INSULIN GLARGINE PRODUCTION PLANT IN SINGAPORE

GENE EDITING TECHNIQUE INFLUENCE AND POSITION TO HUMAN SOCIETY

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By
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On my honor as a University student, I have neither given nor received unauthorized aid
on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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In 1953, James Watson and Francis Crick discovered the structure of DNA, which promoted the molecular-level development of genetics. Since then, according to Nicholl (2008), “intense activity and excitement” were made “as the main features of the gene and its expression were determined” (p. 6), and in the last decade, tremendous progress has been made on areas like industrialization of DNA synthesis, discovery, and development of molecular tools and organisms (Ostrov et al., 2019). Based on these information, the overall goal of this thesis project is to focus more on the application of biotechnology, both on technical and social aspects. To be more specific, the technical topic is about the production of one pharmaceutical product, insulin, and the STS topic, which is loosely coupled with the technical one, is about the gene editing techniques’ position and influence on society, analyzed by the System in Context and SCOT framework.

The Technical and STS parts will be further completed in Spring 2023, and the final draft of these two parts will be completed near the end of April.

INSULIN GLARGINE PRODUCTION PLANT IN SINGAPORE

Insulin production is a vital process, as 72 million people in the world, about 1% of the population, require insulin to treat diabetes (Uildriks, 2021). A contemporary insulin technology is insulin glargine, a slow-releasing insulin product that is beneficial to those that have to take insulin every day. Insulin glargine remains in the bloodstream longer allowing patients to take insulin less often; thus, they need fewer injections every day. The motivation behind exploring this technology now is to study how the sustainability and efficiency of the process can be optimized as diabetes cases continue to rise.

The prevalence of diabetes in Asia is rising; 60% of diabetes cases are in Asia with the majority of these cases in India and China (Ramachandran et al., 2012). It is,

therefore, germane to produce insulin for the Asian market, specifically developing nations as insulin prices can be an obstacle in these regions. More than 80% of Type 2 diabetes cases occur in developing countries where it can be very difficult to manage the high out-of-pocket costs of insulin treatment (Ramchandran et al., 2012). Further, diabetics in lower economic groups spend 25-34% of their income on treatment (Ramachandran et al., 2012). According to a study conducted in China (Liu et al., 2017), 16 day's wages of the lowest paid unskilled government worker is required to purchase a month's treatment of a long-acting basal insulin analog. The study found that these high prices could be attributed primarily to the manufacturer's selling price (MSP). The high selling prices of insulin can be attributed to a variety of factors including the vulnerable population which is willing to pay thousands of dollars for a lifesaving drug, a virtual monopoly in the insulin market, and patent abuse by evergreening (Rajkumar, 2020). Patent evergreening has been used in the insulin industry by the top manufacturers for almost a decade as new formulations continue to be made that provide more reliable control of diabetes. These patents allow for monopoly control of the insulin market hindering biosimilars from entering the market and targeting specific areas of manufacturing; thus, distribution of insulin to lesser developed countries remains difficult. To target lower economic groups and reduce distribution difficulties to developing countries, it is proposed that an insulin manufacturing process be designed in Singapore to serve the developing and developed nations in the surrounding area. Our goal will be design a process to provide a more affordable and accessible insulin glargine product for all people suffering from type 2 diabetes in Asia.

Our insulin glargine product will be slow-release; produced via recombinant DNA technology using a strain of *Escherichia coli* (DrugBank, 2022). Insulin can be

rendered long acting by replacing asparagine with glycine in position 21 of the A-chain and by carboxy-terminal extension of B-chain by 2 arginine residues (Bolli, 1999). The arginine amino acids shift the isoelectric point from 5.4 to 6.7, making the molecule less soluble in physiological blood; this allows the product to crystallize prior to dissolving, rendering it “slow-release”. The unit operations that will be used to manufacture the drug include, but are not limited to: fermentor, centrifuge, incubator, ion-exchange chromatography column, cation-exchange chromatography column, and preparative high-performance liquid chromatography column (Preparative HPLC) (Hwang et al., 2016).

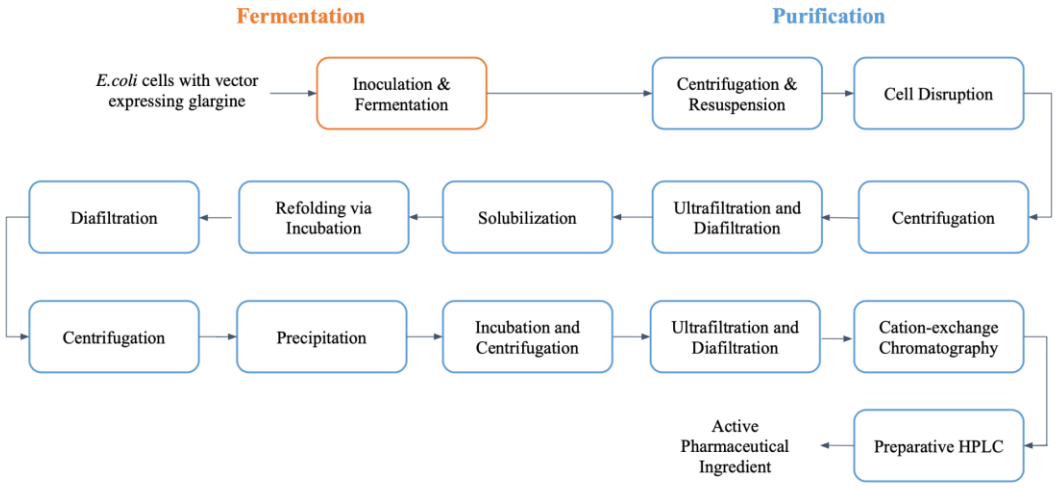

Figure 1: Block Flow Diagram, includes Fermentation in upstream part and Purification in downstream part (Adapted by Ben Ford, Bethany Iudica, Lauren Laughlin, Yixuan Yuan (2022) from Hwang, H. et al, (2016))

The steps in our insulin glargine production process can be seen in Figure 1. In general, the whole process for insulin production includes fermentation, primary recovery, inclusion body solubilization, and chromatography. We will not be addressing formulation in our project. We will use *E. coli* as host cells for our insulin precursor production, purchased already containing the vector expressing glargine. An insulin precursor is produced as a soluble inclusion body which can be used in the

solubilization and refolding steps shown in Figure 1 (Baeshen et al., 2014). *E. coli* is the most widely used host cell for recombinant proteins as it is widely studied and has less associated costs (Hwang et al., 2016). We will use the process and data described in "Recombinant Glargine Insulin Production Process Using *Escherichia coli*" by Hwang et al. as a reference (2016) as well as finding further sources of information and data. We will design a process to produce insulin glargine which will include upstream and downstream processes.We will consult experts in these fields, Professor Michael King, Professor Giorgio Carta, and Professor George Prpich. We will also reference a University of Virginia capstone project from 2015, "Continuous Manufacturing Process for the Economically-Efficient Production of Biosynthetic Analog Insulin Glargine Active Pharmaceutical Ingredient" (Wilson 2015).

This project will be completed by a group of four chemical engineering students over the course of two semesters in CHE 4474 and CHE 4476. We will have weekly group meetings to evaluate our progress and discuss further work to be completed in the following week. The work will then be divided evenly between all group members. We will meet with Professor Eric Anderson, our advisor, every week to discuss our progress. Our project will consist of a design of the system and all equipment in the facility, an economic analysis of the viability of our project, and a discussion of risk, safety, and sustainability in our plant.

GENE EDITING TECHNIQUE INFLUENCE AND POSITION TO HUMAN SOCIETY

Gene editing technology, or gene manipulation, began from 1970 with the successful isolation of the first restriction enzyme, that could be used for the precise

cutting of DNA sequences. After Stanford University generated the first recombinant DNA molecules in 1972, the scientific revolution in gene manipulation started (Nicholl, 2008, p. 6). Various techniques have been developed, and some of them, like recombinant DNA, has been applied to vaccines and pharmaceutical development, after most recombinant drugs were approved by U.S. Food and Drug Administration (FDA) since 1997 (Khan et al., 2016). Also, other newly developed techniques, like CRISPR-Cas9, are promoting "innovative applications from basic biology to biotechnology and medicine" (Hsu et al., 2014).

CRISPR-Cas9 is developed from the CRISPR-Cas system (the acquired immunity systems that were discovered in archaea and bacteria). CRISPR is an enzyme that targets and removes viral DNA, and Cas9 is the protein that carries a guide RNA (gRNA) strand, normally provided by scientists, which helps to target the specific area of DNA strand for cleavage, and further work like inserting DNA can then be facilitated (Ishino et al., 2018). CRISPR-Cas9 helped scientists to modify the gene more effectively, compared with other methods like zinc-finger nucleases, which could take years to design (Bleicher, 2018).

APPLICATION OF GENE EDIT TECHNIQUE

One application of the gene edit technique is gene therapy for clinical trials. Type 2 diabetes is a metabolic disease resulting from insufficient insulin production due to genetic and environmental factors. Although insulin-like agents are now the main therapeutic strategies for Type 2 diabetes, the use of these agents has various adverse effects still exist and pushed the study of new alternative treatments, such as gene therapy (Yue et al., 2019). As part of the pathogenesis of diabetes is the decline of the number of functional insulin secretion beta-cells, studies around beta-cells are mainly focused on areas like how to induce the existing beta-cells' self-renew and

how to reprogram other cells or direct pluripotent stem cells to become beta-cells. The study has shown the success of producing beta-cells from pluripotent stem cells, both in human embryonic stem cells and induced pluripotent stem cells from reprogrammed somatic cells (Millete & Georgia, 2017). Another study also has found at least 75 gene loci for Type 2 diabetes and determined several new therapeutic targets. Moreover, inhibition of certain genes, like gene NLRP3, has shown an effect on preventing Type 2 diabetes development in mice (Yue et al., 2019).

Gene edit technique on gene therapy has the potential that is not limited to diabetes treatment. Adrenal insufficiency, a disease caused by the inability to produce adequate levels of corticosteroids, attempts from gene editing to cure the malfunction of the adrenal gland have been made to test the possibility (Mariniello & Guasti, 2021). Although there are still many steps that need to be accomplished to apply gene editing on cancer treatment, there is a growing number of reports of successful gene-edited cancer cell lines (Biagioni et al., 2018).

Just like other technology, the development of such techniques is always followed by concerns and debate. When recombinant DNA was just developed and applied in 1970s to 1980s, board discussions occurred, topics like whether this technique will create novel organisms and infect the populace after escaping from laboratories, with concerns about scientists' responsibilities on proper usage for their research (Altimore, 1982). And ethical questions arise especially when it is applied to the human gene editing or germline editing (Baumann, 2016).

Back to 1975, molecular biologists, physicians, and lawyers created guidelines for altered DNA living organisms' research at the Asilomar conference (Vogel, 2015). And years later, in 2015, scientists held another Asilomar, which focused on genetically engineered human beings (Jasanoff & Hurlbut, 2018). As scientists have

not understood all the possible side effects of editing germ cells or embryos, also because of existing regulations, the attempts to genetically altered babies are prevented (Vogel, 2015). However, in 2018, Chinese scientist Jiankui He claimed he successfully edited the human embryo of twin girls to resist HIV infection and made the embryo go through an in vitro fertilization pregnancy (Krimsky, 2019). Jiankui He not only violated international consensus about the editing of human embryos but also failed to prove his study had eliminated the potential risk, such as off-target effects, in using CRISPR-Cas9. Moreover, this experiment reaches gene enhancement, which is the most serious ethical problem (Krimsky, 2019).

Because of his action, draft regulations about clinical research that have gene editing, issued by the Chinese government, need to have national approval. Though Chinese researchers welcomed such regulation, they still worried it may slow down other less controversial research, like editing blood cells (Normile, 2019), as sickle cell disease patients may be cured by related gene therapy (Daley, 2021).

For patients, or patients' families, different views on whether gene editing technique can cure their disease or cure their children also exist. While some patients think their lives and success were shaped and affected by their disease, other patients reflect the idea that they will not consider if there is any moral issue in gene therapy, and will not hesitate to cure their diseases if those therapies are applicable (Hayden, 2016). But for patients, other factors like price, are also influencing their attitude toward gene therapy. For instance, patients who take Glybera gene therapy, a therapy that treats adults with lipoprotein lipase deficiency due to the issues gene for lipoprotein lipase (EUROPEAN MEDICINES AGENCY, n.d.), each need to take 19 vials of Glybera on average, and each vial cost nearly $50,000 (Morrison, 2015). Such price creates a burden for patients to take therapy, and uncertainty about the benefits

and risks associated with these treatments are also challenging the patients (Koulianos, 2021).

FRAMEWORK FOR ANALYSIS

The STS paper will try to understand the position of gene editing techniques when it is influencing humans and social production, and where other actors are situated. For a better understanding of how these groups interact with the gene edit technique, Figure 2, a Social Construction of Technology (SCOT) framework (Bijker et al, 1970) will be used to map those relationships, and the social groups are Scientists & Engineers, Government, and Public users. For Scientists & Engineers, their interactions could be mainly focused on developing and using techniques, and may also include regulating the usage of gene edit, just like the government or regulators. And for the Public, it can be patients that need gene therapy.

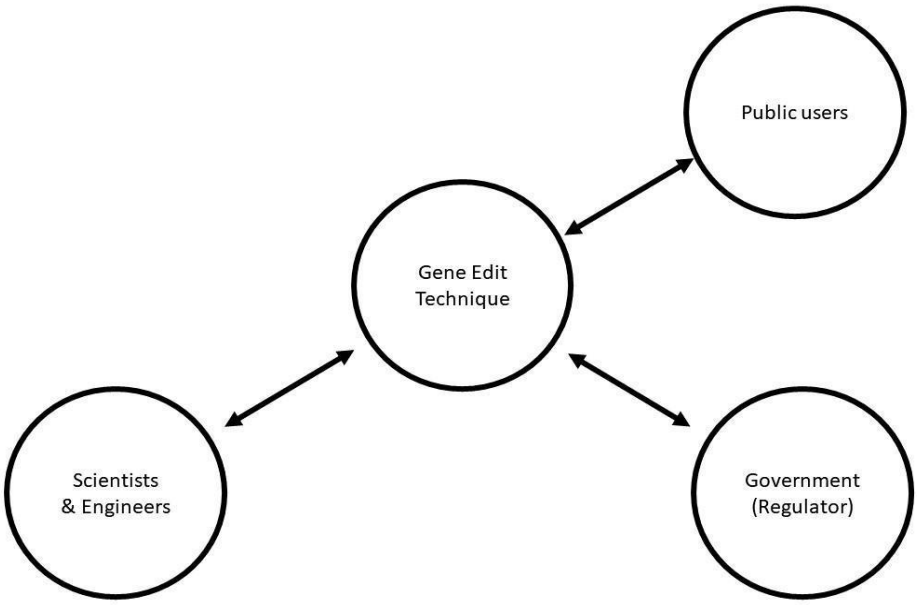

Figure 2: SCOT framework: Middle is gene edit technique, interacting with Public users, Scientists & Engineers, and Government (Adapted by Yixuan Yuan (2022) from Carlson (2009))

In addition, to investigate the position of gene editing techniques on society, the System in Context, shown in Figure 3, will be used. In the circle, is the gene editing

technique, like CRISPR-Cas9, and scientists, engineers, and government are the gatekeepers, as they are either the direct users, developers, and regulators, together with ethic/moral issues and regulations that constrain the technique. Outside the circle, the social context includes the aspects like gene therapy. Finally, far from the technique, the Public, can include patients or people who are not the direct user but are influenced by gene editing.

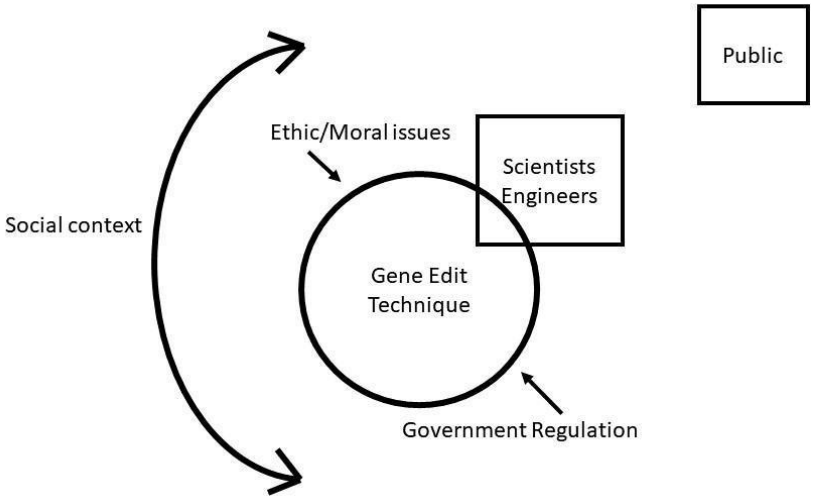

Figure 2: System in Context framework: Scientists and Engineers are gatekeepers with ethic/moral issues and government regulation. Public is separated from gatekeeper, ethic/moral issues and government regulation by Social context(Adapted by Yixuan Yuan (2022) from Carlson (2009))

CONCLUSION

In conclusion, the technical project will investigate the production of insulin glargine, based on data collected from the research papers and the previous UVA capstone project. The whole production line will then be analyzed for investigating the feasibility of building a such production plant in Singapore. As gene edit techniques are one of the major techniques applied for insulin glargine production and could be a possible treatment for clinical trials like diabetes, with tremendous progress has been made on related techniques in recent decades, and some moral

issues, this STS project will help to understand the role of gene edit techniques in society and how they interact with different social groups through SCQT and System in Context framework.

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