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PRODUCTION OF INSULIN USING RECOMBINANT DNA TECHNOLOGY

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Abstract – Biotechnology is a technology-related branch of biology. It has so many applications in the fields of agriculture, pharmaceutical and human medicine. Pharmaceutical biotechnology nevertheless has its cornerstones in fermentation and bioprocessing, but the paradigm shift created through biotechnology and pharmaceutical research has resulted in an up-to-date concept. The biotechnological revolution has redefined the processes of drug research, development, manufacturing and marketing. Insulin is one of the finest inventions in medical science in the field of biotechnology applications. Human insulin was formulated in the laboratory under in vitro conditions using recombinant DNA technology that is used for the treatment of a variety of diseases and is widely used in the clinical research industry. Human recombinant insulin was one of the first products of biotechnology. It has been developed in response to the need for consistent and sufficient global supply. This paper will discuss the production of human insulin formulations and the place of recombinant DNA technology in society.

Keyword - Biotechnology, pharmaceutical, recombinant DNA technology, human insulin

1.0 INTRODUCTION

A. Recombinant DNA in Medical Biotechnology

Molecular cloning of DNA sequence into extrachromosomal DNA elements such as plasmids, that may be promulgated in a bacterial host such as *Escherichia coli* mainly refers to the Recombinant DNA technology [1]. Plasmids are discovered in several bacterial species, and can be transmitted within a species from one organism to another by conjugation that leads to cell transformation. Recombinant DNA technology had developed new therapeutics, to produce engineering crops and to create transport fuels and chemicals for industrial production [1]. Recombinant DNA techniques in medicine can be used to diagnose diseases and examine their pathogenesis. It also can identify carriers of genetic disorders. Recombinant DNA

techniques also can be used for large-scale production of medically significant peptides that would otherwise be in high demand, such as human insulin, growth hormone and interferon. The first idea for recombinant DNA technology originated from the detection of restriction enzymes in bacteria by Werner Arber which ruin foreign viral DNA molecules [1]. From this finding, geneticists have accomplished to "cut" and "paste" DNA molecules, and have evolved and patented novel restriction enzymes for cutting and pasting. The growth of recombinant DNA technology was further progressed in 1972 through the cooperation of Stanley Cohen and Herbert Boyer [1]. Furthermore, they founded the first company that centred on recombinant DNA technology (Genentech) in 1976. The first medicinal biotechnology drugs, which is human insulin, were developed using this technology. The invention of recombinant DNA technology transformed biological development and brought about a series of significant changes. It provided new technological advances to generate a broad variety of therapeutic products with instant consequence in medical genetics and biomedicine by altering microorganisms, animals and plants to produce medically effective substances [2]. Many pharmaceutical biotechnology is recombinant that plays a crucial aspect in human lethal diseases. In 1997, the Food and Drug Administration (FDA) approved more recombinant drugs than in the last few years combined; which consists of AIDS, cancers (Kaposi's sarcoma, leukaemia, and colorectal, kidney, and ovarian cancers), anaemia, Gaucher's disease, haemophilia A, severe combined immunodeficiency disease, and Turner's syndrome and many more [2].

B. Diabetes

Diabetes mellitus is a disease that has been recognized since ancient Egyptian times. Until the early 1920s, the diagnosis of diabetes was equivalent to the death sentence [3]. Physicians Allen and Joslin had endorsed a method to control the diabetic diseases which is fasting and calorie-restricted diet [4]. This led to some change in glycosuria and acidosis, a decrease in coma and a delay in death among children with diabetes. Both patients with diabetes had been advised to reduce their dietary starch and sugar consumption and people who are obese had been recommended to lose weight.

Diabetes mellitus, generally referred to as diabetes, is a metabolic condition that causes elevated levels of sugar in the blood [5]. The insulin hormone converts blood sugar into the cells that are used for storage or energy. With diabetes, the body either does not produce enough insulin or can't use insulin efficiently. Untreated high blood sugar in diabetes can cause damage to the kidneys, nerves, eyes and other organs. There are several types of diabetes, and the first one is Type 1 diabetes, which is autoimmune. The immune system assaults and destroys the pancreatic cells where insulin is stored [5]. It is unclear what triggered the assault and this type is found in about 10% of people with diabetes. Secondly, Type 2 diabetes occurs when the body becomes resistant to insulin, and blood sugar builds up [5]. Third, prediabetes occurs when blood sugar is higher than normal, but not too high to be classified as Type 2 diabetes. Fourthly, gestational diabetes, which during pregnancy is high in blood sugar. Placenta-produced insulin-blocking hormone triggers this kind of diabetes [5].

C. Insulin

Insulin is anti-diabetic hormone in the body that is produced in large quantities only in the β-cells of the Langerhans islet in the pancreas [3]. Insulin deals with the nutrient surplus. During and after meals it is secreted as blood nutrient levels grow. Depending on the type of food eaten, the rate of formation changes with the gastrointestinal hormones and neuronal control. Beta cells will rapidly react to a spike in the glucose concentrations contained in blood by discharging stored insulin; generating more insulin at the same time [6]. Insulin that circulates in the body has a half-life of around 5 minutes. It is easily broken down by enzymes, and eliminated from the blood by the liver or kidneys. Insulin works by binding specific receptors to the surface of insulin-sensitive tissues such as the skeletal muscle, cardiac muscle, fatty tissue and leukocytes [7]. The insulin receptor is a tyrosine kinase activated by insulin binding which results in the tyrosine phosphorylation of a specific set of intracellular proteins. Insulin receptor sensitivity is the main component in preserving normal cell function [6]. Insulin reduces the

blood glucose composition by forming glycogen from converting glucose. Such intervention encourages the preservation at a later date of the remaining nutrients to be used [6]. It also promotes cell growth and cell differentiation.

Insulin antagonizes glucagon so that pre-stored fuel is not used. The cells of the brain, lungs, liver and red blood consume and use glucose without the insulin. Hence insulin also stimulates glycogen synthesis in the liver [6]. Diabetes mellitus, hypertension and other cardiovascular diseases can result when insulin resistance, insufficiency, or failure is present. Maintaining blood glucose levels is important to normal body function [6].

D. Analogue Insulin vs Animal Insulin

The discovery of insulin for patients with diabetes during 1922 has witnessed an improvement in medicine and therapy. Frederick Banting, an orthopedic surgeon, had the concept of isolating pancreatic islet extract by connecting the dog's pancreatic duct and maintaining it alive until the acini degenerated and the islet isolated [4]. Dr Frederick G. Banting and his assistant, Charles H. Best, both from the University of Toronto, began their experiment in 1921 and the result revealed that the dog's depancreatic produced diabetes and that intravenous injection of their pancreatic extract called islet in had decreased blood glucose to normal levels. At the end of 1921, the biochemist J.B. Collip joined the group and helped humans purify the islet. Leonard Thompson, a Canadian boy is the first diabetic human being to receive Banting and Best's crude insulin preparation derived from canine pancreas and consequently in mild blood glucose decline [4]. Over several years, insulin was extracted from pancreatic extract from porcine or bovine. Insulin from porcine is distinguished by only one amino acid from human insulin and is used gradually to replace bovine insulin which contradicts from human insulin by three amino acids [8].

Eli and company started extracting insulin from the animal pancreas but they began to worry about a potential insulin shortage in the early 1970s. Insulin needs in the world were produced almost entirely from pancreas glands of porcine and bovine, which were obtained as a side product from the meat industry. With demand for meat, this supply shifts and is not open to the needs of the diabetic world. Nonetheless, in the United States, the availability of pancreas glands declined rapidly from 1970-1975 and stayed on a plateau at this lower point in the following years [9]. There is no reliable way to divine the availability of future gland supplies while they expected that insulin demand will continue to increase; Eli and the company were worried whether or not the availability of pancreatic bovine and porcine glands would not be adequate to meet the inevitably of insulin-dependent diabetics. Due to the ambivalence of the insulin provider and the estimation of insulin requisite, it seemed not only advisable but also an obligation of the scientific community and insulin manufacturing to find replacement to animal sources for insulin supply to the diabetic world [9].

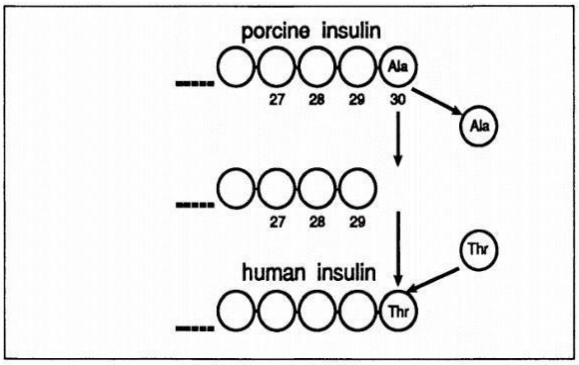

Figure 1: Conversion of insulin from porcine to human (semisynthetic) insulin [9].

Stanley Cohen and Herbert Boyer's revolutionary research, who invented the cloning technique for DNA, marked the advent of genetic manipulation that made it possible for the gene to pass between various biological species. Their discovery led to many recombinant proteins being produced with therapeutic applications such as insulin and growth hormone [10]. In 1978 David Goeddel and his Genentech colleagues used and merged the insulin chains A and B expressed in *Escherichia coli* to prepare the first recombinant DNA human insulin [4]. Recombination refers to the interaction of two DNA molecules in a segment of DNA. Genentech scientists infused the genes for both insulin chains into a K-12 strain of *Escherichia coli*, and after isolation and purification, disulphide bonds connected the A and B chains to generate human insulin. Researchers also employed recombinant DNA technology in the Lily Research Laboratory to

produce human proinsulin, the insulin precursor [9]. The positive human insulin production by recombinant DNA in *E. coli* was declared in 1978. The primarily rDNA-based insulin was marketed in 1982, with Humulin ® R (rapid) and N (NPH, intermediate-acting) [4].

E. Classification of Insulin

Analogue insulin is a sub-group of human insulin. Analogue refers to something similar to something else. Therefore, analogue insulin is an analogue that has been designed to mimic the body's natural pattern of insulin release [11]. Insulins made from synthetics are considered human insulin analogues. Analog is grown in the laboratory but genetically engineered to produce either a more rapid-acting or more consistently acting insulin form [12]. There may have been benefits of controlling blood sugar. Insulin analogue is a type of insulin that is produced using genetic engineering. This includes the utilization of human insulin molecules which offer pharmacokinetic benefits in the absorption, distribution, metabolism and excretion of insulin [6]. Type 1 and Type 2 diabetes are treated with several specific insulins. Whilst insulin is predominantly injected, an inhalation conformation is also acquirable. Oral formulations were not widely used because they deteriorated rapidly [6].

i. Long-acting Insulin

Most long-acting forms of insulin are administered once a day. Insulin glargine (Lantus) is a new long-acting human biosynthetic insulin analogue evolved by Aventis Pharmaceuticals and permitted for use in patients with Type 1 and Type 2 diabetes mellitus in 2000 by the United States Food and Drug Administration and the same year by the European Medical Product Assessment Agency [13]. This analogue outcomes from the elongation of the C-terminal cease of the insulin B chain through two arginine residues, as well as the substitution of the A21 asparagine residue with glycine [13]. These adjustments induced the isoelectric point to shift from pH 5.4 to human insulin 6.7, making insulin glargine less soluble at physiological pH tiers. After subcutaneous injection, insulin glargine precipitates into subcutaneous tissues which hinder its inclusion and prolong its period of action [13].

ii. Rapid-acting Insulin

Rapid-acting insulins are designed for insulin coverage at mealtimes. Several forms are also matched for Type 1 diabetes which covers a particular amount of carbohydrates [6]. This gives convenience for patients with their food. The rapidly-acting insulin is generally injected 5-10 minutes before a meal or at the beginning of a meal. This offers meal coverage, preventing a blood glucose spike. The insulin with rapid action includes: Insulin Lispro U-100 (Humalog and Admelog), Insulin Glulisine (Apidra) and Insulin Aspart (Novolog). They're all available in both disposable pens and vials [6]. Up to 60 units per injection can be used with the disposable pens.

iii. Ultra rapid – acting insulin

Insulin Aspart (Fiasp) is a newer kind of insulin referred to as ultra-rapid acting insulin. This permits for an initiation of only 2.5 minutes compared to the 15-minute onset of rapid-acting insulins (inclusive of the contemporary formulation of Insulin Aspart, marketed as Novolog) [6]. Fiasp can be used as a pen in addition to vials. Ultra-rapid insulin could be the best way for use in insulin pumps due to the fact it is exceptional to reduce high blood glucose speedily [6].

iv. Inhaled (short – acting) insulin

The first configuration of inhaled insulin was branded as Exubera in 2005, but was withdrawn because its larger administration device turned into not easy to manage or use [6]. Afrezza entered the market in 2004 which offered the alternative of insulin without injection [6]. Insulin ought to be treated with a basal insulin and not the only form of insulin used, this is for diabetes Type 1. Afrezza in blister packs provides a lightweight, disposable inhaler and 4, 8, or 12 cartridges. Since the affected person ought to take round doses to the closest 4 dosing units, which is less powerful than other insulins. Relevant inhalation techniques should be found to accurately assess the absorbed extent. The onset and offset of insulin inhaled is faster than with rapidly-acting injectable insulin, a 20% reduction in dose was needed twice each day by way of NPH [6].

v. Older (intermediate – acting) type of insulin

Older forms of insulin include regular insulin (Humulin R and Novolin R) and neutral protamine Hagedorn (or NPH) insulin, also known as isophane insulin [6]. The typical insulin is used as bolus insulin however has been regularly changed by the fast-acting insulin due to its extra penetration and containment, which allows lessen the possibilities of hypoglycemia [6]. Regular insulin often takes more time to become effective and should be given 30 minutes before mealtime. A ratio of one to one is used for regular and rapid-acting insulins. NPH insulin has a shorter time of action and is generally dosed two times a day for Type 1 diabetes. This is known as basal insulin but it has a height that could predispose patients to hypoglycemia.

vi. *U – 500 Insulin*

Usually, the shape referred to as Humulin U -500 is best utilized in patients with intense insulin resistance and who take over two hundred units of insulin day by day [6]. When the frame no longer responds to insulin and glucose storage at a safe degree, extended in blood glucose and insulin resistance is the effect. U-500 insulin is broadly used within the treatment of Type 2 diabetics.

Table 1: Type and structure of insulin analogues [10].

Analog	Structure	Characteristics
NovoSol	Arg(B27)Gly(A21)	Long action, low bioavailability
Basal	Thr(B30)	
Asp(B10)	Asp(B10)	Short acting, rapidly absorbed, increase metabolic potency
Lispro	Lys(B28)Pro(B29)	Short acting, rapidly absorbed
Aspart	Asp(B28)	Short acting, rapidly absorbed
HOE 901	Gly(A21)Arg(B31)Arg(B32)	Long acting, peakless action, low rates of hypoglycaemia
WW99-S32	N(ε)-palmitoyl Lys(B29)	Long action, less variation, highly reproducible pharmacokinetic profile
NN304 (detemir)	Lys(B29)-tetradecanoyl, des (B30)	Long acting, peakless action, less variation

There is likewise the opportunity of insulin resistance formed for Type 1 diabetics specifically if they may be overweight. This kind of insulin is typically dosed 2-4 times an afternoon earlier than meals [6]. This works near the insulin that acts intermediately and ought to take delivery 30 minutes earlier than food or snacks. Because U-500 insulin is five instances extra powerful than other kinds, it has an extended history of dosing errors [6]. Today the U-500 was dedicated to syringes and insulin pens. They dramatically decreased dosing errors of this form of insulin.

2.0 BIOTECHNOLOGY ON RECOMBINANT DNA TO PRODUCE INSULIN

A. Purpose of Recombinant DNA Technology

"Recombinant DNA" refers directly to the combination of two different DNA fragments. However, in common use, the term "recombinant DNA" refers to many biochemical techniques used in the processing of DNA [16]. Recombinant DNA technology involves modifying genetic material outside of an organism to obtain improved and desirable features in or as products of living organisms. Recombinant DNA technology acts as a significant part in health enhancement through the production of new vaccines and drugs. Diagnostic kits, monitoring devices and new therapies also improve treatment initiatives. A

prime example of genetic modification in medicine is the synthesis of synthetic human insulin and erythropoietin by genetically engineered bacteria [2].

B. Process of Recombinant DNA Technology

i. Genes Isolation

In obtaining genes, the important tools are the two main groups of enzymes needed for DNA isolation and preparation of recombinant DNA: DNA ligases and restriction endonuclease. In bacteria, there are restriction enzymes called DNA-cutting enzymes. These enzymes are used to shield bacteria from an infection with the virus [17]. As the bacteria are infected by the virus, the restriction enzymes cleave the virus' DNA molecules entering the bacteria cell. At the same time, the restriction enzymes cut any foreign molecules entering the bacteria's body. Since restriction enzymes only cut within the cell molecules, it is also called restriction endonucleases. To obtain the DNA sequence, it is compulsory to cleave it into smaller fractions [17]. The partly RNA thread is digested by the hybrid RNA-cDNA that has been produced and RNAsaH by consuming the reverse transcriptase obtained from a retrovirus. The resulting cDNA lacks control sequences and introns. The other cDNA chain is synthesized by the primers for polymerase DNA I is from the remaining RNA fragments. It will suffice for short peptides to produce a DNA with a single strand and then hybridize it with a second complementary thread [17]. However, many chemically embodied oligonucleotides are hybridized for long peptides; the ligase and polymerase helps in filling the helix gaps. Clones are moved to the membrane of nitrocellulose, or nylon. The DNA is then denatured at 80^{o}C for nitrocellulose and for nylon is by using UV. After the marked probe has been added, the denatured It is then hybridized with DNA strands. The free probes are then scoured and used for autoradiography. The clear stripe is similar to the desired gene [17].

The polymerase chain reaction (PCR) is a technique used to amplify DNA. Plenty of the selected fragments of the polynucleotide chain are obtained by performing PCR. Regarding the mixture reaction, in addition to DNA, large quantities of two species of short oligonucleotides have been added [18]. The short oligonucleotides act as primers and are compatible with the flanking sequences. Next, the contents of the tube are heated to 94°C to denature the DNA double helix. The content is then cooled down to 50°C-60°C to ensure that the single strand of the oligonucleotide is bound. In order for the Taq polymerase to start reacting and synthesizing the new chain, the content is required the reheated to 74°C.These steps are repeated multiples times to generate millions of copies of the amplified segment [18].

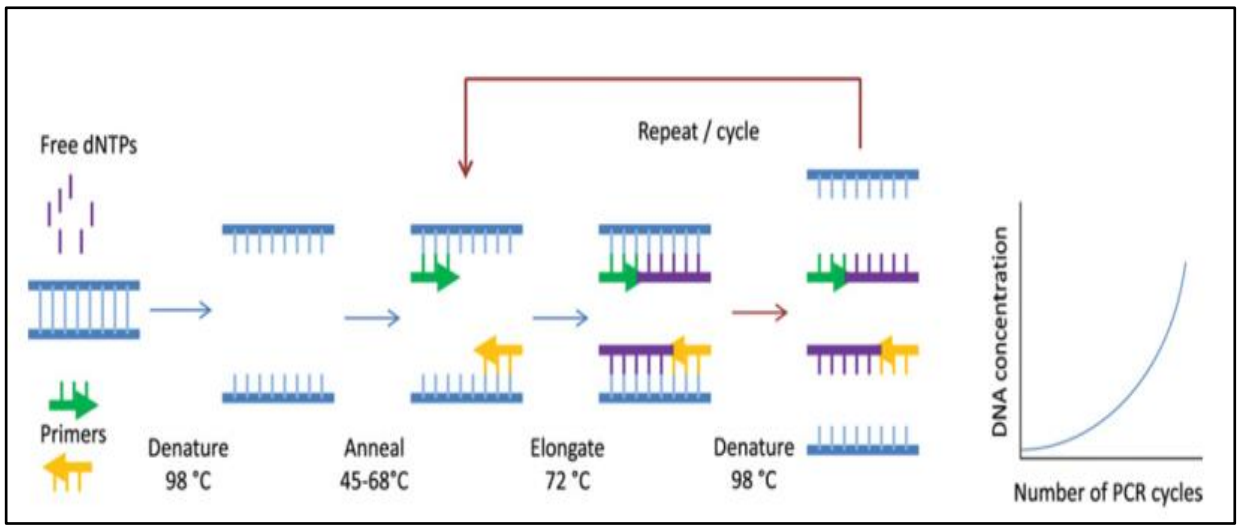

Figure 4: Process of polymerase chain reaction (PCR) [18].

ii. Introduction of a Gene into Vector.

Once the DNA has been amplified, one thing frequently done is to place it and store it in a vector. There are three types of cloning vectors that can be used – a bacterial plasmid symbolizes a spherical autonomous DNA particle, a bacteriophage λ which is a virus and cosmid that bonds both plasmids and bacteriophage [17]. DNA fragments, caused by endonuclease cleaved, sometimes possess the sticky ends needed to link the two DNA molecules. Eventually, complementary homopolymers with a single strand are formed and afterward through the medium of terminal transferase, they are connected with blunt ends until the ends of 3 'and 5' of the second particle chain [17]. Besides that, the alternative technique is to connect linkers, which is one restriction point or more contained in a double – stranded blunt ends sequence. Sticky ends can be created when the appropriate endonuclease digests the ligation that links to DNA.

When a cloned DNA particle and the linker have the same restriction point, adapters will be used. There is a blunt end and a cohesive in adapters [17]. The end 5' in the adapter is limited to phosphoric groups which lead the adapter and the mixture to detach until DNA is linked with the adapter. The phosphoric groups will link with the polynucleotide kinase back to the retained position. This occurs only after the blunt ends of the adaptors and the DNA fractions bind together. After that, the ligation of the DNA tested from the vector DNA is reached [17].

iii. Recombinant Vector Moved into Cell.

To accelerate the entry of the vector into the bacterial cell, the cell needs to be made competent. The cell that is dipped into $CaCl_2$ and then is heated for 2 minutes and 42°C helps it to accept the new DNA from the surroundings. Transformation is when the recombinant plasmid enters the bacterium cells [18]. During the transfection or packaging process in vitro, the bacteriophage is introduced. The transfection process involved production of effective bacteria cells and heated solution but at the same time, introduced the replicative form. The mixture of λ bacteriophage in the protein border is the product of in vitro packing. The capsid proteins and tails are produced by phase genes. Once an adequate amount of protein is obtained, recombined phages (bacteria) are completely formed. The bacteria then attack by infection and then insert their DNA sample into them [17].

iv. Transformant Selection And Recombinant Recognition

A specific base is a place where the bacteria are cultivated after the vector is implemented. The compositions of the peculiar base are based on the factors allowed by the microorganism where the vector has been implanted. In fuel, the plating transformants contain antibiotics which is a sensitive, vaccinated wild strain. Even though there is an earlier presence of a harmful substance, the antibiotic helps in distinguishing the variant cell because it will get bigger. To achieve the largest possible number of recombinants to be created, the incubation period is set at 37^{o}C before the planting for an hour from the beginning of the transformation [18]. Not all vectors, however, contain a cloned gene. An insertion inactivation λ is used to determine which cells are recombinant and have a gene with a litigated vector. In order for cloning of the new DNA part to take place naturally at the gene centre, a gene in the vector must be present with characteristic restrictive sequence. If a cell of the host contains a vector with a slit gene that has encoded resistance proteins to some antibiotic, the cells also will not be immune to it [17].

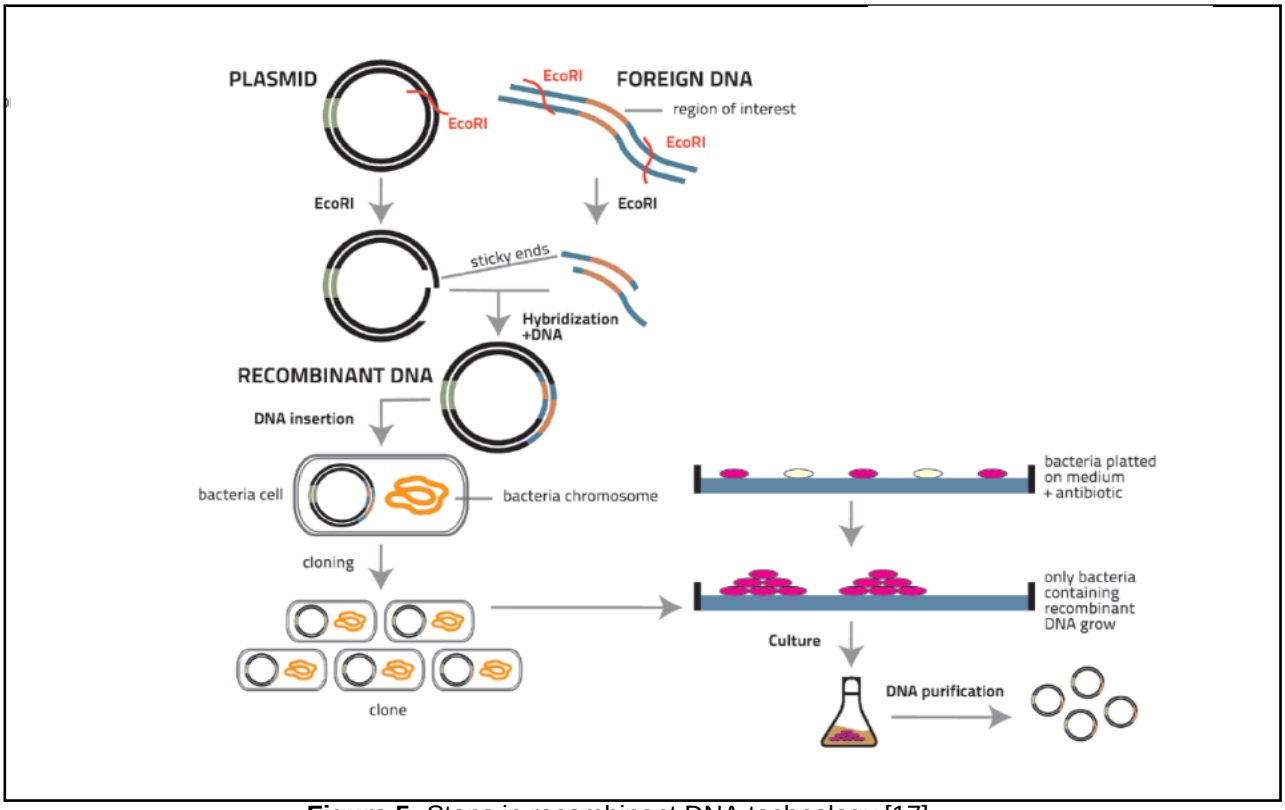

Figure 5: Steps in recombinant DNA technology [17]

C. Human Insulin Production

The β cells of the Islet of Langerhans in the pancreas, the insulin is produced and stored. The storage in the pancreas released the insulin into the bloodstream. Since the early 1920s, diabetics have been receiving insulin purified from the bovine or porcine pancreases. In human therapy, FDA approval in the manufacture of insulin using *Escherichia coli* and yeast is possible due to the advances in recombinant DNA technology [13]. Humulin is currently produced mainly in *E. coli* and *Saccharomyces cerevisiae*. Utilization of the *E.coli* expression system led to the production of the inclusion bodies by the insulin (IP) precursors and the fully functional polypeptides that are ultimately constructed by solubilisation and replication procedures [13].

D. E.Coli Expression System For Production Of Insulin

i. Isolation of Genes

The human insulin gene can be obtained by two general methods. First, in the human pancreatic cells (without intron) is where the complementary DNA (cDNA) in the messenger RNA (mRNA) has been extracted. Second, enzyme reverse transcriptase obtained from a retrovirus treats the mRNA of the chains to create cDNA [17]. Then, using polymerase chain reaction (PCR) the cloning of cDNA on both chains occurred [19].

ii. Insertion of cDNA Into Plasmid

Next, cDNA is inserted into plasmids to form recombinant plasmid. The two DNA molecules can only be inserted into separated plasmids when the bacterial plasmid has been cleaved by specific restriction endonuclease enzymes. At the start of translation, the cDNA is extended at 5' terminus with methionine (ATG) as initiation codon and the sticky ends of *EcoRI* and *BamHI* at 3' for translation termination signal [19]. Both of these vector plasmids are for the cDNA. The enzyme β -galactosidase is encoded when the cDNA is inserted into the plasmids of *EcoRI* and *BamHI* that are beside the lacZ gene. β-galactosidase of the E.coli is the enzyme that controls gene transcription. The insulin gene firstly needs to bind with β-galactosidase to ensure that the

bacteria produce insulin. Specific DNA ligases then help in re-ligated the cut plasmids [19].

iii. Transfection Recombinant

Then the cell is then transferred into a recombinant vector. The recombinant plasmid is introduced to the plasmid-free *E. coli*. To accelerate the entry of the vector into the bacterial cell, the cell needs to be made competent. The cell dipped into $CaCl_2$ and then is heated for 2 minutes and 42°C helps it to accept new DNA from the surroundings [18], [19] .

iv. Fermentation

In the fermentation process, the *E.coli* is placed in an antibiotic-containing culture. The fermentation contained in this process is lactose and ampicillin. The gene in the plasmid that carries the gene of ampicillin resistance by the lacZ gene contain lactose shows that the bacteria cells successfully undergo transformation. Therefore, in the ampicillin environment, the cells can grow and the transcription of the lacZ gene will lead to human insulin chain DNA transcriptions [19].

v. Separation

In order to extract DNA, the cells are separated from the tank and broken open. Cells are lysed utilizing a variety of methods. For example freeze-thaw process, sonication and enzyme digestion [20]. The enzyme digestion typically used is lysosome where the outer layer of the cell wall is digested. Subsequently, a detergent mixture is added to remove the fatty cell wall membrane. Then, the bacterium's DNA is treated by using cyanogen bromide so that the methionine (ATG) chain residue is split [20].

vi. Purification

Centrifugation is carried out to separate the components of the cells from the product and to ensure that only the insulin chains are remaining. Several techniques for purifying the mixture are by chromatographic method or separation techniques that exploit hydrophobicity differences [19].

vii. Synthesis of active insulin

The disulphide bonds formed from two chains (A and B) by using sodium sulphite and sodium dithionite. Then, the reduction – reoxidation reaction with betamercaptoethanol and air oxidation helps the chain join together. Thus, synthetic human insulin is produced [20].

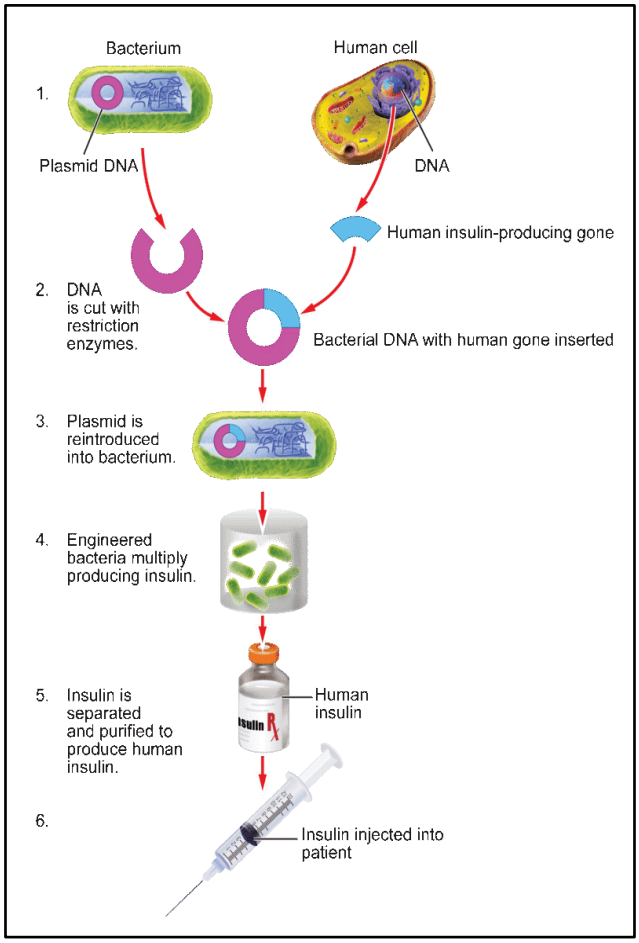

Figure 6: Steps in production of human insulin [21].

3.0 ADVANTAGES OF RECOMBINANT DNA TECHNOLOGY

A. Evolution of Analogue Insulin (Human Insulin)

Since the recovery of recombinant DNA methods in the production of insulin, now there are two choices for those who take insulin either they want to use animal insulin (conventional method) or analogue insulin. Disadvantage of using animal insulin is the dissimilarities in amino acid composition can result in the formation of antibodies which are highly immunogenic. Furthermore, impurities like proinsulin can further stimulate antibody formation [3]. The presence of antibodies will lead to neutralization of the insulin, so the diabetic will need more and more insulin to obtain the same hypoglycemic effect. Other than that, in using insulin of animal origin the pancreas must be removed fresh and must be frozen directly to avoid denaturation of insulin by proteolytic enzyme [3]. So, production of human insulin by recombinant DNA technology helped in removing the dependency on slaughterhouse material.

Human insulin clinical trials indicate its success in managing hyperglycemia [9]. It seems to occur slightly quicker compared to animal insulins. Throughout double- blind animal insulin transfer studies, patients previously treated with mixed beef pork insulin experienced a 70 percent reduction in inbound insulin compared to baseline. Accurate binding of insulin from human, bovine and porcine decreased by 61, 58 and 57 per cent respectively at 6 months [9]. The bound insulin for patients previously treated with animal insulin has declined by 30 percent and 51 percent for patients transferred with human insulin [9].

The theory of using *Escherichia coli* is concerned with the efficiency of host cultivation and safety issues. For safety and containment purposes, Eli Lily & Co selects the *E.coli* K-12 strain for use in recombinant DNA research and development of insulin [3]. Because it is a weakened strain of *E. coli* it does not survive outside the very carefully controlled conditions of the laboratory and production process. It is important to emphasize this because one of the fears that society initially had about this technology when it moved into large scale production was what might happen if some of the material escaped into the environment [3]. In reality, the organism used would not survive more than a few hours. In addition, the bioengineered product inside the *E.coli* is not biologically active until isolated, chemically modified and purified [3].

Insulin analogs consist of pharmacokinetic and pharmacodynamics properties, have more physiological and consistent time-action profiles, and represent a substantial advance in diabetes management. Due to less doses, versatility of the timing of basal analogs, less uncertainty of dose changes, mealtime administration of prandial analogs, along with user-friendly injection systems, analog insulins have been shown to increase patient adherence and care satisfaction. This helps in lower risk of hypoglycemia and improved postprandial and fasting glycemic control [22]. Despite common views, there is also proof of a pharmacoeconomic benefit with insulin analog

primarily attributable to reduced claims linked to hypoglycemia and lower hospital costs. In addition, long-acting insulin analogs, particularly Insulin Detemir, are associated with less weight gain than animal insulin. Both features will also lead to the benefits of healthcare costs [23].

Prandial insulin analogs contain both pharmacokinetic and pharmacodynamics properties that are specifically nearer to normal. For example, prandial insulin analogous causes a faster rise following subcutaneous administrations to higher order of insulin [22]. Then a faster drop compared to normal human insulin occurred, leading to faster onset and offset of insulin effect. Basal insulin analogs have the function of suppressing glucose production between meals and overnight. They give longer periods of action, less volatility, greater probability, less hypoglycemia, and more beneficial weight profile in the case of Detemir. Therefore, they require fewer injections to accomplish their function [22].

Finally, literature includes sporadic reports of insulin allergy, especially to 'older' original animal insulins such as NPH. Localized allergic reactions are documented most commonly and are assumed to happen due to the impurities found in older insulins, especially pro-insulin, C peptide and other peptides [24].

B. Prevent widespread diseases in developing countries due to the ability to mass production of vaccines

The cloning of genes is an effective method for synthesizing protein materials by recombinant DNA techniques to subunit the vaccine. Recombinant subunit vaccines are formed from a protein (antigen) fragment produced in the laboratory using the viral DNA vaccine which is Hepatitis B (HB) [25]. The gene of the hepatitis B virus (HBV), which codes for the antigen, is injected into the yeast genome of baker and then releases the protein antigen [25]. The protein antigen is collected and synthesized for use on the vaccine. This method is also used to investigate a hepatitis C vaccine. Recombinant-DNA techniques can encourage the creation of new principles for subunit vaccine design and production [25]. The recombinant subunit vaccine can also be modified to be safely integrated into Immuno potentiating adjuvant systems using gene-fusion technology [25].

By using rDNA to produce vaccines, the advantage is a low risk of infection. Just fragments of the microbes such as DNA, RNA or protein are used to produce vaccines because Recombinant vaccines do not contain actual pathogens [25]. Recombinant vaccines are indeed securer than traditional vaccines and can be prescribed to people with poor immune systems. Next, is the induction of more coherent immunity [25]. Recombinant vaccines can cause both humoral and cellular immune responses to make vaccination further successful [25].

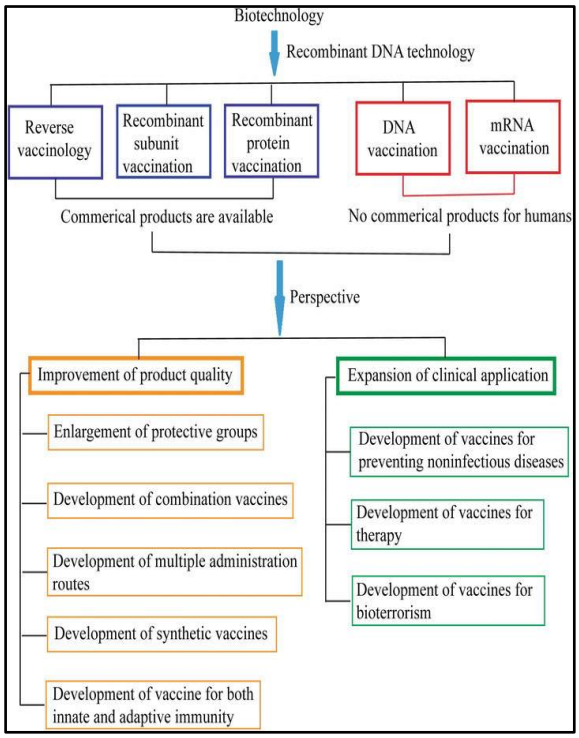

Figure 7: The views of vaccine products by using recombinant DNA based on biotechnologies [25]

The advancement of powerful biotechnology tools (rDNA) implemented to genome-based methods has practically pioneered the development of vaccines [25]. Genome information includes a list of all the possible proteins from which scientists may pick those antigens or antigenic materials that are considered to be further coherent vaccines. The prospects for managing diseases by vaccination along with the advancement of biotechnologies are very convincing [25] Recombinant DNA enables the efficacy of vaccines to be further improved and

the clinical application of vaccines to be dramatically expanded. Furthermore, new vaccine products based on recombinant DNA technology are being accepted on the world market [25].

C. Diagnostic Of Diseases

Recombinant DNA technology has been broadly used in the treatment of diseases using recombinant proteins, in the identification of normal genetic variations, the identification of genetic differences causing diseases and in genetic disease prenatal screening. These methods are highly sensitive and oppressive in the detection of DNA mutations and species differences [26]. These properties signalled the rapid development of DNA applications for hereditary and acquired disease detection [26]. Currently, the main diagnostic implementations of recombinant DNA technology are divided into three main categories which are genetic disorder diagnosis, microorganism identification, and the tumor diagnosis and analysis [27].

D. Diagnosis of Genetic Disorders

Recombinant DNA technology can be used to study Individuals suspected of harboring diseases as a prenatal measure (using villous chorionic biopsy) or by predictive testing (using blood samples) in adult-onset of disease. The latter can also be used for carrier status detection [27]. In which genes pertinent to the disease have been discovered and samples are accessible, a diagnosis may be chosen to make either through straightforward demonstration of an aberrant gene or consequently through linkage analysis of WLPs in overlapping or adjacent DNA sequences [27]. This technology has the benefit of enhanced sensitivity and specificity, and makes a quicker diagnosis possible. For the diagnosis of cystic fibrosis, Duchenne muscular dystrophy, Huntington's disease, myotonic dystrophy and adult polycystic kidney disease, prenatal diagnosis and predictive testing are most frequently needed. Carrier testing for Duchenne and Becker muscular dystrophy, hemophilia and a'-antitrypsin deficiency, and increasingly for cystic fibrosis, on the other hand, is most frequently done [27].

E. Utilized in forensics analysis to identify a criminal suspect

Recombinant DNA technology was also utilized in forensic science to identify a suspect using DNA samples collected at the crime scene from different biological sources; this test is named genetic fingerprinting [28]. The concept first posed by Sir Alec Jeffreys in 1984 was to classify victims and suspects by matching genetic samples to DNA databases [29]. Forensic DNA analysis is used in the case of the 1986 British murder and sexual assault charge against Colin pitchfork for the first time [29]. Since each individual bears unique DNA repeating sequences (minisatellites or microsatellites), the analysis of several such duplicates at a given gene location provides researchers with an identification method to identify a particular individual fingerprint matching DNA [28]. This approach can also be an appliance in paternity tests, pregnancy tests and recognition of human remains [28].

4.0 EFFECT OF RECOMBINANT TECHNOLOGY ON SOCIETY

Billions of people in this modern world have benefited from the advancement of technology. Even if they are not able to contribute, they still understand the science behind the technology. Thanks to recombinant DNA (rDNA) technology, many diseases can be cured due to the production of new medicines and diagnostics. However, the application of RDNA technology in our lives is not only focused on the medical industry but has also already been used by other industries to improve their specialties.

A. Health

Insulin

The discovery of insulin was a milestone in the history of medicine. A dietary restriction was the most effective treatment in the pre-insulin era. Minimizing sugar intake can extend life expectancy for patients by several years. Unfortunately, the patient's condition worsens over time and died of starvation instead. In 1921, an experimental team led by Frederick Banting developed a drug called insulin. The first person to receive insulin

injections was a 14-year-old boy who died of diabetes, but his blood sugar level dropped to normal after 24 hours [30]. Sometime later, in 1923, Eli Lilly produced insulin on a large scale.

However, Banting and Best only discovered short-acting insulin. This insulin lasts only 6 hours, followed by an inevitable and subsequent peak of hyperglycaemia within 24 hours [31]. As a result, continuous efforts have been made to improve the quality of insulin until today. Insulin analogues have been developed over the last decade to enhance the therapeutic properties of the native protein. This is because the pharmacokinetic properties of the rapid, intermediate and long-lasting effects of Humulin make sustained normoglycemia nearly implausible to obtain [31].

Even though insulin remains as the primary treatment required by patients with diabetes, the cost of prescribing insulin has increased for years [32]. Between 2012 and 2018, the price of available insulin increased by an average of 14% per year. The insulin healthcare costs for Type 1 diabetes in 2016 accounted for 31% compared to 23% in 2012. Both patients and society are affected by the rising expense of insulin. One-quarter of diabetics cannot afford their prescribed treatment plans; ration their supplies, which may be dangerous and potentially fatal [32].

The WHO estimates that global sales of insulin will rise from $12 billion to $54 billion over the next 20 years. The dramatic increase in the number of diabetics worldwide and the investigation of alternative insulin administrations methods, like inhalation or oral administration, will increase demand for recombinant insulin soon [13].

Latest manufacturing technology is perhaps incapable of complying with the increasing demand for insulin because of capacity constraints and high production costs. Recombinant human insulin is produced primarily for therapeutic use in humans using *Saccharomyces cerevisiae* and *E.coli.* However, there is an urgent need to increase the production of bioactive insulin and its analogues from E. coli and yeast using the latest innovative and efficient technologies [13].

Nevertheless, insulin is not only used to treat diabetes but is also abused in athletes. Tricker, et al found that 54% of males and 10% of female bodybuilders regularly use steroids [33]. In the United States, more than one million elite and recreational athletes used performance-enhancing drugs, whilst 25% of the anabolic androgenic steroid abusers use insulin at the same time as stated by Rich, et al. The glycogen stores in muscles serve as the main source of carbohydrate during exercise. More muscle glycogen is stored, longer exercise time to exhaustion. Insulin also works in synergy with steroids [33].

B. Food and Agriculture

Genetic Engineered (GE) crops

The development of genetically modified (GM) crops used in agriculture is intended to improve yield and resistance to plant and pest or herbicides. This development has made a significant contribution to both the economic and environmental gains [34]. The adoption of GM crops has helped to boost the substantial net economic benefit for farmers. Farmers profited $14 billion in 2010 and $78.4 billion over the 15-year period (1996-2010). A majority (55%) of farmers' income gains in 2010 went to farmers in developing countries, of which 90% are resource-poor and small farms [35]. In addition, the storage of additional soil carbon from reduced soil processing with biotechnological crops and the use of less fuel has also reduced greenhouse gas emissions. It was equivalent to removing 19.4 billion kg of carbon dioxide from the atmosphere or equal to removing for one year 8.6 million cars from the road [35].

The use of Ti plasmid from the soil bacterium of Agrobacterium tumefaciens in transgenic crops has helped people with malnutrition. As of 1995, 800 million people worldwide had an inadequate diet for macronutrients and micronutrients [36]. The main deficiencies are vitamin A, iron, iodine and vitamin E. To resolve this crisis, Peter Beyer and Ingo Potrykus have inserted genes for a chemical gene called beta-carotene into the DNA of conventional rice which produced Golden Rice. Golden Rice makes a significant amount of β-carotene which is required by humans to produce vitamin A. Therefore, a staple food such as rice, widely consumed worldwide, can be used as a remedy for vitamin A deficiency [37].

C. Environment Biofuels

Renewable powers have recently gained attention and financial support due to expensive crude-oil prices, unpredictable supply of fossil fuel and political and ecological problems related to fossil fuels. March 2008, crude-oil prices reached $100 per barrel, increasing the burden to create a cheaper domestic fuel source [30]. Biodiesel and bioethanol comprise the two types of liquid biofuels used for transport. However, its direct competition with food production is a major drawback to biodiesel, as its sources are limited to seed crops like soybeans and palm oil. The efficiency of production of cellulose bioethanol can be increased by fermentation, which has been improved by the use of genetically modified plants with engineered cell wall composition and microorganisms [39].

In particular, the global aviation industry aims to attain carbon neutrality before 2020 and to lessen carbon dioxide emissions to 50% prior to 2050 then the 2005 releases [38]. Sustainable fuel use is an important strategy to achieve this goal considering it minimizes the environmental footprint and promotes the development of the aerospace field [38]. As the biofuel industry is emerging and portrays an important role in reducing carbon footprint and preserving the environment, United Airlines has invested approximately $30 million in Fulcrum BioEnergy, Inc. The Fulcrum agreement allows United Airlines to purchase 90 million gallons of biofuel per annum at a competitive cost for at least 10 years. This significantly reduces operating costs [38].

5.0 CONCLUSION

The use of biotechnology in medicine allows the human protein to be synthesized and purified in the heterologous cell system, which may be useful for basic research or other medical applications such as diagnostic assay systems. Many proteins, like human preproinsulin, cannot be produced in sufficient amounts from their natural sources due to their low abundance or difficulty in purification using traditional methods from human tissue samples, organs, or cell lines. Therefore, they are developed in the laboratory using recombinant DNA technology to treat various diseases and deficiencies that exist in our body, which are also used by gene manipulation to treat certain genetic disorders. Diabetes refers to the so-called “lifestyle” or “civilization diseases" – it is a significant challenge to human health and well-being around the world. To date, insulin delivery has been the most effective treatment for hyperglycemia.

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