Process Optimization of Inactivated Enterovirus 71 Production with a BIOFLO 310 Bioreactor

Design Project Proposal
BIOE 331 Section 0101

Emily Passaro, Talya Simcox, Valerie Honeycutt

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Statement of the Problem

The global demand for biotherapeutics has surged in recent years due to the increasing prevalence of chronic and infectious diseases. Unfortunately, traditional bioproduction methods struggle to meet this rising demand, resulting in limited accessibility and high costs for patients. This particularly impacts low and middle-income patients, where treatment costs are overly expensive, reducing the availability of life saving drugs.

One such infectious disease is hand-foot-and-mouth disease (HFMD), a viral infection caused primarily by enterovirus 71 (EV71). HFMD causes symptoms such as fever, blisters, and ulcers, and can ultimately lead to neurological and cardiopulmonary complications that result in death (Ventarola et al., 2015). HFMD is commonly found in young children, with the most concentrated number of outbreaks occurring in daycares and nursery schools in the Asian-Pacific region (Yee, 2023). An epidemiological study conducted in China reported a disease incidence of up to 435 cases per 100,000 individuals in 2018 (Wu et al., 2022). After contracting HFMD, only the symptoms of the patients can be treated as no cure currently exists and the only existing preventative measure is a monovalent vaccine available exclusively in China. This can cause significant economic burden, including cost of treatment and impacts on familial income, and a review conducted in Vietnam showed that the corresponding estimated cost of the disease between 2016-2017 was 90 million US dollars (Nhan et al., 2019).

Selected Biological Therapy

The most severe cases of HFMD are caused by infection with EV71. Researchers have postulated that inoculation with an inactivated version of the EV71 virus could potentially be a critical biological therapy to prevent infection (Starr et al., 2003). In 2016, an EV71 vaccine was put on the market in Fujian Province in China. The vaccine takes a copy of the virus and modifies it so that it cannot replicate inside the body. This allows the patients' bodies to develop resistance to the specific virus without actually causing an infection. Compared across 2014, before the vaccine had been implemented, and 2021, five years after introduction of the vaccine, the incidence rate of HFMD decreased from 39% to 11%. Severe cases dropped by 95% and the number of deaths dropped to 0 (Li et al., 2023). Taken together, these results show that an inactivated EV71 vaccine is a promising avenue of HFMD prevention.

Three manufacturers in China produce the inactivated EV71 vaccine, Sinovac Biotech Ltd, Institute of Medical Biology, Chinese Academy of Medical Sciences, and Wuhan Institute of Biological Products. In clinical trials, healthy participants between 6-35 months old were randomized and administered one of the three produced vaccines (Li et al., 2021). Across all groups, not a single patient experienced a severe adverse event as a result of the vaccine, and the most common adverse event reported was a brief fever. Taken together, these results point to the overall safety and efficacy of the vaccine, identifying it as a promising method of minimizing HFMD incidence.

State-of-the-Art Bioreactor Design

Bioreactor design has improved greatly over the last few years, spurred on by the need for efficient and cost-effective systems to meet the ever-growing demand for biotherapeutics. In a recent example, the viral vaccines that were brought about by the COVID-19 outbreak increased the urgent need for efficient bioreactors capable of producing millions of vaccine doses in a short period. As a result, significant focus has been placed on enhancing bioreactor design for viral vaccine production in more recent years. Mammalian and insect cells are commonly

used in systems such as perfusion, batch, fixed-bed, and single-use bioreactors. These advanced systems offer superior control and improved productivity compared to older technologies like roller bottles or tray cultures, due to innovative design and operational strategies. Modern bioreactors incorporate automatic sensing and reduced mechanical stress to enable faster and more versatile operations (Hazari et al., 2024).

Despite the increased need for bioreactors, there are still many challenges associated with their use. Due to the complexity of virus-based particles and extracellular vesicles (EVs), biotherapeutic production is not always straightforward. Factors such as particle stability, variability in size and shape, and maintaining biological activity during purification make large-scale manufacturing difficult. The lack of standardized purification processes adds to these challenges as different biotherapeutic particles require tailored downstream processing (DSP) strategies. Ensuring purity, potency, and compliance with regulatory guidelines while minimizing production costs and processing time further complicates development. Additionally, maintaining aseptic conditions, removing impurities, and optimizing scalability are critical for advancing these therapies from research to clinical application (Moleirinho et al., 2020).

To tackle this, recent advancements in bioreactor design have significantly improved efficiency, scalability, and control in biopharmaceutical manufacturing. Single-use bioreactors offer flexibility and cost savings by eliminating the need for cleaning and sterilization, making them ideal for small to mid-scale production. Perfusion bioreactors enhance productivity by continuously supplying nutrients and removing waste, allowing for higher cell densities to be cultured at a time. Multi-compartment and microfluidic bioreactors enable precise co-culturing and microenvironment control which benefits tissue engineering and drug screening. Innovations like 3D-printed components, miniaturized bioreactors, and in-line monitoring have further optimized performance, reducing costs and enhancing real-time process control. These advancements are driving progress in biopharmaceuticals, regenerative medicine, and industrial biotechnology (Facellitate, 2023). Furthermore, they contribute to reducing environmental impact, improving bioreactor performance, and ensuring consistent product quality across various biotechnological applications (Kumar, n.d.).

Each bioreactor system has its own unique characteristics and are each best suited for different project parameters. One of the main bioreactor systems is a batch culture which involves adding all substrates at the start with no further additions. While this method offers simple operation and low contamination risk, it has limited cell densities, shorter duration, and the accumulation of toxic by-products over time. Fed-batch culture extends batch processing by adding nutrients incrementally to sustain cell growth, resulting in higher cell densities and improved yields. However, the incremental feeding process increases contamination risks, and by-products are not continuously removed. Continuous culture maintains a constant environment by steadily adding nutrients and harvesting cells, which supports consistent cell growth over extended periods. While this process enhances productivity and reduces downtime, it requires careful operation and poses a higher contamination risk. Perfusion culture, a type of continuous culture, retains or recycles cells within the bioreactor, allowing for high cell densities and enhanced productivity in a smaller footprint. Despite its advantages, perfusion culture is technically demanding, has a higher contamination risk, and limits batch traceability (Allman, 2020).

Stirred-tank bioreactors (STRs) are one of the most conventional bioreactors as it is widely used due to their efficient mixing, oxygen transfer, and scalability, and support various impeller designs. However, STRs have drawbacks such as high power consumption, significant

shear forces, and mechanical stability concerns specifically for shear-sensitive animal and plant cells. To address these issues, alternative impeller designs, such as centrifugal impellers, have been developed, enabling successful implementation at volumes up to 20,000 liters. Wave bioreactors, on the other hand, is another common bioreactor that uses a rocking motion to create waves that provide gentle mixing and mass transfer, making them ideal for suspension cultures of various cell lines. They feature disposable, pre-sterilized plastic chambers, minimizing contamination risks and simplifying operations (Zhong, 2010).

Well-known manufacturing companies, like Thermo Fisher, GE Wave Biotech, and Sartorius Stedium, have developed commercial bioreactors meant specifically for viral vaccine production. The advancements in machine learning and computational tools have allowed for software-driven bioreactor modeling, enhancing process simulation and evaluation to produce large-scale vaccine production to be created (Hazari et al., 2024).

Advancements in bioreactor design have significantly enhanced the production efficiency of vaccines against EV71. One is the implementation of microcarrier-based suspension bioreactors, which facilitate large-scale cultivation of Vero cells in serum-free media. In one study the scaling up from a 40 L roller-bottle system to a 200 L bioreactor resulted in an increase three times that in vaccine yield within a shorter production timeline (Wu et al., 2015). Another advancement is the adoption of perfusion culture techniques within these bioreactors to enhance EV71 virus yields by 7 to 14 times compared to traditional single-batch cultures (Fang et al., 2022).

Bioreactor Design Goals

To address the production challenges associated with bioreactors, their design must prioritize scalability, cost-efficiency, and sterility. The system should incorporate advanced monitoring capabilities to ensure precise control over environmental conditions such as pH, dissolved oxygen, and temperature. Bioreactor design goals for the production of vaccines against Enterovirus 71 (EV71) must focus on optimizing several key factors to ensure the best efficiency for manufacturing of the vaccine. First the scalability is very critical, as the bioreactor must accommodate large-scale production to meet overall global demand, especially in high incidence regions like the Asia-Pacific. The system must be able to handle high cell densities while maintaining a controlled environment that optimizes cell growth, virus production, and antigen expression. To meet cost-efficiency goals, the bioreactor should minimize power consumption, incorporate single-use technologies to reduce cleaning and sterilization costs, and ensure a reduction in overall capital investment.

The bioreactor systems must be designed for high-speed production, capable of rapidly producing large quantities of the EV71 vaccine. The design should incorporate advanced monitoring systems for real-time tracking of critical parameters like pH, dissolved oxygen, temperature, and nutrient levels to ensure needed conditions for virus propagation. Durability is also crucial, as the bioreactor must withstand long production cycles without ruining materials or performance. Minimizing contamination risk is essential to ensure product quality and safety for vaccine production that meets stringent regulatory standards.

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