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CRISPR-Cas9: A Promising Therapeutic Approach for Diabetes Mellitus

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Abstract: Diabetes mellitus (DM) is a metabolic condition that raises blood glucose levels (hyperglycemia) without insulin or insulin receptor defects. Insulin is a peptide hormone the pancreatic beta cells produce and controls blood glucose levels. The defective immune system engulfs the beta cells of the pancreas, causing no insulin production or the insulin receptors to become faulty, resulting in insulin resistance. There are two main types of diabetes mellitus: type 1 diabetes mellitus and type 2 diabetes mellitus. In type 1 diabetes mellitus, the beta cells are engulfed by the defective immune system; hence the insulin production stops, and in type 2 diabetes mellitus, there is a defect in the insulin receptor that causes insulin resistance. Both types of diabetes cause uncontrolled blood sugar levels, which can lead to severe damage to vital body organs such as the heart, kidney, limbs, eyes, and nerves. The diseases of these organs are cardiovascular diseases, nephropathy, retinopathy, neuropathy, high blood pressure, and obesity. There are several methods to treat diabetes mellitus, such as islet cell transplant, tablets and medication, insulin pumps, weight loss surgery, insulin, diet and exercise, and emotional support. However, the medicines involved in traditionally treating diabetes mellitus are finerenone, tirzepatide, and, GLP-1 receptor, SGLT-2 inhibitors. These medicines cause severe damage to the body, such as cardiovascular death, transient ischemic attack, end-stage kidney diseases, and limb removal (amputation). Therefore, a new modern gene editing technology such as clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) is involved in treating diabetes. In this technology, gene editing occurs in the human pluripotent stem cells (hiPSCs) taken from the patient's body; these cells are converted into improved pancreatic beta cells. There are different methods involved, including cell-based therapies (such as stem cells and brown adipocytes), targeting specific genes associated with diabetes. This paper presents a critical review of the gene-editing tool CRISPR/Cas9 technology for the treatment of diabetes mellitus.

Keywords: Diabetic mellitus; CRISPR-Cas9; Pancreatic beta cells; Insulin; Gene editing technology; Human pluripotent stem cells

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1. Introduction

Diabetes mellitus is the oldest and most frequent long-term chronic condition in the world [1]. It is characterized as a raised level of glucose (hyperglycemia) which is the disorder of carbohydrate metabolism because of the absence of insulin (converts glucose into glycogen) or a low level of insulin; insulin is a peptide hormone produced from the pancreatic beta cells that need to manage frequently [1]. Globally, diabetes mellitus is the most dominant public health solicitude; it is associated with mortality and increasing prevalence and becomes a socioeconomic burden on the country as well as on the diabetic patient [2]. Diabetes mellitus majorly causes renal failure, blindness, depression, and cardiac-related deaths [3]. According to the World Health Organization, 285 million people experience diabetes throughout the world [4]. Diabetic mellitus causes an annual death rate that can be predicated as 3.96 million people of all ages throughout the world [4]. The more frequent symptoms of diabetes are polyuria, polydipsia, polyphagia, and weight loss [5]. The approved classification system of the World Health Organization the diabetes can be divided into four categories: T1DM (type 1 diabetes mellitus), T2DM (type 2 diabetes mellitus), gestational diabetes, and another type of diabetes [6]. More than 90% of diabetes is caused by type 1 and type 2 [6]. Different methods are involved in the treatment of diabetes such as islet cell transplant, tablets and medication, insulin pumps, weight loss surgery, insulin, diet and exercise, and emotional support [7]. Mainly medicines are used for the treatment of diabetes such as metformin in type 2 diabetes [8]. However, the medicines show adverse effects on the body and may damage the main organs of the body such as the heart, kidneys, limbs, and nerves [9]. The main purpose of this paper is to understand the new gene editing technology the CRISPR-Cas9 involved in the treatment of diabetes mellitus [10].

1.1. Different main classifications of diabetes mellitus

In type 1 diabetes, the defective immune system (T-cells) involves damaging the pancreatic beta cells [11]. The goal of insulin therapy is for hemoglobin A1c (HbA1c) levels to remain above 7.0% in patients with type 1 diabetes [12]. The complications of type 1 diabetes are poor metabolic control, hypoglycemia, weight gain, an imbalance of lipids (hyperlipidemia), reduced uptake is reduced in skeletal muscle as well as insulin action is also reduced in type 1 diabetes [13]. The clinical representation of insulin resistance is metabolic syndrome [14]. Metabolic syndrome is termed as “double diabetes” with the association with type 1 diabetes. The main reasons for insulin resistance in type 1 diabetes are obesity, lack of exercise, and puberty. There is a requirement for high insulin doses to control insulin resistance [15]. The requirement of high insulin dose causes weight gain and hypoglycemia which might be a failure to control glucose [16].

In type 2 diabetes, there is a defect in the insulin receptor which causes insulin resistance [17]. Gestational diabetes is a type of diabetes in which glucose level is not maintained and it appears during pregnancy and is removed after the baby is born [18]. In prediabetes, the blood sugar level is higher than normal but less than the type 2 diabetes [19]. Type 1 diabetes mellitus is more dangerous than type 2 diabetes mellitus because they have a high chance of heart disease and acute metabolic dysfunction. The high blood glucose level increases the threat of heart disease, stroke, nerve damage, kidney damage, eye damage, foot problems, and other complications. The socioeconomic burden is associated with diabetes mellitus, and the increasing order and importance of scientific and technological improvement in searching out new treatment methods for diabetes [20].

1.2. High prevalence of diabetes mellitus in Pakistan

Worldwide approximately 463 million adults are affected by diabetes mellitus mostly type 2 diabetes [21]. In the world after India and China, Pakistan represents a percentage at 3rd in the prevalence of diabetes [22]. The frequency of diabetes in Pakistan in 2016, 2018, 2022, and 2023 was 11.7%, 16.98%, 17.1%, and 26.7% [21]. According to the World Health Organization, the diabetes prevalence in 2023 in Pakistan is 30.8%. Globally the

most recent IDS Atlas analyzed that Pakistan is the third largest country with 33 million people living with type 2 diabetes [23]; and 8.9 million people with another type of diabetes and the remaining population of Pakistan is unrecognized. According to the World Health Organization, the mortality rate is at a peak because of diabetes in 2019 [23]. The people of Pakistan face a high risk of diabetes-related death. The basic variable that causes diabetes in Pakistan are obesity sedentary, way of life genetics, and the consumption of higher sugar control [23].

1.3. Risk factors for diabetes mellitus

Being overweight and obese increases the rate of hospitalization and causes death because of cardiovascular diseases (CVD) and the risk factors for type 2 diabetes are obesity, cigarette smoking, dyslipidemia, and hypertension. Abdominal obesity (excess fat around the abdomen) is linked with the collection of metabolic and cardiovascular risk factors (such as hypertriglyceridemia, abnormal cholesterol level, high blood pressure, low-density lipoprotein (LDL), high-density lipoprotein (HDL) known as the metabolic syndrome [24]. Any above-mentioned disease category of metabolic syndrome increases the chances of cardiovascular syndrome or diabetes mellitus. Upon high heat treatment of foods, browning, and taste compounds are generated by the so-called Maillard Reaction. This reaction produces tasty and flavor-active molecules and health-beneficial compounds. In contrast, it also produces the acceleration of cardiovascular complications in animals and humans with diabetes and produces the factors of diabetes of type 1 and 2 [25].

1.4. Risk of complications in diabetes mellitus

Two basic kinds of complications in patients with diabetes mellitus are microvascular and macrovascular [24]. Diabetic neuropathy is a frequently occurring microvascular complication in diabetic patients, the widespread form is distal symmetrical neuropathy or polyneuropathy which causes disability and morbidity. The physical symptoms of diabetic neuropathy include loss of ambulation, increased risk of foot ulceration and amputation, and severe pain [26]. The adjustable risk factor for diabetic neuropathy is hyperglycemia, the patient's life quality can be improved by glycemia control which reduces the incidence of neuropathy. The nerve conduction (nerve conduction velocity and electromyography) involves diagnosing the neuropathy. Multiple factors are distinguished into two groups: consumption of angiotensin-converting enzyme inhibitors (ACEI), serum lipid level, smoking, method of diabetes control and its quality, with high blood pressure (BP), and sex. Atherosclerosis risk factors involved in diabetic neuropathy, close association of mononeuropathy with hyperlipidemia, cigarette smoking, and high blood pressure (BP). Neuropathy leads to ulceration and amputation [27].

In diabetic patients, diabetic nephropathy is a common microvascular complication. Almost 25% of people with diabetes mellitus type 2 are affected by diabetic neuropathy, which is the reason for leading end-stage renal disease (ESRD) in high-income countries. People who are affected by diabetic nephropathy have a high risk of cardiovascular risk with coronary heart disease [28]. The first-line drugs for diabetic nephropathy are mainly the angiotensin receptor blockers, the renin-angiotensin system (RAS) inhibitors, and the more recently approved aldosterone receptor antagonist finerenone, which slow down the advancement of diabetic nephropathy to end-stage renal disease (ESRD). Traditionally Chinese medicine (TCM) is widely used for the treatment of diabetes nephropathy. Based on TCM theory, the clinical treatment of diabetes nephropathy mainly targets nourishing qi and yin, detoxifying and detumescent, and generating fluid and nourishing blood [29].

People who develop diabetes at a young age show a prolonged revelation of hyperglycemia which leads to a high rate of occurrence and severe complications such as diabetic retinopathy (a complicated condition that affects the eyes leading to damage to the blood vessels in the retina), and even mortality. Therefore, it is

necessary to understand diabetic retinopathy and its danger level for all types of diabetic patients [30]. In the retinopathy or macula edema level assigned, the eye will be sent to a third grader for an edited grade. The grading of the worst eye was based on the level of retinopathy and macula edema [31].

2. Different methods applied for the treatment of diabetes

There are a number of methods for the treatment of diabetes such as islet cell transplant, tablets and medication, insulin pumps, weight loss surgery, insulin, diet and exercise, and emotional support. For the treatment of type 1 diabetes, insulin pumps are used and for the treatment of type 2 diabetes, insulin tablets are used as a good method to control glucose levels as well as a balanced diet and being more active [32]. The other method is islet cell transplant which involves in isolation of healthy beta cells from the demised donor and placing it in the liver of a type 1 diabetes patient. Weight loss surgery involved in type 2 diabetes is the removal of extra obesity or weight in the area of the stomach and intestine such as bariatric surgery. Diet and regular exercise some patients especially those with type 2 diabetes manage their sugar levels without the expenditure of medicines they take a balanced diet and regular exercise for the treatment of diabetes [33]. Diabetes not only changes physical health but also changes the patients emotionally. The factors are stress, sadness, and fatigue. The most common tablet for the treatment of type 2 diabetes is metformin. Insulin can be taken in different forms for the treatment of diabetes such as needles and syringes, insulin pens, and insulin pumps [34].

2.1. Medicines used for the cure of diabetes mellitus

The patient with type 2 diabetes faces a raised risk of cardiovascular and kidney diseases, which causes damaged quality of life and reduces life expectation. Highly increased recognition of these risks and failure to control glucose levels provides regulatory agencies, with risk reduction and researchers have increasingly shifted away from glucose which is a fundamental change. By introducing an effective and new treatment option for the therapeutic goals of diabetes mellitus. Two classes of drugs: (1) glucagon-like peptide-1 (GLP-1) receptor agonists and (2) sodium-glucose cotransporter-2 (SGLT-2) inhibitors, provide benefits for patients of cardiovascular and kidney diseases. Recently an innovative product that serves previously unmet medical needs has been provided to treat patients with type 2 diabetes: a non-steroidal mineralocorticoid receptor antagonist, and dual glucose-dependent insulinotropic polypeptide (GIP) / GLP-1 receptor agonist, finerenone, tirzepatide. The type 2 diabetes finerenone drugs provide benefits for cardiovascular and kidney diseases. For diabetes treatment, recent large industry-funded trials provide older drugs including basal insulin, and sulfonylureas by differentiating their long-lasting cardiovascular effects with liraglutide, a GLP-1 receptor agonist [9].

The most commonly used medicine in type 2 diabetes is metformin, an oral anti-hyperglycemia agent. It reduces basal hepatic glucose production and increases insulin sensitivity in both hepatic and periphilin livers. It also increases the uptake of glucose in skeletal muscles and adipocytes. So, metformin is involved in regulating insulin action and glucose levels. Insulin sensitivity is increased by metformin which leads to a decrease in the requirement of insulin dosage as well as getting fat. The complications of type 2 diabetes and metabolic syndrome can be reduced by incorporating metformin into insulin therapy. Metformin also shows the same results in prediabetes [35]. But in type 1 diabetes, the role of metformin is under debate. The main role of metformin involves decreasing glucose levels and the requirement of insulin decreases the prevalence of metabolic syndrome as compared to insulin without metformin. The above process is not dependent upon blood lipid or weight loss. The weighted average can be increased by using only insulin whereas decreased by the addition of metformin-adjunctive therapy [36].

2.2. Adverse effects of medicines on diabetes mellitus

We judge and measure the effects of medicines as crucial: all causes of death, cardiovascular diseases, transient ischemic attack, end-stage kidney diseases, limb removal (amputation), ST-segment elevation myocardial infarction, gastrointestinal disorders, genital infection, accumulation of ketone bodies, body weight change, severe hypoglycemia [37]. In end-stage kidney disease, we examined kidney dialysis and kidney transplantation. Clinicians for people with type 2 diabetes face challenges in whether to add finerenone and tirzepatide, GLP-1 receptor, or SGLT-2 inhibitors, to their current therapeutic routine treatment. A large network of previous amplification concentrates on GLP-1 receptor agonists and SGLT-2 inhibitors. All provided medications for patients of type 2 diabetes show clinically relevant benefits and harms including finerenone and tirzepatide. A recent new update of the BMJ Rapid Recommendations for the medication of diabetes is involved in designing a network to inform professionals and the health care system [9].

3. Introduction of new technology the CRISPR-Cas9 in diabetes mellitus

Gene therapy is the effective management of diabetes which is clinically demonstrated. A new direction is the CRISPR-Cas9 used for the treatment enforcement of diseases [38]. In recent times, therapeutics of gene-editing with the CRISPR-Cas9 clustered regularly interspaced palindromic repeats have become increasingly common [20]. In prokaryotes (bacteria, archaea) there is a system that works against the virus called the adaptive immune system the "CRISPR-Cas9." The CRISPR-Cas9 system is known as gene editing technology and is used as a tool in gene editing technology in multiple organisms. In eukaryotes, specific genes can be targeted by producing artificially the single guide RNA (sgRNA), which consists of opposite genes of the specific target gene. This process is done by the gene editing technology of the CRISPR-Cas9 system. The tracrRNA DNA nucleases the Cas9 enzyme involved in the double-strand break in the specific gene [39]. In the result of the double-strand break, non-homologous end joining (NHE) the targeted gene is knockout. Moreover, knock-in can be accomplished via homologous direct recombination (HDR) in the presence of a DNA template. The application of CRISPR-Cas9 is used in research as well as the cure for diabetes mellitus and also the tools designed to intercept the challenges of CRISPR-Cas9 technology [10].

3.1. Discovery of CRISPR-Cas9

The palindromic repeats are present in the genome of bacteria and were discovered by scientists in the 1980s [40]. In 1987, Yoshizumi Ishino first described the CRISPR, which describes interspaced short repetitive sequences downstream in the *Escherichia coli* IAP gene. The DNA of bacteria and archaea bacteria contains the CRISPR locus (conserved DNA) which is separated by a sequence of DNA called the interspaced DNA. These unique sequences are present in 40% of bacterial DNA and 90% of archaeal DNA [36].

3.2. Introduction of CRISPR-Cas9 technology

CRISPR-Cas9 Technology which is derived from bacteria. it works in bacteria as an adaptive immune system [41]. This adaptive immune system protects the bacteria from bacteriophages (viruses). In the mechanism of CRISPR-Cas9 technology, the viral genome/foreign DNA is present in plasmids or phages. The bacterial immune system evolved from the acquired system [42].

The new virus attacks the bacteria for the first time (**Figure 1**), the Cas9 enzyme (endonucleases) breaks the viral genome after the detection of viruses protospacer adjacent motif (PAM) sequences, and then inserts the viral genome pieces into spacer DNA of CRISPR locus. As a result of this process, the bacteria attain the ability to memorize the infected virus in the future [43].

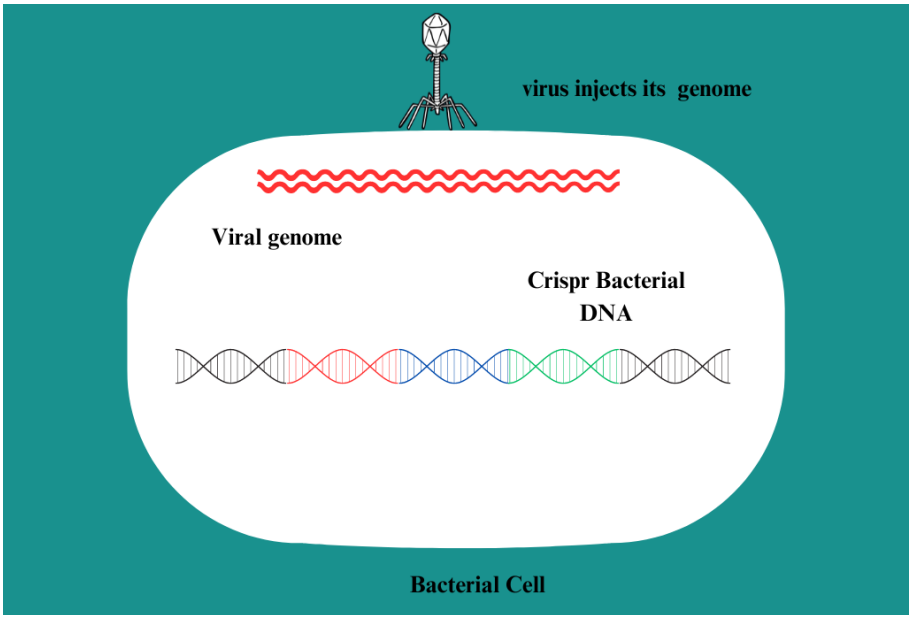

Figure 1. Virus injects its DNA into the bacteria for the first time

The Crispr Cas9 system works in three processes in bacteria (**Figure 2**):

(1) Spacer acquisition: A list of Cas enzymes is involved in cutting the viral genome for example nucleases, helicases, integrase, and endoribonucleases.

(2) crRNA processing: In this process, RNA is formed by the lower strand of DNA that is complementary to the DNA (which contains the CRISPR gene as well as the viral gene).

(3) Interference: in this process, a complex is formed between Cas enzymes and crRNA [43].

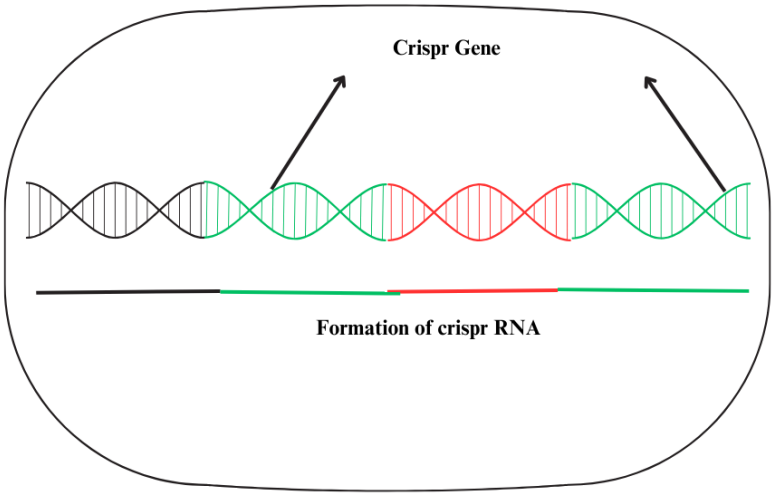

Figure 2. Formation of crRNA from the lower strand of DNA which contains both CRISPR gene and viral gene

4. Types of RNA processing

There are three types of crRNA processing involved in the adaptive immune system of bacteria type I, type II, and type II [44]. In the mechanism of CRISPR-Cas9 technology, the genetic material of the virus is broken down and inserted into the spacer DNA of the CRISPR locus, now the CRISPR locus contains the viral genome into spacer DNA [45]. Then this CRISPR locus is transcribed into CRISPR RNA. Here we use the type II components

which contain: Cas9 enzymes (nucleases), Tracr RNA, and the RNA that is previously transcribed called the CRISPR RNA [46]. All the components of type II are used in *in vitro* technology in the genome-editing field for efficient genetic modification in mammalian cells [43]. **Table 1** shows the roles of different enzymes in the different types of CRISPR RNA processing

Table 1. The roles of different enzymes in the different types of CRISPR RNA processing

Serial no.	RNA processing	Enzymes	Shapes of RNA
1	Type I	Cas6e, Cas6f enzymes	Loops
2	Type II	Cas9 enzyme	Tracr RNA
3	Type III	Cas6 Homolog	Linear RNA

4.1. RNA processing type II of CRISPR involved in genome editing

In the CRISPR-Cas9 RNA processing type II, the most important player is Cas9 which recognizes the sequence of PAM (protospacer adjacent motif) present in the viral genome [47]. The CRISPR RNA recognizes the sequence of viral DNA as it is formed from the viral DNA strand, TracrRNA supports the CRISPR RNA, and the combination of TracrRNA and the Crispr RNA is called the single guide RNA (sgRNA); after recognition, the CRISPR RNA attached to the lower strand of viral DNA, here the DNA-RNA heteroduplex is formed at the sites of the target which gives the signal to the Cas9 enzyme; the Cas 9 enzymes break the viral genome at the site of PAM sequence, this breakage of viral DNA is called the Double Break Genome (breakage of DNA at the same sides) [10]. This type of RNA processing CRISPR-Cas9 technology is used in new CRISPR-based gene editing technologies to knock out or knock in the gene in the genome of living organisms especially humans, for example in the treatment of diabetes mellitus, cancer, leukemia, β-thalassemia, detection of pathogens, and cell engineering. The components of RNA processing type II in CRISPR used in gene editing are shown in **Figure 3**.

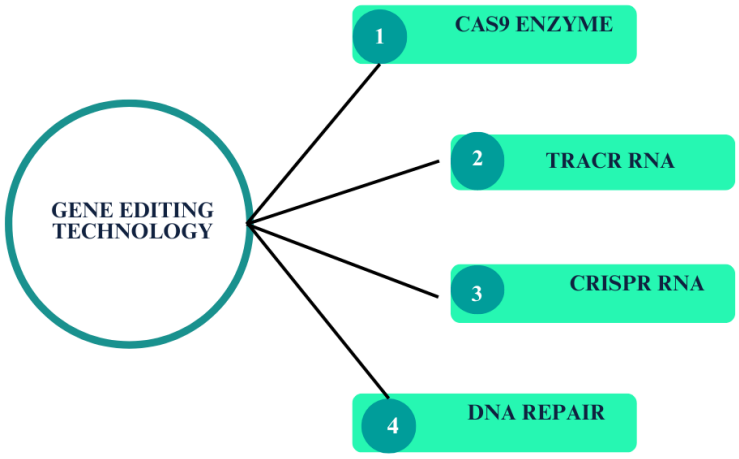

Figure 3. The components of RNA processing type II in CRISPR used in gene editing

4.2. The CRISPR-Cas9 is the dominant application in genome editing technology

CRISPR-Cas9 technology is the dominant application in genome editing technology as it retains essential advantages because of its important player the RNA-guided system [48]. The CRISPR-Cas9 technology is used in important biological functions such as (1) promising therapeutic tool for treating genetic diseases; (2) helping in the development of transgenic animal disease models; (3) specific gene repairing; (4) producing permanent gene

knock out; (5) identifying alternations in import biological process; (6) targeting multiple genes simultaneously; (7) CRISPR is a more accessible editing system. The above-mentioned biological functions convert new desired sites the Cas9 enzyme can be appropriate by changing the sgRNA. CRISPR-Cas9 system direct inference with the target loci, as compared to other technologies that are used in editing, CRISPR-Cas9 can edit multiple genes simultaneously. This is the extra advantage of the CRISPR-Cas9 system. The investigation of the CRISPR-Cas9 technology plays a major role in the clinical treatment of human complications such as diabetes mellitus [10].

5. Involvement of CRISPR-Cas9 for the cure of diabetes

Accurately researchers can edit the mammalian genetic material which is allowed by CRISPR-Cas9 technology [20]. The cutting–edge tools are developed by CRISPR-Cas9 technology which involves gene knock-in and knock-out of genes in the targeted gene expression [49]. CRISPR-Cas9 technology produces the β cells of the pancreas by the human stem cells which cannot be engulfed by the immune system after the insertion of these artificially prepared cells (*in vitro*) in the diabetic patient's body [50]. In many research fields, CRISPR/Cas9 screens have been widely used. For example, researchers used CRISPR in diabetes-related research [51]. Multiple organ and cell communication are involved /proceed in diabetes. Such as in type 1 diabetes the variety within pancreatic islets, and different cells of the immune system. The complex regulatory mechanism for managing blood glucose is difficult for the CRISPR screen. So, the single cell type can be used to study the complications of diabetes which sort out the systematic level of diabetes. For example, study of pancreatic beta cells, these beta cells are derived from iPS cells with GFP receptor under an insulin promoter and also consist of Vitamin D receptor (VDR) as a modulator of inflammation and beta cell survival. It used intracellular insulin immunofluorescence as a quantitative readout and discovered that the cohesion loading complex and the NuA4/Tip60 histone acetyltransferase complex regulate insulin transcription and release. Researchers identify the genes in the laboratory for type 1 diabetes that regulate the pancreatic beta-cell that can be protected from autoimmune processes by the consumption of a genome-wide CRISPR knockout screen. On transplanted pancreatic beta cells, the attack of the immune system (autoimmune system) destroys the beta cells this result gives a strong selection of genes so scientists are capable of discovering many new genes, according to the type of diabetes. Ribonucleotide diphosphate reductase-like domain (rNDP) and genome-wide association study (GWAS) are involved in the protection of pancreatic beta cells from the immune system and reducing intrinsic pressure in beta cells. CRISPR also plays a role in the metabolic research of diabetes [50].

5.1. Genome editing in cell-based therapies with CRISPR-Cas9 to improve the diabetes mellitus treatment

For the treatment of diabetes, human pluripotent stem cells are an excellent source for the production of pancreatic beta cells to produce insulin. The CRISPR-Cas9 technology is an ideal source for genome editing technology which serves to produce the expected cells that are used as a methodology of regenerative medicine and for the cure of multiple diseases such as diabetes [52]. The pre-implantation embryos contain the embryonic stem cells (hiPSCs) inside the inner cell mass. Human pluripotent embryonic stem cells (having limitless capability) are capable of generating all types of cells hence they provide an excellent source for the production of any type of cell of human, and also provide the methodology of regenerative medicine to cure diabetes [53]. Destruction of pancreatic beta cells (the function of beta cells to produce the insulin hormone) occurs in diabetes [53]. So removing these beta cells by artificially producing beta cells produced by the CRISPR-Cas9 technology and human pluripotent embryonic stem cells to treat diabetes [10]. For human beta-cell development, the hiPSCs cells are converted into endocrine cells. For the formation of homologous recombination, researchers use the

clustered regularly interspaced short palindromic repeats (CRISPR). CRISPR-Cas9 associated protein (Cas9 enzyme) which breaks the double-stranded DNA for the specific site generation. In this system, CRISPR-Cas9 RNA is a guiding mechanism for the Cas9 enzyme (endonuclease). The researcher utilizes genome editing as an instrument that works on stem cells with the help of CRISPR-Cas technology [54].

5.2. Human pluripotent embryonic stem cells are converted into improved pancreatic beta cells by genome editing technology

Generating isogenic/recombinant controls in hiPCSc can be formed by genome editing (CRISPR) which is implemented on human pluripotent embryonic stem cells in the presence of a donor DNA template [55]. Various schemes are involved in carrying out the *in vitro* diabetes mellitus diseases that are associated with metabolic syndrome [56]. The hiPSCs stem cells generate the altered isogenic controls which can be differentiated either in relevant target cells or in the pancreatic beta cells. If the diabetic patient's genome contains unfeasible material researchers make some alterations to human embryonic stem cells different gene variants are used. Then these cells are converted into the desired cells. In type 1 diabetes, we obtained the hiPSCs by subjecting the gene variant in *PTPN22* (T1DM) which additionally converted into lymphocytes and the lymphocytes' function. In type 2 diabetes, hiPSCs cells are obtained by the variant *TCF7L2* then the hiPCSc is converted into pancreatic β-cells. More than 500 loci in the genome of humans have been identified for the risk of diabetes diseases [10]. Formation of pancreatic endoderm cell from pluripotent stem cell is shown in **Figure 4**.

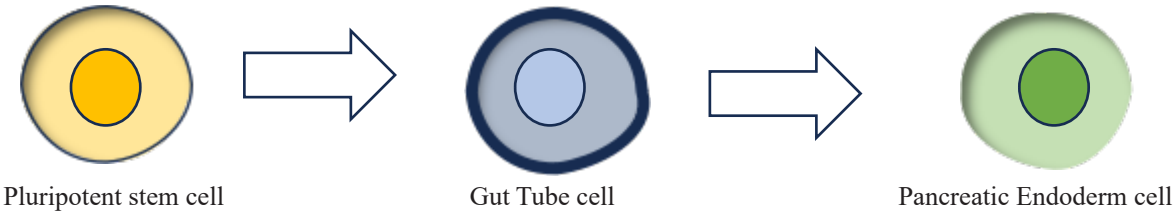

Figure 4. Formation of pancreatic endoderm cell from pluripotent stem cell

5.3. Involvement of CRISPR-Cas9 in clinical diabetes

ViaCyte is a private regenerative medicine company developing cell replacement therapies. CRISPR therapeutics is a pioneering gene editing company involved in the formation of gene-based medicines for serious diseases, its main player is CRISPR-Cas9. Cell therapy by utilizing CRISPR editing and the ViaCyte involved in the cure of diabetes. Gene-edited stem cell-derived medicines which are clinically important [56]. A clinical study to evaluate ViaCyte and CRISPR Therapeutics performed a major clinical role in producing stem cell therapy named VCTX210 which is used to produce the insulin hormone to control the blood glucose level in type 1 diabetes [55]. There is the combination of gene modification and CRISPR-Cas9 technology to produce the VCTX210 in which an allogenic pancreatic endoderm cell (PEC210A) is present and forms a perforated device which is inserted into the patient's body for the maintenance of glucose [20]. **Figure 5** shows the formation of the perforated device which contains VCTX210 cells in which ViaCyte and CRISPR therapeutics are involved. This device will maintain the glucose level after insertion in the patient's body and the blood capillaries will be developed on this.

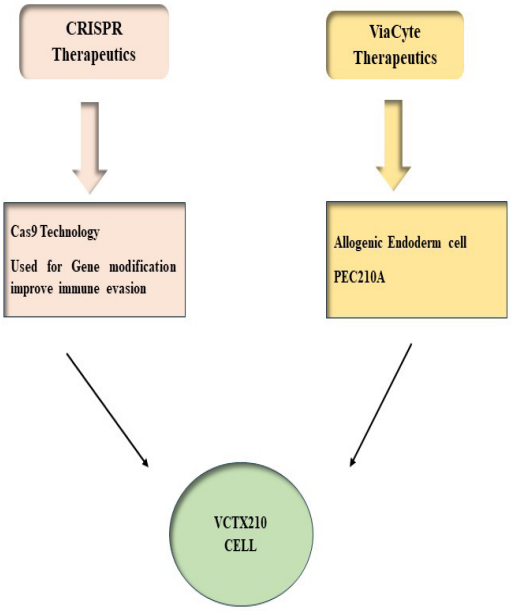

Figure 5. Formation of the perforated device which contains VCTX210 cells in which ViaCyte and CRISPR Therapeutics are involved

6. Conclusion and future prospective

Diabetes mellitus is a commonly identified metabolic condition in which blood glucose level is elevated because of the insulin absence or defect in insulin receptors, and according to the World Health Organization, diabetes mellitus is recognized globally as the ninth leading factor of death. Several methods are applied to treat diabetes, such as islet cell transplant, tablets and medication, insulin pumps, weight loss surgery, insulin, diet and exercise, and emotional support. The traditional method for the treatment of diabetes mellitus is the use of medicines to control the blood glucose level, but these medicines such as finerenone, tirzepatide, GLP-1 receptor agonists, and SGLT-2 inhibitors. These medicines may cause severe damage to the patient's body, such as weight gain, fluid retention, gastrointestinal, lactic acidosis, and bone loss. These complications can arise due to medication's side effects for maintaining blood sugar levels. The pinpoint precision of the genome can be modified at specific sequences of target DNA using CRISPR-Cas9 technology. For treating diabetes, the CRISPR-Cas9 technology holds effective therapies and significant performance. Therefore, a new technology, CRISPR-Cas9 technology, is involved in the production of pancreatic beta cells by pluripotent stem cells in this ViaCyte therapeutic is also involved. Both produce the VCTX210 cells, which cannot be engulfed/destroyed by the immune system. These cells are designed into a perforated device and inserted into the patient's body management of blood glucose levels in type 1 diabetic mellitus.

Disclosure statement

The authors declare no conflict of interest.

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Re-evaluation: Muhammad Zubair, Baqa ur Rahman, Muhammad Farooq
Finalizing: Muhammad Zubair, Muhammad Farooq

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