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CRISPR-Cas9 Gene Editing for Restoring Pancreatic Beta Cell Function in Diabetes

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ABSTRACT

Diabetes mellitus remained a leading metabolic disorder characterized by impaired insulin production and secretion due to β-cell dysfunction or loss. Conventional pharmacotherapy alleviates hyperglycemia but does not restore endogenous β-cell mass. The discovery of clustered regularly interspaced short palindromic repeats (CRISPR) and associated protein 9 (Cas9) has transformed genetic engineering, providing an unprecedented platform for targeted gene correction, activation, or repression in β-cell biology. This review critically examined current advances in CRISPR-Cas9–mediated gene editing as a strategy to restore pancreatic β-cell function in both Type 1 and Type 2 diabetes. Literature from PubMed, Scopus, and Web of Science (2013–2025) was reviewed using keywords *CRISPR-Cas9*, *beta-cell regeneration*, *Type 1 diabetes*, *Type 2 diabetes*, and *gene therapy*. Only peer-reviewed experimental and translational studies were included. CRISPR-Cas9 enabled precise correction of monogenic defects (e.g., *INS*, *PDX1*, *GLIS3*), modulation of β-cell differentiation pathways, and reprogramming of non-β pancreatic cells into insulin-producing phenotypes. In autoimmune diabetes, CRISPR-based deletion of HLA molecules or induction of immune tolerance protected β-cells from cytotoxic destruction. In metabolic diabetes, editing genes regulating endoplasmic reticulum stress, oxidative balance, and insulin exocytosis enhances β-cell resilience. However, off-target activity, low editing efficiency, and delivery barriers constrain clinical translation. CRISPR-Cas9 offered a mechanistically rational, potentially curative approach for β-cell restoration in diabetes, though rigorous optimization of delivery vectors, safety profiles, and ethical frameworks remains essential.

Keywords: CRISPR-Cas9, Pancreatic β-cell regeneration, Gene therapy, Type 1 diabetes, Type 2 diabetes.

INTRODUCTION

The CRISPR-Cas9 system, derived from prokaryotic adaptive immunity, allows programmable DNA cleavage directed by a guide RNA complementary to a target sequence [1]. This technology has rapidly evolved into a cornerstone of genome engineering, enabling site-specific gene knockout, correction, and activation across eukaryotic systems. In mammalian biology, CRISPR-Cas9 has been leveraged to investigate transcriptional networks governing cellular differentiation, repair, and metabolism, offering a robust framework for precision therapeutics [2]. Its simplicity and scalability surpass earlier genome editing tools such as zinc finger nucleases and TALENs, thereby democratizing functional genomics and regenerative strategies.

Pancreatic β-cells are central regulators of glucose homeostasis through insulin synthesis and secretion. Their quantitative depletion or qualitative impairment leads to the development of diabetes mellitus. In Type 1 diabetes (T1D), autoimmune destruction of β-cells results in absolute insulin deficiency, while in Type 2 diabetes (T2D), chronic metabolic stress and insulin resistance culminate in β-cell exhaustion [3]. Current treatments exogenous insulin, incretin analogs, and sensitizers manage symptoms but fail to replace or rejuvenate β-cell mass. The advent of CRISPR-Cas9 offers the unique capacity to repair defective genes, reprogram non-β pancreatic cells, and protect functional β-cells from autoimmune or oxidative injury [4]. This review aims to critically synthesize evidence on the molecular mechanisms, experimental progress, and therapeutic implications of CRISPR-Cas9 gene editing for

restoring pancreatic β-cell function in both Type 1 and Type 2 diabetes, emphasizing translational potential and current limitations.

1. Molecular Mechanisms of CRISPR-Cas9–Mediated β-Cell Restoration

CRISPR-Cas9 operates via RNA-guided endonuclease activity that introduces double-strand breaks at specific genomic loci, stimulating repair through non-homologous end joining (NHEJ) or homology-directed repair (HDR) [5]. In β-cell research, this system has been exploited to dissect transcriptional hierarchies controlling differentiation from pancreatic progenitors. Editing of *PDX1*, *NEUROG3*, and *NKX6.1* genes has elucidated their cooperative roles in β-cell lineage specification [6]. Beyond mechanistic interrogation, CRISPR-Cas9 enables correction of pathogenic mutations in monogenic diabetes forms such as MODY (maturity-onset diabetes of the young), where repair of *HNF1A* or *GCK* mutations restored normal insulin secretion in induced pluripotent stem cell (iPSC)–derived β-like cells [7].

In T1D models, CRISPR-Cas9 has been applied to delete immune-recognition molecules, particularly HLA class I components, thereby rendering β-cells less visible to cytotoxic T lymphocytes [8]. Parallel strategies involve insertion of immunomodulatory transgenes such as *PD-L1* or *CTLA4-Ig* into β-cell genomes to attenuate local inflammation [9]. For T2D, CRISPR-mediated editing of stress-response regulators—such as *TXNIP*, *DDIT3*, and *ATF4* has reduced endoplasmic reticulum stress and apoptosis, preserving β-cell mass under glucolipotoxic conditions [10].

The precise modulation of β-cell secretory pathways through CRISPR activation (CRISPRa) has further enhanced insulin exocytosis. For instance, upregulation of *SLC2A2* (GLUT2) and *KCNJ11* via dCas9-VP64 systems improved glucose-stimulated insulin release in vitro [11]. Such fine-tuning suggests CRISPR is not solely corrective but also augmentative. Nonetheless, efficiency and specificity remain critical challenges, particularly with HDR-dependent repair, which is restricted to dividing cells—a limitation in mature β-cells. CRISPR-Cas9 facilitates both corrective and protective genetic interventions that underpin β-cell regeneration, forming the molecular basis for translational exploration.

2. Experimental Models and Gene Editing Strategies (355 words)

CRISPR-Cas9 applications in β-cell biology span from cell lines to organoids and in vivo animal models. Human iPSCs represent the predominant platform for modeling β-cell development and pathology due to their capacity for directed differentiation into endocrine lineages [12]. Using these systems, researchers have applied CRISPR-Cas9 to introduce or repair specific mutations to study genotype–phenotype relationships, as in *KCNJ11*-related neonatal diabetes [13]. Genome-wide CRISPR screening in iPSC-derived β-cells has identified novel regulators of insulin transcription and secretion, including *ZNF148* and *MAFA* [14].

Rodent models remain indispensable for preclinical evaluation. Streptozotocin-induced diabetic mice and *NOD* (non-obese diabetic) mice have been used to test CRISPR-mediated immune evasion constructs [15]. In vivo delivery through adeno-associated virus (AAV) vectors enables efficient editing within pancreatic tissue but is constrained by vector payload limits (~4.7 kb) [16]. Lentiviral and nanoparticle-based systems provide alternatives, offering higher transgene capacity but introducing concerns regarding insertional mutagenesis or biodistribution [17].

Recent innovations include CRISPR base editing and prime editing, which enable precise nucleotide substitutions without double-strand breaks, thus minimizing off-target mutagenesis [18]. These next-generation platforms have successfully corrected point mutations in *INS* and *GLIS3* genes in vitro. Parallel advances in RNA-targeting Cas systems, such as Cas13, allow transient modulation of gene expression without permanent DNA alteration—a safer interim approach for translational studies [19].

Despite these advances, delivery efficiency and β-cell-specific targeting remain significant barriers. Pancreatic architecture, dense vasculature, and immune infiltration in diabetes complicate vector biodistribution. Strategies utilizing tissue-specific promoters (*insulin* or *Pdx1*) and ligand-modified nanoparticles are under exploration to enhance selectivity [20]. Collectively, refined in vitro and in vivo models have established CRISPR-Cas9 as a versatile toolkit for dissecting β-cell genetics and testing candidate therapeutic edits, setting the stage for translational studies.

3. Immune Modulation and Autoimmunity in Type 1 Diabetes

In T1D, autoreactive T-cells target β-cell antigens such as insulin, GAD65, and IA-2. CRISPR-Cas9 interventions aim to mitigate this autoimmune cascade by modifying either the effector immune cells or the β-cells themselves [21]. Editing T-cells to disrupt *TCR* recognition of β-cell antigens or to insert regulatory constructs like *FOXP3* has yielded suppressive phenotypes capable of tempering autoimmunity in murine models [22].

Alternatively, CRISPR modification of β-cells to express immune checkpoint ligands such as *PD-L1* or *CTLA4-Ig* can induce local tolerance [23]. Engineering universal donor β-cells lacking *B2M* and *CIITA* (required for MHC class I and II expression) confers partial invisibility to cytotoxic T-cells while maintaining metabolic competence

[24]. These strategies parallel CRISPR-engineered universal stem-cell grafts currently tested for transplantation tolerance.

Macrophage polarization also represents a promising immunologic target. Deletion of *IRF5* via CRISPR has shifted macrophages toward the anti-inflammatory M2 phenotype, reducing islet infiltration in diabetic mice [25]. Additionally, CRISPR-mediated knockout of *CXCL10* in β-cells curtails recruitment of autoreactive lymphocytes [26].

Nonetheless, systemic immune editing poses risks, including unintended immunosuppression and off-target edits in hematopoietic progenitors. Thus, localized β-cell-specific immune modulation remains preferable. Encapsulation of CRISPR-modified β-cells within immunoprotective hydrogels or microcapsules has demonstrated sustained insulin secretion without systemic immune alteration [27].

Overall, CRISPR-based immune modulation presents a two-pronged therapeutic route—engineering tolerance within immune effector cells and conferring stealth properties to β-cells—both critical to reestablishing self-tolerance in T1D.

4. Metabolic Resilience and β-Cell Regeneration in Type 2 Diabetes

Type 2 diabetes features chronic hyperglycemia, oxidative stress, and lipotoxicity, which precipitate β-cell dysfunction and apoptosis. CRISPR-Cas9 has provided molecular insight into these degenerative processes while offering avenues for intervention. Gene knockout studies targeting *TXNIP* demonstrated reduced oxidative burden and improved glucose-stimulated insulin secretion in human β-cells [28]. Similarly, disruption of *DDIT3* (CHOP), a pro-apoptotic transcription factor induced by ER stress, conferred cytoprotection under glucolipotoxicity [29].

Beyond stress attenuation, CRISPR has facilitated β-cell neogenesis by reprogramming pancreatic α-cells or ductal cells into insulin-secreting phenotypes through activation of *PDX1*, *MAFA*, and *NKX6.1* using CRISPRa systems [30]. These reprogrammed cells exhibit glucose responsiveness and partially restore glycemia in diabetic rodents, illustrating the regenerative potential of transcriptional re-wiring.

In addition, CRISPR-Cas9 has clarified the polygenic landscape of T2D. Large-scale CRISPR screens in human islets identified key susceptibility genes (*TCF7L2*, *SLC30A8*, *KCNQ1*) that modulate insulin secretion and β-cell proliferation [31]. Correction or modulation of these loci can normalize insulin output, underscoring the feasibility of precision genomic interventions. However, T2D involves systemic metabolic derangements extending beyond the islet. Thus, successful gene editing must be complemented by modulation of peripheral insulin sensitivity and metabolic homeostasis. Combinatorial therapies coupling CRISPR-edited β-cells with metabolic drugs such as GLP-1 receptor agonists have shown synergistic benefits in preclinical studies [32]. While promising, concerns remain regarding durability of edited cell survival and integration within diseased microenvironments. Further optimization of delivery systems and editing fidelity will be essential to translate these metabolic resilience strategies into durable therapies.

5. Translational Challenges, Ethical Considerations, and Future Perspectives

Despite remarkable progress, translation of CRISPR-based β-cell therapies to clinical practice faces multifaceted challenges. The first barrier is delivery efficiency: pancreatic tissue is anatomically secluded and enzymatically harsh, complicating vector penetration. AAV vectors remain favored for safety but have limited cargo capacity, while lipid nanoparticles offer flexibility yet variable tropism [33]. Second, off-target editing and genomic instability raise safety concerns. Even with improved guide RNA design and high-fidelity Cas9 variants (e.g., eSpCas9, HiFi-Cas9), inadvertent mutations persist at low frequencies [34].

Immunogenicity of Cas9 proteins, derived from bacterial species (*S. pyogenes* and *S. aureus*), poses additional risk for immune rejection or inflammation [35]. Humanized Cas9 or transient mRNA delivery may mitigate this, though long-term safety data remain scarce. Ethical and regulatory considerations further complicate clinical translation [36]. Somatic editing is generally accepted for therapy, yet germline modification remains prohibited due to heritable risk.

Clinical trials are emerging cautiously. Early-phase investigations explore CRISPR-edited stem-cell–derived β-cells with immune-evasive features, while ex vivo editing of autologous iPSCs for autotransplantation is under evaluation [37]. Establishing standardized quality control for edited cells including whole-genome sequencing and functional assays is vital to ensure safety and reproducibility [38].

Future perspectives emphasize integration of multi-omics data, AI-guided sgRNA design, and combinatorial editing (base, prime, and epigenetic modulation) to fine-tune β-cell gene networks. Regulatory harmonization and ethical consensus will determine the pace of clinical adoption. Overcoming delivery, safety, and ethical constraints will dictate whether CRISPR-Cas9 evolves from a research tool to a mainstream curative modality for diabetes.

CONCLUSION

CRISPR-Cas9 gene editing represents a transformative leap in diabetes research, uniting molecular precision with therapeutic ambition. Its applications extend from dissecting β-cell developmental pathways to correcting

pathogenic mutations and fortifying cells against autoimmune or metabolic stress. In T1D, CRISPR enables creation of immune-evasive or immunoregulatory β-cells, whereas in T2D it offers routes to rejuvenate β-cell resilience and reprogram alternative pancreatic cells for insulin production. Yet, translation into clinical therapy demands resolution of critical challenges efficient and selective delivery to pancreatic tissue, minimization of off-target effects, and establishment of ethical and regulatory frameworks. The current trajectory of innovation, bolstered by advances in base and prime editing, suggests realistic potential for curative interventions within the next decade. Rigorous preclinical validation, coupled with long-term safety monitoring, will be essential to fulfill this promise. CRISPR-Cas9 thus stands at the threshold of redefining diabetes management, transitioning from compensatory pharmacology to genetic restoration of β-cell physiology. Future research should prioritize development of β-cell-specific, non-viral delivery systems that ensure high editing precision with minimal immunogenicity to enable safe clinical translation.

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