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Title: **A comprehensive review on the evolution of diabetic treatments: from insulin therapy to synthetic biology**

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Abstract

Diabetes mellitus is one of most prevalent diseases in the world and a leading cause of many cardiovascular diseases. Today, there are wide range of options available to treat diabetes. Over the last few decades, the clinical field has gone through a major shift in the direction of genetic engineering and synthetic biology. This has led to the introduction of many new techniques and treatments that can help manage diabetes. The purpose of this paper is to summarize the advancement of diabetic treatments from old conventional methods to the new age treatments which hold the potential to cure diabetes. While reviewing published research and review articles, it was found that over the last few decades, there has been a surge in more handy devices to monitor and manage blood glucose levels. With the advancements in the field of science, techniques such as genetic engineering, nanotechnology and synthetic biology are put to use to design new devices or artificially engineer cells to relieve the symptoms associated with the disease. While the old conventional ways of taking medications or managing glycemic levels using insulin syringes are more common, new age treatments like using insulin patches, synthetic cells and synthetic receptors are likely to gain more popularity in the coming years.

Keywords: Diabetes, insulin, treatments, synthetic cells, synthetic biology

Diabetes, also known as Diabetes mellitus is a chronic metabolic or endocrinological disorder that is known to affect millions of people worldwide. According to a study done by International Diabetes Federation in 2021, more than 537 million adults were affected by diabetes with 3 in 4 from low- and middle-income countries.[1,2] India, per se, accounts for an alarming 17% of the world's diabetic population.[3] Figure 1 highlights the countries with the highest no. of cases of diabetes in the world.

The condition is characterized with hyperglycemia i.e., high blood-glucose levels. It is known that α-cells and β-cells of pancreatic islets closely monitor and control glucose levels in the blood, so when blood glucose levels are low, α- cells release glucagon, and when blood glucose levels are high, β- cells release insulin into the bloodstream to maintain glycemic levels.[4] Type 1 diabetes is an autoimmune disorder that occurs when the immune system mistakenly attacks and destroys the insulin-producing β-cells in the pancreas. This form of diabetes is often diagnosed in

childhood or adolescence, but it can occur at any age. Diabetes can also result from reduced sensitivity of cells to insulin in combination with pancreatic β-cell dysfunction and is called as Type 2 diabetes. Both types of diabetes take a toll on an individual's health and lead to a range of complications such as heart disease, stroke, kidney failure, and blindness.[5,6]

Current diabetic treatments range from medications to specialized glucose monitoring machines. However, with the advent of engineering in the field of healthcare and medicine, many new prospective treatments have come into the limelight. A new area of research has risen to popularity, which is referred to as: "synthetic biology". Synthetic biology is a fast-growing multidisciplinary area which seeks to design new biological systems or redesign current biological systems for practical applications such as biofortification,[7] drug development,[8,9] increasing drug efficacy[10] and other advancements in therapeutics. Synthetic cells or designer cells are a product of synthetic biology. These are artificial cells with simpler genomes that are designed in laboratories to mimic the function of biological cells. Currently, synthetic biology is still in its early stages in the field of diagnostic treatments. However, it is a promising area of research with potential applications in medicine; such as that in treatment of diabetes.[11,12]

Traditional treatments of diabetes

The most common treatments for diabetes focus on insulin replacement therapy with the aim to keep blood glucose levels within the normal range of 70–140 mg per dL.[13] Insulin replacement therapy involves subcutaneous administration of insulin via injections, to regulate blood glucose levels. However, insulin replacement therapy is not always effective, and it can also cause side effects. For instance, excessive glucose in the blood caused by low insulin dose (also referred to as insulin undertreatment) may lead to blindness, renal and heart damage, nerve degeneration, and an increased risk of infection.[14] On the other hand, excessive insulin may result in hypoglycemia, which may cause convulsions, unconsciousness, or even death.[15] In many cases, doctors instruct diabetic patients to take medicines along with insulin therapy, to better modulate blood glucose levels. These medications can work by either of the 3 ways: by decreasing blood glucose levels, producing more insulin or by reducing insulin resistance, and are given to patients orally or subcutaneously .[16]

Despite being widely used, conventional insulin replacement therapy is painful and time-taking.[17] Moreover, maintaining glucose levels within a desirable range has remained exceedingly difficult with insulin due to the delay between glucose measurement and insulin administration, as well as the delayed absorption of insulin after its injection.[18] This is because, after subcutaneous injection, insulin creates hexamers that delay its absorption. In order to form absorbable insulin dimers and monomers, hexameric insulin gradually dissociates, as illustrated in figure 2.[18,19] Regular insulin should be administered around 30 minutes before meal to prevent the rise in blood glucose levels after the meals. But, for many patients, keeping to a 30-minute pre-meal routine is inconvenient and challenging.

New age treatments

Many new technologies have come into picture to enhance both dynamic regulation of blood glucose levels and patient compliance with insulin replacement therapy. These techniques are either non-invasive or minimally invasive, hence more appealing to the general public.

Insulin analogs

As discussed earlier, administration of insulin via insulin therapy has certain drawbacks. As a result, many patients turn to synthetic insulin-like substances called “Insulin analogs”. Insulin analogs are insulin-like molecules that mimic body’s normal physiological insulin release. They are created via amino acid substitutions and structural modifications of insulin in order to increase the ADME (absorption, distribution, metabolism, and excretion) of the molecule. Insulin analogs have several advantages over insulin such as lower risk of hypoglycemia, dosage flexibility, and enhanced glycemic control; all making them a more convenient option for the management of glycemic levels.[20,21] The slight structural changes in insulin analogs gives them unique desirable properties which disrupts hexamer formation when injected under the skin.[22] The slight change in pharmacokinetic characteristics of insulin molecule, affects how quickly the drug/analog is absorbed from subcutaneous tissue.

Insulin analogs are mainly of three types, subject to the time they take to act.[21,22] Rapid-acting insulin analogs are designed to act quickly and have a rapid onset of action, typically within 15 minutes.[22,23] They can be absorbed quickly, which means that they can be taken just before meals. Examples include insulin lispro and insulin glulisine. Long-acting insulin analogs such as

insulin glargine and insulin detemir have prolonged duration of action, typically lasting up to 24 hours.[22,23] Insulin degludec (Tresiba) is another long-acting insulin analog specially known for its ultra-long duration of action, lasting around 48 hours.[23,24]These analogs are designed to cover insulin needs between meals and overnight, offering a more stable blood sugar control. Combination or premixed insulin analogs are a combination of rapid-acting and long-acting insulins and are designed to provide both immediate and long-term blood sugar control.[22]

The dosage of insulin analogs varies from person to person[25], but the frequency of dosage along with the time taken for each type of insulin analog to act has been shown in table 1.

Insulin infusion pumps and Continuous glucose monitoring (CGM)

Mechanical insulin infusion pumps have been created to constantly pump insulin through plastic tubing. These pumps are small and convenient to carry around. They are able to administer substantial insulin dosages when needed for meals in addition to a basal level of insulin throughout the day.[26] Insulin pumps allow for more accurate insulin administration, which helps to maintain more consistent blood sugar levels and lower HbA1c levels.

Continuous glucose monitors (CGMs) are one of the advanced treatment options for diabetic patients. CGMs are externally carried portable devices that continuously measure blood sugar levels with the help of certain sensors. Subcutaneously implanted sensors assess the interstitial fluid's glucose content to continuously estimate blood glucose levels. The real time readings allow the users to monitor glucose levels throughout the day and take necessary steps to control the abnormal glucose levels by making changes to diet and lifestyle. In the most recent developments, closed-loop microcomputer-controlled insulin delivery systems are being developed wherein CGMs and insulin pumps work together to automatically calculate and administer the right amounts of insulin (Figure 3).[18,27]

Nowadays, certain hybrid closed-loop systems are available with integrated automated insulin delivery (AID).[28] AID systems typically consist of an insulin delivery device such as an insulin pump, continuous glucose monitor (CGM) and the control algorithm to interpret CGM data and ensure efficient real time glucose monitoring.[28,29] AID integrated glucose monitoring is thus an advanced automated approach to glucose control while minimizing the risk of hyperglycemia or hypoglycemia. Additionally, from 2013 onwards, diabetes healthcare professionals and patients

have collaborated on several do-it-yourself Open AID systems. These systems, not yet sanctioned by the scientific community, have become accessible to patients worldwide through open-source platforms. Android APS and Loop are some common examples.[29] Additionally, devices like connected glucose meters and insulin pens automatically record insulin injections, displaying dose history, with integrated apps facilitating centralized and accessible insulin data management. These automated options offer an effective and more personalized management of the condition.[30]

Glucose biosensors

Nanotechnology has provided a promising option for early detection and management of diabetes to help improve the quality of life for the patients. [31,32]Recent nanotechnology advancements have resulted in the development of highly specialized glucose biosensors. These nanotechnology-based glucose biosensors offer increased sensitivity since they contain nanoscale materials like quantum dots, graphene and magnetic nanoparticles.[31,33] These biosensors essentially include a detector and a transducer. The transducer functions by transforming the blood glucose level recorded by the detector into a proportionate electric current that can be measured. Continuous glucose monitoring with this technique can provide accurate information and increase patient compliance. Despite their ability to regulate blood glucose levels and increased patient compliance, these recent advances have some disadvantages too. Implanted sensors and tubing raise the patient's risk of infection, while insulin pumps and CGMs are expensive devices. Glucose biosensors are often having drawbacks due to instable enzyme activity or inhomogeneity for which further calibration is essential.[32,34] Therefore, there is a need for technologies to mimic the natural synthesis of insulin, to treat diabetes.

Induced Pluripotent Stem Cells (iPSCs) for insulin production

Induced pluripotent stem cells (iPSCs) are a type of stem cells that can be generated by genetically modifying mature, differentiated cells back into a pluripotent state. This breakthrough in the field of regenerative medicine, has paved a way to produce patient-specific pluripotent stem cells without employing embryonic cells.[35] With the help of mouse models and human derived cell lines, some researchers have demonstrated the ability of embryonic stem cells (ESCs) to generate insulin-producing induced pluripotent stem cells (iPSCs), as shown in figure 4.[36.37] However, unlike biological beta cells, these cells were not sensitive to insulin. To address this issue,

researchers turned to developmental biology to understand the intricate mechanisms of development of pancreatic cells and the transcription factors and morphogens involved. This led to the development of differentiation protocols for ESCs and iPSCs, which use chemicals and growth factors to mimic natural regulation of transcription factors and morphogens such as retinoic acid, activin A, and betacellulin.[38-41] But these protocols are limited by a lack of precise control over intracellular transcription factor levels. This is a significant barrier in revealing the true potential of pluripotent stem cells (PSCs) for generating functional beta cells for the treatment of type 1 diabetes patients.

Hence, there is a need for technologies for more reliable and reproducible generation of functional beta cells for the treatment of type 1 diabetes. This is where the concept of synthetic biology steps in.

Synthetic biology as a prospective treatment

The world of genetic engineering took a drastic turn when Craig Venter and his research team at the J. Craig Venter Institute created the first synthetic cell in 2010.[42] They sequenced the genome of *Mycoplasma mycoides* and created a fresh copy of the genome in lab. This genome was then injected into a separate bacterial cell: *Mycoplasma capricolum*, that had been depleted of its own DNA. The injected synthetic genome successfully took over the host cell, reprogramming it to create a new bacterium that resembled the original species, *Mycoplasma mycoides*, but contained the synthetic genome. Over time, the host cell transformed into a new organism with the traits prescribed by the synthetic DNA.[42]

Synthetic biology combines engineering principles with molecular biology to create novel biological systems with desired functions. Whilst the process of harnessing the benefits of synthetic biology is still in its early phases, it has made significant progress in clinical field. This accomplishment proved the capacity to generate a cell with a totally synthetic genome, paving the possibility for developing new drug delivery systems,[43] cancer therapies, diagnostic tools and markers.[44,45]

CRISPR-Cas9 based treatment

With the efforts of researchers such as Jennifer Doudna and Emmanuelle Charpentier, a revolution has been witnessed in the field of gene editing: CRISPR-Cas9 technology, which is based on the principles of synthetic biology.[46] It has enabled gene editing with unprecedented precision and specificity.[46,47] CRISPR-Cas9 technology, which involves Cas9 protein variants coupled with guide RNAs, has revolutionized genome editing in mammalian cells. It has been used to edit genes associated with diseases such as β-Thalassemia,[47,48] sickle cell anemia[48] and cystic fibrosis.[48,50]

Researchers have used the antibiotic trimethoprim to modulate Cas9 protein degradation and conditionally activate specific genes, resulting in the differentiation of human pluripotent stem cells (hPSCs) into pancreatic progenitor cells (PPCs).[51]

CRISPR/Cas9-based genome editing has also been used in human PSCs to study the process of human pancreatic development and to create beta-like cells effectively for disease modeling purposes. A doxycycline-inducible CRISPR platform (iCRISPR) was created specifically to generate mono- or bi-allelic mutants of several pancreatic genes as well as to tag endogenous genes with fluorescent proteins for in vitro differentiation tracking.[52] The researchers used a dCas9-VPR (a fusion of a catalytically inactive Cas9 and a transcriptional activator) and an inducible system based on the Tet-On 3G technology, which allowed for fine-tuned gene expression regulation by doxycycline (DOX). This system can simultaneously edit multiple genes, with high efficiency and specificity, and can also achieve spatial and temporal control over gene expression. The researchers validated the iCRISPR system by inducing and regulating the expression of endogenous genes such as NANOG, SOX2, and POU5F1 in human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), which are key factors for pluripotency maintenance in hPSCs.[52]

Furthermore, they demonstrated that the iCRISPR system can be used to model human diseases by editing disease-associated genes in hPSCs.[50,53] They induced the expression of oncogenes and confirmed their oncogenic activity, as well as modulated the expression of genes associated with type 1 diabetes, resulting in changes in the glucose-stimulated insulin secretion of cells.

Zhu and others found that the iCRISPR platform can be used to efficiently generate pancreatic progenitor cells and insulin-secreting cells that are depleted in type 1 diabetes.[54] They

showed that the loss of genes such as PDX1 and NKX6.1, that are involved in pancreatic development leads to impaired pancreatic differentiation and hence decreased insulin secretion. They also demonstrated that overexpression of PTF1A gene results in enhanced pancreatic differentiation and increased insulin secretion. They used the iCRISPR system to study the mechanisms underlying the development of type 1 diabetes and edited TCF7L2 gene, which is associated with an increased risk of type 1 diabetes. The results showed that its knockout leads to impaired pancreatic differentiation and decreased insulin secretion, as well as altered glucose metabolism and mitochondrial function.[54]

Overall, these studies provide new insights into the mechanisms of pancreatic development and diabetes, and demonstrates the potential of iCRISPR for modeling human diseases such as diabetes.

Smart insulin patches

Another approach involves a new type of delivering insulin directly to the bloodstream. This could be achieved by designing a system that releases insulin in response to specific stimuli, such as glucose or a drug. With the advancement of technology, people now have access to something which involves a similar approach. Microneedle array patches better known as 'smart insulin patches' are glucose-responsive insulin delivery systems that detect a rise in glycemic levels in blood and secrete doses of insulin into the bloodstream.[55] These microneedle array patches are designed to be minimally invasive and easily applied by patients themselves, eliminating the need for healthcare professionals to administer insulin injections. A glucose sensing polymer is used to create over a hundred microneedles that make up the patch. These needles penetrate the skin and as soon as blood sugar levels exceeds normal range, they release insulin.[55,56] Additionally, because the patches are disposable, there is a lower chance of infection and greater patient convenience.

The technology was successfully tested in diabetic mice, and the blood glucose levels were shown to be adequately regulated by the system, with mice treated with glucose-responsive patches having much lower blood glucose levels than mice treated with conventional insulin injections.[56] This approach has the potential to provide a more precise and targeted approach to insulin delivery, reducing the risk of side effects and improving treatment outcomes.

Synthetic cells

In order to serve as a potential therapy platform for many diseases, synthetic cells (SCs) have been proposed to imitate significant biological functions. Synthetic cells are artificial cells that are created in the laboratory using synthetic biology techniques. Synthetic cells are designed to mimic the capabilities of body cells, including growth,[57,58] ATP synthesis,[59,60] response to stimulus,[61,62] gene expression,[63,64] metabolism.[65] These features have a wide range of applications, in the field of diagnostics and healthcare. Likewise, artificial/synthetic cells can be designed to perform metabolic processes such as insulin production in response to glucose levels.

An approach to use synthetic cells for diabetes treatment involves creating insulin-secreting cells that can be implanted into the body.[66] These cells would be designed to respond to changes in blood glucose levels by producing insulin, thereby effectively mimicking the function of pancreatic cells. The use of synthetic cells also allows for precise control of insulin production, which could help to prevent complications associated with over- or under-dosing of insulin. This approach has been demonstrated in animal models, with synthetic cells successfully regulating blood glucose levels in mice.[66]

In a study done by researchers at the Swiss Federal Institute of Technology in Zurich, the development of β-cell mimicking designer cells that can provide closed-loop glycemic control, was emphasized.[67] This could potentially offer a new approach to treating diabetes.[68] The researchers created synthetic cells from an extra pancreatic human cell line that could respond to changes in glucose levels and mediate insulin secretion. The cells could produce insulin in response to high blood sugar levels, just like β cells do. However, unlike beta cells, the designer cells can be controlled using an external signal, allowing for precise regulation of insulin production.

A synthetic circuit inside human embryonic kidney 293 (HEK-293) cells was developed, which connected glycolysis-mediated calcium entry and signaling, to an excitation-transcription mechanism, that regulates therapeutic transgene expression.[69] The glucose-inducible transcriptional system detects extracellular glucose concentrations and coordinates dose-dependent insulin production. An HEK-293-based assay was developed to assess the stimulus intensity of membrane depolarization. The researchers found that the synthetic promoter PNFAT2, which contains nuclear factor of activated T cells (NFAT) repeats from the murine IL-4 promoter, was the most responsive to chemically induced membrane depolarization. They also found that

co-transfection of a voltage-gated calcium channel amplified the excitation-transcription coupling and increased sensitivity.[68]

The authors then investigated the response of cells to high extracellular glucose levels. They found that glucose uptake led to increased ATP production, closure of ATP-sensitive potassium channels (K-ATP), and CaV1.3-mediated Ca^{2+} influx. This in turn activated the calcineurin signaling cascade, leading to PNFAT-mediated induction of insulin secretion.[69] They were able to induce insulin secretion using synthetic PNFAT promoters in response to extracellular glucose levels. The cells were tested *in vivo* in diabetic mice affected and showed promising results, suggesting that they could be used to regulate blood sugar levels in humans. A simple representation of the synthetic cell model by Xie and colleagues can be seen in figure 5.

In order to mimic the natural cascade of a biological cell, Gu and colleagues in 2018, made artificial beta-cells (ACs) with a multicompartmental 'vesicles-in-vesicle' structure and a glucose-responsive apparatus.[70] The ACs can successfully discriminate between hyperglycemic and normal conditions. Low pH conditions are caused under high glucose levels which induce the fusion of outer and inner vesicle membranes, thereby leading to insulin secretion. Synthetic cells have the potential to revolutionize the treatment of diabetes by providing a more targeted and efficient approach to regulate blood glucose levels. The researchers tested the ACs *in vitro* and found that they were able to produce and release insulin in response to glucose levels, similar to natural beta cells. They also tested the synthetic beta cells in diabetic mice and found that they were able to regulate blood sugar levels and improve glucose tolerance. Gu and others noted that the synthetic beta cells have several advantages over traditional insulin therapy, including the ability to respond dynamically to changes in glucose levels and the potential for long-term glucose regulation without the need for repeated insulin injections.[70] The use of synthetic materials to generate artificial pancreatic beta cells that replicate glucose-responsive insulin release in a robust manner shows promise for improving clinical outcomes in persons with diabetes.

Synthetic gene circuit

Ye and his colleagues developed a synthetic gene circuit that corrects insulin resistance and restores insulin sensitivity.[71] Insulin resistance is a condition in which the cells of body become

resistant to insulin, which is a hormone that regulates blood sugar levels. This can lead to high blood sugar levels and an increased risk of diabetes and other metabolic disorders.[72]

In this gene circuit, an insulin receptor is expressed in this circuit along with a transcriptional module that includes hybrid transcription factor TetR-ELK1 and a construct that directs transgene expression from a TetR-specific promoter. Binding of insulin to insulin receptor initiates the phosphorylation of tyrosine residues on the receptor. This, in turn, phosphorylates proteins like IRS-1 (Insulin receptor substrate-1) which stimulate downstream signaling pathways through activation of Ras and MAPK proteins. Downstream signaling then phosphorylates TetR-ELK1, causing it to move into the nucleus. When TetR-ELK1 binds to a promoter (P_{hCMV-1}), the ELK1 domain of TetR-ELK1 is phosphorylated leading to $P_{hCMV*-1}$-driven expression of the desired transgene. Doxycycline is an antibiotic that inhibits the interaction between the TetR domain of TetR-ELK1 and the operator of P_{hCMV}, hence disrupting the transgene expression. This circuit was designed to produce adiponectin, which is an adipose tissue-derived hormone insulin-sensitizer. As soon as the insulin levels rise above normal, the circuit induces dose-dependent adiponectin expression which then restores insulin sensitivity and reduces insulin resistance in peripheral tissues such as liver, adipose and muscle. This ultimately leads to efficient glucose uptake by body's cells and maintaining blood sugar levels at a healthy range (Figure 6).

The researchers found that the gene circuit was able to correct insulin resistance in cultured human cells and in a mouse model of diabetes.[71] The system was also tested in mice models with diet-induced insulin resistance, and the results showed that it effectively normalized their blood glucose levels along with strong expression of the transgene. The circuit was also found to be self-adjusting and has the potential to reduce the risk of hypoglycemia, meaning that it responded to the level of insulin resistance in mice and adjusted its activity accordingly. The authors suggest that this approach could be used to develop new therapies for type 2 diabetes, which is characterized by insulin resistance.

Synthetic glucose receptors

Synthetic receptors are artificial receptors engineered to change cellular responses by modifying or controlling cellular signaling. Since, carbohydrates are hydromimetic in nature, the biggest challenge faced by researchers while designing synthetic glucose receptors is the inability of the

receptor to distinguish a carbohydrate molecule from water molecules.[73] In 2019, a team of researchers at the University of Bristol created a synthetic receptor with potential uses in glucose sensing and monitoring that can specifically bind to glucose in water.[74]

The enzyme glucose oxidase, which catalyzes the oxidation of glucose, served as a model for creation of synthetic receptor. The receptor was made of a protein scaffold that had two metal ions: Ca^{2+} and Mg^{2+} added to it so that they could associate with glucose molecules alone. Using a variety of methods, including NMR spectroscopy, isothermal titration calorimetry, and X-ray crystallography, the affinity of receptors for glucose was examined. The findings demonstrated that synthetic receptor had a binding affinity that was equivalent to glucose oxidase and could specifically bind to glucose in water. The researchers also showed how the receptor may be used for monitoring and measuring glucose, which is diagrammatically presented in figure 7. They put the receptor into a fluorescence sensor with great sensitivity and selectivity for glucose in solution. They also proved the receptor's capacity to specifically bind to glucose molecules from a complicated mixture, such as human serum, by immobilizing it onto a surface.[74]

A biomimetic receptor for glucose may be useful for controlling diabetes and monitoring blood sugar levels. This study is novel compared to other studies discussed and hence more research is required to improve the structure and functionality of the synthetic receptor for its therapeutic use. However, this study shows that synthetic receptors have the potential to create novel technologies for glucose sensing and continuous monitoring, which might be important for efficient diabetes management.

Conclusions

A number of techniques can be employed as treatment for diabetic patients. Even though insulin replacement therapy is a pretty old way to manage glycemic levels in the blood, it is still one of the most popular methods yet. Many people prefer to use prescription medicines containing alpha-glucosidase inhibitors, sulfonylurea and biguanides. These, however, come with their own set of side-effects such as nausea, dizziness, diarrhea and weight-gain.[75]

Based on the research done so far, it can be stated that synthetic biology could be used to target diabetes and other aspects of diabetes. Synthetic biologists can design and create new molecules or modify existing ones to make them more effective at treating specific diseases.[76] For example,

synthetic cells could be designed to regulate the activity of specific enzymes or transporters involved in glucose metabolism. This approach could help to address the underlying metabolic dysregulation that characterizes diabetes, rather than simply treating the symptoms.

Despite the potential of synthetic cells for diabetes treatment, there are still challenges that need to be overcome. One major challenge is ensuring the safety and efficacy of synthetic cells in human patients. During implantation of synthetically designed cells, immunosuppressive drugs are often used to prevent the immune system from attacking and destroying the transplanted cells. However, long-term use of immunosuppressive drugs particularly glucocorticoids, may have some repercussions, including insulin resistance and impaired glucose metabolism which might lead to the development of Type 2 diabetes.[77,78] Hence, synthetic cells must be carefully designed to minimize the risk of immune rejection and other adverse reactions.

Additionally, even though, synthetic glucose receptors have not been studied rigorously as of now, they can be considered as a prospective way of detecting real time glycemic levels in the blood.

In conclusion, synthetic biology has the potential to revolutionize the treatment of diabetes by providing a more targeted and efficient approach to regulating blood glucose levels. While there are still challenges that need to be addressed, the promising results from *in vivo* animal model studies suggest that synthetic cells could be a game-changer in diabetes treatment.

Conflict of interest: The authors declare that they have no competing interests.

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Tables

Table 1. The different types of insulin analogs, the time taken for them to act and the generally recommended dosage of each type.

S. No.	Type of insulin analog	Examples	Time taken for onset of activity	Frequency of dose
1	Rapid-acting	Insulin lispro, insulin glulisine	10-20 min	Right before or after meals
2	Long-acting	Insulin glargine, insulin detemir, insulin degludec	1-2 h	Once or twice a day
3	Combined/premixed	Neutral protamine Hagedorn (NPH)	1-3 h	Not fixed

Figure legends

Figure 1. Top 10 countries with the highest no. of diabetic patients in 2021 (according to the 10th edition of the IDF Diabetes Atlas).

Figure 2. Subcutaneous administration of insulin leads to formation of hexameric structures that cannot be readily absorbed. These hexamers take time to dissociate to form dimers and subsequent monomers which can be easily absorbed by the bloodstream.

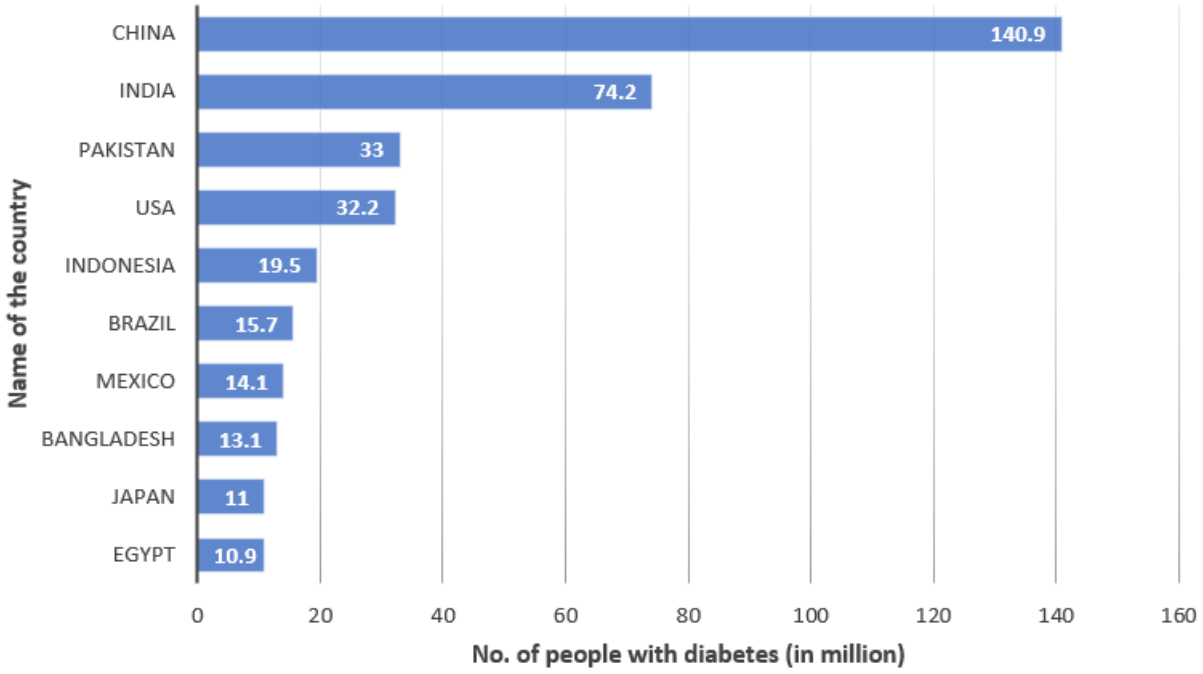
Figure 3. Illustration of a real time Closed Glucose Monitoring system in a diabetes patient using a CGM sensor and an insulin pump.

Figure 4. A simple illustration of how iCRISPR based technology can be used to create pancreatic progenitor cells, expressing genes such as PDX1, NKX6.1 and PTF1A, essential for efficient functioning of the pancreatic cells.

Figure 5. Diagrammatic representation of how hyperglycemia is sensed by a synthetic beta cell, according to Xie and colleagues. Glucose molecules are transported inside the cell via GLUT1/2. The glucose is then converted to pyruvate by glycolysis which subsequently produce ATP in the mitochondria. High ATP concentration blocks ATP dependent K+ ion channels and leads to membrane depolarization. Membrane depolarization activates the voltage gated calcium ion channels, leading to increased calcium influx. This further activates the expression of NFAT, a calcium responsive promoter, which stimulates insulin secretion.

Figure 6. Diagrammatic representation of the synthetic gene circuit for insulin sensitivity as described by Ye et al. (2016), showing how the phosphorylation of TetR-ELK1 in response to insulin signaling, can induce gene expression, connecting insulin sensing to gene regulation,

Figure 7. Biomimetic glucose receptors can detect the high blood glucose levels in diabetic patients. This technology can ultimately aid in designing diabetes treatments by providing continuous glucose monitoring



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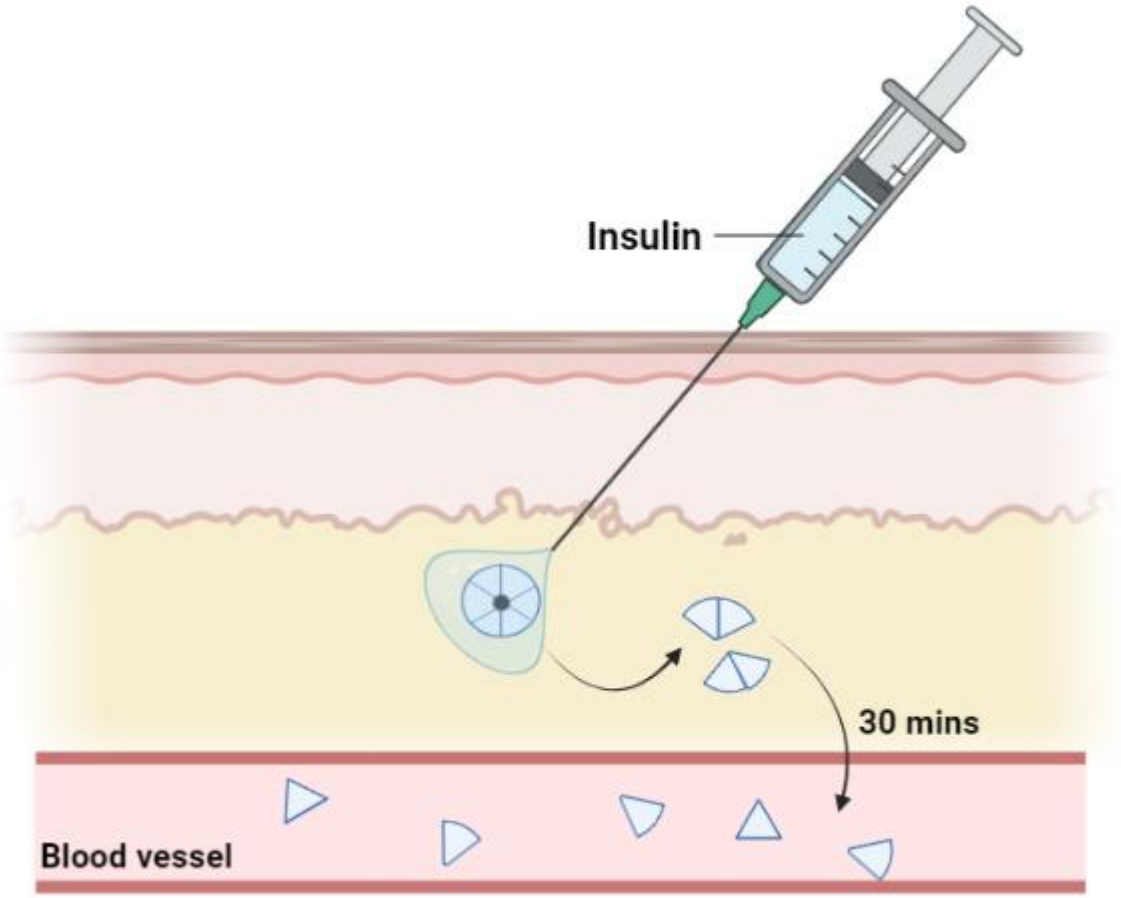

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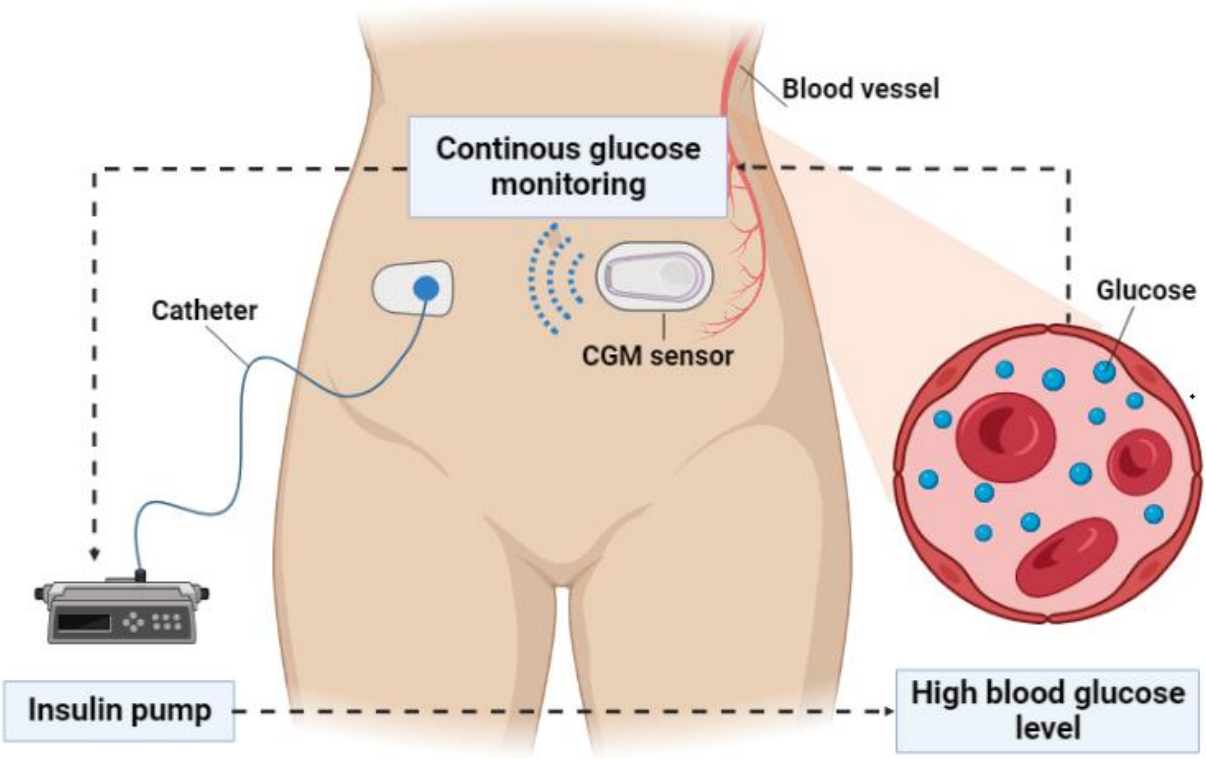

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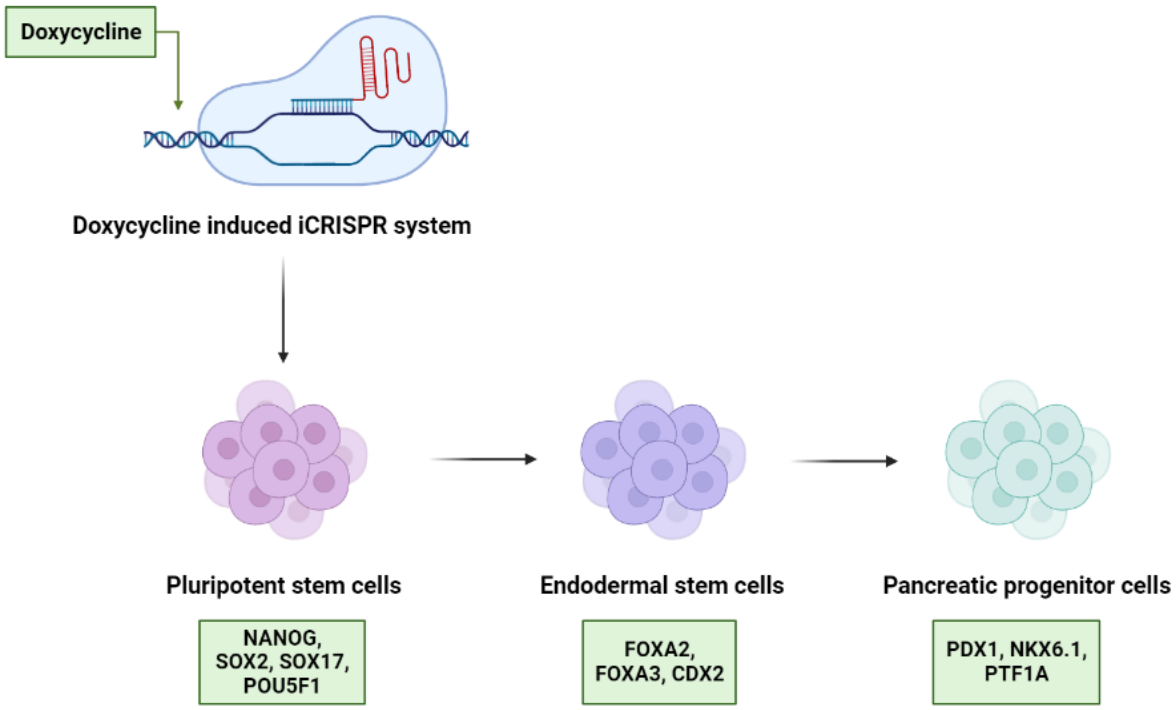

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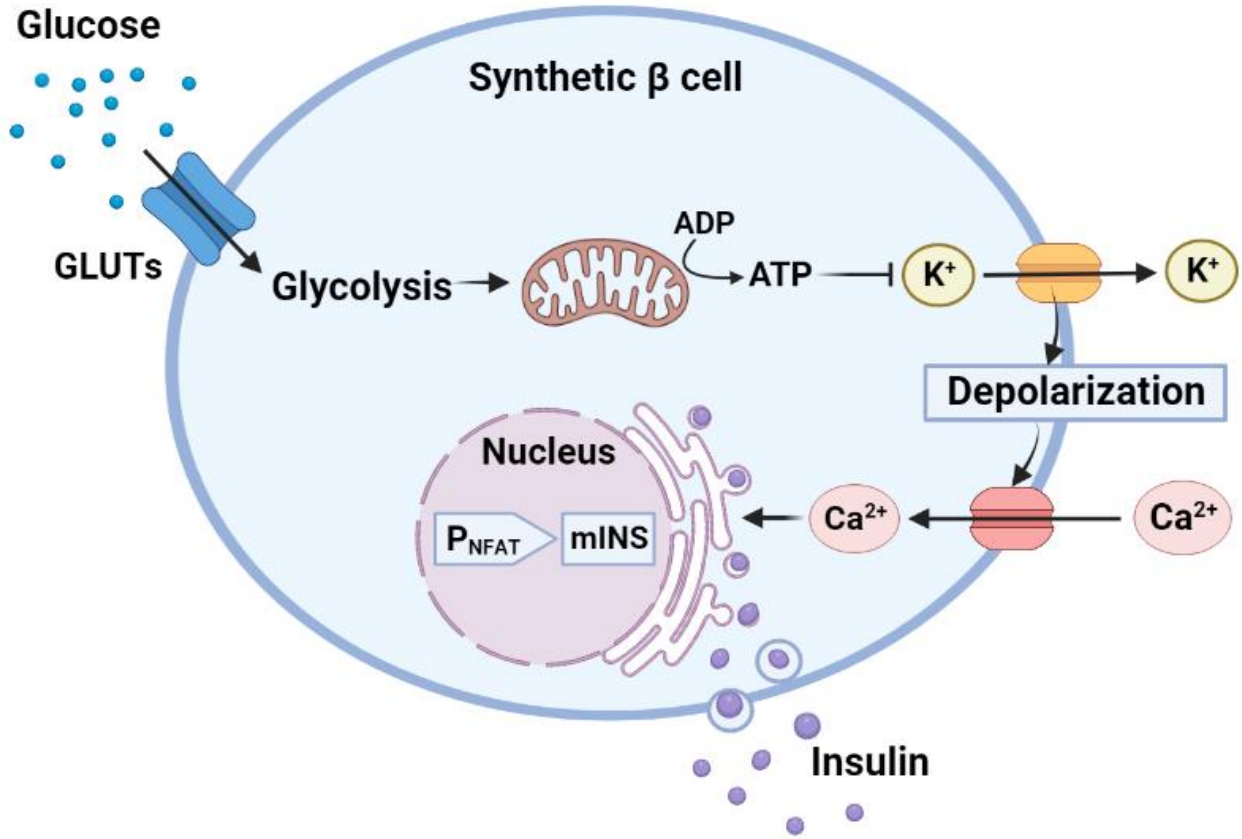

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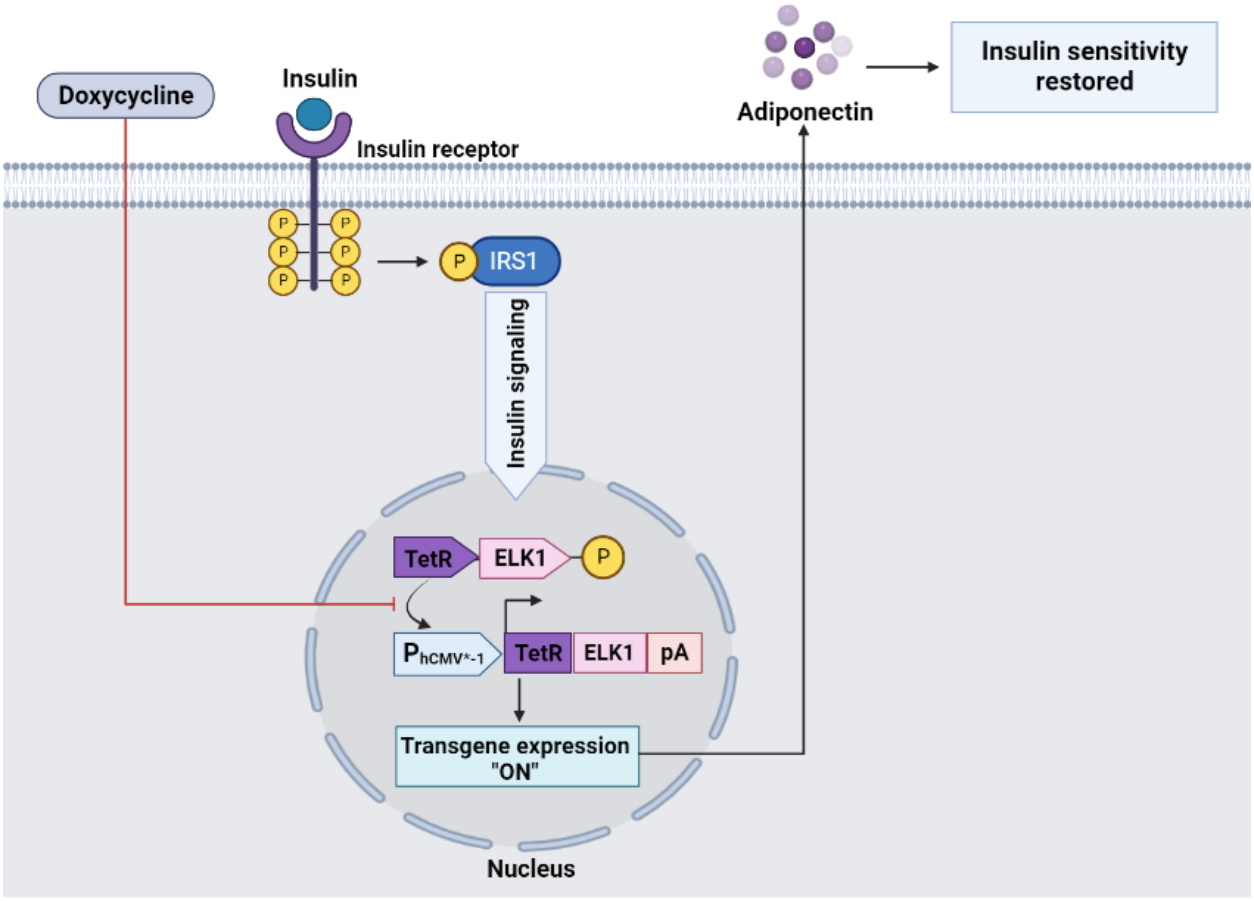

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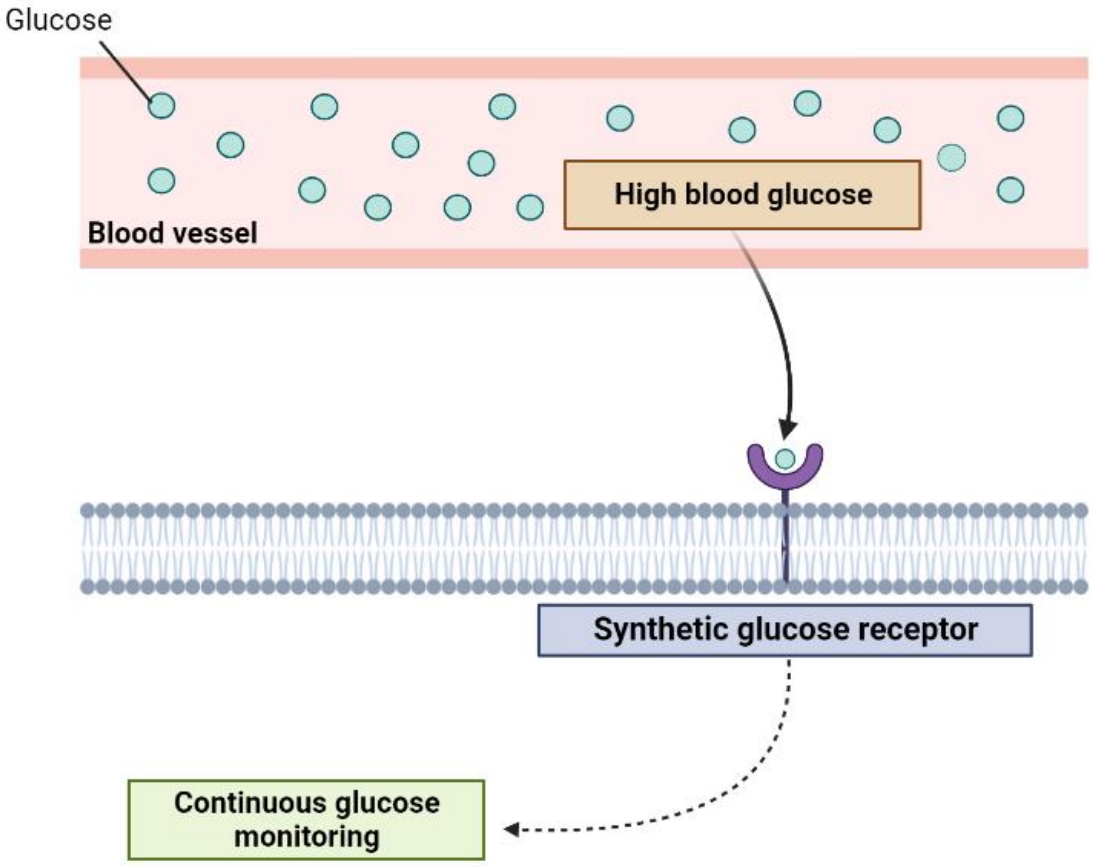
628 Figure 6.



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630 Figure 7.

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