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Review Article

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Recombinant DNA technology: revolutionizing regenerative medicine and empowering nanotechnology strategies

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ABSTRACT

In the past century, the concept of controlling gene expressions to enhance desirable traits in living organisms through recombinant DNA technology was merely a concept. However, in recent times, this field has made significant advancements, offering unique benefits to human life. Recombinant DNA technology allows for the safe, accessible, and abundant production of crucial proteins needed for addressing various health issues. Through laboratory methods of genetic manipulation, scientists generate recombinant DNA molecules by merging genetic material from various origins that wouldn't naturally occur within organisms. Although the chemical structure of DNA is the same across all organisms, the nucleotide sequences vary. The application of recombinant DNA technology extends to diverse fields such as regenerative medicine, nanotechnology, and tissue engineering, allowing for the production of proteins with specific characteristics and effectiveness. This article will delve into the prevalent applications of recombinant DNA technology within fundamental research, highlighting its crucial role in contemporary endeavours across the realms of biological and biomedical sciences, particularly within the fields of regenerative medicine and nanotechnology.

KEYWORDS: Insulin, Regenerative medicine, Nanotechnology, Tissue engineering

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INTRODUCTION

Many years ago, it became clear that certain proteins had the potential to function as therapeutic remedies for addressing human health conditions. For instance, insulin emerged as a treatment for diabetes mellitus, while interferon found applications in combating viral diseases. However, the accessibility of these pharmaceutical products was constrained by the costly and cumbersome procedures required for their extraction. The advent of recombinant DNA technology unveiled the capability to generate an abundant supply of therapeutic agents suitable for human consumption. The commercial production of recombinant DNA (rDNA) technology commenced in the late 1970s, spearheaded by biotechnology firms aiming to manufacture proteins. The progress in recombinant DNA technology revolutionized biological research and opened up new opportunities for creating a diverse array of therapeutic products. This breakthrough has a profound impact on the fields of medical genetics and biomedical sciences, as it allows for the modification of microorganisms, animals, and plants to produce valuable therapeutic agents (Cederbaum *et al.*, 1984; Steinberg & Raso, 1998). This is a foundational technology that harnesses biological agents, like microorganisms or cellular components, to yield advantages for human welfare. It entails combining microbiology, biochemistry, and engineering disciplines to use microorganisms and cultured tissues (Griffith *et al.*, 1999; Hughes, 2001). Scientists can use live creatures, resulting in the

development of transgenic animals or plants. Biotechnology is currently used mostly in agriculture and health, frequently in combination with nanoscience and regenerative technologies (Hoogenboom *et al.*, 1998; Hoet *et al.*, 2005; Bajželj *et al.*, 2014). It is predicted that the global population will reach 9.6 billion people by 2050, with a 60% rise in demand for critical crops (Shaheen & Abed, 2018). Food safety has become a global problem as a result of increased food demand and the challenges posed by climate change, soil degradation, and crop disease expansion, all of which are reducing crop output (Chen *et al.*, 2019). Current efforts are mostly aimed at boosting agricultural yields sustainably while reducing the overuse of pesticides and fertilizers. However, traditional crop enhancement approaches are time-consuming and complex. As a result, novel plant breeding technologies that may overcome the limits of old approaches are required (Puchta *et al.*, 1993; Fiaz *et al.*, 2021). To address this difficulty, novel plant breeding technologies have arisen, including gene knockout/in, epigenetic alterations, and the precise insertion of targeted mutations in specific genomic areas. These developments attempt to simplify and accelerate the crop enhancement process. Nonetheless, there is an urgent need to modernize and improve present agricultural techniques to fulfill the increasing demand for food from the world's expanding population. Genome editing (GE) technology has emerged as a promising approach for addressing challenges in agriculture, enabling the creation of plants with increased yields, enhanced nutritional content, and improved resistance to herbicides, pests, and diseases. Numerous genome editing (GE) techniques have been developed in recent times, with clustered regularly interspaced short palindromic repeats (CRISPR) and nucleases emerging as highly versatile and efficient methods. The fundamental stages of the genome editing (GE) process entail the introduction of transgenes or CRISPR components into plants through specialized gene delivery mechanisms 12-20 (Steinberg & Raso, 1998; Wright *et al.*, 2005; Phillips & Tang, 2008; Christian *et al.*, 2010; Butler *et al.*, 2016; Tang *et al.*, 2017; Anzalone *et al.*, 2019; Manghwar *et al.*, 2019; Lin *et al.*, 2020). When stem cells undergo genetic modification to closely match the host environment, they become valuable for enhancing cell survival post-transplantation. These modified stem cells can be utilized to facilitate the transfer of proteins to adjacent cells, combat cancer cells, or reduce the likelihood of graft-host rejection. Both adult and embryonic stem cells exhibit evident therapeutic potential in terms of cellular repair, replacement, and regeneration. However, one drawback of cell replacement therapy is the high mortality rate of transplanted cells. Genetic engineering plays a crucial role in prolonging the lifespan of engrafted stem cells by incorporating transgenes into the cells to prevent or minimize apoptosis and inflammatory damage. In genetic engineering, a gene cassette is constructed and then inserted into a vector for cell delivery. Within the cell, certain genes expressed by the gene construct are typically activated only once (Johnson, 1983).

Recombinant Human Insulin

For individuals diagnosed with either type 1 or severe type 2 diabetes, insulin replacement therapy stands out as the most effective and recommended treatment option (Ruttenberg, 1972; Mann *et al.*, 1983). The hormone was generated from swine and bovine pancreatic tissue for many years until it was superseded by semi-synthetic human insulin obtained from animal insulin (Obermeier & Geiger, 1976; Morihara, 1990). From 1922 until 1974, animal insulins generated from pancreatic tissue were used for treatment, until semisynthetic human insulin became accessible in limited quantities by altering animal insulin (Markussen *et al.*, 1983; Morihara, 1990; Walsh, 2005; Mayer *et al.*, 2007). This breakthrough was based on determining the structure of human insulin from autopsy material and was chemically manufactured using modest amounts of pure human insulin recovered from post-mortem material (Jonasson *et al.*, 1996). Because of recombinant DNA methods based on microorganisms such as bacteria or yeast, recombinant human insulin has become commercially available on an industrial scale. After post-translational processing and rigorous refinement of the ultimate therapeutic product, the production of biologically active human insulin was successfully accomplished (Miller & Baxter, 1980; Galloway *et al.*, 1982). This achievement involved introducing the genetic code of proinsulin protein into bacteria (*Escherichia coli*) or yeast (*Saccharomyces cerevisiae*, *Pichia pastoris*). Recombinant insulin has higher purity and compatibility than semisynthetic insulin. In 1978, Genentech pioneered the manufacturing of recombinant human insulin (Chance & Frank, 1993), while Eli Lilly pioneered large-scale production (Raskin & Clements, 1991; Ladisch & Kohlmann, 1992). In 1982, the comWWpany obtained commercial authorisation (Humulin®) in the United States and Europe for an *E. coli*-derived medicinal product. In 1991, Novo Nordisk secured marketing approval for recombinant human insulin produced using an alternative biosynthetic approach in yeast (Hilgenfeld *et al.*, 2014; Vasiljević *et al.*, 2020).

Structure of Insulin

Insulin is a hormone secreted by beta cells of Islets of Langerhans in Pancreas. It controls the blood glucose level by catabolizing glucose. It is a boon to diabetic patients whose generally fails normal sugar metabolic function. Insulin possesses a molecular weight of 5808 Daltons (Da) and consists of two chains: Chain A, comprised of 21 amino acids, and Chain B, composed of 30 amino acids, linked together through disulphide bridges (Cheng *et al.*, 1981) (Figure 1).

Initially, beta cells of the pancreas produce a pre-pro insulin molecule of 109 amino acids. The first 23 amino acids of pre-pro insulin act as signalling molecules to pass through the cell membrane and it cleaves and produces proinsulin which contains 86 amino acids. Proinsulin undergoes cleavage by enzymes and produces active Insulin (Figure 2). The signal peptide carries pre-proinsulin to the ER, where it is broken down and converted to proinsulin by the signal peptidase. Three disulphide bonds are formed between cysteine residues in the ER with the help of Protein Disulphide Isomerase (PDI). Proinsulin is transferred from the ER to the Golgi and the trans-Golgi network, where it is converted to active insulin by CPE (Carboxypeptidase E) and PC1/3 (Prohormone Convertase) (Zieliński *et al.*, 2019).

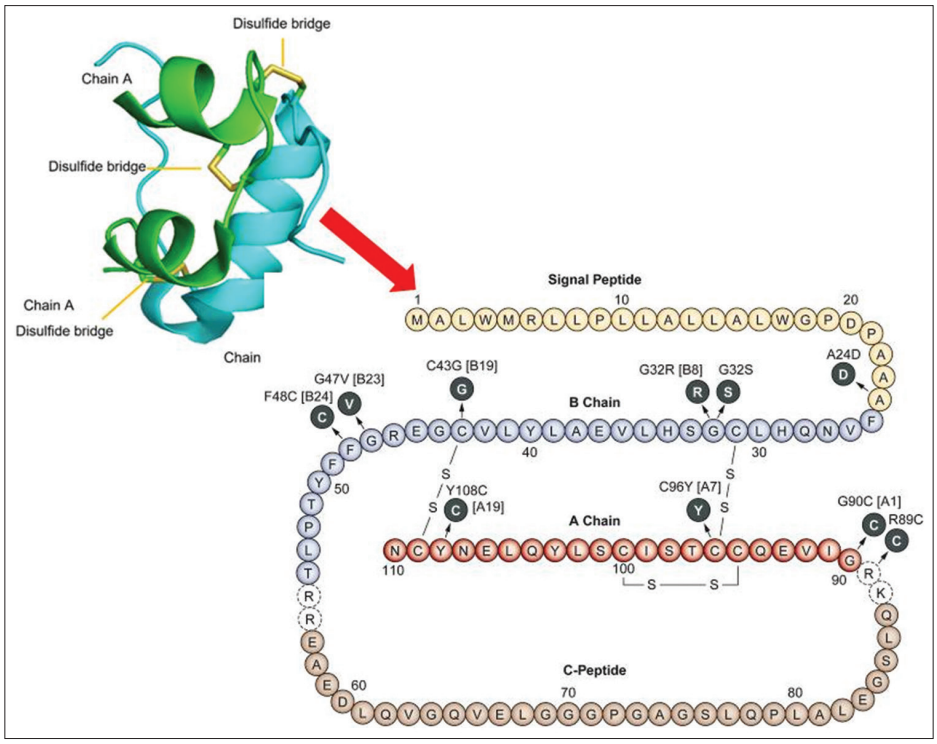

Figure 1: Structure of Insulin

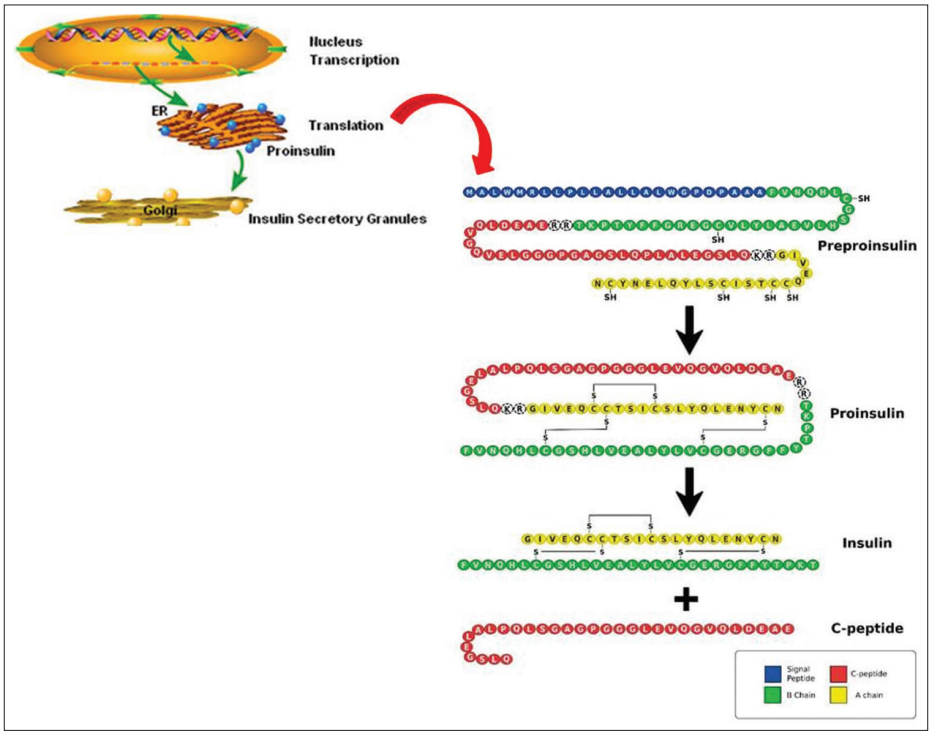

Figure 2: Process of Synthesis of Human Insulin

Production of Human Insulin by rDNA Technology by using *E. coli*

The Institute of Biotechnology and Antibiotics achieved the creation of a fresh bacterial host strain named *Escherichia coli* 20 and introduced a novel pIBAINS expression vector. This vector notably enhances the efficiency of producing recombinant human insulin (Jensen *et al*., 2020). The Human Insulin gene cannot be directly used to produce active insulin in *E.coli* because of the following reasons; The *E. coli* lack systems of

proteolytic processing (post translational modification activity e.g.; phosphorylation, glycosylation) or to process pre-mRNA required to produce mature Insulin from Proinsulin (Regmi *et al.*, 2020; Ramesh *et al.*, 2022) and *E. coli* cannot splice signal sequence (Introns) from pre-pro insulin or to remove the central part (C chain) from Proinsulin. Therefore those steps are bypassed by using separate expression vectors to chemically synthesize coding sequences for the A chain and B chain (Chance & Frank, 1993).

Host Strain

E. coli 20 IBA1 is a laboratory bacterium produced from the gram negative bacterium *E. coli* K-12, which belongs to the *Enterobacteriaceae* family. The *E. coli* strain IBA1 has mutations in two genes rpsL and cytR. The mutation in the rpsL is associated with streptomycin resistance. The cytR gene mutation encodes a transcriptional regulation. *E. coli* is a very efficient host strain to produce recombinant insulin because *E. coli* lacks the cytR repressor (Hallewell *et al.*, 1985).

Construction of Expression Vector

pIBAINS is an expression plasmid derived from plasmid pBR322 (ATCC,31344). The ligation of two HindIII/smaI fragmentsIII resulted in pIBA. Insert a short molecular cloning site (63 nucleotides) and a synthetic transcription terminator trpA into the plasmid that forms pIBA1 with EcoR1/Nde1 cleavage sites using PCR. pIBA2 is created by ligating the promoter coding sequence (945 bp) of deoPIP2 through PCR (Jensen *et al.*, 2020). The deoPIP2 promoter sequence is amplified from the chromosomal DNA of the *E.coli* K-12 strain, and pIBA2 is ligated with Nde1/Xba1, which contains the hybrid protein SOD-INS, which optimizes the plasmid pIBAINS expression for tetracycline resistance. The pIBAINS plasmid carries the SOD-INS gene, which is controlled by the deoPIP2 promoter and functions as an analogue for the human SOD gene sequence, which encodes the A, B, and C chains of recombinant human Insulin. The transcription initiation in *E. coli* is controlled by the deoPIP2 promoter, and the derived plasmid pIBAINS is employed to transform the *E. coli* 20 strain.

Cell Culture

In cell culture, *E. coli* 20 strains containing the plasmid pIBAINS, which carries the human insulin gene, are cultured in shaking flasks. They are grown in a GMS medium supplemented with tetracycline and thiamine for 18 hours at a temperature of 30 °C. The flasks are used as a fermenter inoculum (no antibiotics are utilized). Cells in the inoculum grow in the fermenter for 15-17 hours at 37 °C. Proline and Glucose are provided as carbon sources throughout this growth state of culture until it reaches the stationary phase of development (the growth seen by monitoring the optical density (nm) and medium concentration (mg/mL) (Balsam *et al.*, 2004) (Figure 3).

Inclusion Bodies Isolation

Inclusion Bodies are isolated by centrifuging harvested cells at 15000 g for 15 minutes at 4 °C. The cellular pellet is softly agitated at room temperature for 30 minutes in a lysis buffer composed of the following components: 50 mM tris-HCl (pH 8.0), 500 mM NaCl, 1 mM EDTA, and 0.043% Lysozyme. Then, after 10 minutes, add Triton X-100 to the suspension and stir. The cells were then lysed using a high pressure homogenizer and centrifuged at 18400 g for 15 minutes at 4 °C in the presence of a solution containing Tris HCl (50 mM, pH 8.0), NaCl (500 mM), and 1% Triton X-100. Subsequently, perform two rounds of washing on the pellet using a buffer solution consisting of Tris HCl (50 mM, pH 8.0) and NaCl (50 mM). The suspension containing the inclusion bodies is subjected to centrifugation at 15000 g for 15 minutes at 4 °C. Subsequently, the obtained inclusion bodies are preserved for further processing by freezing them at -30 °C (Jensen *et al.*, 2020).

Dissolution of Inclusion Bodies

To dissolve the inclusion bodies containing insulin, they are placed in a solution of Carbonate buffer, which consists of 12 mM NaHCO3, and EDTA at a concentration of 0.2 mM. The pH of the solution is elevated to approximately 11.9 by adding 5 M NaOH and stirring the mixture for 45 minutes at room temperature. Afterward, the pH is adjusted to 10.8 using 2 M HCl, and the suspension is subjected to centrifugation at 24000 g for 15 minutes at 4 °C to eliminate any remaining insoluble components (Jensen *et al.*, 2020).

Renaturation

Following the dissolution of the inclusion bodies, they are subjected to a folding process, which spans 18 hours and involves vigorous stirring along with aeration. The pH is then modulated to 9.0 by utilizing 2 M HCl as part of the renaturation procedure. The assessment of renaturation occurs at both room temperature and 8 °C (Jensen *et al.*, 2020).

Citraconylation Reaction

Stir in 1 mL Citraconic anhydride and 5 M NaOH to the insulin precursor protein solution (pH 8.7-9.3) for 2.5 hours. It regulates enzymatic processes that create Insulin and inhibit Insulin breakdown. Then add the Ethanolamine solution (2 M) and stir for 45 minutes (Jensen *et al.*, 2020).

Trypsinization

Adjust the pH to 8.8 after citraconylation by adding HCl (2 M) and diluting. Add 1% Trypsin solution and stir for 16-18 hours at room temperature. To suppress trypsinization, add Aprotinin (1 mg/mL) to the solution. The protein sample was subsequently subjected to low-pressure chromatography using DEAE Sepharose. Insulin was eluted from the column using a DE buffer comprising Tris (0.5 M, 20 mM, pH 8.6) and 30% Isopropanol (Zieliński *et al.*, 2019).

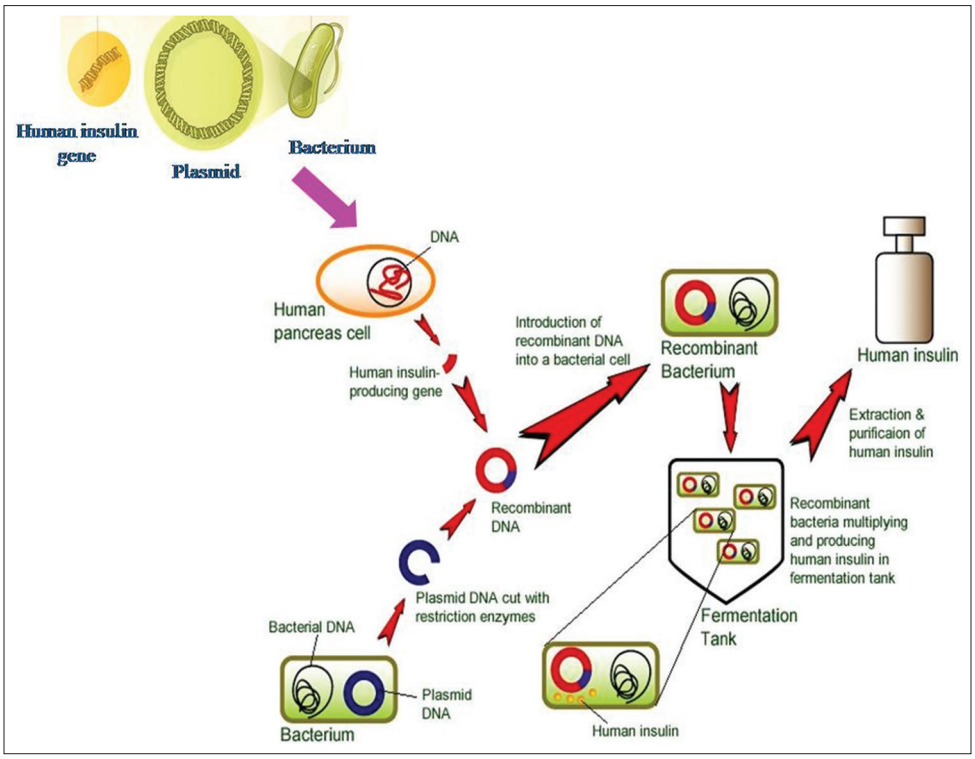

Figure 3: Production of Human Insulin by rDNA technology by using *E.coli*

DE-CITRACONYLATION

The insulin eluted from the column is diluted twice with H_2O, the pH is adjusted to 2.9 with HCl (2 M), and the sample is stirred for 1 hour at 4 °C overnight (Zieliński *et al.*, 2019).

Precipitation of Insulin

First, add 1M $ZnCl_2$ solution to De-citraconylation Insulin elution (pH 5.9) and stir for 1 hour. The material was then centrifuged at 14000 g for 15 minutes at 4 °C. Then collect the precipitate and store it at 4 °C for 30 days. Dissolve the Insulin precipitation in Zinc salt by adding the H_2O and then add Tris (1 M) and EDTA (30 mM, 0.2 M pH 8.6) to the solution. Subsequently, low-pressure chromatography was conducted on Q-Sepharose using a buffer containing 0.5 M Tris and 20 mM Tris at pH 8.6 (Zieliński *et al.*, 2019).

Reaction with Carboxypeptidase B

Following elution from the column, the fraction is diluted by a factor of two with H_2O and then treated with Carboxypeptidase B at a concentration of 2.5 mg/mL in a solution at pH 8.8. After allowing it to stand for 16-18 hours at room temperature for elution, proceed with the elution step followed by RP-HPLC chromatography using an ACE 5C18-300 column and an acetonitrile gradient. Then take the elution off the column and dilute it three times with H_2O. Insulin precipitation will occur with the addition of $ZnCl_2$, and the pH of the insulin suspension will be adjusted to 3.0. Through Sephadex G-25 chromatography, exchange the solution with Ammonium acetate at pH 4.0. Elute insulin using an appropriate buffer. To assess protein content, employ either a spectrophotometer or tandem MALDI-TOF/TOF mass spectrometry, aiming for a purity level of 99% for Recombinant Human Insulin (Zieliński et *al.*, 2019).

Recombinant DNA Technology in Regenerative Medicine of Animals

Stem cells have the remarkable capacity to continuously replicate throughout an individual's lifetime, except for a limited subset that remains undifferentiated. These daughter cells ultimately mature into specialized adult cells with distinct biological functions. Modifying the genes of stem cells prior to their transplantation serves to bolster their survival and elevate their effectiveness in cell therapy applications (Tang *et al.*, 2007). Modifying cells to create functional gene sequences using gene switches, cell specific promoters, and numerous transgenes is what genetic engineering is all about. Gene-modified stem cells are now being utilized to treat neurological illnesses (Parkinson's, Alzheimer's, and spinal cord injury repair), cardiovascular disease, and cancer. Adult stem cells are multipotent and can differentiate into many organs. Stem cells in bone marrow can differentiate into osteocytes, blood cells, and lymphocytes (Messina *et al.*, 2004). Cardiac stem cells may differentiate into any of the cells seen in a healthy heart, including cardiomyocytes and endothelial cells 48-50 (Koransky *et al.*, 2002; Murry *et al.*, 2004; Moretti *et al.*, 2006). It is debatable

whether bone marrow cells may transform into heart cells or any other cell that is not connected to blood (Messina *et al.*, 2004; Tang *et al.*, 2004). Adult and embryonic stem cells both have a high proclivity for cellular repair, replacement, and regeneration. Generally cell replacement therapy has limitations of graft-host rejection, even if they are from a syngeneic population. To overcome these, genetically modified stem cells or transgenes are inserted into the cells to reduce rejection of tissue, apoptosis and inflammatory activities. There are several applications of Gene modified stem cells in various tissues like cardiac, neurological, bone regeneration, diabetes and cancer, etc. Genetic modification of stem cells to increase angiogenesis in ischemic heart disease and myocardial ischemia associated with coronary artery disease leads to mortality (Tang *et al.*, 2005). To cure myocardial ischemia, operative coronary revascularization (CABG) percutaneous transluminal angioplasty (PTCA) procedures are effective for revascularization by using vascular endothelial growth factor (VEGF) (Lange *et al.*, 2005). VEGF expression is not under tight control and thus might cause angioma formation. To develop an approach for safe and effective angiogenesis, the formation of neovascularization and vascular differentiation in ischemic myocardium by homologous mesenchymal stromal cells (MSCs). The bone marrow derived MSCs play an important role in improving blood flow in ischemic myocardium and provide therapeutic angiogenesis by secreting a broad spectrum of angiogenic cytokines, including VEGF (Pillarisetti & Gupta, 2001), bFGF (Farzaneh *et al.*, 2019), HGF (Szaraz *et al.*, 2017), and SDF-1α (Wang *et al.*, 2015). Increased blood supply from neovascularization inhibits necrosis and apoptosis of ischemic myocardium. Furthermore, homologous MSCs have high proliferation and self-renewal capacity, which is important for maintaining effective clinic treatment of patients with substantial atherosclerotic coronary disease MSC transplantation described as "sole therapy" for neovascularization and developed strategies by using MSCs as vehicles for angiogenic gene therapy to enhance the neovascularization by using angiogenic factors produced from the MSCs, which can produce multiple paracrine mediators to enhance revascularization (Pillarisetti & Gupta, 2001).

Paracrine Signalling Mechanism for Ischemic Heart Disease

Research explores several ways of MSC therapy by various factors, both in vivo and in vitro culturing of cardiomyocytes. The paracrine mechanism consists of four major steps for neovascularisation (Figure 4).

1. Differentiation and Engraftment of Cardiomyocytes (Davani *et al.*, 2003; Ding *et al.*, 2015; Chiossone *et al.*, 2016; Najar *et al.*, 2016).
2. Paracrine signalling molecules or mediators from MSCs show an effect on cardiac tissue (Fan *et al.*, 2012; Ju *et al.*, 2018).
3. Endogenous cardiac stem cells (CSC) undergo stimulation for multiplication and the restoration of injured tissues through the mediation of MSC (mesenchymal stem cell) factors (Lei *et al.*, 2004; Poggioli *et al.*, 2016).
4. It stimulates Neovascularization and Immunomodulation (Mangi *et al.*, 2003).

A. Mesenchymal stem cells (MSCs) exhibit a versatile skill set that includes:
i) The capacity to differentiate into endothelial cells and cardiomyocytes. ii) The release of paracrine mediators, notably Vascular Endothelial Growth Factor (VEGF), Insulin-like Growth Factor (IGF), and Hepatic Growth Factor (HGF). iii) Stimulation of the proliferation of endogenous cardiac stem cells (CSCs). iv) Facilitation of neovascularization and immunomodulation processes.

B. The creation of fresh endothelial cells at the site of injury by various MSC mechanism Paracrine mediators have multiple effects in the Myocardium, viz cardiac stem cell stimulation, increase angiogenesis, decrease cardiomyocyte death, reduce fibroblast activation and myocardial fibrosis which plays crucial in cell-mediated immune response. Recent studies revealed that the paracrine activity of MSCs has enormous therapeutic effects. In their research on conditioned media, Nakanishi and colleagues discovered that Mesenchymal Stem Cells (MSCs) secrete soluble substances that play a crucial role in increasing the expression of Cardiac Progenitor Genes (CPGs), such as Myosin heavy chain and Atrial Natriuretic Peptide (ANP) (Ding *et al.*, 2015). These compounds facilitate the growth and migration of cardiac progenitor cells through the activation of paracrine effects. Moreover, Cardiac Progenitor Cells (CPCs) stimulate the overexpression of the Akt 1 gene (Akt-MSC), which serves as a protective paracrine marker released by MSCs. This heightened expression not only reinstates cardiac function but also prevents ventricular remodelling (Li *et al.*, 2016). Additionally, Glycogen Synthase Kinase-3 (GSK-3) prompts MSCs to enhance the rate of cardiomyocyte differentiation and reduce the size of infarctions in the ischemic coronary heart. This cascade results in the generation of bone marrow-derived stem cells and paracrine mediators, including Interleukins-1 (IL-1) and IL-6, which bolster angiogenesis development and stimulate the expression of Vascular Endothelial Growth Factor (VEGF).VEGF, in turn, prevents the death of cardiomyocytes and endothelial cells (Phillips & Sumners, 1998; Kurozumi *et al.*, 2005; Noguchi *et al.*, 2006; Portelius *et al.*, 2010; Blocki *et al.*, 2015; Butler *et al.*, 2017; Chu *et al.*, 2018). Hepatocyte Growth Factor (HGF) plays a pivotal role in mobilizing cardiac progenitor cells while Transforming Growth Factor 1 (TGF-1) acts as a regulator of fibrosis. The coordinated regulation of these cytokine factors induces angiogenesis in the infarcted ischemic heart (Zivin & Pregelj, 2008; Luque-Contreras *et al.*, 2014; Perneczky *et al.*, 2014).

Genetically Modified Stem Cells to Treat Alzheimer's Disease

Alzheimer's disease is a neurodegenerative condition characterized by abnormal protein synthesis within brain tissues, making it the most prevalent form of dementia. It arises due to the formation of amyloid-beta protein plaques. Alzheimer's is a complex neurodegenerative disorder resulting from irregular protein formation within brain cells, involving intricate pathophysiological

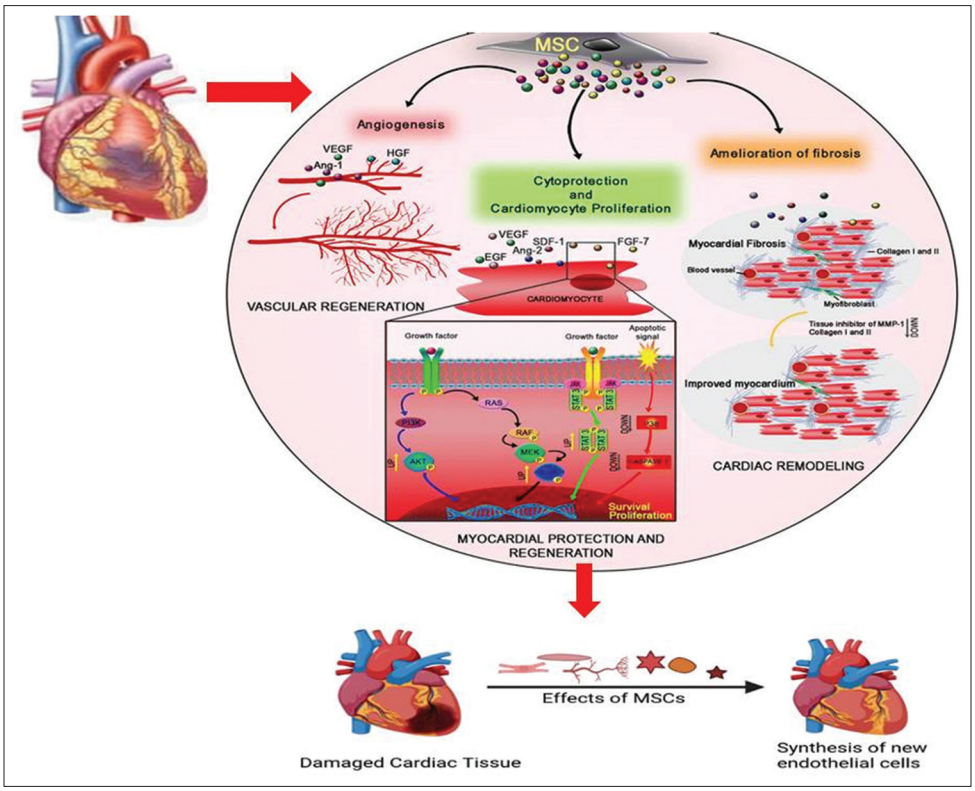

Figure 4: Mechanism of action of MSCs involved in Paracrine signalling

cascades (Blurton-Jones *et al.*, 2014). Pathologically, it is marked by the accumulation of extracellular beta-amyloid (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). Aβ plaques consist of fragments, either 40 or 42 amino acid residues in length, produced through proteolytic cleavage of the Amyloid-beta precursor protein (APP), while NFTs comprise hyperphosphorylated Tau protein. In Alzheimer's disease pathology, two key features stand out, namely TAU and APP proteins (Figure 5). To elaborate, the Amyloid-beta precursor protein (APP) is an integral membrane protein situated at neuronal synapses, where it functions as a cell surface receptor and regulator of synapse formation. APP undergoes two distinct processing pathways: the non-amyloidogenic pathway, in which APP is initially cleaved by α-secretase followed by γ-secretase, leading to cleavage within the Aβ domain, and the amyloidogenic pathway, where APP is successively cleaved by β-secretase and γ-secretase, ultimately releasing Aβ fragments into the extracellular space, typically consisting of 40 (Aβ1–40) or 42 (Aβ1–42) amino acids (Billings *et al.*, 2005; Hosseini *et al.*, 2015; Kwak *et al.*, 2018).

Secondly, 'TAU' is an intracellular microtubule-associated protein that holds a vital role in the structure and function of axons. Tau, expressed in neurons, typically serves to stabilize microtubules within the cellular cytoskeleton and is under regulation through phosphorylation (Figure 6). The presence of hyperphosphorylated Tau is linked to misfolding, aggregation, and the impairment of cognitive functions (Mizumoto *et al.*, 2003; Oddo *et al.*, 2003).

To overcome AD, by increasing the production of the enzyme Neprilysin, the proteolytic enzyme has the most potent activity (Chen *et al.*, 2016; Shaheen & Abed, 2018) which breaks down amyloid-beta plaque formation. Researchers at the University of California, Irvine embarked on a study to explore the potential of reducing amyloid-beta levels in mice brains by introducing neprilysin. The study's lead author, Mathew Blurton-Jones, emphasized the significance of their work, highlighting that neprilysin levels decline with age, increasing Alzheimer's disease risk. If amyloid accumulation is indeed a major contributor to Alzheimer's disease, interventions that either reduce amyloid-beta production or enhance its breakdown could offer promising avenues for AD treatment. One of the challenges in treating neurological conditions like Alzheimer's is the presence of the blood-brain barrier, a protective mechanism that limits the entry of cells and proteins into the brain. This barrier poses difficulties when attempting to deliver therapeutic proteins or drugs to the brain. To address this challenge, the researchers proposed that stem cells could potentially overcome these barriers and serve as effective delivery vehicles for therapeutic agents. To put their hypothesis to the test, the researchers conducted experiments under the oversight of the Irvine Institutional Animal Care & Use Committee, University of California, strictly adhering to National Institutes of Health guidelines. In these experiments, they injected genetically modified neural stem cells into two distinct mouse models (3xTg-AD and Thy1-APP) to assess the expression of Neprilysin. This approach aimed to explore the feasibility of using stem cells as a means to deliver neprilysin and potentially mitigate amyloid-beta accumulation in the brain 87-89 (Bernhardt *et al.*, 2010; Tiwari *et al.*, 2012; Zhang *et al.*, 2019).

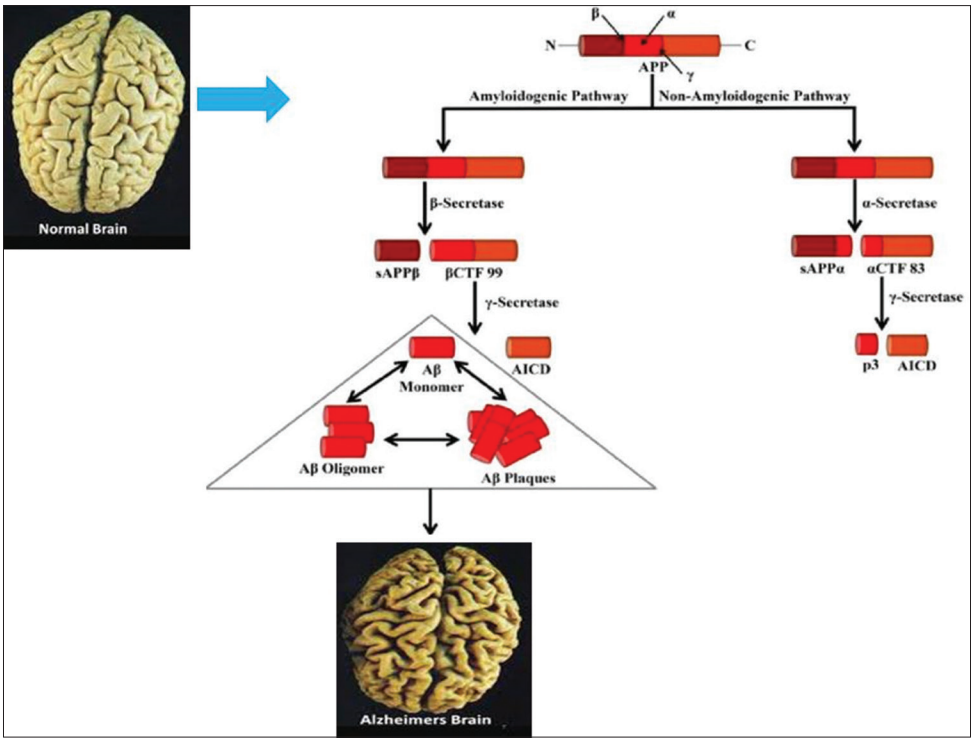

Figure 5: APP cascade pathology- Uncontrolled Proteolytic Processing of Amyloid Precursor Proteins

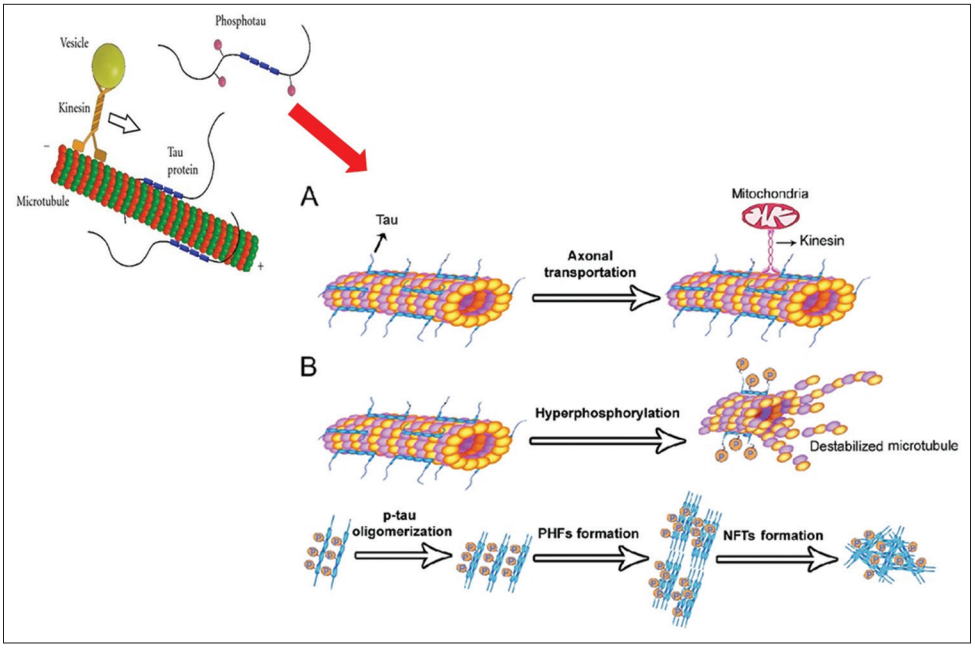

Figure 6: TAU cascade pathology- Cholinergic Hypothesis

Production of Transgenic Mice Models

The 3xTg-AD mice were generated through the introduction of Human APP695 (a sequential amyloid precursor protein with 695 amino acids) carrying the Swedish mutation (KM670/671NL) and Human tau protein featuring the P301L mutation. These genetic components were regulated by the murine Thy1.2 promoter. The mice were produced by micro-injecting these genes into single-cell embryos of homozygous PS1M146V knock-in mice. As for Thy1-APP mice, they were created by performing pronuclear injection of a transgene APP751, which included the Swedish and London mutations (V717I), and this transgene was controlled by the murine Thy1 promoter (Altpeter *et al*., 2016) (Figure 7).

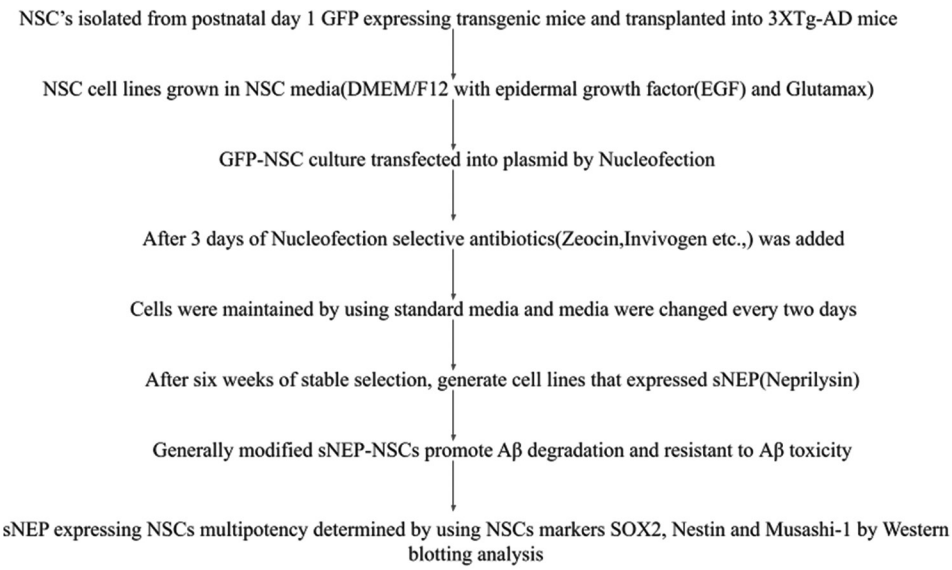

Figure 7: Flowchart for Production of transgenic mice models

Recombinant DNA Technology by Using Nanotechnology Strategies for Plant Genetic Engineering

Nowadays, global agricultural systems are facing tremendous climate changes (extreme temperature, drought conditions and water deficiency) (Giraldo *et al*., 2019) and demand for food requirements is increasing with the increasing global population. The convergence of biology and nanotechnology has significantly progressed the field of nano-engineering, serving as a valuable instrument for enhancing crop yields and realizing ecological sustainability (Jampílek & Kráľová, 2015). Nanomaterials are widely used as fertilizers and pesticides act as specific carriers which facilitates the controlled specific target delivery of agrochemicals for crop improvement. Nanotools, such as nano biosensors, have great potential to sense environmental conditions (detection of toxic metal release), leading to development of advanced agricultural techniques 93-94 (Dubey & Mailapalli, 2016; Kumar *et al*., 2017). The fusion of nanotechnology and biotechnology offers molecular tools for genetic modification and the creation of novel organisms (Shang *et al*., 2019). The use of nanomaterials to achieve organelle targeted delivery will promote the development of plant genetic engineering. Nanobiotechnologies encompass a range of nanoscale materials such as nanoparticles, nanofibers, nanospheres, nanorods, and nanocapsules, which serve as carriers for foreign DNA and chemicals that facilitate targeted gene modification (Cunningham *et al*., 2018). Recent advancements in nanobiotechnology have greatly facilitated researchers in the seamless replacement of genetic material from one species with that of another (de Melo *et al*., 2020). Within the realm of recombinant DNA technology, silicon dioxide (SiO2) nanoparticles have emerged as effective delivery agents for transporting DNA fragments into target species, such as tobacco and corn plants, with minimal unwanted side effects (Nekrasov *et al*., 2013; Chen *et al*., 2019). Additionally, nanoparticle-based delivery techniques have been instrumental in the development of insect-resistant crop varieties. For instance, in gene-gun technology, DNA-coated nanoparticles are employed to bombard cells and transfer desired genes into target plants (Altpeter *et al*., 2016; Hong *et al*., 2020). An essential stage in gene modification involves the introduction of the transgene into the target organism using a specialized gene delivery vehicle (Puchta *et al*., 1993; Mout *et al*., 2017). Nanobiotechnology plays a pivotal role in gene editing by enhancing the efficiency of this process without the need to introduce transgenes. This is achieved through the utilization of the CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats) technique, which enables both transient and stable genetic modifications in a species, resulting in increased efficiency 104-107 (Christian *et al*., 2010; Butler *et al*., 2016; Tang *et al*., 2017; Yin *et al*., 2017). For example, Prevotella and Francisella 1(cpf 1) are used as powerful tools for primer and base editing for the potential genome sequence modifications 108-116 (Feng *et al*., 2017; Dong *et al*., 2020; Fayos *et al*., 2020; Ferrie *et al*., 2020; Imai *et al*., 2020; Lin *et al*., 2020; Li *et al*., 2020; Martínez *et al*., 2020; Zhang *et al*., 2020). So CRISPR-cas technique is a powerful genome modification tool, used for improving various crop species viz; Rice (*Oryza sativa)* (Lei *et al*., 2021; Tian *et al*., 2020), Wheat (*Triticum aestivum*) (Ahmar *et al*., 2020; Rui *et al*., 2020), Maize (*Zea mays*) (Hong *et al*., 2020), Tomatoes (*Solanum lycopersicum)* (Glick & Patten, 2022; Sharma *et al*., 2022), Cotton (*Gossypium hirsutum*) (Tiedje *et al*., 1989), Tobacco (*Nicotiana tabacum*) (Lewis & Potter, 2013), etc. The CRISPR-cas facilitates functional biogenomics of various crop species with efficient, safe and accurate time saving but still, this technique remains a challenging tool for genetic engineering (Paukner *et al*., 2014).

Nanotechnology, in conjunction with molecular biology, has played a substantial role in research. The integration of nanotechnology into the creation of genetically modified organisms (GMOs) is achieved through the utilization of nanoparticles as nanocarriers in combination with the CRISPR/Cas system for delivery into plant cells. The implementation of

nanobiotechnology techniques has significantly enhanced the process of plant breeding. This enhancement is evident in the accelerated selection of new genes, the reduction in the time required to eliminate undesirable genes, and the facilitation of the expression of crucial genes (Wang *et al.*, 1994; Moretti *et al.*, 2006).

Ethical Considerations of rDNA Technology

The development of rDNA technology has a lot of applications in many areas of life. Recombinant DNA technology has been developed to improve beneficial therapeutic approaches to understand diseases. Recombinant DNA is one of the beneficial tools involved in modify the genetic material *in vitro* to obtain the desired characteristics in a given product (García-Granados *et al.*, 2019). However, The main ethical and legal issue of recombinant DNA technology concerns the nature of investigations and their impact on future generations. One of the main issues arising from rDNA technology is the potential side effects that have occurred. For example, the recombinant organisms are clones that may be dangerous and show negative effects on the natural environment and population (Wang *et al.*, 1994; Li *et al.*, 2023). The application of recombinant DNA technology in the production of Genetically Modified Organisms (GMOs) raises concerns about potential health risks. These concerns centre around the possibility of toxic effects on human health, which could heighten the risk of cancer and impact various physiological systems (Moretti *et al.*, 2006). Recombinant DNA technology should follow ethical principles and considerations to prevent violation of human and animal rights and prevent malfeasance of researchers working with this technology.

Future Developments of rDNA Technology

Recombinant DNA research represents a tremendous opportunity to enhance our comprehension of genes by facilitating in vitro analysis and manipulation. Importantly, it empowers the alteration of genes and the cloning of genes using a range of selectable markers tailored for this objective. These advancements in technology have come together to completely transform the landscape of molecular and genetic manipulation and analysis. Recombinant DNA technology has especially excelled in unveiling the structure and arrangement of individual genes (García-Granados *et al.*, 2019; Moretti *et al.*, 2006). The evolution of recombinant DNA technology has ushered in transformative changes in the field of research, paving the way for innovative avenues of exploration. It has been applied in the field of pharmaceutical production and in the manipulation of biosynthetic pathways to create innovative drugs, among which certain drugs exhibit anti-tumour and immunosuppressive qualities (Altpeter *et al.*, 2016). As a tool in gene therapy, recombinant DNA technology holds promise in both preventing and treating genetic disorders. DNA vaccine development, which involves delivering genes coding for pathogenic proteins, aims to confer immunity against various diseases. Clinical trials for treating cancer using human gene therapy are currently underway, with transfection techniques showing minimal toxicity effects. These clinical trials are focused on various types of cancers such as brain cancer, breast cancer, lung cancer, and prostate cancer, as well as conditions like renal fibrosis and other diseases (Farzaneh *et al.*, 2019).

Recombinant DNA technology has already enabled the production of substantial quantities of human hormones, enzymes, and other proteins used as medications. Notable applications include the treatment of diabetes with insulin and the use of clotting factors to manage haemophilia. Regenerative medicine, a field poised to restore the normal function of body tissues damaged by disease or trauma, envisions gene therapy as a key component. This therapy will involve the replacement of abnormally inherited genes with genes that produce normal products. Furthermore, the ability to manipulate genes for the treatment of cancer and autoimmune degenerative disorders is on the horizon, thanks to the emergence of recombinant DNA tools as a potent technology (Li *et al.*, 2023).

Future research in regenerative medicine aims to establish standardized protocols for isolating and differentiating stem cells into various lineages. Additionally, the incorporation of nanomaterials alongside stem cells holds immense potential for brain regenerative studies. Nanomaterials offer a well-suited platform for augmenting the efficacy of stem cell therapies, encouraging genetic alterations to boost stem cell proliferation and specialization, and enhancing the transition of stem cells into neurons during neuronal differentiation. For instance, the combination of curcumin-encapsulated PLGA (Poly Lactic-co-Glycolic Acid) nanoparticles (Cur-PLGA-NPs) in Aβ-treated rats has shown promise in activating the Wnt signalling pathway, this involves the activation of genes essential for the proliferation and differentiation of neural stem cells (Li *et al.*, 2023). Rapidly advancing technology of CRISPR/Cas9 is providing new techniques to overcome the challenges of agricultural food production of GMOs and also will help to develop new varieties and transgene free plants by using DNA recombinant technology.

CONCLUSION

Recombinant DNA technology stands as a pivotal scientific advancement that has significantly simplified human life. In recent years, it has propelled biomedical strategies forward, particularly in the realms of cancer treatment, genetic disorders, diabetes, and various plant-related ailments. Despite the obstacles encountered in enhancing gene-level products, it is imperative to overcome these challenges for the continuous advancement of recombinant DNA technology. Within the realm of healthcare, recombinant technology has evolved into a pivotal instrument for addressing a diverse range of disorders that would otherwise pose significant management challenges. In a nutshell, the development of the innovative pIBAINS expression vector and the introduction of the bacterial host strain *E. coli* 20 have substantially heightened the efficiency of human insulin production via cell culture and protein purification. Regenerative medicine, specifically stem cell therapy, has witnessed remarkable developments, with ongoing

advancements enhancing its therapeutic potential. Gene modification of stem cells represents a key approach to improving their effectiveness. This involves the modification of genes to extend their lifespan, enhance protein secretion, and promote differentiation. Stem cell-based therapy has shown remarkable efficacy in treating Alzheimer's disease (AD) by exerting multiple therapeutic effects that bolster neuro-regeneration and mitigate neurodegeneration through immunomodulatory, anti-amyloidogenic, and neuroprotective mechanisms. Furthermore, the integration of the CRISPR/Cas system with nanoparticles holds the potential to bring about a transformative shift in the field of plant genetic engineering. This approach paves the way for rapid crop improvement, allowing the development of desired traits through the incorporation of nanoparticles using genetic engineering techniques.

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REFERENCES

Ahmar, S., Saeed, S., Khan, M. H. U., Ullah Khan, S., Mora-Poblete, F., Kamran, M., Faheem, A., Maqsood, A., Rauf, M., Saleem, S., Hong, W. J., & Jung, K. H. (2020). A revolution toward gene-editing technology and its application to crop improvement. *International Journal of Molecular Sciences, 21*(16), 5665. https://doi.org/10.3390/ijms21165665

Altpeter, F., Springer, N. M., Bartley, L. E., Blechl, A. E., Brutnell, T. P., Citovsky, V., Conrad, L. J., Gelvin, S. B., Jackson, D. P., Kausch, A. P., Lemaux, P. G., Medford, J. I., Orozco-Cárdenas, M. L., Tricoli, D. M., Van Eck, J., Voytas, D. F., Walbot, V., Wang, K., Zhang, Z. J., & Stewart, C. N. (2016). Advancing crop transformation in the era of genome editing. *Plant Cell, 28*(7), 1510-1520. https://doi.org/10.1105/tpc.16.00196

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., Chen, P. J., Wilson, C., Newby, G. A., Raguram, A., & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature, 576*, 149-157. https://doi.org/10.1038/s41586-019-1711-4

Bajželj, B., Richards, K. S., Allwood, J. M., Smith, P., Dennis, J. S., Curmi, E., & Gilligan, C. A. (2014). Importance of food-demand management for climate mitigation. *Nature Climate Change, 4*(10), 924-929. https://doi.org/10.1038/nclimate2353

Balsam, L. B., Wagers, A. J., Christensen, J. L., Kofidis, T., Weissman, I. L., & Robbins, R. C. (2004). Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature, 428*, 668-673. https://doi.org/10.1038/nature02460

Bernhardt, E. S., Colman, B. P., Hochella, M. F., Cardinale, B. J., Nisbet, R. M., Richardson, C. J., & Yin, L. (2010). An ecological perspective on nanomaterial impacts in the environment. *Journal of Environmental Quality, 39*(6), 1954-1965. https://doi.org/10.2134/jeq2009.0479

Billings, L. M., Oddo, S., Green, K. N., McGaugh, J. L., & LaFerla, F. M. (2005). Intraneuronal Aβ causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron, 45*(5), 675-688. https://doi.org/10.1016/j.neuron.2005.01.040

Blocki, A., Beyer, S., Dewavrin, J. Y., Goralczyk, A., Wang, Y., Peh, P., Ng, M., Moonshi, S. S., Vuddagiri, S., Raghunath, M., Martinez, E. C., & Bhakoo, K. K. (2015). Microcapsules engineered to support mesenchymal stem cell (MSC) survival and proliferation enable long-term retention of MSCs in infarcted myocardium. *Biomaterials, 53*, 12-24. https://doi.org/10.1016/j.biomaterials.2015.02.075

Blurton-Jones, M., Spencer, B., Michael, S., Castello, N. A., Agazaryan, A. A., Davis, J. L., Müller, F. J., Loring, J. F., Masliah, E., & LaFerla, F. M. (2014). Neural stem cells genetically modified to express neprilysin reduce pathology in Alzheimer transgenic models. *Stem Cell Research and Therapy, 5*(2), 46. https://doi.org/10.1186/scrt440

Butler, J., Epstein, S. E., Greene, S. J., Quyyumi, A. A., Sikora, S., Kim, R. J., Anderson, A. S., Wilcox, J. E., Tankovich, N. I., Lipinski, M. J., Ko, Y. A., Margulies, K. B., Cole, R. T., Skopicki, H. A., & Gheorghiade, M. (2017). Intravenous allogeneic mesenchymal stem cells for nonischemic cardiomyopathy: Safety and efficacy results of a phase II-A randomized trial. *Circulation Research, 120*(2), 332-340. https://doi.org/10.1161/CIRCRESAHA.116.309717

Butler, N. M., Baltes, N. J., Voytas, D. F., & Douches, D. S. (2016). Geminivirus-mediated genome editing in potato (Solanum tuberosum L.) using sequence-specific nucleases. *Frontiers in Plant Science, 7*, 1045. https://doi.org/10.3389/fpls.2016.01045

Cederbaum, S. D., Fareed, G. C., Lovett, M. A., & Shapiro, L. J. (1984). Recombinant DNA in medicine. *Western Journal of Medicine, 141*(2), 210-222.

Chance, R. E., & Frank, B. H. (1993). Research, development, production, and safety of biosynthetic human insulin. *Diabetes Care, 16*(S3), 133-142. https://doi.org/10.2337/diacare.16.3.133

Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual Review of Plant Biology, 70*, 667-697. https://doi.org/10.1146/annurev-arplant-050718-100049

Chen, Y. W., Lee, H. V., Juan, J. C., & Phang, S. M. (2016). Production of new cellulose nanomaterial from red algae marine biomass Gelidium elegans. *Carbohydrate Polymers, 151*, 1210-1219. https://doi.org/10.1016/j.carbpol.2016.06.083

Cheng, Y. S., Kwoh, D. Y., Kwoh, T. J., Soltvedt, B. C., & Zipser, D. (1981). Stabilization of a degradable protein by its overexpression in Escherichia coli. *Gene, 14*(1-2), 121-130. https://doi.org/10.1016/0378-1119(81)90154-2

Chiossone, L., Conte, R., Spaggiari, G. M., Serra, M., Romei, C., Bellora, F., Becchetti, F., Andaloro, A., Moretta, L., & Bottino, C. (2016). Mesenchymal stromal cells induce peculiar alternatively activated macrophages capable of dampening both innate and adaptive immune responses. *Stem Cells, 34*(7), 1909-1921. https://doi.org/10.1002/stem.2369

Christian, M., Cermak, T., Doyle, E. L., Schmidt, C., Zhang, F., Hummel, A., Bogdanove, A. J., & Voytas, D. F. (2010). Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics, 186*(2), 757-761. https://doi.org/10.1534/genetics.110.120717

Chu, J., Shi, P., Yan, W., Fu, J., Yang, Z., He, C., Deng, X., & Liu, H. (2018). Pegylated graphene oxide-mediated quercetin-modified collagen hybrid scaffold for enhancement of MSCs differentiation potential and diabetic wound healing. *Nanoscale, 10*(20), 9547-9560. https://doi.org/10.1039/c8nr02538j

Cunningham, F. J., Goh, N. S., Demirer, G. S., Matos, J. L., & Landry, M. P. (2018). Nanoparticle-mediated delivery towards advancing plant genetic engineering. *Trends in Biotechnology, 36*(9), 882-897. https://doi.org/10.1016/j.tibtech.2018.03.009

Davani, S., Marandin, A., Mersin, N., Royer, B., Kantelip, B., Hervé, P., Etievent, J.-P., & Kantelip, J.-P. (2003). Mesenchymal progenitor cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a rat cellular cardiomyoplasty model. *Circulation, 108*(10_S1), 253-258. https://doi.org/10.1161/01.cir.0000089186.09692.fa

de Melo, B. P., Lourenço-Tessutti, I. T., Morgante, C. V., Santos, N. C., Pinheiro, L. B., de Jesus Lins, C. B., Silva, M. C. M., Macedo, L. L. P., Fontes, E. P. B., & Grossi-de-Sa, M. F. (2020). Soybean embryonic axis transformation: Combining biolistic and Agrobacterium-mediated protocols to overcome typical complications of in vitro plant regeneration. *Frontiers in Plant Science, 11*, 1228. https://doi.org/10.3389/fpls.2020.01228

Ding, R., Jiang, X., Ha, Y., Wang, Z., Guo, J., Jiang, H., Zheng, S., Shen, Z., & Jie, W. (2015). Activation of Notch1 signalling promotes multi-lineage differentiation of c-Kit^{POS}/NKX2.5^{POS} bone marrow stem cells: Implication in stem cell translational medicine. *Stem Cell Research and Therapy, 6*(1), 91. https://doi.org/10.1186/s13287-015-0085-2

Dong, O. X., Yu, S., Jain, R., Zhang, N., Duong, P. Q., Butler, C., Li, Y., Lipzen, A., Martin, J. A., Barry, K. W., Schmutz, J., Tian, L., & Ronald, P. C. (2020). Marker-free carotenoid-enriched rice generated

through targeted gene insertion using CRISPR-Cas9. *Nature Communications, 11*, 1178. https://doi.org/10.1038/s41467-020-14981-y

Dubey, A., & Mailapalli, D. R. (2016). Nanofertilisers, nanopesticides, nanosensors of pests and nanotoxicity in agriculture. In E. Lichtfouse (Eds.), *Sustainable Agriculture Review* (Vol. 19, pp. 307-330) Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-319-26777-7_7

Fan, D., Takawale, A., Lee, J., & Kassiri, Z. (2012). Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. *Fibrogenesis and Tissue Repair, 5*, 15. https://doi.org/10.1186/1755-1536-5-15

Farzaneh, M., Rahimi, F., Alishahi, M., & Khoshnam, S. E. (2019). Paracrine mechanisms involved in mesenchymal stem cell differentiation into cardiomyocytes. *Current Stem Cell Research and Therapy, 14*(1), 9-13. https://doi.org/10.2174/1574888X13666180821160421

Fayos, I., Meunier, A. C., Vernet, A., Navarro-Sanz, S., Portefaix, M., Lartaud, M., Bastianelli, G., Périn, C., Nicolas, A., & Guiderdoni, E. (2020). Assessment of the roles of SPO11-2 and SPO11-4 in meiosis in rice using CRISPR/Cas9 mutagenesis. *Journal of Experimental Botany, 71*(22), 7046-7058. https://doi.org/10.1093/jxb/eraa391

Feng, D., Wang, Y., Wu, J., Lu, T., & Zhang, Z. (2017). Development and drought tolerance assay of marker-free transgenic rice with OsAPX2 using biolistic particle-mediated co-transformation. *Crop Journal, 5*(4), 271-281. https://doi.org/10.1016/j.cj.2017.04.001

Ferrie, A. M. R., Bhowmik, P., Rajagopalan, N., & Kagale, S. (2020). CRISPR/Cas9-mediated targeted mutagenesis in wheat doubled haploids. In L. M. Vaschetto (Eds.), *Cereal genomics: Methods and Protocols* (pp. 183-198) Humana, US: Springer.

Fiaz, S., Khan, S. A., Anis, G. B., Gaballah, M. M., & Riaz, A. (2021). CRISPR/Cas techniques: A new method for RNA interference in cereals. In K. A. Abd-Elsalam & K.-T. Lim (Eds.), *CRISPR and RNAi systems* (pp. 233-252). Amsterdam, Netherlands: Elsevier. https://doi.org/10.1016/B978-0-12-821910-2.00032-1

Galloway, J. A., Root, M. A., Bergstrom, R., Spradlin, C. T., Howey, D. C., Fineberg, S. E., & Jackson, R. L. (1982). Clinical pharmacologic studies with human insulin (recombinant DNA). *Diabetes Care, 5* (S2), 13-22. https://doi.org/10.2337/diacare.5.2.s13

García-Granados, R., Lerma-Escalera, J. A., & Morones-Ramírez, J. R. (2019). Metabolic engineering and synthetic biology: Synergies, future, and challenges. *Frontiers in Bioengineering and Biotechnology, 7*, 36. https://doi.org/10.3389/fbioe.2019.00036

Giraldo, J. P., Wu, H., Newkirk, G. M., & Kruss, S. (2019). Nanotechnology approaches for engineering smart plant sensors. *Nature Nanotechnology, 14*, 541-553. https://doi.org/10.1038/s41565-019-0470-6

Glick, B. R., & Patten, C. L. (2022). *Molecular biotechnology: Principles and applications of recombinant DNA*. New Jersey, United States: John Wiley & Sons.

Griffith, A. J. F., Gelbart, W. M., Lewontin, R. C., & Miller, J. H. (1999). Modern genetic analysis. US: W. H. Freeman & Co Ltd.

Hallewell, R. A., Masiarz, F. R., Najarian, R. C., Puma, J. P., Quiroga, M. R., Randolph, A., Sanchez-Pescador, R., Scandella, C. J., Smith, B., Steimer, K. S., & Mullenbach, G. T. (1985). Human Cu/Zn superoxide dismutase cDNA: Isolation of clones synthesising high levels of active or inactive enzyme from an expression library. *Nucleic Acids Research, 13*(6), 2017-2034. https://doi.org/10.1093/nar/13.6.2017

Hilgenfeld, R., Seipke, G., Berchtold, H., & Owens, D. R. (2014). The evolution of insulin glargine and its continuing contribution to diabetes care. *Drugs, 74*, 911-927. https://doi.org/10.1007/s40265-014-0226-4

Hoet, R. M., Cohen, E. H., Kent, R. B., Rookey, K., Schoonbroodt, S., Hogan, S., Rem, L., Frans, N., Daukandt, M., Pieters, H., van Hegelsom, R., Neer, N. C., Nastri, H. G., Rondon, I. J., Leeds, J. A., Hufton, S. E., Huang, L., Kashin, I., Devlin, M.., . .Ladner, R. C. (2005). Generation of high-affinity human antibodies by combining donor-derived and synthetic complementarity-determining-region diversity. *Nature Biotechnology, 23*(3), 344-348. https://doi.org/10.1038/nbt1067

Hong, W.-J., Kim, Y.-J., Kim, E.-J., Chandran, A. K. N., Moon, S., Gho, Y. S., Yoou, M.-H., Kim, S. T., & Jung, K.-H. (2020). CAFRI-Rice: CRISPR applicable functional redundancy inspector to accelerate functional genomics in rice. *The Plant Journal, 104*(2), 532-545. https://doi.org/10.1111/tpj.14926

Hoogenboom, H. R., de Bruıne, A. P., Hufton, S. E., Hoet, R. M., Arends, J. W., & Roovers, R. C. (1998). Antibody phage display technology and its applications. *Immunotechnology, 4*(1), 1-20. https://doi.org/10.1016/s1380-2933(98)00007-4

Hosseini, A. M., Megges, M., Prigione, A., Lichtner, B., Toliat, M. R., Wruck, W., Schröter, F., Nuernberg, P., Kroll, H., Makrantonaki, E., Zoubouliss, C. C., & Adjaye, J. (2015). Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks. *BMC Genomics, 16*(1), 84. https://doi.org/10.1186/s12864-015-1262-5

Hughes, S. S. (2001). Making dollars out of DNA: The first major patent in biotechnology and the commercialization of molecular biology, 1974–1980. *Isis; an International Review Devoted to the History of Science and Its Cultural Influences, 92*(3), 541-575. https://doi.org/10.1086/385281

Imai, R., Hamada, H., Liu, Y., Linghu, Q., Kumagai, Y., Nagira, Y., Miki, R., & Taoka, N. (2020). In planta particle bombardment (iPB): A new method for plant transformation and genome editing. *Plant Biotechnology, 37*(2), 171-176. https://doi.org/10.5511/plantbiotechnology.20.0206a

Jampílek, J., & Kráľová, K. (2015). Application of nanotechnology in agriculture and food industry, its prospects and risks. *Ecological Chemistry and Engineering S, 22*(3), 321-361. https://doi.org/10.1515/eces-2015-0018

Jensen, E. T., Bertoni, A. G., Crago, O. L., Hoffman, K. L., Wood, A. C., Arzumanyan, Z., Lam, L. K., Roll, K., Sandow, K., Wu, M., Rich, S. S., Rotter, J. I., Chen, Y. I., Petrosino, J. F., & Goodarzi, M. O. (2020). Rationale, design and baseline characteristics of the Microbiome and Insulin Longitudinal Evaluation Study (MILES). *Diabetes, Obesity and Metabolism, 22*(11), 1976-1984. https://doi.org/10.1111/dom.14145

Johnson, I. S. (1983). Human insulin from recombinant DNA technology. *Science, 219*(4585), 632-637. https://doi.org/10.1126/science.6337396

Jonasson, P., Nilsson, J., Samuelsson, E., Moks, T., Ståhl, S., & Uhlén, M. (1996). Single-step trypsin cleavage of a fusion protein to obtain human insulin and its C peptide. *European Journal of Biochemistry, 236*(2), 656-661. https://doi.org/10.1111/j.1432-1033.1996.00656.x

Ju, C., Shen, Y., Ma, G., Liu, Y., Cai, J., Kim, I.-M., Weintraub, N. L., Liu, N., & Tang, Y. (2018). Transplantation of cardiac mesenchymal stem cell-derived exosomes promotes repair in ischemic myocardium. *Journal of Cardiovascular Translational Research, 11*, 420-428. https://doi.org/10.1007/s12265-018-9822-0

Koransky, M. L., Robbins, R. C., & Blau, H. M. (2002). VEGF gene delivery for treatment of ischemic cardiovascular disease. *Trends in Cardiovascular Medicine, 12*(3), 108-114. https://doi.org/10.1016/s1050-1738(01)00158-x

Kumar, S., Bhanjana, G., Sharma, A., Dilbaghi, N., Sidhu, M. C., & Kim, K.-H. (2017). Development of nanoformulation approaches for the control of weeds. *Science of The Total Environment, 586*, 1272-1278. https://doi.org/10.1016/j.scitotenv.2017.02.138

Kurozumi, K., Nakamura, K., Tamiya, T., Kawano, Y., Ishii, K., Kobune, M., Hirai, S., Uchida, H., Sasaki, K., Ito, Y., Kato, K., Honmou, O., Houkin, K., Date, I., & Hamada, H. (2005). Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. *Molecular Therapy, 11*(1), 96-104. https://doi.org/10.1016/j.ymthe.2004.09.020

Kwak, K.-A., Lee, S.-P., Yang, J.-Y., & Park, Y.-S. (2018). Current perspectives regarding stem cell-based therapy for Alzheimer's disease. *Stem Cells International, 2018*, 6392986. https://doi.org/10.1155/2018/6392986

Ladisch, M. R., & Kohlmann, K. L. (1992). Recombinant human insulin. *Biotechnology Progress, 8*(6), 469-478. https://doi.org/10.1021/bp00018a001

Lange, C., Bassler, P., Lioznov, M. V., Bruns, H., Kluth, D., Zander, A. R., & Fiegel, H. C. (2005). Hepatocytic gene expression in cultured rat mesenchymal stem cells. *Transplantation Proceedings, 37*(1), 276-279. https://doi.org/10.1016/j.transproceed.2004.11.087

Lei, J., Dai, P., Li, J., Yang, M., Li, X., Zhang, W., Zhou, G., WangzhenGuo, L. X., & Liu, X. (2021). Tissue-specific CRISPR/Cas9 system of cotton pollen with GhPLIMP2b and GhMYB24 promoters. *Journal of Plant Biology, 64*, 13-21. https://doi.org/10.1007/s12374-020-09272-4

Lei, Y., Haider, H. K., Shujia, J., & Sim, E. S. K. (2004). Therapeutic angiogenesis: Devising new strategies based on past experiences. *Basic Research in Cardiology, 99*, 121-132. https://doi.org/10.1007/s00395-004-0447-x

Lewis, T., & Potter, E. (2013). *Ethical consumption: A critical introduction*. (1st ed.). London, United Kingdom: Routledge.

Li, J., Wang, Z., He, G., Ma, L., & Deng, X. W. (2020). CRISPR/Cas9-mediated disruption of *TaNP1* genes results in complete male sterility in bread

wheat. *Journal of Genetics and Genomics, 47*(5), 263-272. https://doi.org/10.1016/j.jgg.2020.05.004

Li, L., Chen, X., Wang, W. E., & Zeng, C. (2016). How to improve the survival of transplanted mesenchymal stem cells in ischemic heart? *Stem Cells International, 2016*, 9682757. https://doi.org/10.1155/2016/9682757

Li, M., Sun, X., Yin, M., Shen, J., & Yan, S. (2023). Recent advances in nanoparticle-mediated co-delivery system: A promising strategy in medical and Agricultural Field. *International Journal of Molecular Sciences, 24*(6), 5121. https://doi.org/10.3390/ijms24065121

Lin, Q., Zong, Y., Xue, C., Wang, S., Jin, S., Zhu, Z., Wang, Y., Anzalone, A. V., Raguram, A., Doman, J. L., Liu, D. R., & Gao, C. (2020). Prime genome editing in rice and wheat. *Nature Biotechnology, 38*(5), 582-585. https://doi.org/10.1038/s41587-020-0455-x

Luque-Contreras, D., Carvajal, K., Toral-Rios, D., Franco-Bocanegra, D., & Campos-Peña, V. (2014). Oxidative stress and metabolic syndrome: Cause or consequence of Alzheimer's disease? *Oxidative Medicine and Cellular Longevity, 2014*, 497802. https://doi.org/10.1155/2014/497802

Manghwar, H., Lindsey, K., Zhang, X., & Jin, S. (2019). CRISPR/Cas system: Recent advances and future prospects for genome editing. *Trends in Plant Science, 24*(12), 1102–1125. https://doi.org/10.1016/j.tplants.2019.09.006

Mangi, A. A., Noiseux, N., Kong, D., He, H., Rezvani, M., Ingwall, J. S., & Dzau, V. J. (2003). Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nature Medicine, 9*, 1195-1201. https://doi.org/10.1038/nm912

Mann, N. P., Johnston, D. I., Reeves, W. G., & Murphy, M. A. (1983). Human insulin and porcine insulin in the treatment of diabetic children: Comparison of metabolic control and insulin antibody production. *British Medical Journal, 287*, 1580-1582. https://doi.org/10.1136/bmj.287.6405.1580

Markussen, J., Damgaard, U. L., Pingel, M. A., Snel, L., Sørensen, A. R., & Sørensen, E. (1983). Human insulin (Novo): Chemistry and characteristics. *Diabetes Care, 6*(S1), 4-8.

Martínez, M. I. S., Bracuto, V., Koseoglu, E., Appiano, M., Jacobsen, E., Visser, R. G. F., Wolters, A.-M. A., & Bai, Y. (2020). CRISPR/Cas9-targeted mutagenesis of the tomato susceptibility gene PMR4 for resistance against powdery mildew. *BMC Plant Biology, 20*, 284. https://doi.org/10.1186/s12870-020-02497-y

Mayer, J. P., Zhang, F., & DiMarchi, R. D. (2007). Insulin structure and function. *Biopolymers, 88*(5), 687-713. https://doi.org/10.1002/bip.20734

Messina, E., De Angelis, L., Frati, G., Morrone, S., Chimenti, S., Fiordaliso, F., Salio, M., Battaglia, M., Latronico, M. V., Coletta, M., Vivarelli, E., Frati, L., Cossu, G., & Giacomello, A. (2004). Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circulation Research, 95*, 911-921. https://doi.org/10.1161/01.RES.0000147315.71699.51

Miller, W. L., & Baxter, J. D. (1980, June). Recombinant DNA—A new source of insulin. *Diabetologia, 18*, 431-436. https://doi.org/10.1007/BF00261696

Mizumoto, H., Mizumoto, K., Shatos, M. A., Klassen, H., & Young, M. J. (2003). Retinal transplantation of neural progenitor cells derived from the brain of GFP transgenic mice. *Vision Research, 43*(16), 1699-1708. https://doi.org/10.1016/s0042-6989(03)00235-9

Moretti, A., Caron, L., Nakano, A., Lam, J. T., Bernshausen, A., Chen, Y., Qyang, Y., Bu, L., Sasaki, M., Martin-Puig, S., Sun, Y., Evans, S. M., Laugwitz, K. L., & Chien, K. R. (2006). Multipotent embryonic *isl1*$^{+}$ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell, 127*(6), 1151-1165. https://doi.org/10.1016/j.cell.2006.10.029

Morihara, K. (1990). Enzymatic semisynthesis of human insulin: An update. *Journal of Molecular Recognition*, *3*(5-6), 181-186. https://doi.org/10.1002/jmr.300030502

Mout, R., Ray, M., Yesilbag Tonga, G., Lee, Y. W., Tay, T., Sasaki, K., & Rotello, V. M. (2017). Direct cytosolic delivery of CRISPR/Cas9-ribonucleoprotein for efficient gene editing. *ACS Nano, 11*(3), 2452–2458. https://doi.org/10.1021/acsnano.6b07600

Murry, C. E., Soonpaa, M. H., Reinecke, H., Nakajima, H., Nakajima, H. O., Rubart, M., Pasumarthi, K. B., Virag, J. I., Bartelmez, S. H., Poppa, V., Bradford, G., Dowell, J. D., Williams, D. A., & Field, L. J. (2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature, 428*, 664-668. https://doi.org/10.1038/nature02446

Najar, M., Raicevic, G., Fayyad-Kazan, H., Bron, D., Toungouz, M., & Lagneaux, L. (2016). Mesenchymal stromal cells and immunomodulation: A gathering of regulatory immune cells. *Cytotherapy, 18*(2), 160-171. https://doi.org/10.1016/j.jcyt.2015.10.011

Nekrasov, V., Staskawicz, B., Weigel, D., Jones, J. D. G., & Kamoun, S. (2013). Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nature Biotechnology, 31*, 691-693. https://doi.org/10.1038/nbt.2655

Noguchi, H., Xu, G., Matsumoto, S., Kaneto, H., Kobayashi, N., Bonner-Weir, S., & Hayashi, S. (2006). Induction of pancreatic stem/progenitor cells into insulin-producing cells by adenoviral-mediated gene transfer technology. *Cell Transplantation, 15*(10), 929-938. https://doi.org/10.3727/000000006783981431

Obermeier, R., & Geiger, R. (1976). A new semisynthesis of human insulin. *Biological Chemistry, 357*(6), 759-767. https://doi.org/10.1515/bchm2.1976.357.1.759

Oddo, S., Caccamo, A., Shepherd, J. D., Murphy, M. P., Golde, T. E., Kayed, R., Metherate, R., Mattson, M. P., Akbari, Y., & LaFerla, F. M. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular Aβ and synaptic dysfunction. *Neuron, 39*(3), 409-421. https://doi.org/10.1016/s0896-6273(03)00434-3

Paukner, S., Kohl, G., & Lubitz, W. (2004). Bacterial ghosts as novel advanced drug delivery systems: Antiproliferative activity of loaded doxorubicin in human Caco-2 cells. *Journal of Controlled Release, 94*(1), 63-74. https://doi.org/10.1016/j.jconrel.2003.09.010

Perneczky, R., Alexopoulos, P., & Alzheimer's Disease euroimaging Initiative. (2014). Cerebrospinal fluid BACE1 activity and markers of amyloid precursor protein metabolism and axonal degeneration in Alzheimer's disease. *Alzheimer's and Dementia, 10*(S5), S425-S429.e1. https://doi.org/10.1016/j.jalz.2013.09.006

Phillips, M. I., & Sumners, C. (1998). Angiotensin II in central nervous system physiology. *Regulatory Peptides, 78*(1-3), 1-11. https://doi.org/10.1016/s0167-0115(98)00122-0

Phillips, M. I., & Tang, Y. L. (2008). Genetic modification of stem cells for transplantation. *Advanced Drug Delivery Reviews, 60*(2), 160-172. https://doi.org/10.1016/j.addr.2007.08.035

Pillarisetti, K., & Gupta, S. K. (2001). Cloning and relative expression analysis of rat stromal cell derived factor-1 (SDF-1): SDF-1 α mRNA is selectively induced in the rat model of myocardial infarction. *Inflammation, 25*, 293-300. https://doi.org/10.1023/a:1012808525370

Poggioli, T., Vujic, A., Yang, P., Macias-Trevino, C., Uygur, A., Loffredo, F. S., Pancoast, J. R., Cho, M., Goldstein, J., Tandias, R. M., Gonzalez, E., Walker, R. G., Thompson, T. B., Wagers, A. J., Fong, Y. W., & Lee, R. T. (2016). Circulating growth differentiation factor 11/8 levels decline with age. *Circulation Research, 118*(1), 29-37. https://doi.org/10.1161/CIRCRESAHA.115.307521

Portelius, E., Dean, R. A., Gustavsson, M. K., Andreasson, U., Zetterberg, H., Siemers, E., & Blennow, K. (2010). A novel Aβ isoform pattern in CSF reflects γ-secretase inhibition in Alzheimer disease. *Alzheimer's Research and Therapy, 2*(2), 7. https://doi.org/10.1186/alzrt30

Puchta, H., Dujon, B., & Hohn, B. (1993). Homologous recombination in plant cells is enhanced by in vivo induction of double strand breaks into DNA by a site-specific endonuclease. *Nucleic Acids Research, 21*(22), 5034-5040. https://doi.org/10.1093/nar/21.22.5034

Ramesh, G., Wood, A. C., Allison, M. A., Rich, S. S., Jensen, E. T., Chen, Y. I., Rotter, J. I., Bertoni, A. G., & Goodarzi, M. O. (2022). Associations between adherence to the dietary approaches to stop hypertension (DASH) diet and six glucose homeostasis traits in the Microbiome and Insulin Longitudinal Evaluation Study (MILES). *Nutrition, Metabolism, and Cardiovascular Diseases, 32*(6), 1418-1426. https://doi.org/10.1016/j.numecd.2022.03.014

Raskin, P., & Clements, Jr., R. S. (1991). The use of human insulin derived from baker's yeast by recombinant DNA technology. *Clinical Therapeutics, 13*(5), 569-578.

Regmi, D., Al-Shamsi, S., Govender, R. D., & Al Kaabi, J. (2020). Incidence and risk factors of type 2 diabetes mellitus in an overweight and obese population: A long-term retrospective cohort study from a Gulf state. *BMJ Open, 10*(7), e035813. https://doi.org/10.1136/bmjopen-2019-035813

Rui, Y., Varanasi, M., Mendes, S., Yamagata, H. M., Wilson, D. R., & Green, J. J. (2020). Poly(beta-amino ester) nanoparticles enable nonviral delivery of CRISPR-Cas9 plasmids for gene knockout and gene deletion. *Molecular Therapy Nucleic Acids, 20*, 661-672. https://doi.org/10.1016/j.omtn.2020.04.005

Ruttenberg, M. A. (1972). Human insulin: Facile synthesis by modification of porcine insulin. *Science, 177*(4049), 623-626. https://doi.org/10.1126/science.177.4049.623

Shaheen, A., & Abed, Y. (2018). Knowledge, attitude, and practice among farm workers applying pesticides in cultivated areas of the Jericho District: A cross-sectional study. *Lancet, 391*(S3). https://doi.org/10.1016/S0140-6736(18)30328-3

Shang, Y., Hasan, M. K., Ahammed, G. J., Li, M., Yin, H., & Zhou, J. (2019). Applications of nanotechnology in plant growth and crop protection: A review. *Molecules, 24*(14), 2558. https://doi.org/10.3390/molecules24142558

Sharma, R., Patil, N., & Sivaram, A. (2022). Ethical and safety concerns of recombinant DNA technology. In N. Patil & A. Sivaram (Eds.), *A complete guide to gene cloning: From basic to advanced* (pp. 159-165) Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-030-96851-9_10

Steinberg, F. M., & Raso, J. (1998). Biotech pharmaceuticals and biotherapy: An overview. *Journal of Pharmacy and Pharmaceutical Sciences, 1*(2), 48-59.

Szaraz, P., Gratch, Y. S., Iqbal, F., & Librach, C. L. (2017). *In vitro* differentiation of human mesenchymal stem cells into functional cardiomyocyte-like cells. *Journal of Visualized Experiments, 126*, e55757. https://doi.org/10.3791/55757

Tang, X., Lowder, L. G., Zhang, T., Malzahn, A. A., Zheng, X., Voytas, D. F., Zhong, Z., Chen, Y., Ren, Q., Li, Q., Kirkland, E. R., Zhang, Y., & Qi, Y. (2017). A CRISPR–Cpf1 system for efficient genome editing and transcriptional repression in plants. *Nature Plants, 3*, 17018. https://doi.org/10.1038/nplants.2017.18

Tang, Y. L., Shen, L., Qian, K., & Phillips, M. I. (2007). A novel two-step procedure to expand cardiac Sca-1+ cells clonally. *Biochemical and Biophysical Research Communications*, *359*(4), 877-883. https://doi.org/10.1016/j.bbrc.2007.05.216

Tang, Y. L., Zhao, Q., Qin, X., Shen, L., Cheng, L., Ge, J., & Phillips, M. I. (2005). Paracrine action enhances the effects of autologous mesenchymal stem cell transplantation on vascular regeneration in rat models of myocardial infarction. *Annals of Thoracic Surgery, 80*(1), 229-236. https://doi.org/10.1016/j.athoracsur.2005.02.072

Tang, Y. L., Zhao, Q., Zhang, Y. C., Cheng, L., Liu, M., Shi, J., Yang, Y. Z., Pan, C., Ge, J., & Phillips, M. I. (2004). Autologous mesenchymal stem cell transplantation induces VEGF and neovascularization in ischemic myocardium. *Regulatory Peptides, 117*(1), 3-10. https://doi.org/10.1016/j.regpep.2003.09.005

Tian, Y., Chen, K., Li, X., Zheng, Y., & Chen, F. (2020). Design of high-oleic tobacco (*Nicotiana tabacum* L.) seed oil by CRISPR-Cas9-mediated knockout of *NtFAD2–2*. *BMC Plant Biology, 20*, 233. https://doi.org/10.1186/s12870-020-02441-0

Tiedje, J. M., Colwell, R. K., Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., & Regal, P. J. (1989). The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. *Ecology, 70*(2), 298-315. https://doi.org/10.2307/1937535

Tiwari, J. N., Tiwari, R. N., & Kim, K. S. (2012). Zero-dimensional, one-dimensional, two-dimensional and three-dimensional nanostructured materials for advanced electrochemical energy devices. *Progress in Materials Science, 57*(4), 724-803. https://doi.org/10.1016/j.pmatsci.2011.08.003

Vasiljević, J., Torkko, J. M., Knoch, K.-P., & Solimena, M. (2020). The making of insulin in health and disease. *Diabetologia, 63*, 1981-1989. https://doi.org/10.1007/s00125-020-05192-7

Walsh, G. (2005). Therapeutic insulins and their large-scale manufacture. *Applied Microbiology and Biotechnology, 67*, 151-159. https://doi.org/10.1007/s00253-004-1809-x

Wang, X., Zhen, L., Miao, H., Sun, Q., Yang, Y., Que, B., Lopes Lao, E. P., Wu, X., Ren, H., Shi, S., Lau, W. B., Ma, X., Ma, C., & Nie, S. (2015). Concomitant retrograde coronary venous infusion of basic fibroblast growth factor enhances engraftment and differentiation of bone marrow mesenchymal stem cells for cardiac repair after myocardial infarction. *Theranostics, 5*(9), 995-1006. https://doi.org/10.7150/thno.11607

Wang, Y., O'Malley, Jr., B. W., Tsai, S. Y., & O'Malley, B. W. (1994). A regulatory system for use in gene transfer. *Proceedings of the National Academy of Sciences of the United States of America, 91*(17), 8180-8184. https://doi.org/10.1073/pnas.91.17.8180

Wright, D. A., Townsend, J. A., Winfrey, Jr., R. J., Irwin, P. A., Rajagopal, J., Lonosky, P. M., Hall, B. D., Jondle, M. D., & Voytas, D. F. (2005). High-frequency homologous recombination in plants mediated by zinc-finger nucleases. *The Plant Journal, 44*(4), 693-705. https://doi.org/10.1111/j.1365-313X.2005.02551.x

Yin, K., Gao, C., & Qiu, J. L. (2017). Progress and prospects in plant genome editing. *Nature Plants, 3*, 17107. https://doi.org/10.1038/nplants.2017.107

Zhang, H., Demirer, G. S., Zhang, H., Ye, T., Goh, N. S., Aditham, A. J., Cunningham, F. J., Fan, C., & Landry, M. P. (2019). DNA nanostructures coordinate gene silencing in mature plants. *Proceedings of the National Academy of Sciences of the United States of America, 116*(15), 7543-7548. https://doi.org/10.1073/pnas.1818290116

Zhang, J., Zhang, X., Chen, R., Yang, L., Fan, K., Liu, Y., Wang, G., Ren, Z., & Liu, Y. (2020). Generation of transgene-free semi dwarf maize plants by gene editing of *gibberellin-oxidase20–3* using CRISPR/Cas9. *Frontiers in Plant Science, 11*, 1048. https://doi.org/10.3389/fpls.2020.01048

Zieliński, M., Romanik-Chruścielewska, A., Mikiewicz, D., Łukasiewicz, N., Sokołowska, I., Antosik, J., Sobolewska-Ruta, A., Bierczyńska-Krzysik, A., Zaleski, P., & Płucienniczak, A. (2019). Expression and purification of recombinant human insulin from *E. coli* 20 strain. *Protein Expression and Purification, 157*, 63-69. https://doi.org/10.1016/j.pep.2019.02.002

Zivin, M., & Pregelj, P. (2008). Prolonged treatment with donepezil increases acetylcholinesterase expression in the central nervous system. *Psychiatria Danubina, 20*(2), 168-173.