Evolution of Genetic Engineering in Medicine: Recombinant DNA and CRISPR Gene Editing Technologies

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Abstract

Genetic engineering is a new and upcoming field constantly developing. This field has so many possibilities and opens up new doors for ideas and solutions we have not seen before. Gene editing allows us to make edits, deletions, and substitutions in sequences. Recombinant technologies allow us to use aspects of other organisms' DNA. Gene editing technology has the potential to improve or possibly reverse symptoms of inherited or acquired genetic diseases. There are two predominant modes of genetic engineering in medical biotechnology: recombinant DNA and CRISPR gene editing. Recombinant DNA technology uses another organism as a host to create the desired outcome for the source. To perform edits using CRISPR, the utilization of recombinant technologies is important. Recombinant DNA technology allowed for the manipulation of sequences while often using hosts like bacteria. CRISPR-mediated gene editing is a technology that has grown from and is also partially dependent on recombinant DNA technology. Gene editing is constantly evolving and adapting to new needs and possibilities. There are many promising discoveries and the ongoing development of recombinant and gene editing technologies allows for new possibilities and future advancement in medical biotechnology. I will review recombinant DNA technology and its development into CRISPR gene editing technology in this article and then describe several real-world applications of these technologies to improve human health.

Recombinant DNA technology

The foundation of medical biotechnology is recombinant DNA. This subfield was a transformative point in DNA technologies, which propelled newer more advanced technology. Recombinant DNA technology was developed in the 1970s by Paul Berg, Stanley Cohen, and Herbert Boyer at Stanford University. Recombinant DNA technology was the genesis of genetic engineering in not only medicine but agriculture as well (Gill et al., 2023). Recombinant DNA works by isolating a specific gene or parts of DNA from one organism and inserting them into the DNA of another, allowing scientists to combine DNA from different host sources and generate new compositions of DNA that wouldn't occur naturally. This process can apply to different species and organisms. Recombinant DNA technologies are growing in many fields, like medicine and agriculture.

There are several steps in creating recombinant DNA. First, the desired DNA fragment, which encodes for a protein, is isolated from a donor species via polymerase chain reaction (PCR). Invented in 1983 by Kary Mullis, PCR works to rapidly copy and amplify a targeted piece of DNA repeatedly (Kaunitz et al., 2015). Next, restriction enzymes, or other techniques, assemble the fragments. Enzymes like DNA ligase are used to “glue” the fragments together. Restriction enzymes cut DNA at specific sequences. Using polymerase chain reaction and restriction enzymes, the fragment is isolated and cut. The DNA fragment is assembled into a circular construct known as a plasmid. This allows for the replication inside of a host species, commonly yeast or *E. coli*. The recombinant plasmids are then grown up. The copies inside the host species are purified. Lastly, the recombinant DNA is introduced into the host organism, where the plasmid is transcribed and translated to produce the desired protein (Figure 1). There are

numerous ways for the DNA to be inserted back into the host such as electroporation, microinjection, virus transduction, or lipid nanoparticles. The technological development opened new doors and possibilities.

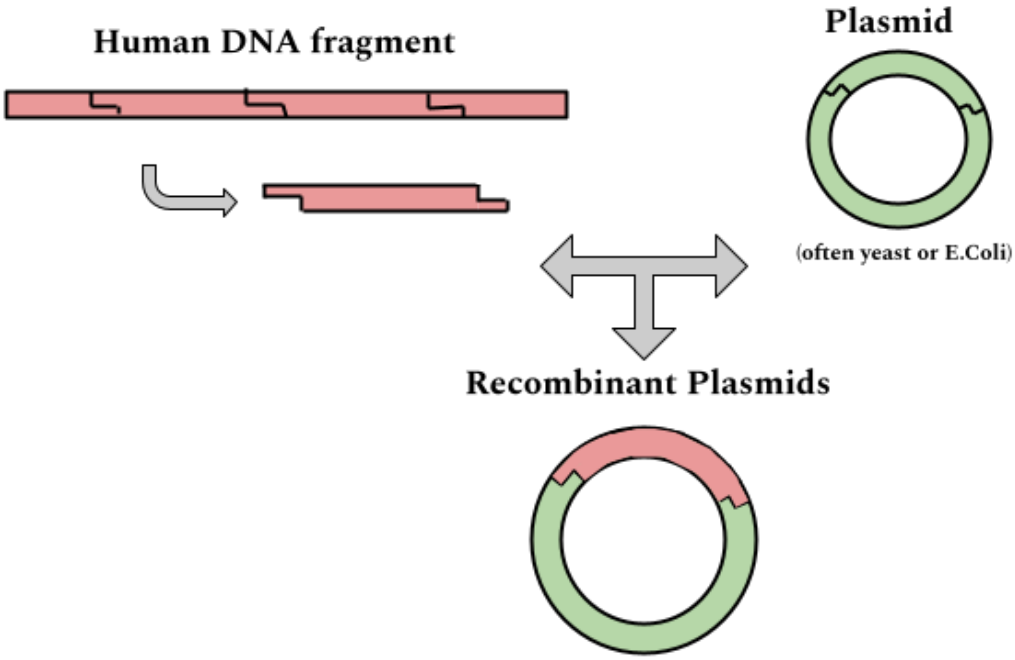

Figure 1. The desired DNA fragment, or trait, is amplified from a donor species via PCR. Restriction enzymes or other techniques are used to assemble the fragments. Using PCR, the fragment is isolated and cut. The DNA fragment is assembled into a circular construct known as a plasmid. Replication occurs inside a host species, commonly yeast or *E. coli*. Then, the recombinant DNA is introduced into the host organism, where it's replicated and produces the desired trait or protein, such as insulin.

Going back to the first step in creating recombinant DNA, Polymerase Chain Reaction works to copy and amplify a targeted DNA segment repeatedly. PCR requires the DNA you intend to make copies of, DNA primers, nucleotides, and heat-resistant DNA polymerase enzymes like taq polymerase, as outlined in Figure 2.

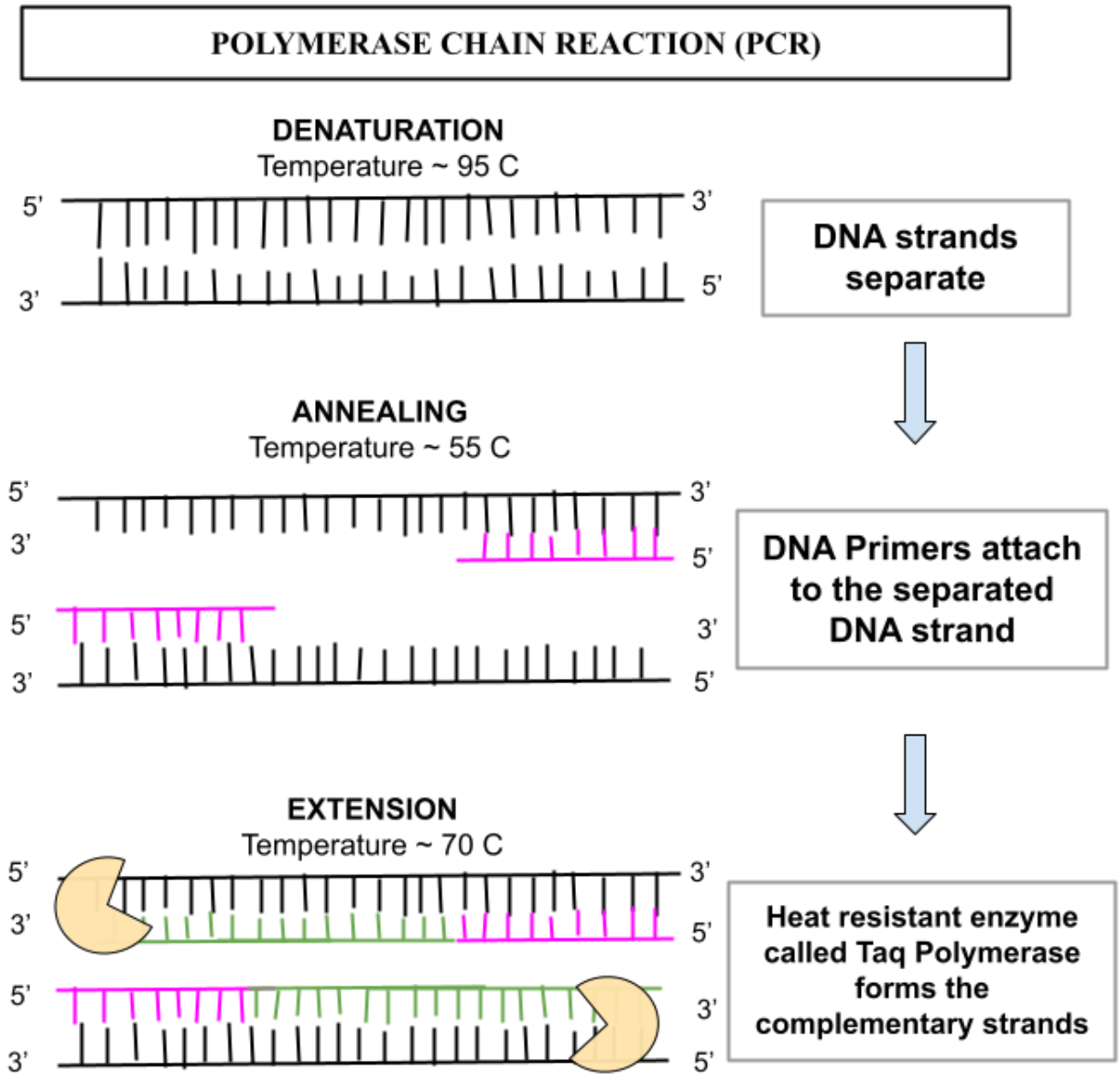

Figure 2. Overview of PCR. To begin, the first step involves denaturing which causes the double helix structure to untwist and separate at the base pairs. Next, the two strands that normally form the double helix separate due to denaturing or “melting”. Denaturing in this case means to heat the DNA sample to a high temperature that breaks the hydrogen bonds present between nucleotides on the opposite sides of the strands. The next step, which is called annealing, lowers the temperature. During this step, short sequences of DNA called primers, which are complementary to the base pairs, bind to the complementary sequences on the single-stranded DNA. Lastly, in extension, the temperature is raised once again. A DNA polymerase enzyme like Taq polymerase is then used to synthesize or create a new DNA strand complementary to the single-stranded template (Zhu, 2020). This results in two new double-stranded DNAs that match

the original target DNA fragment. When the reaction is repeated, those two pieces double to 4, and so on.

The simplicity and productivity of PCR have made it a vital tool for various uses and it continues to be essential in many critical applications such as diagnostics, fingerprinting, and virus detection. Polymerase chain reaction has transformed so many fields. Although the main focus is DNA amplification, as it can amplify DNA at rapid rates and efficiently, there are many uses for PCR. These applications allow scientists and researchers to synthesize functional DNA constructs to produce a product of interest inside of the host. Some of the main applications seen throughout other fields are genetic research, mutation detection, environmental science, medical research, forensics, and the development of medication and drugs (Zhu et al, 2020). Recombinant DNA is utilized in numerous areas of medicine and biotechnology. A prominent example is synthetic insulin for the treatment of Type I diabetes. In the 1920s, when insulin was first discovered as a treatment for diabetes, the hormone had to be extracted from live, non-human animals. This process of procuring naturally-produced insulin was time-consuming and laborious, yet it remained the standard practice for nearly 50 years (Quianzon et al., 2012).

In the late 1970s, Herbert Boyer and a team of scientists from the University of California San Francisco and Stanford University used recombinant techniques to develop synthetic insulin. PCR was not developed at this time as it was later invented in 1983. Researchers assembled the entire insulin gene sequence using 3-base-pair fragments, one at a time in a specific order. Next, they incorporated this into a plasmid using restriction enzymes (Beutler, 1978). This required inserting human insulin-encoding DNA into yeast or bacteria cells. These cells can then be grown to high numbers and instructed to produce human insulin which could be then isolated from organisms and used in human patients (Vajo, 2001). This large-scale application of recombinant DNA technology to the medical field laid the foundation for what would become one of the nation’s most prolific biotechnology companies: Genentech. The use of genetic engineering to produce synthetic insulin afforded a more consistent, safer, and scalable drug development process. Importantly, the use of synthetic insulin hormones eliminated the risk of adverse, allergic reactions that were associated with animal-derived insulin (Quianzon et al., 2012).

Indeed, Boyer's achievement at Genentech was a major milestone and changed the treatment of diabetes immensely (Quianzon et al., 2012). As recombinant technology further developed it opened the possibilities for novel, more targeted treatments in which specific properties of a drug could be tailored to a patient's needs. This resulted in increased drug effectiveness and safety.

Similarly, vaccine development has been enhanced by recombinant technology. Traditionally, vaccines require involved use of weakened or attenuated forms of a whole pathogen. Recombinant DNA technology enabled the production of subunit vaccines, whereby only select proteins or antigens from the pathogen are supplied by the vaccine rather than the whole form. This removes the risk of virulence or infection upon vaccination while maintaining the desired immune response (Nascimento et al, 2012).

Another application of recombinant technology is in diagnostics. Diagnostic tests are used to detect the presence of pathogens or disease biomarkers in a patient sample such as blood or urine. Recombinant technology aids in this detection by identifying the specific binding sites in which antibodies bind to antigens (Ebrahimi et al, 2022). Advanced tools like these have shown great potential. These technologies have not only revolutionized research but have pushed to open a range of wide possibilities.

Although it primarily focuses on treating genetic disorders, an alternate approach provides patients with “treatment” not a permanent cure. Recombinant DNA technologies work in mitigating and managing the symptoms. More advanced technologies like CRISPR-Cas9 have the potential to possibly cure genetic diseases by making direct edits to the DNA fragment within the genome of the host organism. The crucial transition between recombinant and editing technologies is the idea of treatment vs cure. Both technologies have had a significant impact and are constantly developing.

CRISPR-Based Gene Editing

Gene editing and recombinant DNA technology are both approaches to biotechnology, the two have developed and grown off of each other. Gene editing is a more precise, targeted approach to modifying specific areas of the genome. Gene editing is a molecular biology technique that is used as a powerful tool to get more specific and targeted modifications of an organism's DNA. It allows for the modification of a specific sequence with precision and accuracy. Gene editing is altering a living organism by replacing, removing, or adding a DNA sequence to improve or correct something (Doudna, 2020).

One of the most central forms of gene editing is CRISPR-Cas9. “CRISPR" stands for Clustered Regularly Interspaced Short Palindromic Repeats and "Cas9" means the CRISPR-associated protein 9. CRISPR technologies evolved from fighting viral DNA in bacteria, an immune system in bacteria, to being modified for all DNA types. CRISPR-Cas9 was a revolutionary piece of gene editing technology. The beginnings of CRISPR go back to the 1980s when a research team led by Yoshizumi Ishino discovered repetitive DNA sequences in *E. coli*. Later on in the 2000s, Francisco Mojica also started noticing repetitive sequences. In 2005, Philippe Horvath and Rodolphe Barrangou found CRISPR sequences and connected the idea to bacterial immune response. Then in 2012, Jennifer Doudna and Emmanuelle Charpentier discovered that the Cas9 protein could be programmed with an RNA molecule to target specific DNA sequences (Jinek et al., 2012). This opened up numerous possibilities for the use of CRISPR-Cas9. To build on this, in January 2013, Feng Zhang, who was a researcher at the Institute of MIT and Harvard was the first to adapt CRISPR-Cas9 for editing in eukaryotic cells such as humans (Cong et al, 2013).

Many have found this technology exceptionally remarkable due to its capability to act as molecular scissors (Figure 3). For a CRISPR gene edit to proceed, the researcher must first identify the specific sequence of DNA that they desire to edit and then design a guide RNA that matches the target sequence to tell the Cas enzyme where to cut. CRISPR can act as a “scissor” and make a simple cut. It relies on the gRNA which is designed to match the DNA sequence being modified. The gRNA and Cas9 protein are then introduced to the cell and gRNA guides the Cas9 to where the edit needs to be made (Swartjes, 2020). The Cas9 “scissor” then

makes the cut and the ends are joined again. To further describe the “edit”, gRNA directs the Cas9 where the cut will be made and the cell has repair pathways that fix the “cut” in the DNA. One of the pathways is non-homologous end joining which is error-prone. When errors occur, things like mutations can be introduced which disrupt the normal function of the gene and can lead to a non-functioning protein (Gostinska, 2022).

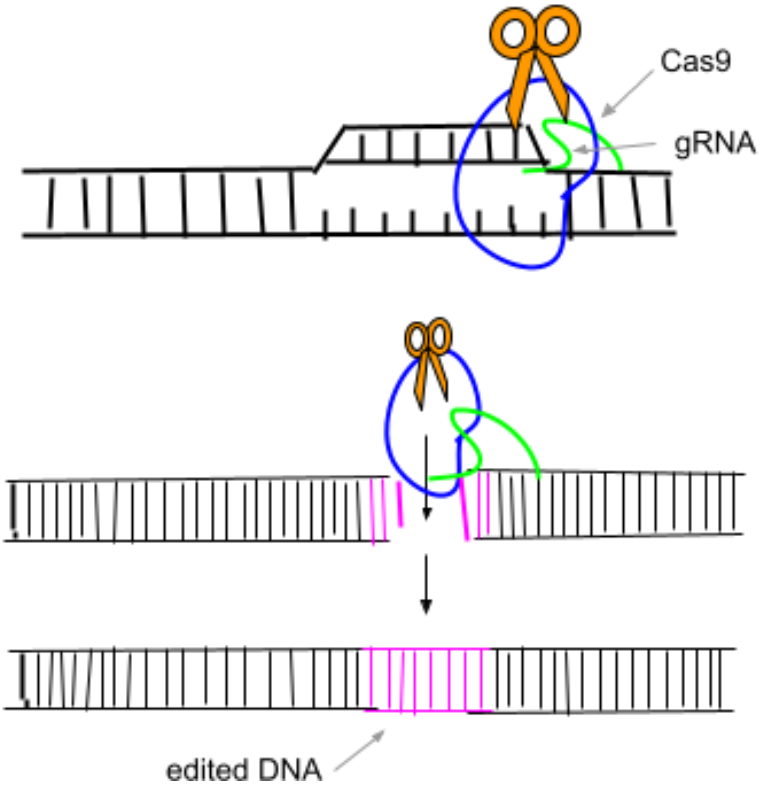

Figure 3. A guide RNA guides the Cas-9 where to cut. The cell itself repairs the cut in a way that leads to a change in the DNA. The Cas-9 protein acts as “molecular scissors” and can make edits efficiently and directly.

Similarly, single base edits involve making a change in a single base, for example changing a cytosine to an adenine. Importantly, base editors utilize a variant of Cas9 that is often “kinase-dead” which prevents them from cutting through both strands. The base editor converts the targeted base to a different base pair which creates a single base pair without affecting the surrounding sequence (Yadav, 2020). To summarize, CRISPR/Cas9 can be used to create a targeted mutation in a sequence, add new genetic information, or perform very precise edits to change the DNA sequence in a targeted way. These strategies have opened the door for many real-world applications of CRISPR gene editing, as will be discussed in the following section.

Applications of CRISPR gene editing

CRISPR is being used to test for COVID-19 RNA. The test is conducted by first acquiring a respiratory or nasal sample from the patient. The RNA from the sample is extracted to test for the presence of COVID-19 (Avery et al., 2022). Reverse transcription is used to convert RNA into DNA so that the CRISPR/Cas9 complex can recognize it. The DNA is amplified through polymerase chain reaction. CRISPR-Cas9 system uses a gRNA which is designed to use a

specific sequence of DNA if it is present. If the gRNA encounters the target sequence, the CRISPR-associated enzyme is then guided there to the target sequence. When the CRISPR system has binded to the targeted area it activates the cleavage activity which then cuts a reporter molecule that releases a fluorescent signal which signals the detection of viral DNA. The absence of fluorescence is usually an indication of no viral DNA, and the presence of fluorescence indicates positive viral DNA results. Recently, this method has been further developed into a more direct CRISPR-based test that doesn't require reverse transcription and instead uses Cas proteins to identify and cut RNA instead of DNA (Gaanbatar, 2021).

Another application of CRISPR gene editing is to treat sickle cell anemia. Sickle cell anemia is caused by a single nucleotide mutation in the hemoglobin gene, resulting in abnormal hemoglobin. This causes the red blood cells to become deformed and less flexible (Martin et al., 2006). CRISPR technologies can first target the hemoglobin gene and directly modify the faulty gene. In sickle cell anemia, the goal is to specifically target the mutated gene that is the cause of the abnormal hemoglobin being produced. Then the CRISPR-Cas9 binds to the location, and the Cas9 enzyme cuts out the DNA at the specific area. After its cut, the cell can naturally repair the cell. Similarly, another approach to CRISPR involvement in sickle cell includes targeting a negative repressor fatal hemoglobin instead of the mutant gene itself (Park,2021). A repair template is then needed to “fix” the break in the DNA which allows for any mutation to be corrected. This is a new field of opportunities, and it's currently being researched regarding its effectiveness and safety.

Huntington's disease is also an area with potential applications for CRISPR. Huntington's is a neurological disease caused by a mutation in the Huntington gene. The mutated Huntingtin protein builds up in the brain, which leads to progressive damage in the nerve cells, and it causes other cognitive and psychiatric problems. Similar to the process of CRISPR being used in sickle cell, first, it targets the gene or the mutated region. The gRNA binds them to the targeted area and then cuts the DNA, forming a double-stranded break. The fix uses DNA repair systems as mentioned previously, to insert the sequence for normal Huntingtin protein production. The result is that cells no longer produce the mutant protein, which could reverse the pathology of the disease. A challenge particular to this condition is its complexity which requires delivery of CRISPR/Cas9 system to the brain tissue which is impossible or extremely invasive. In contrast, when treating something like sickle cell, you must edit blood cells which can be acquired through draw or marrow and then reintroduced to a patient (Park, 2021). Current efforts and research are being directed to explore delivery methods that are not as invasive such as lipid nanoparticles or antibody-directed approaches.

Ethical Implications of Genetic Engineering in Medicine

Many issues can occur in gene editing. Pleiotropic effects occur when a change to one element impacts the functionality of some seemingly unrelated function. This relates to the debate of whether new advancements in gene editing are safe for humans. A single change can have other off-target effects on the rest of the genome. Changes like insertions, deletions, and substitutions can have other unanticipated effects. CRISPR-Cas9 can be used to make edits at specific parts of the DNA. Off-target editing can occur even with technologies such as CRISPR-Cas9. An off-target sequence can be recognized by the CRISPR/Cas9 and it can

cause unexpected edits and potential mutations in the genome. Specifically, if the off-target site is in a growth factor receptor it can promote cancerous growth.

Many ethical concerns have also risen in the debate on whether or not gene editing is okay to implement. Many ideas like consent, medical problems, future effects, inequality, positive medical potential, and unintended effects are critical in the debate regarding ethics and hesitancy (Joseph, 2022). A concern that comes up in the debate is the effect on future generations, it debates whether or not the genetically modified effects should pose a concern to the next generation inheriting them. Changes in genetic material that is passed down have concerns like safety as well as consent. It raises questions on long-term impacts that we haven't fully assessed and have not been tested yet. On one hand, equal access is another key point that has repeatedly been brought up, people question if genetic modifications should be allowed if they are not equally accessible to all levels of social class. Since not everyone can afford this relatively new and pricey technology, it is unfair for people of the richer class to be able to genetically modify a genome (Joseph et al, 2022). Regulation and consideration are imperative in balancing scientific evolution and ethical concerns. Similarly the term “designer babies” is an umbrella term for talking about creating offspring that are genetically modified or edited for specific traits, it also raises numerous ethical questions and challenges on what it means to have diversity and express human values. Many believe that humans should not be genetically altered because they have been biologically created to be unique and person (Gostinska, 2022). However, genetic modification has been showing promising results for many fields of medical biotechnology, with new solutions that would not have been possible otherwise. Others argue that gene editing should be used solely for medicine and health (Gostinska, 2022). Genetic engineering has the potential for finding cures and new research for disease, illness, and even personalized medicine.

Conclusion

Overall, the field of medical biotechnology has encompassed a vast area of possibility and development and achieved numerous milestones in science, genetics, and medicine. Medical biotechnology constantly evolves through advancements such as insulin advancement, CRISPR-Cas9, and more precise technologies. Although there are many upcoming advances and discoveries this topic still raises futuristic questions relating to ethics, acceptability, and impact which are all important to consider. Rapidly advancing ideas and work are opening up new research to cures for genetic diseases, cancer therapies, neurological disorders, and even enhanced human traits. Though there are medical and ethical concerns, advancements could lead to newly progressing technologies and applications. Gene editing continues to evolve and grow to new levels while simultaneously adapting to individualized projects and research.

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