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Messenger RNA Vaccine Production

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Introduction

Recently, messenger RNA (mRNA) vaccine production has increased dramatically with the COVID-19 pandemic. More than 11 billion vaccine doses will be produced by the end of 2022, with December production alone set to reach almost 1.5 billion doses [1]. If it is possible to improve the production, cost, or effectiveness of these vaccines in any way, it will have a massive impact on the number of resources being used, the money and time spent on producing these vaccines, and the effectiveness on the vaccine.

Messenger RNA vaccines deliver mRNA directly to the cytoplasm, where ribosomes translate it. The mRNA does not enter the nucleus and cannot be incorporated into the genome. Its presence in the cell is transient, and it is quickly metabolized and eliminated via cellular processing mechanisms [2]. Since the mRNA is swiftly eliminated by cellular processes, the vaccine is not as effective as it could be. Changing the immunogenicity of the vaccines will allow for better effectiveness. Immunogenicity is the ability of cells to provoke an immune response due to a suspected foreign body. If the immunogenicity is lowered the vaccine will decay less and the translation efficiency will be greater.

Process Description

1. Starts with plasmid DNA (pDNA) template with a DNA-dependent RNA polymerase promoter and the sequence for the mRNA construct [3]
2. Linearization serves to make the pDNA the template for the RNA-polymerase to make the mRNA [4]
3. Linearized pDNA is transcribed to mRNA [4].
4. *In vitro* transcription (IVT), pDNA acts as DNA template [4]
5. Following IVT, the pDNA template and contaminating bacterial DNA are digested by DNase [4]
6. Purification using chromatography or tangential flow filtration (TFF) [5]. TFF allows for the separation of impurities not retained by the membrane
7. Formulation using combination of lipids and polymers to carry the mRNA and protect it from degrading [5]
8. Appropriate distribution of the vaccine occurs after the final product is made

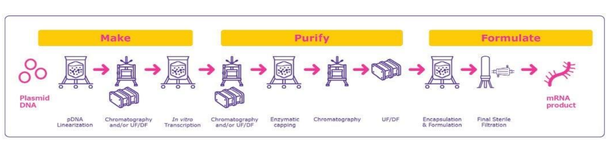

Figure 1: Manufacturing process of mRNA vaccines [5].

Sensitive Unit

One of the main problems with using *in vitro* transcription (IVT) is the way its delivered and how that contributes to higher levels of immunogenicity compared to other vaccination methods.

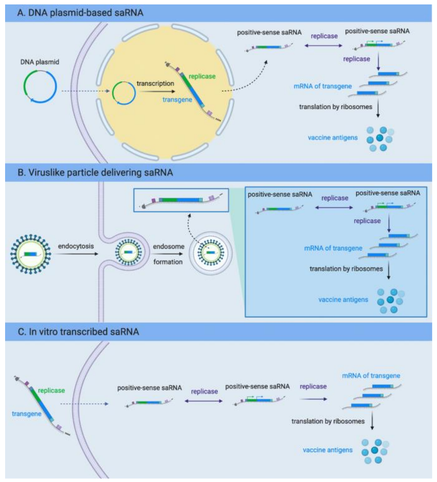

Figure 2: mRNA vaccine method [6].

Because the mRNA is produced IVT (in a lab, outside of the body), the relevant receptors recognize the strand [6]. When a foreign object enters a body, in this case the mRNA from the vaccine, the hosts immune system attacks that object. For some medical therapies, such as tumor recognition, that is the goal; however, for this kind of vaccine to work properly, the mRNA must get into the target cell without detection to produce the antigens necessary to induce immunity.

Possible alterations and solutions to the immunogenicity problem are; shortening the "U-rich" sequences, replacing traditional nucleotides with pseudo nucleotides (altering the structure, making it less recognizable), extending the Poly A tail, and increasing GC rich codons [6].

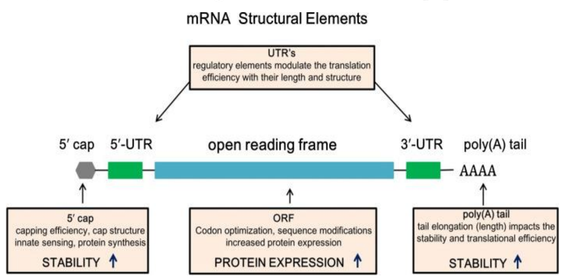

Figure 3: Key structural components of mRNA [7].

Because the mRNA itself is naturally unstable, even with all the suggestions above, it requires a vesicle of some form. "Scientists have developed lipid-based delivery, polymer-based delivery, peptide-based delivery, virus-like replicon particle delivery and cationic nanoemulsion delivery, or even injected directly." [6].

Overall, the goal is to lower the immunogenicity to improve the effectiveness of the vaccine. There are a multitude of ways to do this.

Research

Hypothesis:

- Messenger RNA vaccines that reduce immunogenicity by reducing the length of U-rich sequences, increasing GC-rich sequences, and pseudo-nucleotides will improve the translation and effectiveness of the vaccine

Methods:

- Measure the antibody titer concentration of animal test subjects
- Measure the levels of antigen production within the animal test subjects
- After viewing what individual components elicit a strong immune response, try different combinations of the alternatives
- Test further in clinical trials on humans
- Alter single components of different mRNA Vaccines, one at a time
  - Potential areas for alterations are
    - Replacing uracil with pseudo uracil [8]
    - Replacing cytidine with 5-methylcytidine (m5C) [8]
    - Replacing Uridine with pseudouridine
    - Increasing GC-rich sections
    - Varying the length of the Poly-A tail
    - Different delivery methods, pictured below

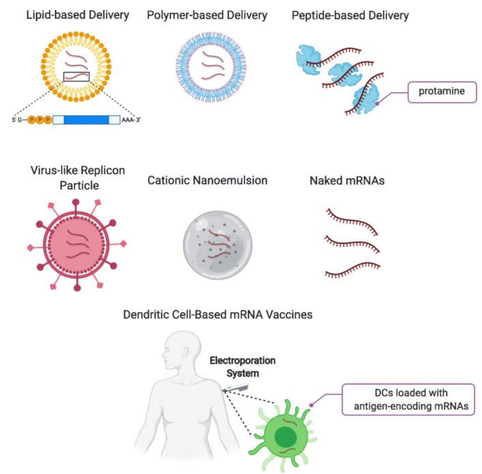

Figure 4: Delivery methods of mRNA vaccines [6].

Analysis:

- The first test round will determine what components help trigger the best immune response
- The second round of tests will determine the impact the alternatives have together
- If successful in the animal and lab stages, scientists could implement the new alternatives into a vaccine given to human test subjects

Potential Ecological Impact

- RNA-based vaccine production is faster than vector vaccine production - currently the most common vaccine – making it take less energy to manufacture [9]
- 99% of the ecological footprint from mRNA vaccines is from air and ground transportation and delivery [9]
  - Emissions due to the transportation sector increase the amount of greenhouse gases, such as Carbon dioxide, in the earth's atmosphere
- The remaining 1% accounts for the manufacturing of the vaccine and the disposal of one-time-use materials such as glass and plastic
- Currently, it is hard to measure just how much mRNA vaccines impact the earth accurately
  - One dose of a COVID-19 mRNA vaccine supposedly has a CO2 footprint of 0.01-0.02 kg, but this value can vary [9]

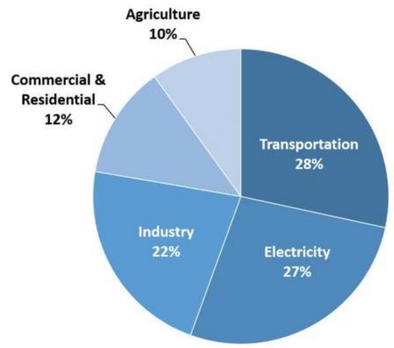

Figure 5: Total U.S. greenhouse gas emissions in 2018 by sector [10].

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