Columbia Engineering Design Challenge: Vaccine Scale-Up

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1 Plant-Based Vaccines: A Brief Introduction

Plant-based vaccines are derived from antigens produced in transgenic plants and harvested from raw plant biomaterial. This technology was first introduced in 1986 with the creation of transgenic sunflower and tobacco plants that produced nopaline synthase and human growth hormone[1]. Since then, there has been an explosion of research into this practice, particularly in the search for a new method of antigen production. There are many advantages to using plant-based vaccines, including sustainability, generation of both a systemic and a mucosal immune response, stability, cheaper production costs, and oral delivery. Since most plant material is edible and the cell wall provides natural bioencapsulation for the antigen proteins[2], much of the lengthy and expensive purification process necessary for traditional vaccines may be bypassed. In addition, because primary production is as simple as cultivating plants, it is much easier to distribute and relocate means of production to areas of low access, allowing for a more decentralized means of production. Our mission was to explore the scalability of plant-based vaccine production on the order of a global pandemic response, to which we assigned an arbitrary goal of $1B$ doses per year.

2 Process Overview & Justifications

2.1 Transgenetic Lettuce

We searched for plants characterized by a high protein-to-weight ratio, short time to maturity, long growing seasons, self-pollination, and small footprint, that were edible with minimal processing (preferably raw). We eventually settled on Simpson Elite lettuce, a commercially available strain of lettuce used by Su et al. in 2015 to induce immune tolerance to coagulation factor IX (FIX) in mice[3]. This particular brand of lettuce is relatively fast growing (50-55 days to maturity) and is heat-tolerant, allowing it to be harvested for a month longer than conventional lettuce.

We were particularly interested in chloroplast genome transformation due to high protein production and maternal inheritance, addressing concerns of transgene escape and cross-contamination[4]. Chloroplast expression would allow for multiple copies of the transgene in each cell, increasing its protein output up to 70% of total leaf protein. Furthermore, the matrilineal propagation of chloroplast genes prevents transgene escape and eliminates a need for the meticulous breeding practices required to prevent cross-contamination.

2.2 Hydroponics Farming

One of our major goals was to make vaccine production more financially and environmentally efficient. We propose using existing infrastructure from the growing "vertical farm" industry and its use of hydroponics. Hydroponics involves growing crops in a nutrient-rich medium in a controlled, closed fluidics system. Vertical farming utilizes stacking layers of hydroponic growing environments in a controlled, indoor space.

The benefits of vertical farming are many. First, the equipment for vertical farming already exists and is in use all around the world. Converting it to plant-based vaccine production is as simple as planting the seeds and adjusting the system to meet the needs of the plant. Hydroponic systems are fully automated, and require less manual labor and resources to grow, lowering the entire process' carbon footprint and creating a more sustainable, reliable manufacturing system.

2.3 Lettuce Lyophilization

Lyophilization, or freeze-drying, is the main processing step in converting the antigen-containing plant material into a stable vaccine. Research has shown that proteins "bio-encapsulated" in lyophilized plant cells maintain their functional folding within the cell and maintain their potency in this form for up to two year at ambient temperatures (and likely longer), thus bypassing an expensive and time-consuming cold chain. The extended lifetime also allows for significant vaccine stock-piles to prepare for high demand periods. Practically, lyophilization also increases the protein-to-volume ratio, allowing more room in a capsule for other components.

2.4 Vitamin E TPGS

After safely transporting the protein to the gut, the secondary issue lies in maximizing intestinal wall absorption via microfold (M) cells and other routes of antigen capture[10]. Vitamin E TPGS is a water-miscible combination of a hydrophobic vitamin E fused to a hydrophilic PEG chain, and serves as a surfactant to significantly increase drug absorption through biological barriers. In a nanocrystal repaglinide form that remains stable for up to 4 years at room temperature, TPGS was able to enhance oral bioavailability up to 15.0-fold compared with free drug[12].

2.5 Enteric Capsules

As an added layer of protection, our lyophilized plant tissue will be encapsulated in commercial enteric-coated capsules to ensure the protein vaccine reaches the gut epithelial tissue. Enteric capsules are specially formulated with a polymer-based coating resistant to gastric dissolution, allowing capsules to reach the small intestine, where the rate of absorption is highest, before releasing their contents[11].

2.6 Mechanical Processes: Grind & Pour

In their paper, Su et al. used a Hamilton Beach coffee grinder to process their lyophilized lettuce leaves[3]. However, we did not exactly see hundreds of thousands of coffee grinders as the pinnacle of scalable approach. Instead, we looked at existing solutions for high-throughput processing and pulverizing. One potential solution we found was an industrial-grade flour mill to grind freeze-dried

leaves to powder rather than grain to flour. One prospect we looked at was the Meadow Mills Steel Burr Commercial Grain Mill at $\sim$ \$3000, reported to be able to process over 18 kg of material per hour, allowing it to easily handle the load requirements for an entire factory and much more. Once ground and checked for antigen concentration, we would be able to use a simple automated capsule filler to portion out the correct amount of lyophilized lettuce powder and vitamin E TPGS into each pill.

3 Production Volume

In order to calculate rough estimates of the resources required to produce on the scale of $1B$ doses per year, we chose the influenza virus vaccine as our model due to its consistent demand over time. The majority of CDC-approved recombinant vaccine products contain 15 µg of HA protein per dose for patients older than 6 months (GlaxoSmithKline) and 4 years (Seqirus)[5]. We found that both antigenic proteins were very similar in molecular weight ($\sim$ 55 kDa). Assuming that our adjuvant would be similar in molecular weight to CTB ($\sim$ 10 kDa), our fusion protein would be approximately 20% heavier than the pure HA used in current vaccines.

Additionally, considering the flu vaccine is administered intramuscularly, we needed to account for the difference in bioavailability between IM and oral delivery. Typical oral bioavailability is below $1-2\%$, while the intramuscular $f\%$ ranges from $75-100\%$[6]. However, these figures on oral bioavailability do not take into account the aforementioned absorption enhancing properties of vitamin E TPGS, shown to increase oral bioavailability by 4 to 15-fold, the combined effect of enteric capsules and natural plant cell wall in preventing premature degradation of the antigenic drug. With these additions, we felt that an oral bioavailability $f\%$ of 10% was realistically achievable.

Then, we used an upper bound of 100% for intramuscular absorption and lower bound of 10% for oral $f\%$ in our calculations. Therefore, with a 10% rate of absorption and a 20% heavier protein, we would require 180 µg of protein per dose. Again, referring to the figures from Su et al., we expect a yield of $\sim$ 43.5 kg of lettuce (in dry weight) per 1000ft^2 per year[3]. From this, we expect target protein yields of 927.02 µg/g to 1478.54 µg/g of dry weight after storage times of 24 months and 2 months, respectively. This translates to $\sim 224000-357000$ doses per 1000ft^2 per year, depending on projected storage time. In the beginning of this design challenge, we set an arbitrary goal of $1B$ doses per year. With the expectation that new vaccines would be consumed within 2 months of production, we determined that $1B$ doses per year would require $\sim$ 0.1005mi of hydroponic farming area[7].

4 Projected Costs

4.1 Fixed Costs

The question of scalability also relies heavily on low variable costs. However, it is also important to consider the initial investment in infrastructure. For this, we examine the Fraunhofer cGMP Plant-Based Vaccine Factory, launched in April 2010 and located in Newark, Delaware. This factory is "fully automatic and houses lighted, irrigated growth modules and processing stations. It

is equipped with Fraunhofer CMI designed, robotically operated, custom engineered machines (including seeding and harvesting machines)"[7] and was built for $\$15M$. At 13000ft^2, this factory would be capable of producing $4.64M$ doses per year. At that rate, in order to reach our goal of $1B$ doses per year we would require approximately 215 similarly sized factories to reach our goal. This comes out to an initial investment of $\sim \$3.225B$. Although a seemingly large figure, this is less than 10% of a large pharmaceutical company like GlaxoSmithKline's annual revenue (in 2019) and would likely be subsidized by local and federal governments - the Fraunhofer factory was granted $\$5M$ by the state of Delaware towards construction and $\$4.4M$ by DARPA towards research.

Furthermore, these cost estimates are limited by the internal growing space of each factory. Considering the speed of lyophilization (72 hours) and the relatively negligible time required to process, grind, and fill capsules, we believe it is safe to assume that the growing rate of the lettuce, on the scale of 50-55 days, would be the primary bottleneck in production. Since we are using chloroplast transformation in our plants, the risk of genetic cross-contamination, transgene escape, and/or loss of transgene expression is next to none. Therefore, outdoor farms and privately-owned greenhouses would largely have the necessary infrastructure in place, provide an attractive supplemental source of greenery, and potentially boost local economies, all while significantly cutting down on the number of required factories. Consider this: a single average U.S. farm of 444 acres, according to the USDA National Agricultural Statistics, could supply enough lettuce for 70% of our $1B$ dose target, and rigorous quality control and processing at the factories would maintain cGMP standards. Although additional time and costs would be spent in transportation, additional quality checks, and labor, it is not a stretch to imagine cutting down the figure of 215 factories by 50-75%, at the very least.

4.2 Variable Costs

We now turn to the final component - variable costs. We do not have significant information on the overhead and operating costs of such a cGMP factory - however, according the financial statements of the aforementioned factory's parent organization, Fraunhofer-Gesellschaft, the total operating budget in 2014 of their Life Sciences Group was $\$175M$ for 7 facilities. Assuming, naively, a uniform distribution of costs across their facilities, this comes out to a $\$25M$ operating budget per facility per year, or \$5.39 per dose at maximum capacity.

The material cost per pill boils down to an enteric capsule, a dose of vitamin E TPGS, and a dose of lettuce-based vaccine. On Amazon, GlaxoSmithKline sells enteric coated capsules for a unit price of \$0.046. The FDA-approved dosage of 15mg of vitamin E TPGS can be procured from Sigma Aldrich for \$0.14 a serving. According to AgFunder Network, the going market rate is currently around \$0.30 per kg of greens in a conventional outdoor farm, and \$1.06 in a hydroponic greenhouse[8]. Since 870 kg of fresh lettuce corresponds to approximately 43.5 kg dry weight which produces $\sim$ 357000 doses, a single dose of lettuce would cost \$0.00073 from a farm or \$0.0026 from a greenhouse. Summing these figures gives us an estimated cost of \$0.1886 per pill (using greenhouse lettuce) in material components, and $\sim$ \$5.58 including operating costs.

Without the need for a cold chain distribution network and trained personnel to administer the vaccine, our vaccine can be distributed significantly faster at a fraction of the cost. Assuming negligible distribution and marketing costs per-unit, and discounting the sunk costs of the initial

vaccine R&D and clinical trials, a company that builds all 215 factories, produces exclusively in hydroponic greenhouses, and prices at $\frac{1}{2}$ the $\sim$ \$40 sticker price of the average flu shot, could recoup their construction costs in $\frac{\$3.225B}{\$20-\$5.58} = 223.6M$ doses, or about 22.3% of their yearly production capacity.

5 Risks & Concerns

The risks associated with our proposal for plant-based vaccines are generally similar to all new vaccine proposals. FDA approval and clinical trials are always a long and arduous process, and always present some risks to participants. However, we would classify our product as a lower risk product than traditional vaccines. Although there are always uncertainties, all components of our vaccine are generally regarded as safe (GRAS). The most significant risk is the potential for ineffective oral vaccines to instead induce immune tolerance of the viral antigen. However, this would likely be due to the repeated insufficient exposure to the antigen caused by low bioavailability of the oral vaccine and ineffective immune response to our adjuvant, both crippling issues to a formulation that would never make it out of clinical trials.

In addition to technical risks, there is the potential for social pushback against the use of genetically modified organisms (GMOs). Despite a dearth of evidence linking GMOs to harmful effects, social pressures in 2016 forced the FDA to add special labels to GMO produce and products[9]. Some encounter the GMO label and express concern towards the product's safety, largely due to miseducation. This sentiment has decreased over time, but still remains, and public fear associated with GMOs may pose a barrier to public acceptance of plant-based vaccines. To address this pushback, it would be necessary to direct marketing and advocacy efforts towards ensuring the public that the product is safe to consume, a requirement that traditional vaccines also must address.

6 References

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