Poster 1

**Population balance equation-based model to understand the crystallization of AAV-based gene therapies**

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Purification of genome-loaded recombinant adeno-associated viral (full rAAV) capsids from empty capsids is challenging. We developed an efficient method for purification of full rAAV based on preferential crystallization. In order to design an efficient crystallization-based method, we need to understand the capsid crystallization process in depth. We developed a population balance equation (PBE)-based model to understand the crystallization mechanism, time scale, kinetics and other physical properties of the crystals.

Poster 2

**Ex Vivo Expansion and Differentiation of Erythroblasts: From Culture Dishes to Stirred Tank Reactors for the Production of Red Blood Cells**

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Transfusion of donor-derived red blood cells (RBCs) is the most common form of cell therapy. Nevertheless, it faces challenges such as emerging blood-borne diseases, and supply limitations; for instance, in low-income countries, or for chronically transfused patients requiring special blood groups. Production of cultured RBCs (cRBCs), in which erythroid precursors (erythroblasts) are cultured from hematopoietic progenitors and subsequently
differentiated into transfusion-ready erythrocytes, is a potential alternative. However, the large number of cRBCs required for a single transfusion unit requires major innovations in the culture process.

Stirred bioreactors are conventionally used for other mammalian cell cultures. In this culture system, turbulence is a critical parameter, due to the reported detrimental effects of shear stress and energy dissipation due to mixing in the growth of some mammalian cell lines. Furthermore, the bioreactor materials can also influence the performance of the cell cultures, as the conventionally used stainless steel can leach metal ions that impact cell growth and viability. The aim of our work is to scale-up the expansion and differentiation of erythroblasts for the production of cRBCs using stirred bioreactors. In addition, we aim to compare the performance of two stirred bioreactor types (conventional glass and stainless steel autoclavable bioreactors and single-use plastic bioreactors).

We have successfully performed the expansion of erythroblast cultures using Applikon MiniBio 500 mL and single-use Applikon AppliFlex ST 0.5 L stirred tank bioreactors, following a repeated batch cultivation strategy. Erythroblasts produced in these systems have shown the same proliferation potential and similar expression of erythroid surface markers (CD235, CD71) as those cultured in conventional static conditions, while maintaining a high viability during the cultivation, suggesting that the potential negative effects of excessive shear rates and turbulence have been avoided.

In addition to proliferation, the differentiation of cultured erythroblast into mature reticulocytes was also tested in the stirred bioreactors. Slightly lower cell yields were observed in the stirred cultures compared to static conditions, in agreement with previous reports on the effect of shear stress on the differentiation dynamics of erythroblast cultures. During erythroblast differentiation, cells lost expression of CD71 and CD49d at a comparable rate to cells kept under static conditions (culture dishes). These results show that the expansion and differentiation of erythroblast cultures is feasible in stirred bioreactors, and indicate that the single-use AppliFlex ST platform is adequate for the transition to GMP production of cRBCs.
Harvesting lentiviral vectors from HEK293T cell culture using a high-throughput multiplexed spiral inertial microfluidic cell retention device

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Successfully commercializing emerging gene and cell therapies will require enhanced viral vector yields. Thus, viral vector manufacturing and harvesting strategies must be improved to meet the rising demand for viral vectors. Research into novel cell retention devices that can efficaciously separate viral vectors from host cells is paramount to making gene and cell therapies commonplace and accessible to patients. Current membrane filter cell retention devices face challenges including membrane fouling and undesired retention of dead cells and products. To circumvent these challenges, we employ spiral inertial microfluidic technology in a cell retention device that carries out downstream processing of terminal batch cultures and perfusion cultures. The spiral inertial microfluidic device is a high-throughput, multiplexed unit, assembled by stacking 50 individual devices. The assembled unit divides fluid entering its inlet across 200 spiral channels to support higher input flow rates by parallelizing the separation of cells from viral vectors. We harvested lentiviral vectors from transfected human embryonic kidney (HEK)293T cell batch cultures at processing rates exceeding 1L/min, testing how the device supports a variety of input flow rates. Vesicular stomatitis virus G glycoprotein (VSV-G) and baboon envelope (BaEV) pseudotyped lentiviral vectors, each with a green fluorescent protein (GFP) transgene, were harvested from cells using the stack of 50 spiral inertial microfluidic devices. The harvested viral vectors were used to transduce Jurkat T cells, and transduction efficiency was measured as the proportion of GFP+ Jurkat T cells identified with flow cytometry. We observed the highest transduction efficiencies at input flow rates of 1350 mL/min for VSV-G and BaEV pseudotyped lentiviral vectors. Future work will apply these findings to continuously process viral vectors produced in perfusion culture.
Mechanistic Modeling of Viral Particle Production

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Viral systems such as wild-type viruses, viral vectors, and virus-like particles are essential components of modern biotechnology and medicine. Despite their importance, the commercial-scale production of viral systems remains highly inefficient for multiple reasons. Computational strategies are a promising avenue for improving process development, optimization, and control, but require a mathematical description of the system. This poster reviews mechanistic modeling strategies for the production of viral particles, both at the cellular and bioreactor scales. In many cases, techniques and models from adjacent fields such as epidemiology and wild-type viral infection kinetics can be adapted to construct a suitable process model. These process models can then be employed for various purposes such as in-silico testing of novel process operating strategies and/or advanced process control.

Establishing a Testbed Setup for Upstream and Downstream Processing of Continuous Biomanufacturing of Monoclonal Antibodies

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Monoclonal antibody (mAb) drugs have been FDA-approved for the treatment of indications such as cancer, rheumatoid arthritis, leukemia, and others. The regulatory framework emphasizes a drug’s critical quality attributes (CQAs), characteristics of the drug that are important for ensuring efficacy and reducing adverse event. For example, glycoforms
attached to a mAb’s Fc region affect the drug’s ADCC (antibody-dependent cellular cytotoxicity) activity and half-life in the bloodstream. Undesirable mAb glycosylation profiles can also lead to adverse events in the patient.

Manufacturers ensure that their products consistently meet their desired CQAs by controlling critical process parameters (CPPs) that significantly impact process performance and product quality. By using continuous manufacturing, a bioreactor can be maintained at a stable operating state using advance process control strategies to ensure consistent product quality.

We are building novel first principles- and data analytics-based mathematical modelling tools to support advanced process control. To experimentally validate these modelling tools and to fully understand the impact of model choice on product quality, we built a fully instrumented and integrated continuous mAb manufacturing testbed. The testbed upstream consists of 4 parallel 3L bioreactors equipped with ATF perfusion devices. Each bioreactor is equipped with instrumentation to fully characterize the process, including in-reactor probes for temperature, pH, dissolved oxygen, optical density (Optek), viable cell density (Aber Futura), and Raman spectroscopy (Kaiser RamanRXN2). To provide further at-line process and product characterization, each upstream assembly is equipped with two MAST sample pilots for automated sampling of both the bioreactor contents and the ATF perfusate line. The MAST system also delivers samples to a Nova FLEX2 cell culture analyzer for key metabolite quantification as well as verification of in-reactor sensors including pH. For at-line characterization of CQAs, the cell-free perfusate samples are collected in a Gilson GX-271 liquid handler, purified using at-line purification using Protein A chromatography, and delivered automatically via MAST to either an Agilent 1260 Bio-Inert HPLC for assessment of aggregation and titer or to an Agilent 6545XT LC/QTOF for characterization of glycosylation profiles using mass spectrometry. One bioreactor assembly is integrated with a fully continuous downstream system including Protein A and ion exchange chromatography (Sartorius BioSMB), in-house-designed continuous viral inactivation, and concentration and buffer exchange (Sartorius SartoFlow).
Mathematical modeling to optimize rAAV production to insect cells

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Efficient manufacturing of recombinant adeno-associated virus (rAAV) is of utmost importance for the successful delivery to patients of cutting-edge gene therapy treatments. Still, current rAAV manufacturing processes with both mammalian and insect cells present severe limitations in terms of efficiency, and lead to production of a significant amount of empty capsids. In this work, we develop a mathematical model for rAAV production with the baculovirus expression vector system to shed insights on the extracellular and intracellular bottlenecks for filled capsid synthesis. The model features 117 ordinary differential equations and 30 parameters, describing the physical and chemical phenomena occurring during baculovirus infection and rAAV formation. The intracellular phenomena considered by the model include baculovirus transport to nucleus, DNA replication, transcription, rAAV capsids and Rep proteins synthesis, transgene rescue and amplification, and transgene encapsidation. The model predictions show good agreement with experimental measurements of intermediates and filled capsids concentration across several batches. We use the validated model to carry out a digital analysis of the process to investigate process conditions and genetic modifications that can increase productivity.
Production of defective interfering particles in high cell density perfusion cultivations with continuous virus harvesting

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Since many years, defective interfering particles (DIPs) derived from influenza A virus (IAV) have been discussed for their use as antivirals. DIPs are virus mutants harboring an internal deletion in one of their viral RNA (vRNA) segments. Due to these deletions, DIPs can only replicate in the presence of infectious standard virus (STV), compensating for the missing gene function. In a co-infection, the defective vRNA interferes with STV genome replication and stimulates the immune response, and thus has therapeutic potential. In the present study, we established an automated perfusion process for production of a DIP, called DI244, using a VHU® Perfusion system comprising tubular membrane modules for cell retention and for continuous virus harvesting into the permeate.

Long-acting Microparticle Vaccine Platform for Single Injection Hepatitis B Vaccination

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Many vaccines—such as the polio, COVID-19, tetanus, and diptheria vaccines—require at least one booster shot often administered 1 month later to complete an immunization. Limited patient access, due to geographical barriers or compliance issues, can prevent high vaccine coverage when multiple vaccinations are required for immunity. To address this, we developed long-acting vaccines which require only one shot containing both the prime and
boost doses. In fact, we can deliver up to 11 boosts over a 6-month period. We utilize a novel microparticle delivery system called SEAL that encapsulates, stabilizes, and releases labile vaccines at extended time points many weeks after initial injection. Here, we show the application of this technology for a long-acting hepatitis B vaccine with CpG adjuvant. We demonstrate that long-acting SEAL vaccines for hepatitis B release active HBsAg antigen and CpG at approximately 4 weeks after administration and are immunogenic in vivo equivalent to 2 bolus injections. This technology would allow, for the first time, multiple doses of vaccine to be formulated into a single injection, thereby simplifying vaccine administration and increasing coverage.

Cell Therapy Process Development with a 2 mL Perfusion Bioreactor

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With six chimeric antigen receptor (CAR) T cell immunotherapy products currently approved by the FDA for the treatment of hematological cancers, there are increasing efforts to develop novel and better manufacturing technologies and processes for cell therapies to improve efficacy, reduce variability, and reduce cost. Knowledge in the field about how to optimize cell expansion for consistent and reproducible cell-based treatments is improving, but major challenges still exist in experiment reproducibility and robustness during process development. For autologous processes, donor and patient variability can confound experiment results. Materials are also costly, including peripheral blood mononuclear cells (PBMCs), growth factors, viral vectors, and serum or chemically defined media. Therefore, process development experiments in cell therapy are typically done in milliliter sized, often static, cultures with minimal environmental control. While these systems can generate data quickly, the lack of control and monitoring can result in variabilities that may prove difficult to translate to larger culture systems. To address this gap, Erbi Biosystems has developed the Breez™ True Perfusion™ bioreactor. This fully closed sterile single-use perfusion bioreactor operates at a 2 mL working volume and achieves industrially relevant cell densities in excess of 100e6 cells/mL while also reducing labor and bench space requirements. Using the Erbi Breez™ bioreactor, we implement in-place activation, transduction, and expansion. We show that with improved media exchange rates, cell expansion performance is improved, achieving nearly patient dose levels with more than 300-fold expansion from 0.6 million cells at the point of transduction to more than 100e6
cells/mL at 14 days post-transduction compared to 10^6 cells/mL for the 24-well G-rex, with a similar total media consumed per cell ratio. Transduction efficiency was also comparable between the bioreactor and spinoculation on a retronectin-coated 24-well plate. We then looked at intensified donor and media screening performance between the two platforms to show how higher performance can provide more resolution into donor performance and variability, achieving cell densities of 200e6 cells/mL in the best case. Additionally, on-line data showing optical density, pH, and dissolved oxygen will be presented. These data show that the Breez™ platform is well suited for both cell therapy process development and manufacturing for lower dose size therapies.

**Poster 10**

**Scalable, High-Titer Production of Clinical Grade Adeno Associated Virus in the Gibco™ CTS™ AAV-MAX Helper-Free AAV Production System**

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Production of high-titer AAV at clinical scale remains a significant challenge for the gene therapy industry. Developers need to understand how to optimize, scale, and qualify their manufacturing process for AAV production under the tightest of timelines. Due to high demand in the gene therapy space, effective solutions to address these challenges must be developed and implemented. Until recently, AAV production took place primarily in adherent cultures using 293T cells in the presence of fetal bovine serum; however, such adherent systems suffer from a number of significant drawbacks including difficulty in scaling up, the presence of the SV40 large T antigen in the producer cell line as well as cost, consistency and regulatory considerations stemming from the use of animal sera. To address these shortcomings, we present data on the Gibco™ CTS™ AAV-MAX Helper-Free AAV Production System, a chemically defined, suspension-based AAV production system that allows for scalable, high-titer production of AAV viral vectors in a non-293T cell lineage. The AAV-MAX system comprises all of the components required for scalable AAV production in mammalian cells including: (1) a clonally-documented, high-titer 293F-derived producer cell line (VPC2.0), (2) a chemically defined growth and expression medium, (3) a production enhancer, (4) a cationic lipid-based transfection reagent and booster, (5) a protein-free complexation buffer, and (6) a Polysorbate 20-based lysis buffer. The AAV-MAX system delivers AAV titers at ≥5x10^10 viral genomes per mL (vg/mL) across multiple AAV serotypes with excellent scalability from multi-well plates to stirred tank bioreactors making the AAV-MAX system an ideal production platform for generating clinical grade AAV.
Filtration Insights in Gene Therapy Processing

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The gene therapy industry is growing rapidly, with over 20 therapies now approved worldwide and hundreds more in clinical trials. As the field reaches a wider range of indications with larger patient populations, some added focus must be placed on developing more efficient, scalable manufacturing processes.

Early gene therapy manufacturing has largely borrowed technologies developed for recombinant protein processing. This has generally translated well, but there is still significant room for improving yield and product quality. In this work, we focus on filtration technologies including depth filtration, tangential flow filtration, sterile filtration, and viral filtration. We will discuss how well each has translated into processing of adeno-associated virus (AAV) and lentivirus, two of the largest classes of gene therapies, and highlight areas for improvement.

Mediating Cell Adhesion Using Surface Microtexture

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There is a need for surfaces that lower cell adhesion strength while maintaining cell growth to enable the next generation of cell culture surfaces for delicate primary cells and automated, high throughput workflows. In this study, we investigated the use of microtexture alone to control cell adhesion. We developed a fast, simple, and inexpensive process for creating microtextured polystyrene surfaces. These cell culture surfaces enable decreased cell adhesion strength while maintaining high cell viability and proliferation, through a simple reduction in the cell-surface contact area. Cancer cells grown on microtextured polystyrene showed significant changes in cell morphology compared with cells grown on flat polystyrene. Using image analysis to quantify cell morphology changes, we found that surface textures decreased cell area by half and led to much
more elongated cell shape compared to flat surfaces. We designed a microfluidic shear force measurement platform to quantify the removal of cells from these surfaces, and showed that significantly more cells were removed from the microtextured surfaces than the flat surfaces, demonstrating that our surfaces lead to decreased cell adhesion.

Poster 13

Rational Design of rAAV Production via Mechanistic Modeling

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Recombinant adeno-associated virus (rAAV) is one of the most commonly used platforms for in vivo gene therapy treatments. The reduced toxicity, robust and long-term transgene expression, and ability to transduce both dividing and non-dividing cells as well as target a wide range of tissues have made rAAV the most widely used viral vector. However, the standard method of producing rAAV via transient transfection of mammalian cells, specifically human embryonic kidney 293 (HEK293) cells, typically has low yield and generates a high portion of empty particles, laying extra burden on downstream processing. To elucidate the mechanisms of rAAV synthesis in HEK293 suspension-adapted cells, we have developed a mechanistic model based on the published understanding of the underlying biology and existing data. Quantitative analysis suggests the misaligned dynamics of capsid and viral DNA production result in the high ratio of empty particles. Through a model-based strategy, we explored a novel transfection method using low-dose multiple transfections in HEK293 cell culture that successfully increased the ratio of full-to-empty capsids in the viral harvest without compromising the viral titer. Molecular analysis through a next-generation rAAV production model attributed the improvements to changes in the kinetics of viral protein expression and DNA replication. Here, we demonstrate that the use of multiple transfection times is a practical method for increasing the genome titer and improving the percentage of full capsids for rAAV production. Our results also demonstrated the capability to manipulate product composition from an operational standpoint.
Capturing bubbles and preventing foam using aerophilic surfaces

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In most bioreactors and biomanufacturing facilities, gas is introduced into the culture medium through sparging to create the necessary growth conditions. In this process, foam is formed and stabilized by proteins and other surfactants in the media. Foam is unwanted as it leads to cell death, loss of sterility, decreased operational volume, and other unwanted outcomes. To combat this, the authors propose nano-engineered surfaces that can capture bubbles before reaching the air-water surface, eliminating the formation of foam. When a rising bubble approaches a horizontal surface, the thin liquid film separating the bubble and the surface needs to be drained for the bubble to be captured by the surface. The authors design aerophilic surfaces that can efficiently drain liquid films upon bubble contact, and show how their ability to catch bubbles relates to their texture parameters. The authors develop a physical model, based on surface slip length and liquid properties, to determine when the film would drain. Then they use these findings to design a plastron encased surface comprising textures across three length scales, which enables capture upon contact and a reduction of two orders of magnitude in capture time relative to a flat surface. Finally, the authors use these principles to create an easily scalable device that passively captures a rising flow of bubbles in a surfactant rich solution. This technology can be utilized to prevent foam from forming in bioreactors and fermentation tanks and eliminate the use of antifoaming agents.
Cell and gene therapies (CGT) are at the forefront of innovation and developments to treat, prevent, and potentially cure severe diseases including cancer. Ingredients and manufacturing processes of these therapeutic products are very susceptible to contamination with adventitious agents such as viruses and bacteria. These contamination events, albeit very rare, not only take millions of dollars to recover from but also lead to patients not receiving therapies. The safety of these products is critical, especially since the recipients are immunocompromised and highly vulnerable to infections.

To ensure safe and effective production, there is a vital need for methods that can reliably assess products for contamination with high sensitivity. Sterility testing, a crucial step in product release, is currently a huge bottleneck in the timely delivery of CGT products to patients. The traditional compendial sterility tests take up to four weeks for a result. The absence of a label-free, rapid assessment method for microbial safety impedes the widespread adoption of cell therapy products. In short, high-throughput methods with minimal sample preparation for the detection and characterization of individual viruses are desired.

Nanopore sensors have the potential to be the ultimate detectors of charged biological species. An analyte is electrophoretically driven through a nanoscale pore submerged in an electrolytic solution. Based on the underlying principle of 'resistive pulse sensing', an electric pulse signature that contains the characteristic geometrical and chemical information of the analyte is generated. Using a combination of experiments and multi-scale simulations, we are designing nanopores for selective, label-free, and rapid fingerprinting of the target virus species.

This work presents a novel strategy to make biomanufacturing safer through at-line detection of adventitious agents in the manufacturing process. The technology can enhance safety and efficacy in the development of therapeutics for a broad range of diseases. Importantly, it will reduce the time required for sterility testing from four weeks to just a few hours, thereby cutting down the overall manufacturing timeline by 50%.
Towards a Scalable AAV5 capsid Production using Microbioreactor for Gene Therapy Application

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Adeno-associated virus (AAV) is a commonly preferred vehicle to deliver the gene of interest (GOI) in gene therapy application. Current AAV production methods do not scale on cost basis, and this impedes the reach of gene therapy technology for treating rare genetic disorders. Here we demonstrate AAV5 production using Erbi Breez™ microbioreactor via triple plasmid polyethyleneimine transfection of HEK293 cells. Erbi Breez™ is a 2mL microbioreactor that enables high cell density culture (reaching up to ~100 million cells/mL) in a perfusion mode. In principle, Erbi Breez™ can produce a single therapeutic dose of AAV5 at high volumetric efficiency and is a very promising technology for personalized medicine. However, a major challenge is to optimize the capsid production process at high cell densities, which is currently lacking. In order to develop a conceptual understanding of AAV capsid production at high cell densities, here we demonstrate AAV5 capsid production at low (~ 1.5 million cells/mL) and medium cell densities (~ 10 million cells/mL) using microbioreactor in perfusion mode. We observed that dynamics of capsid production and their corresponding metabolic states of producer cells were significantly different under these two conditions. However, per cell productivity and filled capsid fraction were comparable to that of shake flask experiments at similar cell densities. Currently, we focus on improving the existing process by exploiting the capabilities of microbioreactor, developing new transfection strategies, and engineering metabolic pathways of producer cell lines to meet critical quality attributes of AAV5 capsids. Our multidisciplinary approach will pave the way for democratizing gene therapy technology across various spheres of genetic disorders and can accelerate the bench-to-bed clinical translation.
Thermal Imaging-based State Estimation of a Cell Thawing Process

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This work describes observer design of a cell thawing process used in cell therapy in which frozen cells need to be thawed before injection. The first-principles model is formulated based on the Stefan problem and dynamically simulated via a finite difference scheme with the method of lines solved over a moving grid. A Luenberger observer is designed based on the continuous-time moving-boundary moving-grid equations and information from real-time thermal imaging. Simulation and experimental results show that the integrated numerical model and observer can precisely predict the spatiotemporal evolution of thawing with errors of 1–2% throughout the cell thawing process despite sensor noise, offering a reliable approach for monitoring and control of cell thawing.
A novel g-TUBE®-based sample pretreatment method to increase the sensitivity of adventitious virus detection using third-generation sequencing

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An increasing number of diseases are becoming treatable with CAR-T cell therapy and the risk of virus contamination during T-cell biomanufacturing poses serious concerns for the safety of patients. The use of High Throughput Sequencing (HTS) recently emerged as a promising alternative to in vivo adventitious agent testing approaches because it enables a more sensitive and pathogen-agnostic microbial detection. However, a significant obstacle to microbial detection using HTS is the excess abundance of background nucleic acid from the human or mammalian host, which decreases the sensitivity of microbial detection using current HTS technologies.

The g-TUBE® is a plastic tube marketed as a nucleic acid shearing tool, with an inlet portion and a channel upstream from the shearing region designed to maintain relatively constant pressure during the sample flow through the shearing region. In a preliminary experiment, we observed a significant decrease in cell density and viability after centrifuging a T-cell suspension using a g-TUBE®. Then, we hypothesized that performing RNA/DNA digestion following g-TUBE® centrifugation would robustly degrade the cell-free nucleic acid while maintaining the viral capsid. To test our hypothesis while developing a new sample pretreatment method, T-cell preparations were artificially contaminated (or spiked) with viruses at a known cell-to-virus ratio. The novel sample pre-treatment method consisted of centrifuging the sample in a g-TUBE®, followed by DNA/RNA degradation using nucleases. In addition to the novel pre-treatment method, we evaluated pre-treatments using the g-TUBE® or nuclease as a control. We synthesized the second strand from extracted nucleic acid, prepared the DNA library, and performed sequencing using the GridION sequencer from Oxford Nanopore Technologies. We ran bioinformatic analyses using epí2me’s Fastq-custom and What’s In My Pot (WIMP) to estimate the abundance of viral reads and human DNA reads. Total nucleic acid was quantified using a Qubit™ fluorometer and host/viral specific nucleic acid was quantified via ddPCR for evaluating quality control.

Our results demonstrate that pre-treatment of spiked samples with the g-TUBE® lyses T-cells, releasing their genomic content, which, in turn, is digested by non-specific nucleases.
In addition, we observed that pre-treatment with g-TUBE® did not affect the averaged absolute concentration of representative DNA and RNA viruses. Following sequencing, the total number of reads analyzed ranged between 330,087 and 7,463,518 reads. We did not detect any reads from the spiked virus in the untreated control sample due to the high abundance of human background DNA and RNA. Interestingly, we observed the highest proportion of viral reads in the samples pre-treated using our novel method.

In summary, we report that centrifuging the sample in g-TUBE® and performing subsequent cell-free DNA/RNA degradation is an efficient pre-treatment that increases adventitious virus detection sensitivity using Oxford Nanopore sequencing. Our novel sample pre-treatment method can be incorporated into sequencing pipelines of other sequencing technologies for the sterility testing of cell-based therapy products.

**Case Study: Production and Purification of CD19CAR-AAV6 at the 50L Scale in the Gibco™ CTS™ AAV-MAX Helper-Free Production System**

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Given the tremendous interest in the use of viral vectors for gene therapy applications, there is a critical need to scale AAV manufacturing processes to deliver products able to fulfill clinical and commercial needs. Due to the speed with which sponsors look to move their therapies into clinical testing, the ability to have robust, off-the-shelf solutions for AAV production, as well as detailed guidance for how to produce and purify AAV using those systems at large scale, is of utmost importance. Here, we present data on the production and purification of a CD19CAR-AAV6 using the Gibco™ CTS AAV-MAX Helper-Free AAV Production System from the shake flask up to the 50L single-use bioreactor scale. Bioreactor control and process parameters are presented for optimized AAV production at the 2L, 10L, and 50L production scales, including inoculation strategies, agitation, pH control, and plasmid DNA complexation parameters. Additionally, we present a scalable downstream process for purification of the 50L CD19CAR-AAV6 production run. This purification strategy utilizes depth filters for supernatant clarification, tangential flow filtration (TFF) for concentration and buffer exchange prior to loading of the AAV onto the POROS™ CaptureSelect™ AAVX Affinity Resin. Utilizing this process, we achieved ~44% total process recovery of rAAV6 vectors demonstrating feasible end-to-end production of AAV vectors using the AAV-MAX Production System.
Accelerating the manufacture and scale up of virus-like particle vaccines

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The recent global health crisis demonstrates the need for preparedness in responding to emerging and evolving viral pathogens and demonstrates a pressing need for plug-and-play platforms for rapid development of safe and effective vaccines. In the current race for COVID-19 vaccines, most of the approved or authorized vaccines candidates are mRNA and viral vector-based vaccines which target the viral spike (S) protein. However, as COVID-19 continues to evolve, current vaccines become less effective against emerging variants. Additionally, mutations occur in all SARS-CoV-2 proteins, illustrating the risk of depending upon a single vaccine target. Since SARS-CoV-2 patients also exhibit immune response against Nucleocapsid (N) and Membrane (M) proteins, a vaccine that contains all part of the virus will be a more promising long-term solution.

VLPs are nanoparticles comprised of self-assembled viral proteins and host lipid membranes that resemble the original virus, but are non-infectious due to the absence of a viral payload. Their conformation and epitope presentation are more similar to the native virus; therefore, VLPs are expected to elicit a stronger immune response than other types of vaccines. We propose an integrated approach to develop a virus-like particle (VLP)-based vaccine platform that includes an HEK-293 transposon-based expression system and advanced process development for intensified production of the VLP product. This comprehensive platform will result in rapid, streamlined VLP vaccine development and manufacturing that can speed development of emerging pathogenic viruses.

The flexible platform features a modular approach to designing plasmids for the protein components of a candidate VLP vaccine. Candidates are screened by transient transfection into the producer cell line, and then scaled-up in manufacturing. The project team combines expertise in synthetic biology, biological process modeling and control, and manufacturing process development, including extensive experience with continuous biologics manufacturing.
This project will demonstrate a platform for rapid development of VLP vaccines that can potentially confer broader protective immunity than spike only-based protein subunit vaccines. This approach will have broad applicability to vaccine manufacturing generally. Our process will incorporate model-based product and process development that can accelerate identification of candidates with manufacturability and immediate evaluation of the potential for process intensification. This approach will allow expansion of the VLP modality in the arsenal against emerging viruses.