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Vaccines have moved into the spotlight in recent years and are now the subject of intense discussion around the world. From preventing deadly diseases to stopping the spread of illnesses, vaccines have made a profound impact on human health and have saved countless lives.

Each new vaccine development and the ongoing study of vaccine effectiveness expand scientific knowledge and contribute to research into these life-saving treatments. Numerous vaccine technology platforms have evolved over the past decades. As a result of their proven track record in successfully combating many diseases, traditional whole pathogen vaccine platforms have achieved wide acceptance. While these vaccines still require cultivation of the pathogen, the newer generation of vaccines, such as recombinant-protein and nucleic-acid vaccines only require the genetic sequence of the pathogen.

These breakthrough platforms can significantly accelerate development and manufacturing processes as well as unlock new potential to address a broad range of indications at an unprecedented speed. After all, to meet the growing need for new vaccines, they must be developed quickly and cost-effectively.

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Stirred-tank Bioreactors and How They are Used in the Development of Vaccines

Abstract

Increasing demands from governmental vaccination programs and pandemic events such as the COVID-19 outbreak require scientists to work under pressure to shorten the time-to-market of developed vaccines. The current global vaccine market valuation of approximately 50 Billion USD, with 80% of the market on human vaccines. Altogether, a need for new methods to increase speed and yield, and to produce new vaccines in a cost-effective manner in order to remain competitive is a constant concern for scientists. Although the competition on the vaccine market is high, big vaccine manufacturers are more and more forming and maintaining collaborations with young companies and former competitors to speed up vaccine development. Additionally, we see a trend towards collaboration with the biotechnology industry in order to accelerate the research, development and large-scale production of new vaccines.

Advanced technologies in upstream bioprocessing enhance the efficiency of vaccine development

Parallel processing	Single-use technology	Scalable systems	Process intensivation	Efficient use of data

Figure 1: Advanced technologies in upstream biotechnology.

Bottlenecks for production arise from the use of two-dimensional T-flasks and roller bottles. Therefore, a shift to stirred-tank biological control systems is essential in order to increase productivity. By enabling the parallel control of several bioreactors at the same time, monitored and controlled by powerful software solutions, vaccine development processes can be optimized in small scales and the parameters transferred in order to scale-up to large production volumes.

Parallel Processing - Learn from failures and optimize the bioprocess

Process optimization consumes time when experiments are running individually and sequentially. And these experiments are very costly. By utilizing scale-down strategies and single-use bioreactors, the consumption of resources can be reduced. Parallel bioprocess control systems are well suited for scale-down approaches and offer the possibility to change individual parameters in several bioreactors at the same time, while monitoring and comparing the effect of the changes in parallel (Fig 2).

Figure 2: DASbox® Mini Bioreactor
Parallel operation of up to 24 vessels. The optimal tool for PAT, DoE and scale-down approaches.

Single-Use solutions – A step ahead of cross contaminations

Process optimization and development includes significant manual interactions, increasing the risk of contaminations. Traditional glass or stainless-steel bioreactors need to be carefully cleaned and sterilized after each run before they can be reused.

Especially nowadays, where time is crucial to find a cure against COVID-19, the use of single-use bioreactors offer the potential to speed up a bioprocess and prevent the loss of a whole run due to contamination.

Scalable systems

Nearly five million people have been infected by the novel corona virus so far. Due to its high infection rate, this number is expected to increase tremendously before a new vaccine will be available. In order to produce enough doses of vaccines to help develop immunity at a global scale, easy parameter and technology transfer if needed when scaling up from bench- to pilot- and production is needed. However, developing a scale-up strategy is time-consuming and cost-intensive. High titer, robustness of the process, constant product quality, fast turn-around times, and scalability are some of the success factors that need to be considered. It is important to work with bioreactors that are comparable with bench- and pilot- and production sized bioreactors. Keeping in mind critical scalability-related engineering parameters like proportional vessel/impeller geometry, oxygen transfer rate (OTR), impeller power number (Np) and the impeller power consumption per volume (P/V) helps to optimize a scale-up strategy.

Experience the power of data

One of the major benefits of working with advanced bioreactor control systems is the use of powerful SCADA software. A powerful software suite, monitoring all critical parameters, automatically adapting feeding speed, gassing conditions, and many more parameters, is the heart of each process. With the help of software, limiting factors can be detected and eliminated to efficiently optimize a process. Thanks to the digitalization, the global lock-down did not affect international collaboration of scientists and manufacturers. Like the scientists and manufacturers around the globe are communicating with each other, it is also important that the software is able to understand all the information delivered by the various sensors connected to a bioreactor. This is especially true when they are manufactured by different suppliers. Modern communication protocols such as OPC UA enable the seamless communication among devices, allowing the independent implementation into a process while being safe and stable.

Conclusion

Stirred-tank bioreactors are one of the key technologies needed on the journey of developing and producing a new vaccine (Fig.3). They are optimal tools during each step in upstream biology. Working with bioreactors enables for the parallel control of several bioreactors resulting in a more efficient and reproducible optimization of various process parameters. The quality of the produced product greatly benefits from the possibility to program automated responses such as feeding cycles or pH control. And finally, large systems are available on the market that are suitable to operate in cGMP environments to produce vaccines.

Figure 3. Scalable systems – Total upstream solutions.
Eppendorf bioprocess products cover the whole upstream bioprocess workflow from early research to large scale production volume in development.

For more information about Bioprocessing in Vaccine Development and Manufacturing visit the Eppendorf website.

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WHITE PAPER No. 23

Vero Cell-based Vaccine Production: Cell lines, Media and Bioreactor Options

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Executive Summary

The recent Covid-19 pandemic introduced new challenges for the vaccine industry, it has also brought in new innovations in vaccine development including DNA/RNA based vaccines. The pandemic also increased demand for well-established cell-based vaccine production technologies.

We hereby review the strategies for optimizing Vero cell based vaccine production using rabies and influenza as examples. The Vero cell line is one of the most satisfactory vaccine production hosts based on its infectability, stability and well-documented performance in quality and quantity of viral yield. It is one of the first cell lines that received FDA approval for vaccine use and is used throughout the world. Cell culture media technology has advanced drastically in recent years, and a number of serum free and protein free options are available through commercial suppliers. Because serum tends to bind toxins and contaminants, its elimination calls for adoption of the highest quality reagents and careful monitoring of culture conditions in order to achieve optimal performance.

Vero cell-based vaccine production often utilizes micro carriers or similar types of cell attachment matrix. The advancements of 3D cell culture matrix such as Fibra-Cel® have been important additions to the range of possible choices for optimizing in vitro production systems.

With a wide array of bioreactor options available, high-density attachment cell culture will continue to be one of the most productive methods for vaccine production.

In this era of daunting challenges from the Covid-19 pandemic, Eppendorf is constantly upgrading and expanding its cell cultivation technologies to meet the demands of this unprecedented public health emergency.

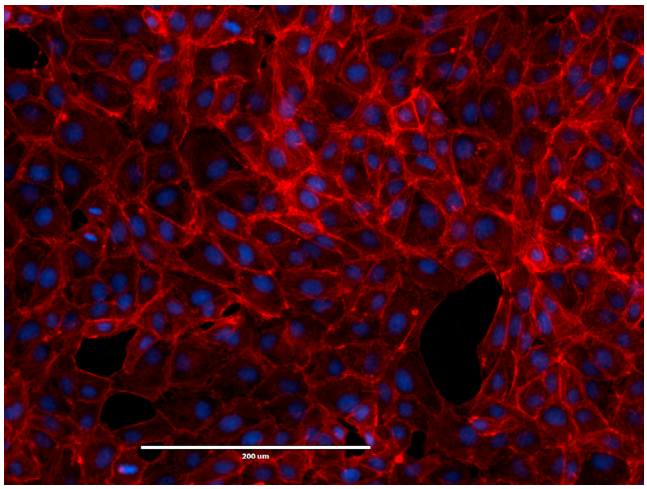

Fig. 1. Fluorescent image of confluent Vero cells DAPI-stained nuclei appear blue, and actin filaments stained with rhodamine-conjugated phalloidin appear red (Eppendorf Inc.).

Introduction

Long before Covid-19, various viral diseases, including rabies and influenza, have been worldwide challenges to the international biomedical community. WHO notes that in 1998 over 32,000 deaths due to rabies were reported, while influenza has been responsible for millions of deaths worldwide over the course of the last century [1].

Rabies is often transmitted to humans from infected domestic animals. Dogs infected with rabies can become extremely aggressive and attacks on humans are widespread, especially in certain Asian countries where using unleashed dogs for home security is common, and the dogs were often not vaccinated. The virus is spread through their saliva and bites by infected animals can be fatal. In China, the disease is referred to as "Kuang Quan Bing", i.e. "Mad Dog Disease". The annual number of deaths caused by rabies had grown to about 59,000 worldwide according to the World Health Organization (WHO) [2].

Since the 18th century, vaccination has proven to be the most successful (and perhaps the only) route to the elimination of viral diseases. The history of smallpox is well known, as is the introduction of the use of cowpox virus from lesions in infected animals by Jenner in 1796 [3]. Despite his work and that of others, smallpox epidemics continued throughout the 19th century, due to improperly applied or non-existent vaccination regimes. The work of Louis Pasteur and others toward the end of the 19th century put vaccination on a sound scientific footing [4].

Influenza has been a permanent worldwide scourge long before Covid-19 virus. The CDC Influenza Division reported an estimated range of deaths between 151,700 and 575,400 individuals resulting from the 2009 H1N1 virus infection during the first year that the virus circulated [5]. Annual deaths in the United States reached 61,000 in the 2018 season with over 800,000 hospitalizations according to CDC statistics [6]. Anti-viral drugs are employed for acute treatment, but vaccination remains far and away the most effective approach for combating viral illnesses.

There has been for years a constant, underlying concern regarding the possibility of the emergence of a truly deadly virus strain, on a level with the 1918 influenza outbreak, the "Spanish Flu" that caused ~50 million deaths throughout the world. This catastrophic possibility was realized this year (2020) with the appearance and pandemic spread of the Covid-19 virus, now responsible for over 50 million cases and over 1.3 million deaths worldwide in its first year alone.

Currently numerous Covid-19 vaccine development programs are underway throughout the world, many of them utilizing the tried and true cell culture methods, including Vero, to produce Covid-19 virus fragments and/or spike protein for vaccine development purpose. The Vero cell line has been used for years in various virus vaccine development, and is recognized as a safe and efficient production tool. With the current demands of pandemic vaccines development, we believe the need for bioreactor-based Vero cell culture will continue to grow.

Biological Systems for Viral Cultivation

Today the expanding demand for vaccine products has necessitated the development of a range of techniques for growing large quantities of antigenic proteins. Traditionally, viruses have been grown in embryonated hen's eggs, but numerous shortcomings compromise their utility. These include a bottleneck in the availability of high quality, pathogen-free eggs, as well as low titers of emerging viruses [7]. Another major concern is that when viruses are cultivated through extended passages in hens' eggs, there is an evolutionary process in the amnion and allantoic cavity of the egg resulting in the selection of certain viral subpopulation, antigenically and biochemically distinct from the original inoculum. Because of these and other factors, well characterized permanent cell lines are coming to dominate the field.

As an alternative to egg-based vaccine production, the advantages of mammalian cell culture systems have been widely recognized. Cultured cells provide much shorter lead times, a more controlled production process that takes advantage of closed-system bioreactors, a reduced risk of microbial contamination, and the opportunity to cultivate viral stocks without significant egg passage-dependent antigenic changes [8].

A WHO conference some years ago expressed concern regarding the rapid emergence of pandemic viral strains. It was concluded that insufficient time would be available to generate the large quantity of high quality, fertile hens' eggs that would be required to the demands of a worldwide pandemic. In recent years, situation has only exacerbated. Thus, the cell culture alternative provides a flexible and scalable platform that can make use of the well-established biopharmaceutical bioreactor cell culture infrastructure for vaccine production.

Cell Line Options

Over the years, a number of stable cell lines have been approved by regulatory authorities for influenza virus production, such as the Spodoptera frugiperda insect cell line (Protein Sciences/Sanofi [9]), the Madin-Darby canine kidney (MDCK) and the Vero cell line from African green monkey, one of the most widely used. Another example is the PER.C6® cell line, which was recently announced for use in a Covid-19 vaccine development program from J&J [10]. It is noteworthy that certain cell lines may provide an environment favoring selection of viral subpopulations, and these types may be inappropriate for vaccine production. Anez et al attempted production of Dengue virus vaccine candidates using FRhL-2 diploid fetal rhesus monkey lung cells [11]. However, passage in this cell line resulted in the accumulation of a mutational variant which was responsible for reduced infectivity and immunity in Rhesus monkeys. This phenomenon was not observed in viruses passaged in the Vero cell line. Other lines of investigation support the Vero cell line as the candidate of choice for viral vaccine production, including: efficiency of primary virus isolation and replication to high infectivity titers; genetic stability of the hemagglutinin molecule, while maintaining the antigenic properties of human-derived viruses; and similarities in the pattern of protein synthesis and morphological changes between virus-infected Vero and MDCK cells [12,13]. Given the regulatory acceptance as well as the abundance of vaccines already successfully developed on Vero platform (Table 1), The attachment culture of Vero cells remains to be one of the most attractive options for cell based viral vaccine production. The continued interest has also driven the scientific community towards further development of Vero cells into suspension cell lines, further expands the capability of the Vero cells in the vaccine development and production market [14].

Media Alternatives

There are a variety of different Vero isolates available from commercial suppliers (Vero, Vero 76, Vero E6, Vero B4), but all are quite similar, and their nutritional needs are comparable [17]. The search for the ideal mammalian cell culture medium began in the 1950s, with the holy grail being an economical, protein-free, serum-free medium that would provide strong growth support and have the property of scalability to large volumes, up to thousands of liters, while coming in at an affordable price.

Table 1. Anti-viral vaccines using Vero cell culture production technologies. Modified from Barrett et al [15], and Kiesslich and Kamen [16].

Anti-viral Vero Cell-Based Vaccines			
Study (year)	**Disease**	**Vaccine Type**	**Genus**
Wang et al (2008)	Chikungunya Fever	Live attentuated	Alphavirus
Howard et al (2008)	Chikungunya Fever	Inactivated	Alphavirus
Blaney et al (2008)	Dengue Fever	Live attenuated or live chimeric	Flavivirus
Tauber et al (2008)	Japanese encephalitis	Inactivated	Flavivirus
Valneva Austria GmbH (Ixiaro, 2019)	Japanese encephalitis	Inactivated	Flavivirus
Ruis-Palacios et al & Vesikari et al (2006)	Rota gastroenteritis	Live attenuated	Rotavirus
GSK (RotaRix, 2008)	Rota gastroenteritis	Live attenuated	Rotavirus
Montagnon (1989)	Polio	Live attenuated Inactivated	Picornovirus
Aycardi E (2002)	Rabies	Inactivated	Lyssavirus
Spruth et al (2006) Qu et al (2005) Qin et al (2006)	Severe acute respiratory syndrome	Inactivated	Cornovirus
Monath et al (2004)	Smallpox	Live attenuated	Orthopoxvirus
Lim et al (2008)	West Nile Encephalitis	Inactivated	Flavivirus
Monath et al (2006)	West Nile Encephalitis	Live attenuated	Flavivirus
Baxter International Inc. (PREFLUCEL)	Influenza	Inactivated	Orthomyxovirus
Chan and Tambyah (2012)	Influenza	Inactivated	Orthomyxovirus
Merck & Co. (Ervebo 2019)	Ebola	Live-attenuated	Zaire Ebolavirus
Wu et al., (2015)	Hand-foot-and-mouth disease	Inactivated	Non-polio enterovirus
Pereira et al., (2015)	Yellow fever	Inactivated	yellow fever virus

Serum provides a protective function to cultured cells and binds toxins and other contaminating materials. Thus serum-free media must be extremely carefully formulated. Albumin can be substituted for serum, but it may impede the downstream steps of purification [18].

Chen et al. have tested five different serum free media, combined with Cytodex 1 microcarriers [19]. The following were evaluated: OptiPro SFM (Invitrogen®), VPSFM (Invitrogen), EX-CELL® Vero SFM (SAFC® Biosciences), Provero-1 (Lonza®) and HyQ SFM4MegaVir (HyClone®). The EX-CELL Vero SFM gave one of the highest cell densities, demonstrating that the use of serum free media has become routine for Vero cell cultivation. Comparable results were observed with a commercial serum-free medium MDSS2N (manufactured under the name AXCEVIR-Vero™ by Axcell Biotechnologies). In this case, Vero cells were compared with MDCK cells grown in T-flasks and microcarrier cultures.

Rabies Virus Cultivation Strategies

The Brazilian group led by Frazatti-Gallina has been active in the field of Rabies vaccine production [20]. Using Vero cells adhered to microcarriers and cultivated in a bioreactor with serum-free medium, they generated an effective rabies vaccine. With the aid of tangential filtration, they purified the Rabies virus by chromatography and inactivated it using beta-propiolactone.
Their protocol states that 350 cm^2 T-flasks were harvested and inoculated into a 3.7 liter New Brunswick™ CelliGen® bioreactor, at a proportion of 16 cells per microcarrier (Cytodex® 3-GE), yielding an initial seeding of 2.5×10^5 cell/ml. The cells were grown in serum-free MDSS2 medium (Axcell Biotechnologies). The serum-free VP-SFM medium, according to the manufacturer, was developed for Vero, BHK-21 and CHO cell growth. This medium drives the adherence of the Vero cells to the microcarriers. After 4 days of cultivation in VP-SFM medium, the cells were infected with PV rabies virus (multiplicity of infection = 0.08). The harvests of the culture supernatant were carried out 3 days after the virus inoculation and four times thereafter at intervals of 24 h. During this period, culture conditions were maintained at 60 rpm at a pH of 7.15 and 5% dissolved oxygen. Only the temperature varied from 36.5 °C in the cellular growth phase of the culture to 34°C after virus inoculation. In the course of the program, seven batches of virus suspensions were produced in the bioreactor (16L per cycle) at a mean viral titer of 10^4. FFD50/0.05 mL.

The effectiveness of the preparation was demonstrated by immunizing mice with three doses of the new vaccine (seven batches), comparing it with the commercial Verorab and HDCV (Rabies vaccine). Mean titers of neutralizing antibodies of 10.3-34.6, 6.54 and 9.36 IU/mL were found, respectively.

The choice of the serum-free medium was fortunate. In this case the amount of contaminating DNA was very low, and tolerable, less than 22.8 pg per dose of vaccine. The authors argue that this protocol is especially applicable in the developing world, where rabies is a constant hazard and a major public health problem.

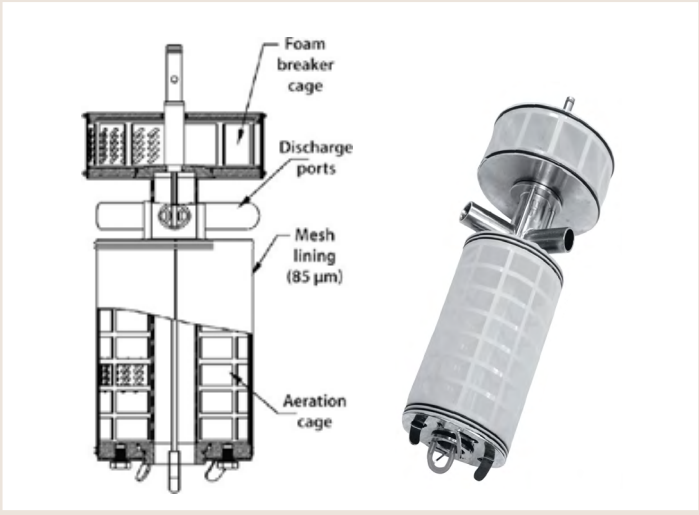

Fig. 2. New Brunswick Cell Lift Impeller (Eppendorf Inc). Patented design consists of three discharge ports located on the impeller shaft to provide uniform circulation without traditional spinning blades for conducing microcarrier cultures under ultralow-shear conditions. The flow is driven by centrifugal force, the rotation of the three ports creates a low-differential pressure at the base of the impeller shaft, lifting microcarriers up through the hollow shaft and expelling them out through its ports (The discharge ports must be submerged during operation). Bubble-sheer is eliminated by the Cell Lift impeller, which utilizes a ring sparger generating bubbles only within the aeration cage, so that the oxygenation works without any bubbles coming into contact with the cells.

Yu et al. sought methods to increase yield in Vero cell culture systems over that obtained using roller bottles [21]. In a recent review, they summarized the production technology developed over the course of the last seven years. They have adopted the 30 L New Brunswick BioFlo® 4500 fermenter/bioreactor.
The cells were cultivated in media containing 10% serum, first grown as a monolayer, and when the cell density reached 1.0–1.2 × 10^6 cells/mL, they were transferred to the bioreactor containing 25 g/L of Cytodex-1 for perfusion culture. The virus preparations, also cultured in roller bottles, were infected with the PV2061 virus strain, harvested and transferred to the bioreactors.

Wang et al have described a purified Vero cell rabies vaccine that has been widely produced in China, which is responsible for almost two-thirds of the total rabies vaccines used in Asia [22]. This successful offering is a purified Vero cell vaccine, referred to as ChengDa Vaccine (Liaoning ChengDa Biological Co., Ltd., Shengyang, China [23]). It is grown on a Vero cell line utilizing the L. Pasteur 2061 strain of rabies virus, inactivated with β-propiolactone, lyophilized, and reconstituted in 0.5 mL of physiological saline. It fulfills the WHO recommendations for potency.

The process used at ChengDa was developed by Aycardi [24]. A single Eppendorf bioreactor was capable of producing one million dose of rabies vaccine per year. The method uses ultra-high density microcarrier cell cultures adapted to a 30 L New Brunswick CelliGen bioreactor equipped with a patented Cell Lift Impeller (Figure 2), specifically configured for a perfusion system to feed the growth media into the bioreactor. A specially designed decanting column (New Brunswick Scientific) was used to prevent perfusion loss of microcarrier and keep the cells in high concentration. The system delivers high oxygen transfer, high nutrient level and low shear stress, thus allowing cell growth up to 1.2 x 10^7 million cells/mL under continuous perfusion for up to 20 days.
ChengDa Vaccine was licensed by the Health Ministry of China and the State Food and Drug Administration of China (SFDA) in 2002 and has been marketed throughout the country since that time.

Influenza Virus Cultivation Strategies

The application of Vero cells for the propagation of influenza virus in animal-derived component free (ADCF) media was extensively described by Wallace et al in their US patent (no. 7,534,596 B2) [25]. The patent application includes the steps of attaching ADCF-adapted cells to a microcarrier (SoloHill® Engineering Inc.) and infecting the cells with vaccine media, producing virus within the cells and harvesting of the virus. The influenza viruses produced by this method achieved higher titer than that of the egg produced vaccine (Table 2).

Table 2. Comparing egg-based influenza production with Vero-cell–based production using Hillex II microcarriers (SoloHill Engineering).

Production System	Panama H1N1 Titers (log10 TCID50/mL)
Egg	7.8
Vero: Serum-containing	7.9
Vero: Serum-free ADCF	8.0

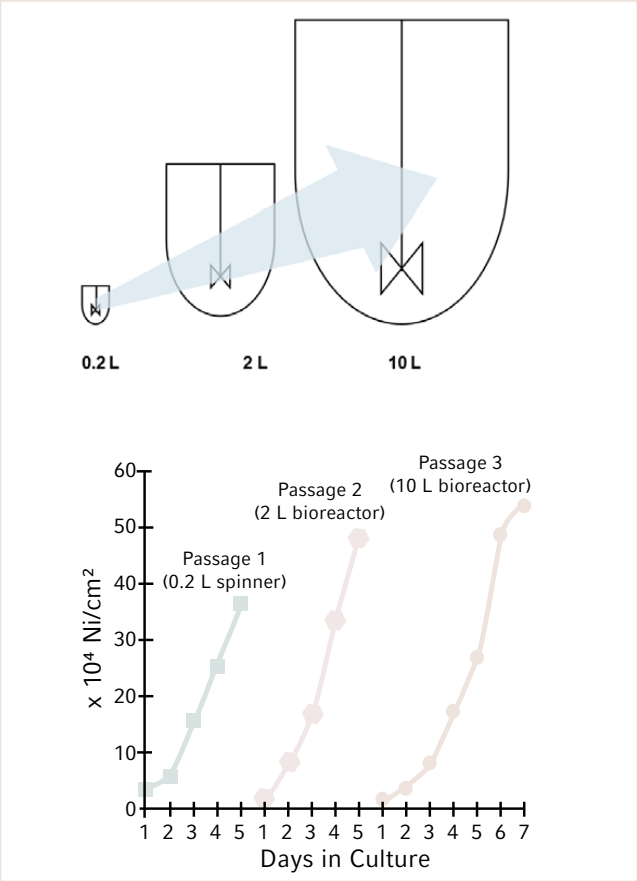

Fig. 3. Vero-based expansion on microcarriers; seed train of Vero cells cultured on Hillex II micocarrier beads (SoloHill Engineering).
Top: Diagram detailing bioreactor based expansion scheme;
Bottom: Scale-up from Spinner flask to industrial bioreactors.

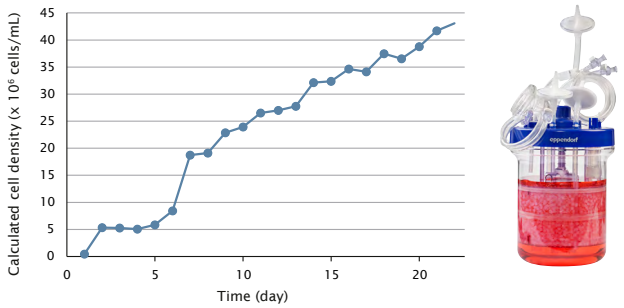

Fig. 4: High-density Vero cell culture in BioBLU 5p Single-Use vessel pre-packed with Fibra-Cel.

A method for microcarrier-based expansion of cells from a 0.2 L spinner culture to a 2 L and 10 L bioreactor culture was developed (Figure 3). A New Brunswick CelliGen 310 bioreactor with a 5 L vessel was used for the 2 L culture stage. The vessel was equipped with a ring sparger, spin filter, 3-segmented pitched blade impeller (up-pumping), and 4-gas control at 100 mL/min (Air, N_2, CO_2, and O_2). This expansion strategy couple with the demonstration of viral productivity represents an ideal closed system platform for vaccine production.

A similar method using Vero cell line for influenza vaccine production was demonstrated by chen et al [26]. Using Cytodex 1 microcarrier beads, these investigators were able to achieve cell densities of 2.6 x 10^6/mL in serum free, protein free medium. These findings were obtained using a 250 ml Bellco microcarrier spinner flask equipped with a paddle impeller, inoculated with 2.5 10^5/mL Vero cells in 5% CO_2 atmosphere. In a subsequent expansion phase, starting from an initial number of 5 x 10^5 /mL, the cells were expanded in a 3L bioreactor. After 24 hours the cells had adhered to the microcarriers and the virus was added together with fresh medium. Using these procedures, the authors were able to obtain high virus titers up to 10 Log10 $TCID_{50}$/mL. They conclude that their approach could serve as a basis for large scale commercial production of influenza virus.
In 2011, Baxter International Inc. announced the approval for PREFLUCEL, the first Vero Cell based seasonal influenza vaccine, available for 13 participating European Union countries, including Germany, Spain, UK and the Scandinavian countries. Preflucel is comprised of purified, inactivated split influenza virions, manufactured using Baxter's adaptation of the Vero cell platform. Although not approved for sale in the United States, data from a U.S. Phase III study with over 7,200 healthy individuals has shown that Preflucel provided 78.5% protective efficacy against subsequent culture-confirmed influenza infection, and robust immune responses against the three viral strains contained in the vaccine.

Recent Examples of Vero Cell Cultivation in Bioreactors

Although bioreactor based Vero cell culture has been widely used in vaccine production, the cultivation methods were typically guarded by vaccine manufactures. With increasing demand from our customer base, Eppendorf bioprocess applications lab developed a number of bioreactor application notes for attachment Vero cell culture.

In stirred-tank bioreactors, including both in R&D and in actual vaccine production, Vero cells are often grown on microcarriers or 3D support matrix such as Fibra-Cel. We have evaluated our Fibra-Cel disks as an attachment matrix because of their auspicious surface to-volume ratio. We find that they provide an optimal three-dimensional environment, protecting cells from damaging shear forces, allowing the realization of much higher cell densities by enabling perfusion culture. In perfusion bioprocessing, it is possible to constantly add nutrients and to remove byproducts, while retaining the cells in the bioreactor. Therefore, higher cell densities can be reached than in conventional batch or fed-batch processes. We cultivated the cells in perfusion mode, which ensures a consistent supply of nutrients and the removal of toxic byproducts. We cultivated Vero cells in BioBLU® 5p Single-Use vessels pre-packed with Fibra-Cel, regulating the process with a BioFlo 320 bioprocess control station. We achieved high cell densities, up to 4.3 x 10^7 cells per mL (Figure 4) [27]. We believe that this provides strong support for Vero-cell-based vaccine production using Fibra-Cel packed-bed vessels (Table 3).

Table 3. Comparison of growth surfaces of different cell culture vessels.

Vessel	Total growth surface (cm2)	Growth surface equivalent to (number of BioBLU 5p vessels)
BioBLU 5p Single-Use Vessel	180,000	1
T-25 flask	25	7200
T-175	172	1028
Roller bottle	850	212
10-layer stacked plate	6300	29

In another program to provide a method to inoculate larger bioreactors packed with Fibra-Cel, we have evaluated spin filter as the means to increase inoculation yields. The spin-filer allows easy perfusion of microcarrier based cell culture without the need for acquiring external perfusion devices [28] We cultivated Vero cells on Cytodex 3 microcarriers at a density of 10 g/L in an Eppendorf 3 L glass vessel using a microcarrier spin filter coupled with a pitched-blade impeller. This device is a cylinder-shaped cage that spins with the impeller shaft and is covered with a large 75-micron screen designed to prevent microcarriers from being collected with the waste media (Figure 5). The process was controlled with a BioFlo 320 bioprocess control station without needing any additional perfusion devices. The design of the spin filter permits cultivation of anchorage-dependent Vero cells in perfusion mode on microcarriers while ensuring a consistent supply of nutrients and the removal of toxic byproducts.
At a modest microcarrier loading density of 10 g/L, we achieved attachment Vero cell culture density of 8.0×10^6 cells/mL in 9 days [28], sufficient for inoculation of a 40 L CelliGen 510 Fibra-Cel packed-bed bioreactor designed for vaccine production. The CelliGen 510 has become the leading Rabies vaccine production platform in China, and the method, including CelliGen 510 itself, has been written into Chinese FDA guidelines as part of their production method recommendations. However, due to the very high surface capacity, it typically requires several stacked-plate culture devices to produce enough cells for inoculation. We believe this simple spin-filter perfusion platform has great potential to replace stacked plates for inoculation of larger bioreactors in vaccine production.

Fig. 5: Microcarrier specific spin filter with pitched-blade impeller.

Conclusions

The combination of advances reviewed here provides strong support for the use of cell culture systems for virus vaccine production. The fact that Vero cells have been approved for vaccine products represents an important step on the road to technologies that do not rely upon hens' eggs for generation of adequate quantities of viruses. Advances in culture media enable the elimination of serum, thus driving the rapid and efficient purification of proteins. Whereas serum-containing media may continue to occupy a default position, it is now generally recognized that serum-free media are the optimal choice. The use of microcarrier or Fibra-Cel add to the efficiency of the culture technology, allowing greatly increased cell densities to be reached. Finally, improvements in bioreactor design combined with these various technological advances result in a greatly improved and more functional production train.

The tumultuous events of the past year, dominated by the Covid-19 pandemic, have brought us the overarching need for a Covid-19 vaccine. The range of Eppendorf bioreactors offers important opportunities for vaccine research, development as well as production. Our vaccine-enabling cell culture technologies support on developing production strategies are not limited to Vero cells, but can be used for other attachment mammalian cell culture and vaccine production as well. According to our existing vaccine customer base, the Eppendorf packed-bed platform is the most effective attachment cell-based production platform on the market, achieving well over 100 million cells/mL in mammalian cell culture. With our expertise and specialized equipment, ranging from microcarrier spin filter, cell-lift impeller, packed-bed bioreactors, and single-use solutions, we offer a wide range of technologies well suited for vaccine research and development.

Acknowledgments

Our thanks to Ms. Alain Fairbank and Dr. Mark Szczypka from SoloHill Engineering, Inc. for their assistance and content support. Special thanks go to K. John Morrow Jr. Ph.D, from Newport Biotech, Newport, KY, USA.

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APPLICATION NOTE No. 471

HEK293 Bioreactor Transfection for Vaccine Applications Using the Eppendorf SciVario® twin Bioprocess Controller: An Example with COVID-19 Spike Protein Production

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Abstract

Bioreactor suspension cell culture platforms are essential tools for vaccine production. They can support very high cell densities, allowing for much higher production yields and reduce the challenges associated with the scalability process of adherent cell culture. Bioreactor transfection is a method of deliberately introducing plasmids into large numbers of cultured cells for protein production. With the current global COVID-19 health crisis caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it is imperative to produce large quantities of vaccine components such as spike proteins, the predominant antigen of SARS-CoV-2 S vaccines.

To demonstrate feasibility, production of the SARS-CoV-2 S HexaPro spike protein was conducted using the suspension-adapted HEK293 cell platform Expi293F™ transfected with spike-protein-encoding plasmid DNA. To achieve high yield, the transfection was performed using a BioBLU® 1c Single-Use Bioreactor controlled by the SciVario® twin bioreactor control system. During the cell culture run, parameters like cell density and viability, as well as metabolite concentrations were monitored. In the end, the transfection strategy reached a spike protein titer of around 4 mg/L, which is in line with previous reports [1].

Introduction

Vaccines are valuable tools to minimize the risk of infectious diseases, such as the COVID-19 pandemic that started in 2020. Many vaccines subtypes exist, all with the goal to train the immune system to fight certain infectious agents and thus provide protection for future infections.
One such subtype is the protein subunit vaccine which contains only components or antigens rather than the whole pathogen. An example for an antigen used for subunit vaccines is the COVID-19 spike protein (S-protein).

After delivering this antigen to the body, it is recognized by the immune system and stimulates immune responses, such as antibody production (Figure 1).
Adjuvanted spike protein vaccines are available on the market, such as the Novavax COVID-19 vaccine NVX-CoV2373 that recently received the emergency use authorization from the FDA [2].

Furthermore, recent market analyses have concluded that the global subunit vaccine market is anticipated to grow at a significant Compound Annual Growth Rate of 10.9 % during the forecast period of 2022-2028 [4].
In order to advance vaccine production and to enable sufficient protection against prevalent and future pathogens, scalable vaccine production strategies are needed. Bioreactors offer a platform to develop such strategies.
In this application note, a bioreactor-based SARS-CoV-2 S HexaPro spike protein production was developed using the suspension cell culture platform Expi293F (Thermo Fisher Scientific). In previous studies, this cell line demonstrated efficient expansion to high densities [5] and was employed for the production of Adeno-associated virus capsids [6], an important component of vaccination but also in the emerging field of gene therapy.
The bioprocess conditions were controlled and monitored by the SciVario twin bioreactor control system in conjunction with the BioBLU 1c Single-Use Bioreactor. Parameters like cell growth, viability, and metabolic activity (glucose, ammonia, and lactate levels within the medium) were analyzed throughout the run. In addition, the SARS-CoV-2 S HexaPro spike protein titer was determined at different time points after transfection by ELISA, and its purity and molecular weight were analyzed by SDS-PAGE.

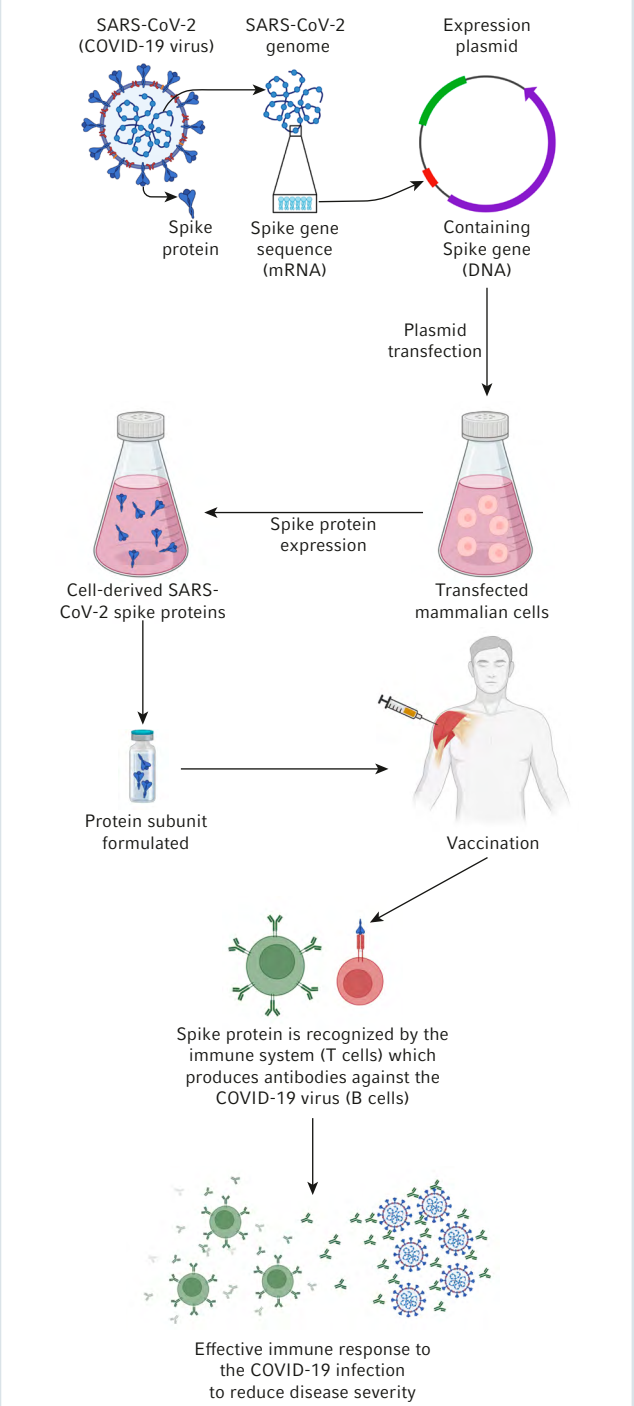

Figure 1: Schematic representation of SARS-CoV-2 S spike protein production and potential immunization procedure. Created with BioRender.com

Material and Methods

SciVario twin bioreactor control system and BioBLU 1c Single-Use Bioreactors

The SciVario twin bioreactor control system was used to perform two batch culture runs simultaneously using BioBLU 1c Single-Use Bioreactors equipped with a single pitched-blade impeller. Each bioreactor control system is equipped with three universal port connectors for pH and dissolved oxygen (DO) sensors, a temperature control block that combines electrical heating and water cooling, agitation control and a gas module that includes a Thermal Mass Flow Controller (TMFC) with standard gas flow rates of 0.1 – 1,200 sL/h (resulting in an ultra-high turndown ratio of 1:12,000), as well as four solenoid valves (see Figure 2).

Figure 2: The SciVario twin bioreactor control system allows the control two glass or single-use bioreactors, either individually or in parallel, at the same time across a wide range of vessel sizes from small- to bench-scale. It was developed for both cell culture and microbial fermentation applications.

Sensor calibration

Prior to the preparation of the BioBLU 1c Single-Use Bioreactors, ISM® gel-filled pH sensors (Mettler Toledo®) were connected to the SciVario twin bioreactor control system where they were automatically detected by the software. The calibration process was performed according to the operations manual using buffer solutions of pH 7 and pH 4 as "zero" and "span", respectively. Hereafter, the pH sensors were disconnected and sterilized in an autoclavable pouch.

BioBLU 1c Single-Use Biorector preparation and process parameters

Each BioBLU 1c Single-Use Bioreactor was equipped with magnetic drives. The previously sterilized pH sensors were then inserted in a spare PG 13.5 port under aseptic conditions in the biosafety cabinet. In addition, the polarographic DO sensors (Mettler Toledo) were fitted *via* a non-invasive sensor sleeve into both bioreactors. DASGIP® peltier exhaust condensers were connected to each bioreactor and the sparge line (from the controller) was connected to the submerged sparge filter on the bioreactor. Three liquid addition ports were used on each bioreactor: one for inoculation/medium addition, one for base addition and another for the addition of 0.1% of Sigma Aldrich® Antifoam C Emulsion (Merck). Then, the BioBLU 1c Single-Use Bioreactors were placed in their respective temperature control block to keep the system at a constant temperature. Finally, each bioreactor was filled with HEK ViP NX cell culture medium (Sartorius) supplemented with GlutaMAX™ (Thermo Fisher Scientific) and conditioned for at least 24 hours using the parameters and setpoints listed in Table 1.

Table 1: Process parameters and setpoints of the batch culture experiments.

Working volume	1 L
Agitation	155 rpm (tip speed 0.4 m/s)
Temperature	37 °C
Inoculation density	0.4 × 10^6 cells/mL
Cell culture medium	HEK ViP NX cell culture medium
DO setpoint	40 % (P = 0.1; I = 3.6/h)
pH setpoint	7.0 (deadband = 0.2), cascade to CO_2 (acid) cascade to 0.45 M sodium bicarbonate (base)
Gassing range	0.1 SLPH – 60 SLPH
Gassing cascade	Set O_2 % at 30 % controller output to 21% and at 100 % controller output to 100%. Set flow at 0 % controller output to 0.1 SLPH, and at 100 % controller output to 60 SLPH

SARS-CoV-2 S spike protein production workflow

An overview of the entire workflow schematic for SARS-CoV-2 S spike protein production is shown in Figure 3. The individual steps are detailed below.

SARS-CoV-2 HexaPro expression vector

Recombinant SARS-CoV-2 S HexaPro spike protein was produced by transfecting the target cells with SARS-CoV-2 S HexaPro expression vector obtained in-house from a HexaPro variant plasmid in *E. coli* (Addgene, 154754). Briefly, *E. coli* strain DH5 alpha cells were cultured in shake flasks with LB medium (Merck) supplemented with the appropriate selective antibiotic (ampicillin, Merck) for 18 hours at 37 °C and 200 rpm using an Innova® S44i shaker incubator. After reaching the log phase, the cells were pelleted by centrifugation at 11,000 rpm (16,639 × g) for 5 min at 4 °C. Plasmid DNA was purified from culture using the PureLink™ HiPure Plasmid Filter Maxiprep Kit (Thermo Fisher Scientific). Finally, purified plasmids were eluted from the HiPure column by gravity flow after adding 15 ml of elution buffer. The absorbance at 260 nm and 280 nm was determined by using a BioSpectrometer® Kinetic D30 spectrophotometer. The ratio of A260/A280 estimates sample purity.

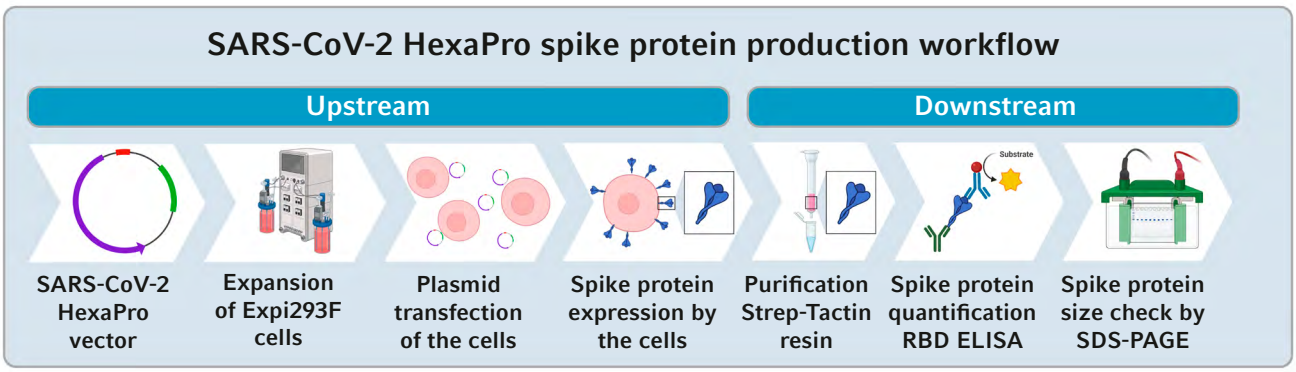

Figure 3: The SARS-CoV-2 S HexaPro spike protein production workflow. Created with BioRender.com

Ratios between 1.8 and 2 are commonly accepted as pure DNA. Furthermore, an A260 value of 1 translates to ~50 ng/µL of pure double-stranded DNA. Hence, the concentration and purity of the plasmid were 2.58 mg/mL and 1.85, respectively.

Suspension Cell Line (Expi293F)

In order to produce the SARS-CoV-2 S HexaPro spike protein, the suspension adapted HEK293 cell line Expi293F (Thermo Fisher Scientific) was used for plasmid transfection. In previous transfection experiments for the production of Adeno-associated virus capsids, this suspension cell line demonstrated sufficient protein production and high cell density [5,6].

Expi293F cell inoculum preparation for the BioBLU 1c Single-Use Bioreactor

The cell's expansion process was performed as shown in Figure 4. Cells were cultured in HEK ViP NX Cell culture medium supplemented with GlutaMAX in a New Brunswick S41i CO_2 incubator shaker at 37 °C, 8% CO_2 and at an agitation speed of 125 rpm. During the expansion process, the inoculation density, percentage fill of the shake flasks and other parameters were kept constant. Finally, 200 mL inoculum containing 400 × 10^6 cells in HEK ViP NX cell culture medium was added to each inoculation bottle.

Expi293F cell culture in BioBLU 1c Single-Use Bioreactors

Both BioBLU 1c Single-Use Bioreactors were inoculated with the inoculum from the inoculation bottles (see section "Expi293F cell inoculum preparation for the BioBLU 1c Single-Use Bioreactor") for a total working volume of 1 L with a cell density of ~0.4 × 10^6 cells/mL and more than 95% cell viability. In both cases, the temperature was set to 37 °C and the DO setpoint of 40 % was controlled by a cascade (Table 1). To control foam formation, Antifoam C Emulsion was added as needed. The pH setpoint was controlled using a cascade of CO_2 (acid) and 0.45 M sodium bicarbonate (base) (Table 1).

Transfection procedure of Expi293F cells in BioBLU 1c Single-Use Bioreactors

The transfection cell density of approximately 1 – 1.2 × 10^6 cells/mL was reached at two days after inoculation in both bioreactors. Then, 1 µg of HexaPro vector was diluted in 50 mL of Gibco™ Opti-MEM™ I Reduced Serum Medium supplemented with GlutaMAX (tube 1).

The transfection mix was prepared by adding 1 µl of FectoVIR or FectoPRO per µg of plasmid DNA to 50 mL of Opti-MEM medium (tube 2). The content of tube 1 was filtered through a 0.22 µm syringe filter into tube 2 and mixed by inversion. Then, the transfection mix solution was incubated for 20 minutes at room temperature and finally added onto the cells in the bioreactor.

Cell viability and metabolic activity

Samples were collected twice a day from the bioreactors to determine the cell viability, cellular density, and the concentration of metabolites (glucose, ammonia (NH_3) and lactate), by connecting a sterile 5 mL syringe to the Luer Lock sample port. Then, 3 mL of dead volume were discarded and another 3 mL were collected again (using a new 5 mL sterile syringe) as a viable sample for analysis.

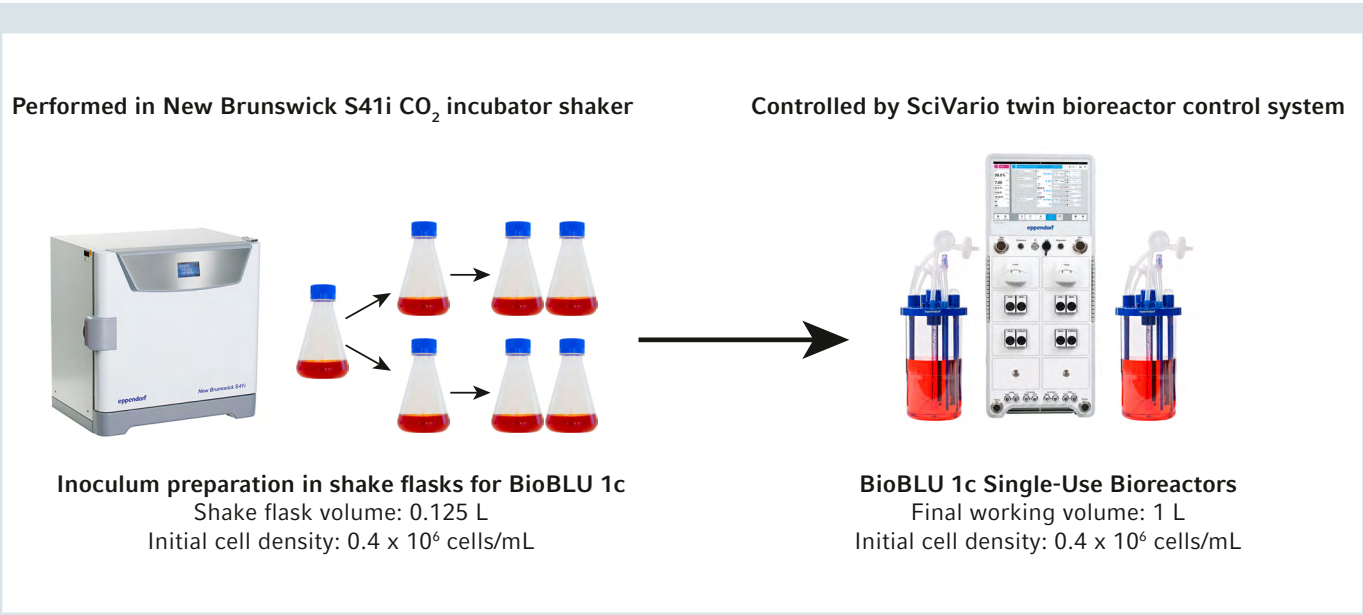

Figure 4: Expi293F cell culture scale-up from shaker to bioreactor for inoculum preparation.

Cell density and viability were measured (via the trypan blue exclusion method) using a Vi-CELL® XR Viability Analyzer (Beckman Coulter®). pH values were monitored offline by using an Orion Star™ 8211 pH-meter (Thermo Fisher Scientific). Using the offline pH value, the pH calibration on the controller was restandardized daily to prevent any discrepancy between online and offline measurements. Glucose, ammonia and lactate were measured using a CEDEX® Bio Analyzer (Roche).

SARS-CoV-2 S HexaPro spike protein purification and titration

Every day (until day 5) after transfection, 60 mL of sample containing Expi293F cells and medium were collected using a Labtainer™ BioProcess Container (Thermo Fisher Scientific) with line sets. The cells were centrifuged at 300 × g in a centrifuge 5430R for 5 minutes to separate the cell pellet from the supernatant. SARS-CoV-2 S spike proteins from the supernatant were purified by affinity chromatography. Briefly, 2 mL of Strep-Tactin® Superflow® resin (as 50% suspension, IBA LifeSciences) were added to Poly-Prep® Chromatography Columns (Bio-Rad). The resin was equilibrated with 5 mL of Strep-Tactin wash buffer (IBA LifeSciences) (five column volumes). The supernatant was then added to the columns followed by another washing step using Strep-Tactin wash buffer as above. Finally, 4 mL of Strep-Tactin elution buffer (IBA LifeSciences) were added to the column before the eluate was collected in a single tube and concentrated using a 30 kDa cutoff spin concentrator (Amicon® Ultra-15 Centrifugal Filter Units, Merck) at 4000 x *g* for 5 min (4 °C).
After the purification step, the pure SARS-CoV-2 S HexaPro spike proteins from the supernatant were titrated through the Invitrogen™ Human SARS-CoV-2 RBD ELISA kit (Thermo Fisher Scientific). This ELISA antibody pair detects the SARS-CoV-2 regional binding domain (RBD) of the S1 subunit of the spike protein. Then, the assay was conducted according to the manufacturer's protocol instructions.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed with the Invitrogen™ NuPAGE™ 4 to 12%, Bis-Tris, 1.0–1.5 mm, Mini Protein Gels (Thermo Fisher Scientific) under non-reducing conditions.
SARS-CoV-2 S HexaPro spike protein samples were diluted in Invitrogen™ NuPAGE™ LDS Sample Buffer (4x) (Thermo Fisher Scientific) and 15 µl per sample were loaded onto the gel. Gels were run in Invitrogen™ NuPAGE™ MES SDS Running Buffer (20x) (Thermo Fisher Scientific) at 200 V and the electrophoresis was completed in approximately 20 minutes. Then, gels were washed with DI water to remove the SDS and buffer salts, stained with Invitrogen™ SimplyBlue™ SafeStain (Thermo Fisher Scientific) and incubated for 1 hour at room temperature with gentle shaking (30 rpm) using a New Brunswick S41i CO_2 incubator Shaker.

Finally, the gels were washed twice with DI water for 1 hour, and photographs were taken with a clear background using an Edvotek™ White Light Box (Thermo Fisher Scientific). A pre-stained protein standard (Invitrogen™ Novex™ Sharp Pre-stained Protein Standard, Thermo Fisher Scientific) was used for accurate molecular weight estimation in a range of 3.5 to 260 kDa.

Results and Discussion

SARS-CoV-2 S HexaPro spike protein production in BioBLU Single-Use Bioreactors

The expansion and transient transfection of Expi293F cells were carried out in a 1 L BioBLU 1c Single-Use Bioreactor culture. For transfection, approximately 1 µg of our in-house-produced HexaPro expression vector was used for 10^6 cells. Total volumetric DNA-to-transfection reagent (FectoVIR or FectoPRO) ratio was 1:1.

Both bioreactors were inoculated with a cell density of approximately 0.4 × 10^6 cell/mL. Transfection was carried out at day 2 post inoculation with a cell density of approximately 1-1.2 × 10^6 cells/mL. As shown in Figure 5, two different growth profiles were obtained. When using FectoVIR, a rapid increase of cell growth was observed up to day 7 of culture, reaching a peak in viable cell density at 9.5 × 10^6 cells/mL. After transfection with FectoPRO, slower cell growth was observed, and the culture reached 6.6 × 10^6 cells/mL at day 8. It is worth pointing out that both bioreactors showed lower cell growth after transfection compared to the Expi293F cells growth in a 1 L bioreactor under the same conditions but without transfection (data not shown, peak viable cells density was approximately 13 × 10^6 cells/mL).

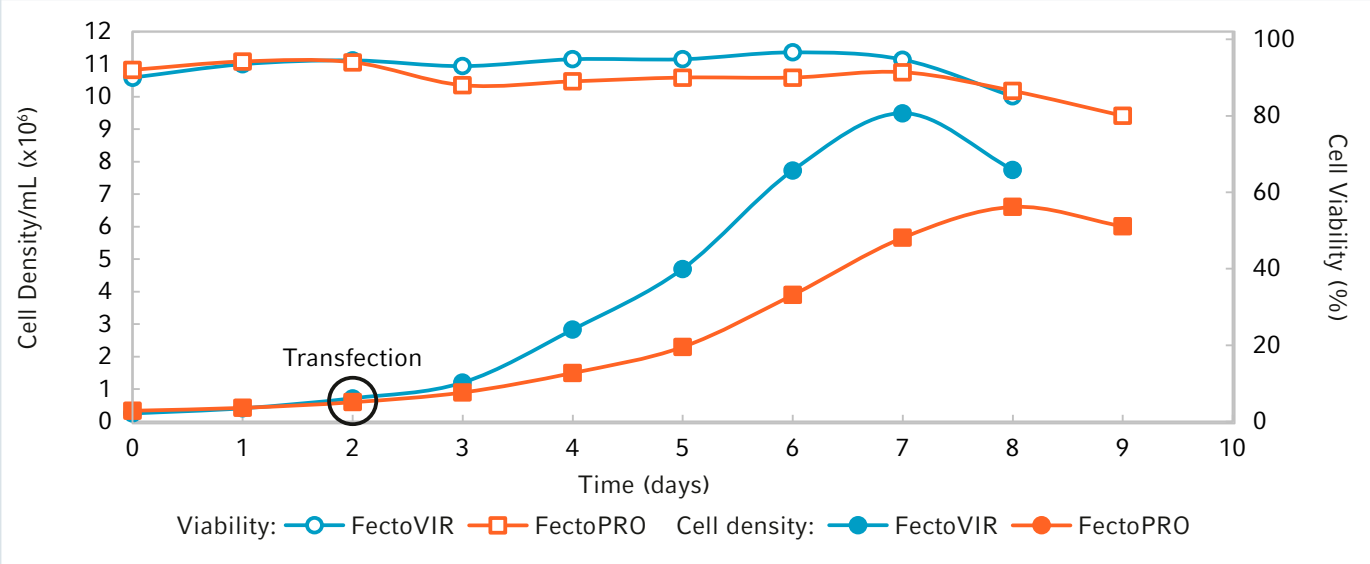

Figure 5:. Expi293F growth profile in BioBLU 1c Single-Use Bioreactors. Expi293F cell density and viability of two different bioreactors containing different transfection mixes were shown.

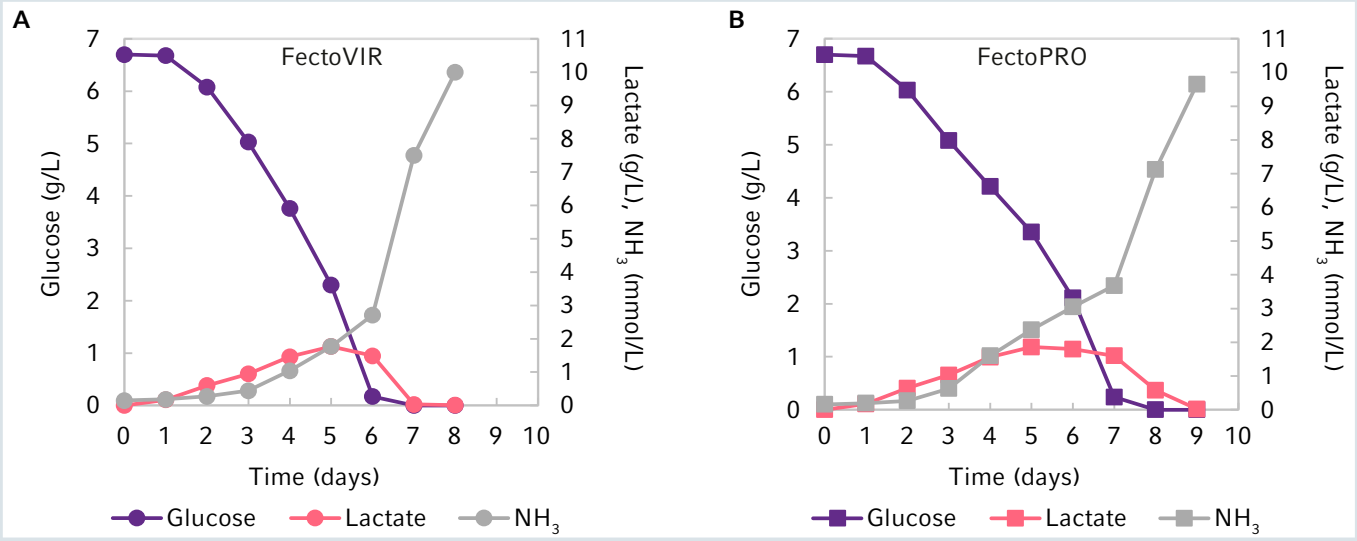

Figure 6: Metabolic profile. A: Expi293F cells transfected using FectoVIR in the transfection mix. B: Expi293F cells transfected using FectoPRO in the transfection mix.

Furthermore, the concentration of glucose, lactate and ammonia (NH_3) was analyzed on a daily basis. The lactate concentration level were below 2 g/L in both bioreactors throughout the run. NH_3 concentrations were between 2 and 3 mmol/L until day 5 in both bioreactors and increased drastically to toxic levels after day 6 towards the end of both runs (Figure 6).

60 mL of sample was harvested daily post-transfection until day 5 to determine the SARS-CoV-2 S HexaPro spike protein titers in the supernatant by ELISA (Figure 7A). Spike protein concentrations were reaching 3.5 mg/L for the FectoVIR-transfected and 4 mg/L for the FectoPRO-transfected cells.

Thus, the spike protein concentration in the supernatant from cells transfected with FectoPRO as part of the transfection mixture was noticeably higher (about 4 mg/L) compared to the bioreactor in which FectoVIR was part of the transfection mixture (3.5 mg/L) despite its lower growth post transfection.

Finally, SDS-PAGE was used to analyze the SARS-CoV-2 S HexaPro spike protein produced in both bioreactors. As shown in Figure 7B, both samples showed high purity with apparent molecular weight of ~ 190 kDa as expected [7]. Molecular weight standards in kDa are indicated on the left side of Figure 7B.

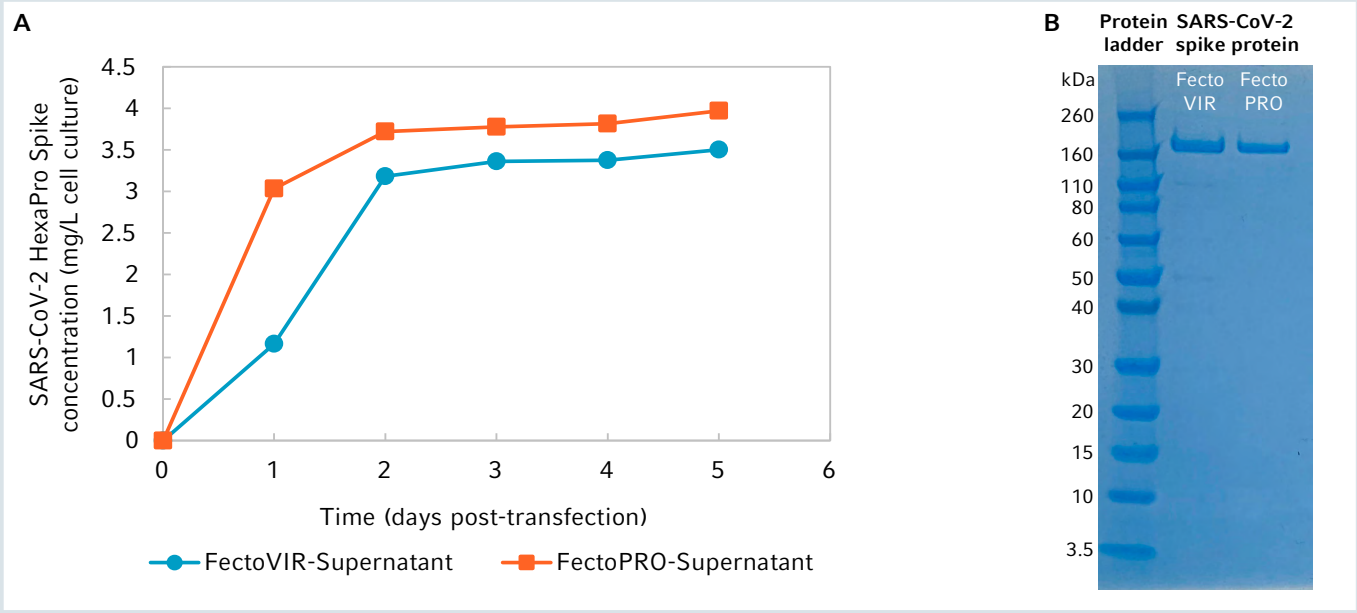

Figure 7: Characterization of purified SARS-CoV-2 S HexaPro spike protein. A: Spike protein concentration obtained every day (until day 5 post-transfection) in the cell culture supernatant of transfected cells. B: SDS-PAGE gel of SARS-CoV-2 S HexaPro spike protein after elution from the Strep-Tactin column.

Conclusion

This study successfully demonstrated the feasibility of bioreactor-based plasmid transfection in BioBLU 1c Single-Use Bioreactors under control of the SciVario twin bioprocess controller.
COVID-19 spike proteins were produced from the plasmid transfection, representing an important compound of protein subunit vaccines. The suspension cell culture approach allows a more straightforward scale-up into larger bioreactors and offers much desired simplicity, as well as access to a greater variety of production systems over attachment culture-based methods.

The efficient setup of the SciVario twin enabled precise control of the cell culture environment, resulting in robust spike protein titers of 3.5 to 4 mg/L. To conclude, the Expi293F cell line together with the advanced suspension stirred-tank bioreactors and controllers offered by Eppendorf provide an efficient platform for the development of suspension cell-based protein production at various scales.

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MACHEREY-NAGEL

NucleoSpin® 96 Plasmid Transfection-grade

Purification of plasmid DNA with transfection-grade purity using the platform ep*Motion*® 5075vt

Introduction

Transfection of cultured cells is one of the most common applications for isolated plasmids and requires highly pure DNA. The main impurities in plasmid DNA preparations derive from endotoxins. Endotoxins are lipopolysaccharides derived from the bacterial cell wall that have cytotoxic effects and negatively influence cell viability and transfection efficiency. Additionally, endotoxins are known to influence gene expression in cell cultures, leading to false results in gene expression analysis. The efficient isolation of plasmid DNA from bacterial cultures is essential for a variety of molecular applications utilized by many research laboratories.

MACHEREY-NAGEL has developed a 96-well kit, NucleoSpin® 96 Plasmid Transfection-grade, for the isolation of endotoxin reduced plasmid DNA based on silica membrane technology. The kit combines a fast processing with novel endotoxin removal wash buffers, enabling convenient and time saving isolation of transfection-grade DNA (≤ 50 EU/µg DNA, endotoxin units per µg DNA).

This application note describes the automated process on the liquid handling workstation ep*Motion*® 5075vt using the NucleoSpin® 96 Plasmid Transfection-grade kit from MACHEREY-NAGEL. The novel optimized protocol allows the processing of a variable sample number in multiples of 8 (8–96). The processing of 96 samples takes approximately 100 minutes excluding cultivation and harvesting.

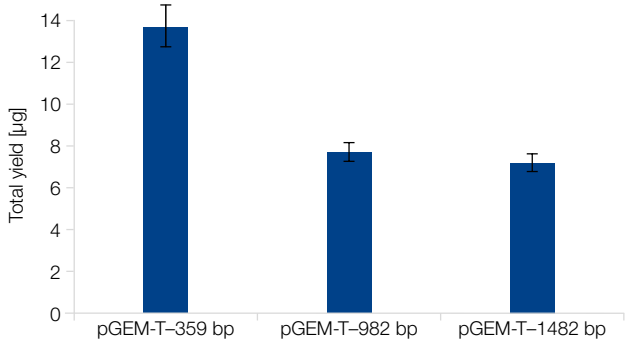
Product at a glance

NucleoSpin® 96 Plasmid Transfection-grade	
Technology	Silica membrane and endotoxin reduction technology
Sample material	Up to 5 mL bacterial culture (*E. coli*, high-copy plasmids)
Preparation time	Approx. 100 min for 96 samples (excluding cultivation and harvesting).
Format	Variable sample number in multiples of 8 (8–96)
Typical yield	5–20 µg
Elution volume	100–200 µL
Binding capacity	20 µg

Material and methods

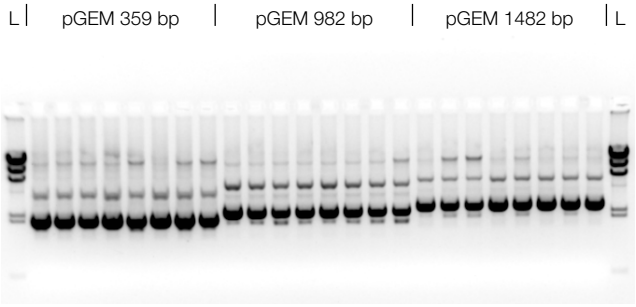
The optimized protocol is programmed to process up to 96 samples in parallel (Variable sample number in multiples of 8) and developed for the ep*Motion*® 5075vt platform. Cultivation and harvesting of bacterial cells is recommended to perform according to the NucleoSpin® 96 Plasmid Transfection-grade user manual. Bacterial cell pellets from up to 5 mL cultures are resuspended in Resuspension Buffer A1 and subsequently lysed by addition of Lysis Buffer A2 for 5 min at room temperature. Following lysis and neutralization by addition of Buffer A3, all subsequent steps are performed on the ep*Motion*® 5075vt. The NucleoSpin® 96 Plasmid kit utilizes two different 96-well filter plates in order to achieve a precise separation as well as high yield and quality of plasmid DNA. Lysate clearance and Plasmid DNA binding is performed by vacuum. Crude lysates are cleared by the NucleoSpin® 96 Plasmid Filter Plate, removing cellular debris as well as chromosomal DNA. Nucleic acids are subsequently bound to the silica membrane of the NucleoSpin® 96 Plasmid Binding Plate during the binding step. Contaminants, such as salts or proteins, are then removed from the silica membrane by three washing steps, and highly pure plasmid DNA is finally eluted under low ionic strength conditions in a slightly alkaline Elution Buffer AE.

Application data



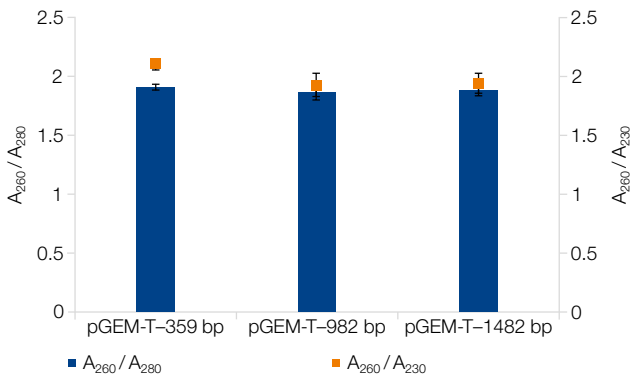
Isolation of transfection-grade plasmid DNA from bacterial cultures

Plasmid DNA of three different bacterial strains, transformed with plasmids containing either a 1482 bp, a 982 bp or a 359 bp insert, was isolated from 1.5 mL of bacterial cultures (*E. coli* DH 5α, high-copy plasmid pGEM®-T Easy; n = 24) using the NucleoSpin® 96 Plasmid Transfection-grade kit on the ep*Motion*® 5075vt. Total yield was determined by UV spectrometry (dark blue bars). All measured endotoxin contents showed significant less than 50 EU/µg DNA (EU = endotoxin units)



Reproducible yields of plasmid DNA

Plasmid DNA of three different bacterial strains, transformed with plasmids containing either a 1482 bp, a 982 bp or a 359 bp insert, was isolated from 1.5 mL of bacterial cultures (*E. coli* DH 5α, high-copy plasmid pGEM®-T Easy). The reproducibility and integrity was analyzed by gel electrophoresis (10 µL per eluate; 1 % TAE gel; Marker (L): GeneRuler™ 1 kb DNA Ladder – Thermo Scientific).



Purity of transfection-grade plasmid DNA from bacterial cultures

Plasmid DNA of three different bacterial strains, transformed with plasmids containing either a 1482 bp, a 982 bp or a 359 bp insert, was isolated from 1.5 mL of bacterial cultures (*E. coli* DH 5α, high-copy plasmid pGEM®-T Easy; n = 24) using the NucleoSpin® 96 Plasmid Transfection-grade kit on the ep*Motion*® 5075vt. Total purity was determined by UV spectrometry (A_{260}/A_{280}: dark blue bars; A_{260}/A_{230}: orange squares). All measured endotoxin contents showed significant less than 50 EU/µg DNA (EU = endotoxin units; data not shown).

Speed up and automate your transfection grade plasmid DNA extraction

MACHEREY-NAGEL and Eppendorf® deliver a fully automated solution for your high throughput plasmid DNA extraction in transfection-grade purity. We adapted the NucleoSpin® 96 Plasmid Transfection-grade kit on the ep*Motion*® 5075vt to speed up your nucleic acid purification workflow.

- Endotoxin removal wash buffer and optimized filter plates for highly pure plasmid DNA with less than 50 endotoxin units per µg DNA.
- Flexible sample numbers (multiple of 8) and fast processing of 96 samples within 100 minutes (excluding cultivation and harvesting).
- Reliable performance and excellent yields using NucleoSpin® 96 Plasmid Transfection-grade kit on the ep*Motion*® 5075vt.

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® 96 Plasmid Transfection-grade	Kit based on silica membrane technology for the isolation of transfection-grade plasmid DNA from bacterial cultures in 96-well format	1 x 96 / 4 x 96 / 24 x 96	740491.1 / .4 / .24
ep*Motion*® 5075vt	Basic device incl. vacuum system, gripper, vac frame 2, vac frame holder, Eppendorf ThermoMixer®, epBlue™ software, mouse, waste box, 100–240 V ±10 % / 50–60 Hz ±5 %, 0.2 µL–1 mL	1	5075000304

Knowledge Gain Through High Parallel Bioprocess Setups

The perfect tool for Design of Experiments

Sebastian Kleebank studied Bioengineering at the University of Applied Sciences in Juelich, followed by his Ph.D. at the RWTH Aachen, Institute for Molecular Biotechnology.
In his actual position as a Product Life Cycle Manager at Eppendorf SE, Bioprocess Unit, he is responsible for the small-scale product portfolio.
During his 15 years in the bioprocess industry, he gained a lot of experience in small scale upstream process development, including several years working in the lab.

Contact: bioprocess-experts@eppendorf.com

ABSTRACT

In the Quality by Design (QbD) and Process Analytical Technology (PAT) era, the generation and validation of a large amount of data have become standard. Parallel bioreactor control systems offer the possibility to test and optimize different process parameters at the same time, thus saving time and resources.

INTRODUCTION

Working with bioprocess control systems that help to develop and optimize processes in terms of time and cost efficiency is crucial. In the Quality by Design (QbD) and Process Analytical Technology (PAT) era, the generation and validation of a large amount of data have become standard. This data is not generated by random trial and error experiments. Experiments are carefully designed with the information gathered from previous experiments. This Design of Experiment (DoE) approach allows profound insights into the process. Parallel bioreactor control systems offer the possibility to test and optimize different process parameters at the same time, thus saving time and resources. This is especially important in the fast-changing biotechnology industry.

We spoke with Dr. Sebastian Kleebank about the DASbox® Mini Bioreactor System, a compact and flexible parallel bioprocess controller for the control of up to 24 bioreactors.

What was one of the most significant experiences in your laboratory life?

Sebastian:
Everybody who works in a laboratory running long, work-intensive, and expensive experiments knows the thrilling feeling, and the question in mind: "How does my experiment look like when I will be back In the lab after the weekend?". One of my most prominent incidents for me was, that I stepped into the lab and found my culture everywhere on the floor as a consequence of a foam out.
This was the first time I realized that continuous monitoring of the process is crucial and reliable process control is essential.

What are the major challenges in running a bioreactor control system?

Sebastian:
The goal of each scientist is to gain as much process knowledge as possible in a short time. This is especially true for scientists working in industrial laboratories, where the time-to-market is important. To get to know your process, one needs to perform many tests. To save resources, this is mainly done in small working volumes of less than a liter. And here a precise and reliable process control is key to gain high-quality results. Regarding the foam out mentioned above it is a very comfortable feeling to know that the DASbox system can prevent this. We developed a very sensitive level sensor that can identify even the slightest foam formation and distinguishes precisely between air bubbles and liquid. With a sensitivity in a range between 1 – 20000 µS, foam can be reliably detected and destroyed, especially when you are investigating unknown, new processes.

During fed-batch, perfusion, or continuous cultivations, it can happen that the user underestimates the amount of substrate that the organisms would need. This could lead to exceeding the maximum working volume of the connected bioreactor. With the combination of our very precise pumps in the DASbox system and the powerful DASware software suite, we offer a solution that mitigates the risk of such errors.
The software of our DASbox system sums up the added and removed liquid volumes and automatically stops the feeding if the maximum working volume is reached.

How can the DASbox help to optimize all the different steps of a process?

Sebastian:
From the inoculation of the bioreactor to harvesting, the DASbox and its control software offer solutions to support you in each individual step. After inoculation, the oxygen requirement for aerobic cultures increases steadily.
With our oxygen cascade (flexible change of stirrer speed, oxygen concentration of the input gas and/or gas flow rate) the oxygen demand of the culture can effectively be covered.
The end of the batch phase can, for example, be reliably identified by the so-called substrate consumption peak of the dissolved oxygen concentration and the substrate pump can be started automatically. When you add an exhaust gas analyzer, the substrate can even be added in relation to the metabolic activity of the cells. Our precise pumps ensure that the substrate is added continuously even at very small feed rates of down to 0.3 mL/h. In combination with submerged liquid addition, this results in a very smooth DO (Dissolved Oxygen) signal that enables a reliable process control.
And even the right time for harvesting can be defined for example by reaching a certain number of cells (e.g. when using a DASGIP OD sensor) or other measured variables and the harvest pump can be started automatically.
With our scripting functionality, there are virtually no limits to the flexibility of the control strategy. Controller inputs and outputs can be freely configured. Signals from balances, external sensors e.g. for methanol or glucose, as well as internal process values can be selected.

What are the benefits of highly parallel approaches?

Sebastian:
The obvious correlation is quite simple: the more experiments are performed in parallel, the more results are collected at the same time. The real advantage of highly parallel approaches is that you can systematically investigate your process and thus increase the knowledge gain. The knowledge about the process is therefore the actual benefit here. The basis for this is that a reliable and reproducible process control can be ensured and that the results can be transferred to large scales. Both can be achieved with the DASbox system. One way to systematically investigate your process can be to consider the fact that many processes are divided into two parts. The first part being the growth phase and the second part being the production phase. The tricky part is that the optimal growth conditions may not be the optimal conditions for the production phase.
When using design of experiments (DoE) you can easily start the growth phase in all setups under the same standard conditions (resulting in the same amount of cells after the end of the growth phase) and then switch the individual conditions to analyze the impact of factors like temperature, pH, and dissolved oxygen concentration on the product concentration and product quality.
But highly parallel setups also own the risk of manual handling errors. For example, a certain setpoint could be assigned to the wrong bioreactor unit. To avoid such programming errors, our DASware design software helps you to import complex experimental designs and automatically assigns the correct setpoints.

How do you keep an overview of all the many process results?

Sebastian:
In high parallel experiments, it is crucial to identify which setup worked as expected and which did not. Otherwise, the wrong conclusions are made. DASware control gives a very good overview on every single process using customizable online charts. Thus, you can easily identify the setups which worked as expected. For example, when using a pH setpoint of 7.0 you can easily check if the process value was also 7.0 over the relevant process time. The DASware control software is capable of doing this for all relevant process parameters of up to 24 vessels in parallel.
Additionally, the strength of the software is to compare individual runs, also from past experiments. With DASware control, you can start the inoculation time for each vessel individually. This feature simplifies the comparison of relevant process parameters like pH, DO, temp. profiles between different units and even between different historical runs.
I don't know how much time I spent manually synchronizing the time axis of different runs to be able to compare them when I was still actively working in the lab. Now the DASware control software does this with one mouse click.

If I am experienced in bioprocessing, but not in programming, how can I benefit from the scripting possibility of the DASware software?

Sebastian:
We have a collection of scripting-templates that can easily be applied by beginners or being modified by expert users with almost no limitation. Just ask our field service and benefit from our long-term experience in the industry.

Where do you see the biggest advantage of the DASbox system?

Sebastian:
The biggest advantage of the DASbox system is its compactness combined with the high precision of process control. Together with the powerful DASware control software, the DASbox system is the ideal tool for screening and process development. Up to 24 vessels can be connected to one process computer allowing for highly parallel setups requiring only 1.80 meter/6 feet of bench space and the integrated storage options for accessories ensure that everything is stored where you need it.

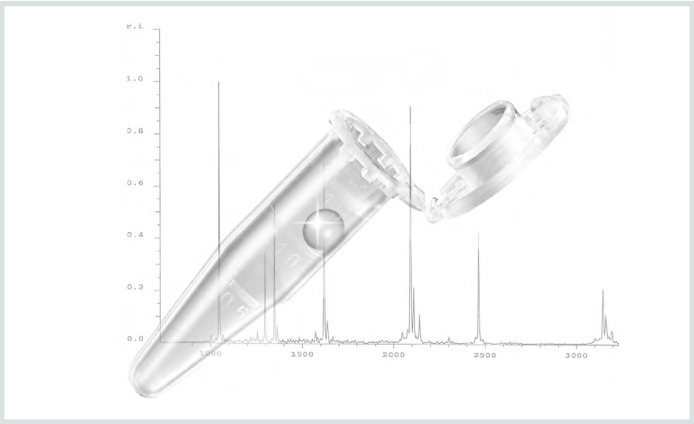
For more information about DASbox® please visit the Eppendorf website.

Although the DASbox is a very compact system it uses industry-standard ports (PG13.5) that enable the use of standard sensors with an outer diameter of 12 mm.
It offers the ability to use both single-use and glass vessels side-by-side or go completely single-use or glass for certain runs. This is a major advantage as the glass vessels allow users to make impeller changes and accessory adjustments. With the large variety of accessories and modular upgrades, you can easily react to changing process requirements. For example, if there is the need to add further features like exhaust gas analysis, these devices can easily be added to the systems. The installation is comparatively simple, as only a power supply and the individual gas connections are required on site. There is no cooling water needed for temperature control due to our Peltier technology, which we also use for exhaust gas condensation. With this, we keep the evaporation at a minimum and effectively prevent clogging of the exhaust filters. With easy workflow guidance on the one hand and options to deeply adjust controller settings, the DASbox system is the optimal tool for beginners and experts alike

Eppendorf Protein LoBind® Tubes – Your Excellent Choice for Handling and Storage of Viral Samples (in Vaccine Production Workflows)

Dr. Rafal Grzeskowiak – Application Specialist
Brigitte Klose - Global Marketing Manager Consumables

Large quantities of virus particles are often used in gene therapy and vaccine production (i.e. SARS ..). For downstream purification of viruses and proteins various protocols are used: filtration, ion-exchange chromatography and gradient centrifugation.
All of these techniques rely on high quality plastic labware for handling and storage of viral samples. During vaccine production and purification viral samples are rather diluted and viral particles are large. Therefore the unspecific adsorption to plastic labware often poses a major problem and leads to sample loss. The solution to this problem may be the use of high quality Protein LoBind tubes.



ScienceDirect® - "The leading platform of peer-reviewed literature that helps you move your research forward" – published the "Journal of Chromatography A", Volume 1142, Issue 1, 16 February 2007 with the detailed description of a study on "Sorption processes in Ion-exchange chromatography of viruses" by E.I. Trilisky, A.M. Lenhoff, Department of Chemical Engineering, University of Delaware, Newark, USA.

The authors of this article tested nine tubes from various manufacturers in the ion-exchange purification protocols. They clearly showed, that only by using Eppendorf Protein LoBind Tubes the concentration of viral particles remained stable during entire storage time of 120 hr. In all other tubes tested the concentration of samples declined down to 60% – 18% of the initial one (Fig. 1).

Noticeably, it was also shown that concentration of viral samples stored in Eppendorf Protein LoBind Tubes remained stable under usage of various buffer systems (HEPES, phosphate, Tris buffers) and broad range of ionic strength conditions: NaCl concentration between 0 and 3 mol/L (Fig. 2, adapted from [1]).

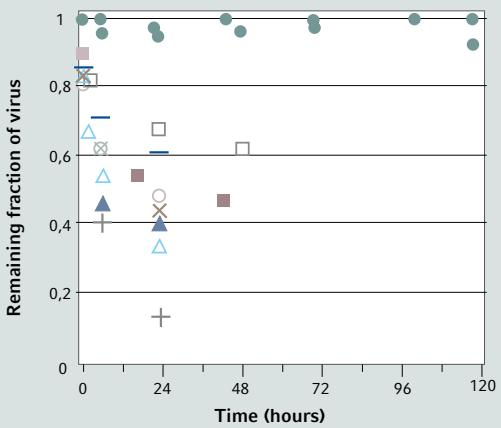


Fig. 1: Virus loss in different containers (20mM HEPES, 150 mM NaCl. Ph 7.8: initial Ad5 concentration was approximately 6 x10^{10} p/mL ≈ 0.02 g/L).
● Eppendorf Protein LoBind Tubes and 8 competitor tubes.
Adapted from [1], page 7, Copyright 2007 by Elsevier B.V.

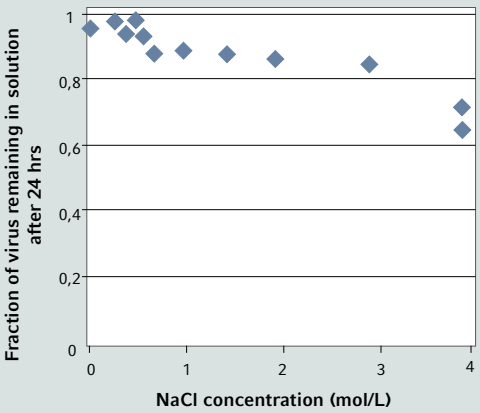


Fig. 2: Virus loss due to binding to the container as a function of ionic strength (0.5 mL LoBind tubes, 20 mM HEPES, pH 7.8, 400 µL solution per tube with an initial concentration of 1.3×10^{10} p/mL ≈ 0.04 g/L, data were collected after 24 and 48 h with no significant differences between the two sets of time points).
Adapted from [1], page 7, Copyright 2007 by Elsevier B.V.

The authors conclude that of all containers tested, only one type – Eppendorf Protein LoBind Tubes – did not bind viral particles and is recommended for collection and storage of viral samples.

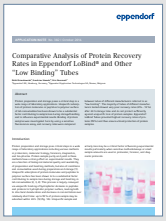
Find more information on the above described article
https://pubmed.ncbi.nlm.nih.gov/17240385/

Literature

[1] E I Trilisky, A M Lenhoff, Sorption processes in ion-exchange chromatography of viruses. J Chromatogr A. 2007 Feb 16; 1142(1): 2-12

Learn more about LoBind Tubes
»How it works – Eppendorf LoBind®«

Watch video



Application Notes 382
»Comparative Analysis of Protein Recovery Rates in Eppendorf LoBind® and other ›Low Binding‹ Tubes«

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Application Note 404
»Total Sample Recovery in Eppendorf Protein LoBind Conical Tubes«

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About Eppendorf

Since 1945, the Eppendorf brand has been synonymous with customer-oriented processes and innovative products, such as laboratory devices and consumables for liquid handling, cell handling and sample handling. Today, Eppendorf and its approximately 5,000 employees employees serve as experts and advisors, using their unique knowledge and experience to support laboratories and research institutions around the world. The foundation of the company's expertise is its focus on its customers. Eppendorf's exchange of ideas with its customers results in comprehensive solutions that in turn become industry standards. Eppendorf will continue on this path in the future, true to the standard set by the company's founders: that of sustainably improving people's living conditions.

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