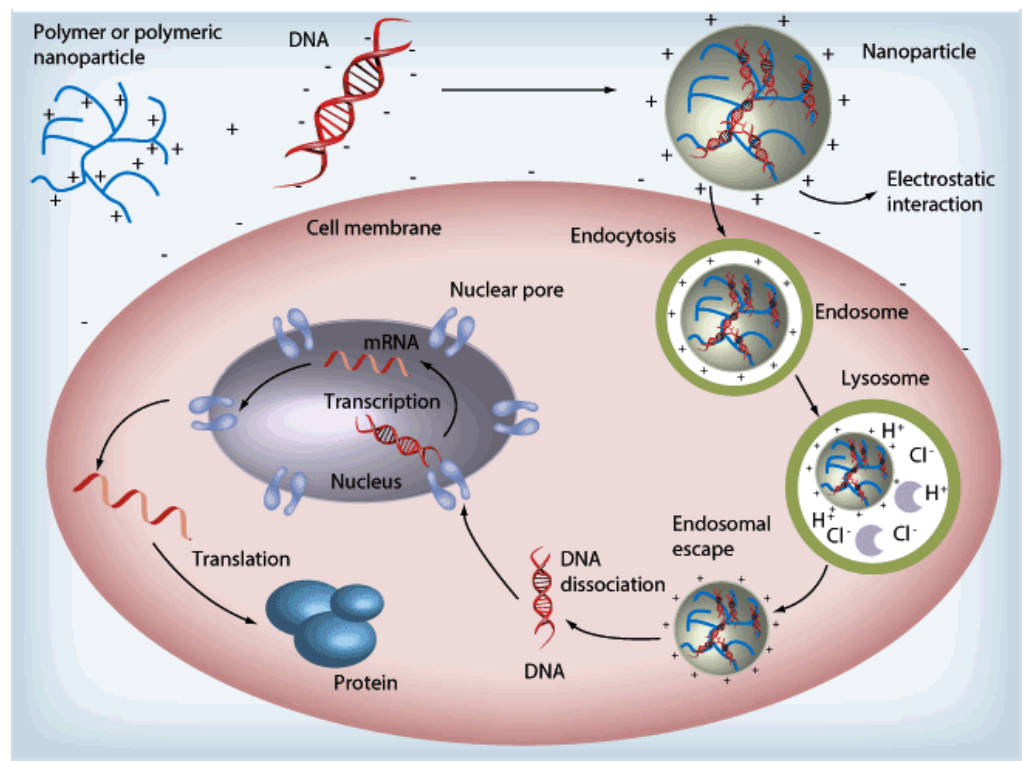


Figure 2. Gene delivery process by polymers. Reproduced with permission from [52].

problems regarding cationic lipids are described in more detail elsewhere [49,57–59]. Cationic polymers, in turn, can condense DNA molecules to a relatively small size and are more stable when compared with cationic liposomes giving origin to polyplexes [50,51]. Among cationic polymers, polyethyleneimine is considered one of the most effective polymer-based transfection agents, due to its ability to escape from the endosomal membrane [49,50]. However, it may be associated with toxicity issues with increase in its molecular weight [49].

Gene therapy strategies for T1DM

• Correction of hyperglycemia

Several strategies have been employed aiming to control the main feature of T1DM, the hyperglycemia. This is a suitable approach to control the high blood glucose levels trying to minimize their side effects, such as neuropathy, glaucoma, nephropathy and cardiomyopathy, among others. Most of the current therapies intent to compensate the deficiency or lack of insulin production by the external administration of insulin but it is not a permanent solution and patients need to resort to its administration frequently. Thus, insulin gene therapy to restore the insulin production levels to a lasting solution, is currently the most selected therapy for the treatment of T1DM.

Studies on streptozocin (STZ)-induced diabetic mice treated with furin cleavable insulin (*INS-FUR*) gene, delivered intrapancreatically by recombinant Ad (rAD) vector, showed favorable results with successful correction of hyperglycemia, ketoacidosis and glucose tolerance [60]. In recent studies, a synthetic promoter for HD-Ad vector has been developed, which regulates the insulin gene expression in the liver in response to the changes in the blood glucose levels [61,62]. After the intravenous administration of Ad virus-expressing rat *INS-FUR* with the synthetic promoter into diabetic *Lepr db/db* mice, it was possible to successfully reduce the blood glucose levels to normal levels on day 14, which was maintained until 50 days. Moreover, the enhancement in glucose tolerance and insulin sensitivity was also demonstrated [61].

Ren *et al.* have successfully shown the long-term correction of diabetes in rats [63] and long-term reversal of diabetes in nonobese diabetic mice [64]. In both studies, they used *INS-FUR*, which was cloned into the site of LV HIV/MSCV (HMD) to produce HMD/*INS-FUR* construct.

The viral vectors were delivered to the liver via the portal circulation, by intervallic fusion in the full flow occlusion (FFO) technique. In STZ-induced diabetic rats, they were able to decrease the insulin to subnormal levels, which was maintained for 500 days (experimental end point), without the elevation in liver enzyme levels as compared with the controls. Similarly, in nonobese diabetic mice, they were also able to decrease the blood glucose to normal levels in 24 h, which was maintained for 150 days (experimental end point). Also, after 5 months of the treatment with *INS-FUR-HMD*, cytoplasmic secretory granules in the liver, which released insulin in a glucose-dependent manner, were observed by immunoelectron microscopy and was compared with insulin secreting granules in Nit-1 β cells. No granules were seen when treated with empty vector and nontreated animal group. Moreover, in both cases they were also able to show regulated insulin secretion after a bolus intravenous dose of glucose along with expression of β -cell transcription factors and pancreatic hormones was also observed [63,64].

Similar studies using *INS-FUR* delivered by lentiviral transduction to liver cells of autoimmune diabetic insulin dependent DM (LEW.1AR1/*Ztm-iddm*) and STZ-induced rats via portal vein have also been reported [65]. Normalization of the blood glucose levels after 5–7 days of injection was observed and it remained stable for 1 year of the observation period. In contrast with the study by Ren *et al.*, the viral vectors produced by Elsner *et al.* lack the HIV transcription factor ‘*tat*’, which can cause undesirable adverse effects [65].

Other studies using nonviral methods to control the hyperglycemia and increase insulin levels were also employed. Sato *et al.* injected a solution with circular plasmid pmaxGFP DNA which contained the green fluorescent protein gene, directly into the pancreatic parenchyma. The administration was done by electroporation using a square-pulse generator and green fluorescent protein expression was observed 1 day after gene delivery with its expression reduced to baseline within 1 week [66]. Other examples using this combination also obtained increased levels of insulin [67,68].

An improvement in the injection method is the hydrodynamic method used by He *et al.*, who intravenously administrated an insulin expressing plasmid to mice to check its effects in glycemia control. The results were very positive with the

integration of the plasmid by the liver cells which increased the plasma insulin levels and reduced the blood glucose levels. The hyperglycemic complications were also improved [69].

Palizban *et al.* have used a 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) liposome to deliver the insulin gene. The complex was successfully transfected by injecting into K cells *in vivo*, a cell type that produces glucose-dependent insulinotropic peptide (GIP). This process regulation as well as the timing of hormone release is comparable with β cells, and thus, K cells may be a good alternative to the production of insulin in the absence of viable β cells [70].

Niu *et al.* transfected NIH3T3 cells *in vitro* with chitosan nanoparticles containing insulin plasmid and observed a significant increase in insulin levels in the medium. The *in vivo* administration by lavage and colocalization of the chitosan nanoparticles containing insulin plasmid resulted in the reduction in the fasting state blood glucose levels after 5 days when compared with the control, proving the efficiency of chitosan as a delivery agent [71]. They also tried to deliver the polyplex through the GI tract where they obtained identical results [72].

Similar to the other studies described above with cationic lipids, Rasouli *et al.* investigated the ability of K and L cells to produce insulin in diabetic mice [73]. Through oral delivery, chitosan nanoparticles containing the insulin gene were administered in diabetic mice. The glucose levels decreased and a higher insulin expression was observed in K cells compared with the L cells [73].

• Promote β -cell survival & proliferation

The correction of hyperglycemia with insulin therapy has been the most commonly employed method. However, with exception of hyperglycemia, other diabetes-related complications are neither prevented nor treated with insulin therapy. One possibility for the treatment of T1DM is the transplantations of the islets; however, this is severely limited by complications associated with the transplantation and scarcity of donors [74]. Another alternative approach would be promoting the β -cell (islets) regeneration and proliferation, which can be effectively used to restore the β cells, and may lead to endogenous production of insulin in response to fluctuations in blood glucose levels. Pdx-1, Ngn-3 and NeuroD are some crucial transcription factors identified that influence the development of the islets and the pancreas [75].

Taniguchi *et al.* used adenovirus-mediated delivery of Pdx-1 and Ngn-3 to the pancreas of the diabetic mouse through the retrograde intracommun bile duct injection. With Pdx-1, they were able to show the formation of neoislets and proliferation of the pancreatic ductal cells, however significant antidiabetic effect was not observed. However, in the case of Ngn-3, only a weak induction in the production of β cells was observed [76]. Similarly, Wang *et al.* also studied the codelivery of two pancreatic transcription factors genes, Ngn-3 and Pdx-1, to the liver using rAd (AdVhFIX) in STZ-induced diabetic mice. This study demonstrated that both the immunogenic response of host elicited by the Ad capsid along with the expression of the two pancreatic transcription factors are essential for inducing insulin production by liver cells and improving the glucose tolerance [77]. Li *et al.* have successfully shown neoislet formation in the liver of diabetic mice by treatment with Ngn-3 gene therapy using HD-Ad vectors. They were also able to demonstrate that the intravenous coadministration of Ngn-3 and Betacellulin (an islet growth factor), with the help of HD-Ad vector induce the formation of new islets in the liver of the diabetic test mice along with the reversal of hyperglycemia [78].

Protein kinase B, also known as serine/threonine kinase Akt, is a direct target of PI3K, the signaling pathway involving these is considered to be highly relevant to the therapy of T1DM. Several studies suggest that Akt1 has a pivotal role in glucose metabolism and maintenance of β cells (proliferation and survival), thereby making it an attractive target for protection of the β cells for T1DM therapy [79–81]. In a study by Bone *et al.*, a fiber-modified infectivity-enhanced Ad vector, Ad5RGDpK7, was used to deliver rat insulin promoter-driven (CA-Akt1) into β cells and to evaluate its effect both *in vitro* and *in vivo*. In case of *in vitro* studies, efficient promotion of human islet cell survival and β -cell proliferation was observed. The intravenously administered systemic vector led to endogenous Akt expression and activation in islets showing beneficial bystander effect in STZ-induced diabetic mice *in vivo*. The vector was also administered into the pancreas via bile-ductal injection; however, they resulted in adverse effects locally [82]. Another example of regeneration of pancreatic islets is the study that involved the administration of a gene cocktail comprising of cyclin D2, CDK4 and GLP-1, to nonhuman primate models using

ultrasound targeted microbubble destruction. The administration of the gene cocktail led to induction of islet regeneration and restoration of the β -cell mass [83]. Thus, all these studies suggest the potential of using gene therapy-based pancreatic β -cell survival and proliferation for T1DM therapy.

• **Modulating the immune response & protection from inflammation**

T1DM results from the inflammation of the pancreatic islets with the presence of leukocyte infiltrates, also known as insulinitis. The islet infiltrates are mainly composed by CD8⁺ T cells, along with B cells, macrophages and NK cells [84,85]. The CD8⁺ T cells target different islet antigens such as insulin, preproinsulin, glutamic acid decarboxylase and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) [84,86–87]. Studies have shown that immunosuppressive cytokines such as IFN- γ , TGF- β 1, and IL-10 and IL-4 can be used to suppress the progression of autoimmune destruction of pancreatic β cells [88–92].

Some studies report the injection of genetic material in the muscle in order to treat or prevent diabetes through the administration of IL-10 [91,93]. Nitta *et al.* injected IL-10-encoded plasmid into NOD mice. For more than 2 weeks after the injection, IL-10 was still detectable in the serum of mice with the incidence of diabetes markedly reduced as compared with control and nontreated NOD mice [91]. Zhang *et al.* also injected an IL-10 expression plasmid intramuscularly to evaluate its effect in autoimmune diabetes. They showed that in mice injected with plasmid, the delay-type hypersensitivity responses were suppressed, with the number of CD4⁺ or CD8⁺ lymphocytes and the glucose levels markedly decreased in comparison with the control group [93].

Another study performed by Zhang *et al.* used rAAV vector for of IL-10 gene delivery (rAAV-IL-10). A single intramuscular injection of rAAV-IL-10 was given 4 weeks before renal capsule delivery of 650 syngeneic islets in NOD mice. With high doses of rAAV-IL-10, 60% of the test mice maintained normal blood glucose level for 117 days. However, with low doses, normoglycemia was only maintained for 17 days, indicating dose dependency in the protection of grafted islets. With enhanced survival of grafted islets, a decrease in the intensity of the autoimmunity was also observed [92].

Similar to other studies already presented in this review, IL-10 production was also evaluated when a plasmid encoding for each cytokines were intravenously administrated using a cationic polymeric carrier, poly(α -[4-aminobutyl]-L-glycolic acid), in NOD mice. As seen previously, there was evident reduction in the prevalence of severe insulinitis compared with the control and nontreated groups [94]. Ko *et al.* reported a similar study with the intravenous administration of the same complexes to NOD mice. In this study, it was reported that after 6 weeks after the injection 75% of the observed islets were intact compared with less than 3% in the control group, and only 5% of the islets were severely infiltrated by the lymphocytes compared with over 30% in the control group. After a long follow-up of the glucose levels (20 weeks), it was demonstrated that the combination of the polyplexes prevented the development of diabetes in 75% of the treated animals [95]. A recent study made by Mandke *et al.* corroborates these findings using a low molecular weight chitosan instead of poly(α -[4-aminobutyl]-L-glycolic acid) [90].

Hou *et al.*, in turn, tried a novel gene therapy strategy, which, besides the immunotherapy, also combined the regeneration of β cell. For this, a plasmid encoding proinsulin and pancreatic regenerating (Reg) III protein (pReg/PI) was made. After intramuscular injection, there was an increase in the insulin amounts in the serum of STZ-induced diabetic mice which reduced the incidence of diabetes. It also promoted the balance between different lymphocytes with an increase in the regulatory cells, which may attribute to the establishment of self-immune tolerance [96]. Despite the positive results shown, due to the small amount of material that can be administrated by this method, it is usually only used for vaccination [50]. An ideal alternative to the injection technique for delivery of naked DNA could be the gene gun method. This method is based on the delivery of heavy metal particles coated with DNA that are accelerated to high speed by pressurized inert gas to penetrate into target tissue cells [49,50]. A study using this gene gun technique showed that the vaccination of auto-antigen-encoding pDNA administration preferentially induced IL-4 secreting immunoregulatory T cells (CD4⁺ T cells), which significantly delayed the onset of diabetes [87].

Recently, the first successful clinical trial with engineered plasmid expressing proinsulin

(BHT-3021) as gene-based T1DM therapy was performed by Roep *et al.* [97]. The trial comprised 80 subjects diagnosed with T1DM, in which different doses of BHT-3021 were administered intramuscularly every week for 12 weeks, along with BHT placebo. The study showed that with the engineered DNA plasmid encoding proinsulin, the insulin-specific CD8⁺ T cells were reduced in T1DM patients, leading to preservation of β -cell function. Moreover, improvement in the C-peptide levels was observed with no serious adverse effects related to BHT-3021 [97]. The first successful clinical trial showed the potential of the gene delivery systems for the T1DM therapy by specifically targeting the autoimmune response towards pancreatic islets.

• Insulin secretion from nonpancreatic cells

The gene therapy approach can also be utilized in T1DM therapy by differentiating nonpancreatic cells to insulin secreting cells or by surrogating other cells such as epidermal, intestinal, hepatocytes and myocytes to deliver insulin [98–101]. Fodor *et al.* studied the *ex vivo* transdifferentiation of primary hepatocytes to form insulin secreting cells using lentivirus vector mediated *Pdx-1* delivery [102]. After *in vivo* transplantation, sufficient insulin secretion to regulate glucose homeostasis over at least 8 weeks was observed. However, despite of significant improvements in the hyperglycemic state of the mice, moderate hyperglycemia was still observed in the fed state, which could be due to the limited numbers of cells that were transfected [102].

Falqui *et al.* [101] constructed a retroviral vector L-x-SN encoding a furin-cleavable human proinsulin, which was released by retroviral engineered human fibroblasts. The biological activity of the insulin produced demonstrated biological activity *in vitro* that was comparable to human insulin. Furthermore, blood glucose levels were efficiently lowered by transplantation of insulin-producing primary human fibroblasts in STZ-induced diabetic mice. However, cell overgrowth was observed leading to hypoglycemia, indicating the absence of regulated insulin production [10,101]. An *ex vivo* therapy approach using lentiviral vector for transduction of epidermal keratinocytes has also been studied [103]. After 2–4 h of the intraperitoneal injection of rapamycin (exogenous ligand), insulin secretion was detected with a significant decrease in the blood glucose levels in diabetic

athymic mice, which were transplanted with 3FM-hppI4-HD keratinocytes [103].

Tian *et al.* developed lentiviral vectors that produces bioactive insulin from keratinocytes, by the fusion between the furin-cleavable proinsulin and the self-dimerization mutant of FK506-binding protein. Thus, the produced bioactive insulin was secreted only after exogenous administration of rapamycin. The authors were able to show decrease in serum glucose levels to normal levels in diabetic mice with severe hyperglycemia, with similar results from repeated administration of rapamycin [103].

Conclusion & future perspective

T1DM is a global epidemic with an alarming number of patients suffering from the disease worldwide. The main goal of any therapy directed towards T1DM is achieving normal blood glucose levels. Gene therapy is one of the many approaches utilized to accomplish this in a safe, specific and efficient manner. Gene delivery for the treatment or therapy of T1DM has been pursued for many years now. Since the first cloning of insulin gene, the gene therapy approach for the treatment of T1DM has been widely investigated. Gene therapy for the treatment of T1DM encompasses a broad area, from targeting noninsulin producing cells (e.g., hepatocytes, fibroblasts and myocytes) for stimulating the production and storage of insulin, and to genetically manipulate β cells to avoid autoimmune destruction. With this technique, is not only possible to ‘control’ the T1DM disease, but also to cure this disability either by restituting the insulin gene that is missing or by diminishing the self-immune response against the pancreatic β cells.

Several systems of viral and nonviral vectors were discussed and presented in this review, each one with their own advantages and drawbacks, making the choice for any of them to be a compromise relation between the efficacy and the toxicity of the systems. However, despite all the efforts made so far, the treatment/therapy of T1DM is still far from the reality.

In the future, for efficient therapy of T1DM, there should be an adequate control of the production, storage and release of insulin. For this, a careful choice regarding the type of the vector to be used is needed. Despite of the proven efficiency of viral vectors, they are always associated with adverse effects such as host immunogenicity (Ad) and insertional mutagenesis (RV).

Therefore, optimizations of the viral vectors are still needed to make them safer for T1DM applications. In the case of nonviral vectors, the selection of the components along with the construction of such carriers should be done critically in order to improve the transfection efficiency and to increase the usability of these systems in the future. Moreover, the determination of the specific mechanism of cell endocytosis will also be crucial to improve the escape of the genetic material from the endosomes of the cells, particularly for polyplexes. Also, a targeted therapy to specifically deliver the genetic material will also improve this kind of therapy. Possibly, a more effective treatment could also be obtained by a combination of gene therapy with stem cell therapy.

In conclusion, there are many exciting, interesting and promising results around the gene therapy for the treatment of T1DM that should encourage the continuous research in this field in order to make this therapy a possibility one in a near future.

Financial & competing interests disclosure

HA Santos acknowledges financial support from the Academy of Finland (decision no. 252215), the University of Helsinki Funds, the Biocentrum Helsinki, and the Finnish Center for International Mobility (grant no TM-13-9048). Financial support from the European Regional Development Fund (ERDF) through the Programa Operacional Factores de Competitividade– COMPETE, by Portuguese funds through Fundação para a Ciência e a Tecnologia (FCT) in the framework of the project PEst-C/SAU/LA0002/2013, and co-financed by the North Portugal Regional Operational Programme (ON.2– O Novo Norte) in the framework of project SAESCTN-PIIC&DT/2011, under the National Strategic Reference Framework (NSRF), is also acknowledged. F. Araújo thanks the FCT for financial support (SFRH/BD/87016/2012). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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