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Investigation of XCell ATF® Perfusion Technology for virus manufacturing process intensification at MSD Animal Health

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Summary

Over the last decades many viral manufacturing processes have been transferred from the time consuming and higher risk adherent culture system in roller bottles to more scalable suspension cell culture or microcarrier culture in bioreactors. Suspension cell culture was often made possible with the introduction of animal component free (ACF) media or even chemically defined media. The advantage of suspension growth cell cultures is its ease of scalability and opportunity to operate under closed-system conditions in bioreactors. Growing demand for vaccines (subunit / inactivated / live attenuated) and the need for efficient and economic manufacturing processes drives the continuous search for innovations in the vaccine business. Perfusion has been proven as an important and reliable way of culturing cells at high cell density while retaining cell growth and cell quality. The current availability of large-scale single-use filters to retain cells in culture while refreshing culture medium has significantly enhanced the interest for perfusion-based culturing by large pharma companies. Studies have been conducted at MSD Animal Health (Boxmeer, the Netherlands) to evaluate the XCell ATF® (Alternating Tangential Flow) Technology supplied by Repligen Corporation (Repligen). One of the studies focused on the intensification of virus propagation on suspension cells. The XCell ATF® perfused cultures yielded a 5-fold increased cell density at the used medium refreshment. The perfused virus culture also yielded up to 5-fold higher viral output when compared to the reference batch process. Scalability of the XCell ATF® Perfusion Technology was shown by evaluating a large working volume perfused virus culture using the XCell ATF® 10 Device. The normalized viral output showed a comparable relative output between the small-scale and the large-scale perfusion batches. In a third study, XCell ATF® Technology was used to evaluate a different cell line at the N-1stage of virus production. The XCell ATF® perfused cultures yielded a 3-fold increase in cell density. The resulting batch process also yielded up to 3-fold higher viral output when compared to the reference batch process. XCell ATF® Technology was found to be a suitable, high performing and scalable platform which imposes low shear stress to the cells and results in increased viral titers and similar product quality, thus providing an intensified production system for virus manufacturing. Implementation of this technology in redesigned commercial vaccine processes allowed for significant productivity gains thereby improving plant capacity utilization, mitigated facility expansion needs, and lowered CAPEX.

MSD Animal Health

About MSD Animal Health

For over 130 years, MSD has been inventing for life, bringing forward medicines and vaccines for many of the world's most challenging diseases. MSD Animal Health, a division of Merck & Co., Inc., Kenilworth, N.J., USA, is the global animal health business unit of MSD. Through its commitment to

The Science of Healthier Animals®, MSD Animal Health offers veterinarians, farmers, pet owners and governments one of the widest ranges of veterinary pharmaceuticals, vaccines and health management solutions and services as well as an extensive suite of connected technology that includes identification, traceability and monitoring products. MSD Animal Health is dedicated to preserving and improving the health, well-being and performance of animals and the people who care for them. It invests extensively in dynamic and comprehensive R&D resources and a modern, global supply chain. MSD Animal Health is present in more than 50 countries, while its products are available in some 150 markets. For more information, visit www.msd-animal-health.com and connect with us on LinkedIn and Twitter.

Challenge of high quality, high volume viral antigen manufacturing

With MSD seeing a growing market demand for vaccines, manufacturing has been growing their capacities over the years. Typically, capacity is increased by adjusting the batch sizes or by increasing the frequency of batch manufacturing. Bioreactor sizing has increased over the years as well, but this is limited because of regulatory thresholds. At a certain timepoint, manufacturing capacity will become limited both in floor space and in cleanroom occupation, leading to the need for manufacturing facility expansion or transfer of operations to other manufacturing sites. To use the available capacity most efficiently, the pharma business trends towards the use of intensified manufacturing processes like perfusion technologies. With the help of perfusion technology, cell cultures can be maintained at higher cell densities while maintaining nutrient supply and waste metabolite removal. In theory, perfusion technology allows for operation at higher cell densities in existing process equipment while the high quality of the biological production system is maintained. MSD Animal Health (Boxmeer, the Netherlands) tested the perfusion principle for several viral manufacturing processes with the aim to increase the output per batch. The choice was made for an established perfusion system that supports the small-scale to large-scale operation range and has the option to work with single use perfusion filters within that same range. This paper describes the evaluation of perfusion methodology for viral manufacturing processes. The initial tests were performed with the use of the XCell ATF® 2 Device (Repligen) in small-scale bioreactors. Large-scale testing was done with one of the viral antigens using the XCell ATF® 10 Device connected to a large-scale bioreactor.

XCell ATF® Technology

The XCell ATF® System is the global standard cell retention technology used in the intensification of cell culture bioprocesses. It has been widely adopted across industry for delivering process efficiency, achieving ultra-high cell densities and corresponding increases in volumetric productivity of monoclonal antibodies, recombinant proteins, viruses, VLPs, stem cells and other biologics. The XCell ATF® Technology is also highly versatile, enabling a number of applications including perfusion in the production (N) bioreactor, seed train intensification, fed-batch intensification and a novel harvesting technique called high productivity harvest. Because of significant improvements in yield with technology, more compact, flexible, single-use facilities and more efficient process workflows are being implemented across the bioprocess industry. The XCell ATF® Technology is linearly scalable and has been successfully scaled from 1 L up to as high as 5000 L production bioreactors and is currently being used in at least 50 commercial manufacturing processes.

XCell ATF® Device

The XCell ATF® Device utilizes a diaphragm pump to provide alternating tangential flow through a hollow fiber filter, back and forth between the device and the bioreactor. The diaphragm movement is constant and uninterrupted, creating a low shear flow for the cell suspension. The alternating motion (illustrated in Figure 1) results in an inherent backflushing effect on the filter membrane throughout the run, therefore minimizing biofilm build-up, keeping the filter clean, and enabling a longer perfusion process. The device flow rate is controlled by a dedicated XCell™ Controller, which

relies on pressurized air and vacuum utilities to drive the diaphragm. A range of flow rates is possible and can be optimized to the requirements of a particular cell line and the intended intensified cell culture process.

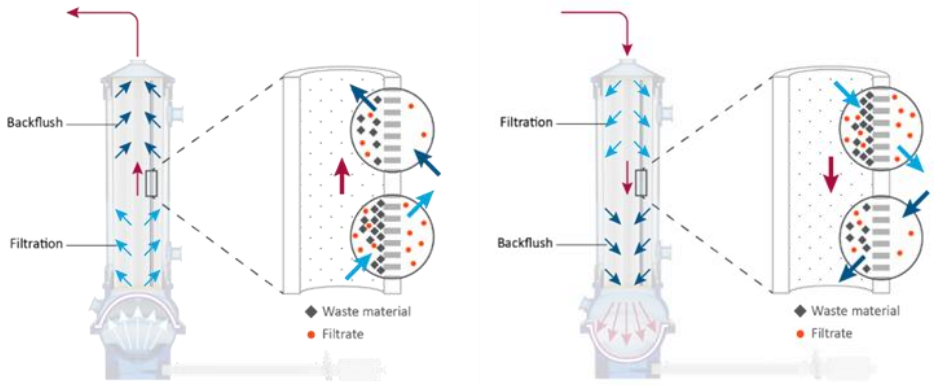

Figure 1. XCell ATF® Technology extends filter lifetime by utilizing a diaphragm pump to provide alternating tangential flow through a hollow fiber filter, back and forth between the device and the bioreactor.

XCell ATF® Device sizes range from the bench scale XCell ATF® 1 Device designed for cell culture development work at 500 ml - 2 L working volumes to the large scale XCell ATF® 10 Device meant for GMP manufacturing at 200 L to 1000 L and beyond. Most devices are available in both stainless steel as well as single-use formats and can be connected to almost any type of bioreactor.

XCell ATF® evaluation experiments

Experiment #1: growth of animal suspension cells and production of virus in small-scale perfusion culture

Methodology

To assess the potential use of XCell ATF® Technology for the suspension culture of animal cells, initial studies were performed using a small-scale glass bioreactor (Applikon) equipped with a marine impeller. Temperature (37° C) was controlled via an external water bath (Neslab®) connected to the glass water jacket. Growth parameters (temperature, pH, oxygen, and stirrer speed) were controlled using an in-Control or ez-Controller (Applikon). The XCell ATF® 2 Device (0.13 m^2, PES, pore size 0.2 µm, lumen 1 mm, Repligen) was connected with an A2B silicon sanitary connection line to a dip-tube (80% submerged) in the headplate of the bioreactor. The XCell ATF® Device flow was generated by an XCell™ C24 Controller connected to the stainless steel (SS) device pump housing. The XCell ATF® Device flow rate was operated at a flow rate recommended by Repligen. Permeate removal and fresh

medium addition were controlled via peristaltic pumps that were connected to the bioreactor controller. The medium level in the bioreactor was maintained using a level sensor. The refreshment rates expressed as vessel volumes per day (VVD) were gradually increased to the maximum used VVD as cell density increased The medium refreshment was either controlled manually by gradually increasing the permeate pump speed, or by automatic pump speed increase using an algorithm. The cells were cultured to the desired viable cell density and diluted back to lower densities prior to reaching stationary phase at the maximum used medium refreshment rate. Cell counts were done with use of a NucleoCounter® NC-100 (ChemoMetec) device.

Increased cell culture densities were obtained, so the next step was to evaluate whether XCell ATF® Technology would also support virus amplification on the cell culture. Thus, cells from experiment 1 were used to perform a virus culture under perfused conditions. After sufficient cell density was obtained virus was added at a standardized multiplicity of infection (MOI). Perfusion with virus culture was maintained throughout the remainder of the culture. Bioreactor parameter settings were adjusted to accommodate optimal growth for the virus of interest. Samples were taken at daily intervals to perform viable cell count, culture viability and live virus titer. Live virus titration was done using a TCID50 titration method. Specific staining was applied to determine specific live virus titer. At the end of the virus culture, fresh medium supply was stopped, samples were taken from the cell/virus suspension. Samples were taken from the harvest and the permeate to determine live virus titer and virus recovery.

Results:

To assess the potential use of XCell ATF® Technology for the support of animal suspension cell growth in ACF culture medium, a total of 13 perfusion cell culture runs were performed and compared with the reference batch process, see Figure 2. The perfused cell cultures continued to grow in an exponential trend. The trend line of the perfusion cell curve runs parallel to the slopes of the batch process, indicating equal growth performance and cell quality. The cell viability throughout the perfusion process remained high and was comparable to typical cell viability under normal batch conditions. The cell suspension showed nice single cells and some small clumps, typical for the used cells. There were no issues with filter blockage or membrane fouling during clean cell operation. Earlier explorative studies with comparable 1 mm lumen filters operated under traditional tangential flow had resulted in blocked filter lumen and reverse flow was needed to maintain operation. The XCell ATF® Device operating principle was found to be better suited for the cell lines explored, mainly because they do not grow in perfect single cell appearance. Longer cell growth or multiple cell passages within the same bioreactor will generate some cell accumulation on the liquid surface of stirring rods and sidewalls. The clumps of cells and cell debris can come loose during normal operation and clog the membrane entrance which has a typical lumen size of 1mm. During XCell ATF perfusion cultures, this was not an issue due to the alternating technology.

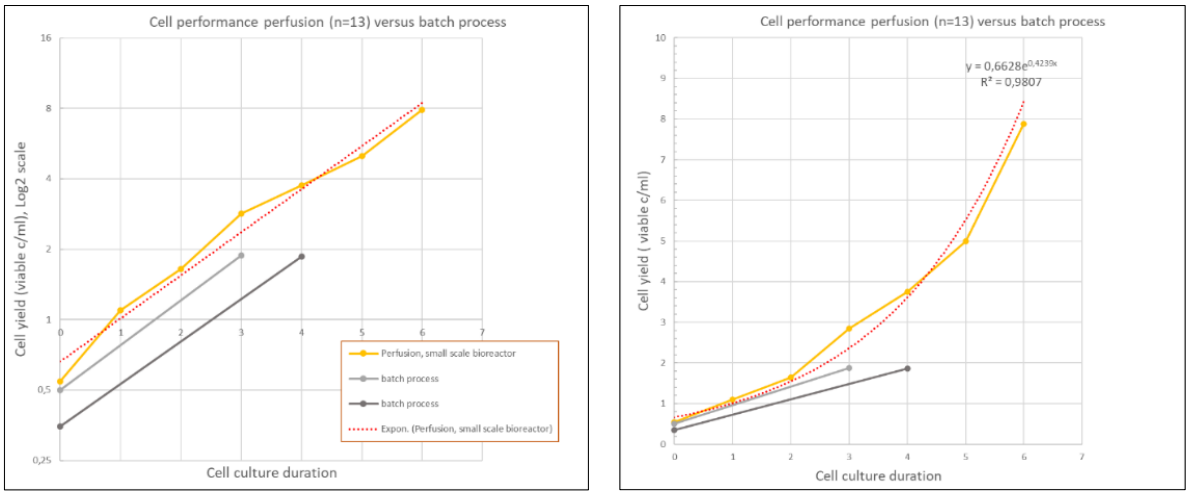

Figure 2a and 2b. Cell growth curve showing viable cell yield as Log2 scale (2a, left) and linear scale (2b, right) versus cell culture duration (x-axis, days post cell seeding) of the reference batch processes (culture without medium refreshment) versus perfused cells. The dotted line represents the exponential trend line of the perfused cultures.

To assess the virus producing quality of the cells from perfusion culture, several virus cultures were performed in small-scale bioreactors. Live virus titration was done on samples during the virus culture process and during the downstream process (DSP). The live virus titer is considered as one of the critical quality attributes for the manufacturing process. The average cell viability of 13 perfusion virus cultures was compared to the average cell viability of 13 reference batch processes, see figure 3. The cell viability kinetic is an indication of the process comparability between the batch process and perfusion process. In general, there is high comparability between the averages of both processes, where during the perfusion process the viability tends to be slightly higher but also has a sharper drop-off towards the last day of culture. Figure 3 shows the starting cell densities and the viral harvest yields of batch process versus perfusion XCell ATF® process. Data were normalized against the reference batch process. The graph shows that at a 5-fold higher starting cell density, the viral output showed an approx. 5-fold output. This indicated that with the used refreshment regime, the perfused cells are of good quality and behave very similarly in the virus culture with a linear viral output versus the increased starting cell density. The 5-fold increased cell density did not result in any practical issues with bioreactor parameter settings, membrane fowling of the perfusion device or DSP operations. The loss of virus via the permeate (waste stream) was very low and considered acceptable. This is remarkable, as based on membrane pore size and virus particle size more loss of virus was expected. The gentle tangential flow and backflush prevented significant loss of virus during

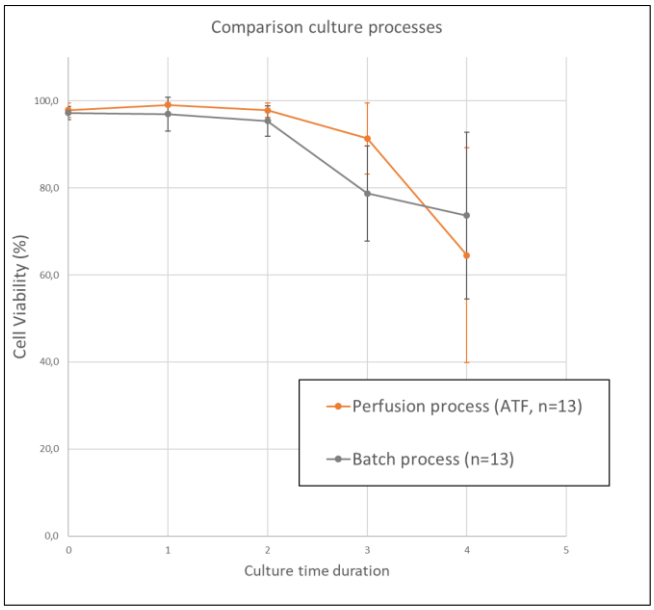

Figure 3. Cell viability (Y-axis, %) during virus culture versus incubation time (x-axis, culture time duration) of the reference batch process (without medium refreshment) versus small scale perfused virus culture. The curves are the averages of 13 batches of each process, vertical bars represent the standard deviation.

Discussion and conclusions

The tested cell line showed very good cell performance in the small-scale XCell ATF® Perfusion System. The perfusion process resulted in significantly higher cell densities at the applied refreshment rates. Cell-viability and cell-growth rates are considered as main quality indicators for the clean cell cultures and were comparable to the growth rate and viability from the reference batch culture process. The XCell ATF® 2 Device worked very well in the tested small-scale bioreactor. The low shear applied to the cell and the alternating flow resulted in robust operation and high-quality cell suspensions.

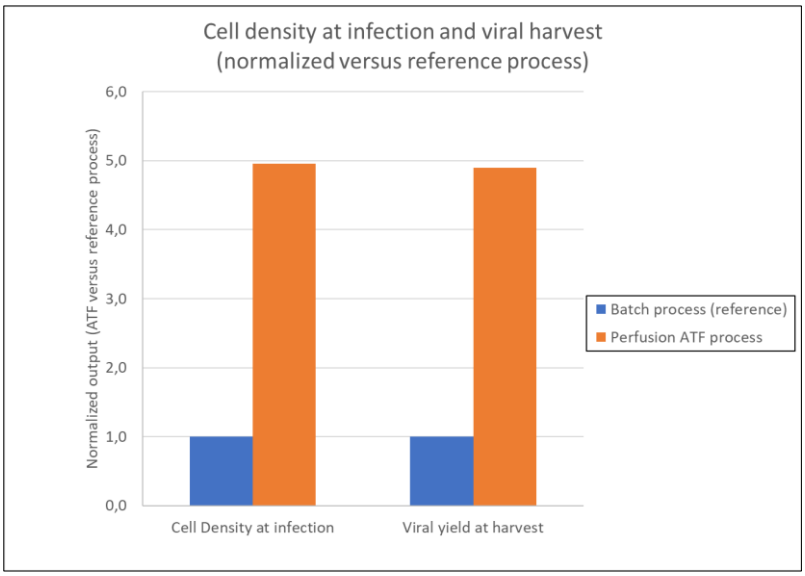

Figure 4. Bar-plot showing the cell density at infection and the viral yield at harvest of the reference batch process (blue bar) versus the XCell ATF® perfusion process (orange bars). Values were normalized to the reference process. Presented are the average values of 13 batch cultures versus 13 XCell ATF® perfusion batches.

Cells from the XCell ATF® perfusion culture were subsequently used in virus culture experiments. The perfusion was continued during virus culture. The perfusion process behaved similarly versus the batch process with regards to cell viability and cytopathogenic effect (CPE) progression. With the tested cell/virus combination there was acceptable loss of virus via the permeate waste. Because of this, the XCell ATF® Perfusion Device could be used to reduce the harvest volume while maintaining high titer viral output. This offers additional process benefits because no additional equipment for viral concentration is needed.

Experiment #2: Scale-Up of intensified upstream process

Methodology

To assess the scalability of the XCell ATF® Perfusion System, viral growth on cell culture was done in a large-scale bioreactor. According to the scale-down calculations from Repligen, the culture volume used with an XCell ATF® 2 Device had a comparable backflush efficiency and a comparable membrane fowling per m^2 membrane surface. A large-scale bioreactor connected to an Applikon DCU controller was used. A single-use XCell ATF® 10 Device was connected to the bioreactor according to the instructions given by Repligen by double 1" ID silicon tubing with Aseptiquik® Single-Use Connectors (CPC). The XCell ATF® flow was controlled with an XCell™ C410v3 Controller (Repligen). The cell- and virus-culture parameters were the same as for the small-scale culture. The medium level in the bioreactor was maintained using the load cells and the DCU controller. The refreshment rate gradually increased to the maximum used VVD. The medium refreshment was controlled on a daily base by manually increasing the permeate pump speed over time. The cells were cultured to a 5-fold target density. Cell medium was replaced by virus medium by first removing the spent medium via the XCell ATF® permeate line at elevated pump speed. Virus medium was added to final culture volume and virus was added at a standardized MOI. Samples for cell count and live virus titration were taken throughout the virus culture process.

Results

To investigate the scalability of the XCell ATF® perfusion process, the small-scale runs were compared to the large-scale manufacturing run. The results of the cell culture performance are shown in Figure 5a (Log2 Y-scale) and 5b (linear Y-scale). Both curves were highly comparable and the exponential trend lines showed high R2 values of ±0.98. The x-coefficients were 0.438 and 0.4671, indicating good similarity between small scale and large-scale XCell ATF® Perfusion Systems. The viability of the cells remained high at average 98% (data not shown).

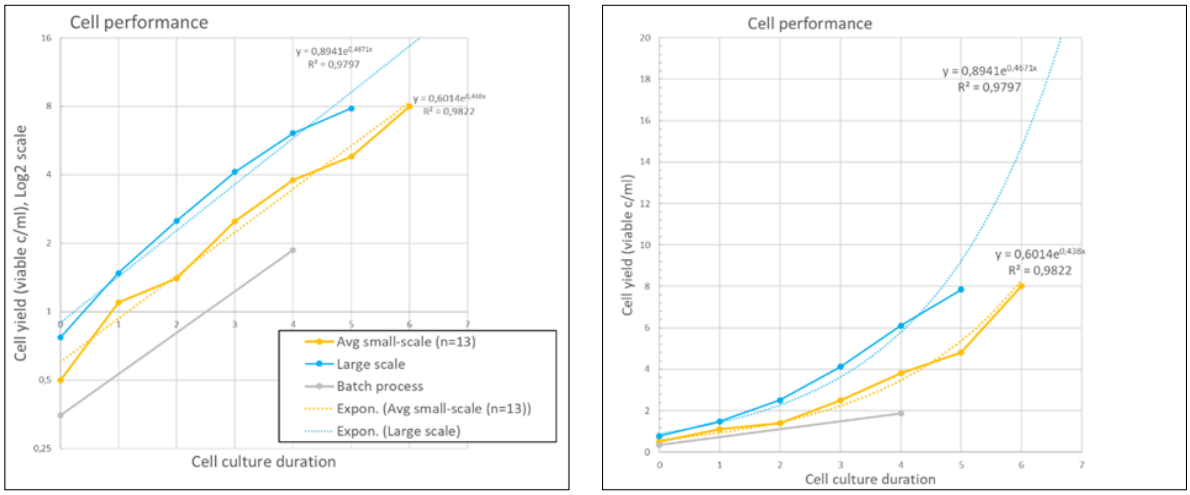

Figure 5a and 5b. Cell growth curve showing viable cell yield as Log2 scale (5a, left) and as linear scale (5b, right). The blue curve represents the large-scale perfusion batch, the yellow curve the average of small-scale cultures (n=13). The dotted lines represent the exponential trend lines of the perfused cultures. Reference batch process is plotted in grey.

Cells were grown in perfusion mode at large scale until the targeted cell density and then infected. Figure 6 shows the normalized cell density at start virus infection, and the normalized viral yield. Values were normalized versus the average values of the small-scale perfusion cultures.

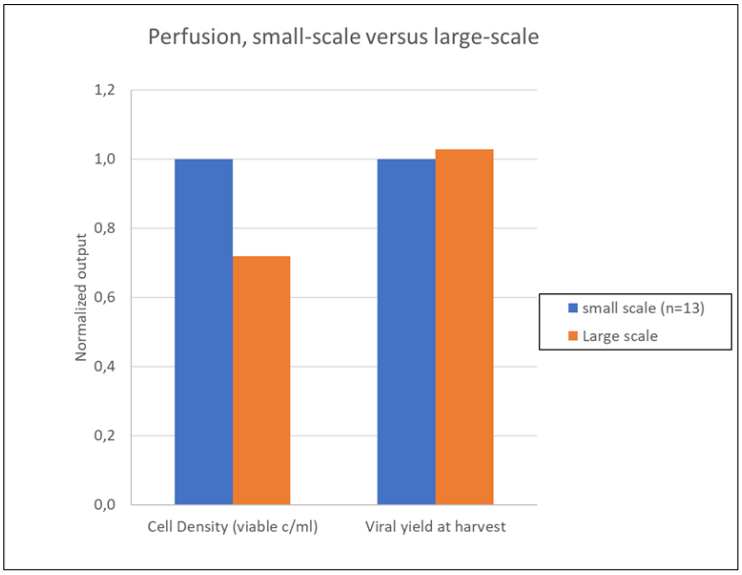

Figure 6. Bar-plot showing the cell density at start of the infection and the viral yield of the average small scale perfusion cultures (n=13, blue bar) versus the large-scale XCell ATF® perfusion process (orange bars). Values were normalized to the small-scale perfusion process.

Discussion and conclusion

The virus production process was tested at large scale using the single-use XCell ATF® 10 Device. The cell performance was comparable to the small-scale perfusion process. The viral culture on the cells in the large-scale bioreactor resulted in the expected increase in live virus titer output and the scale-up from small scale to large scale was successful. This offers the opportunity to develop a more

efficient manufacturing process with lower cost of goods, higher volumetric output per batch, better plant utilization, and with equal viral antigen quality.

Experiment #3: growth of a different cell line in perfusion mode and production of virus on perfused (N-1 stage) cells

Methodology

Another experiment aimed to maintain a different suspension cell culture operated using XCell ATF® Perfusion Technology, that could continuously supply cells to perform virus culture in batch mode (N-1 bioreactor stage cells). Initially, a study was performed in a small-scale bioreactor equipped with a marine impellor. Growth parameters were controlled using bioprocess controllers. The XCell ATF® 2 Device (0.13 m^2, PES, pore size 0.2 µm, lumen 1 mm, Repligen) was connected with an A2B silicon sanitary connection line according to manufacturer instructions. The XCell ATF® Device flow was generated by an XCell™ C24 Controller connected to the filter device. Permeate removal and fresh medium addition were controlled via small peristaltic pumps that were connected to the bioreactor controller. Medium level in the bioreactor was maintained using equal flow rates for permeate removal and medium addition. The refreshment rate was gradually increased to the maximum used VVD. The cells were cultured to an approx. 3-fold density versus reference culture and diluted back to lower densities when reaching stationary phase at maximum medium refreshment.
To assess cell quality of perfused N-1 cells, viral growth was done at several cell densities. After sufficient cell density was obtained cells were separated by centrifugation and inoculated into a virus bioreactor with culture medium. A 3-fold higher cell inoculation versus the reference process was applied. Virus was added and no changes were made to the virus culture parameters. Samples were taken to perform viable cell count and antigenic mass.

Results

To assess the potential use of XCell ATF technology for the support of suspension cell growth, a total of 14 perfusion cell culture runs were performed and the average cell growth was compared to reference batch processes, see Figure 7. The perfused cell cultures continued to grow to approx. 3-fold viable cell density versus the reference batch culture in an exponential trend. The slope of the perfusion cell curve runs parallel to the slope of the batch process, indicating equal growth performance and cell quality. The cell viability throughout the perfusion process remained high (> 95% for both the reference process and the perfusion process). No filter blockage or membrane fouling during clean cell operation was observed.

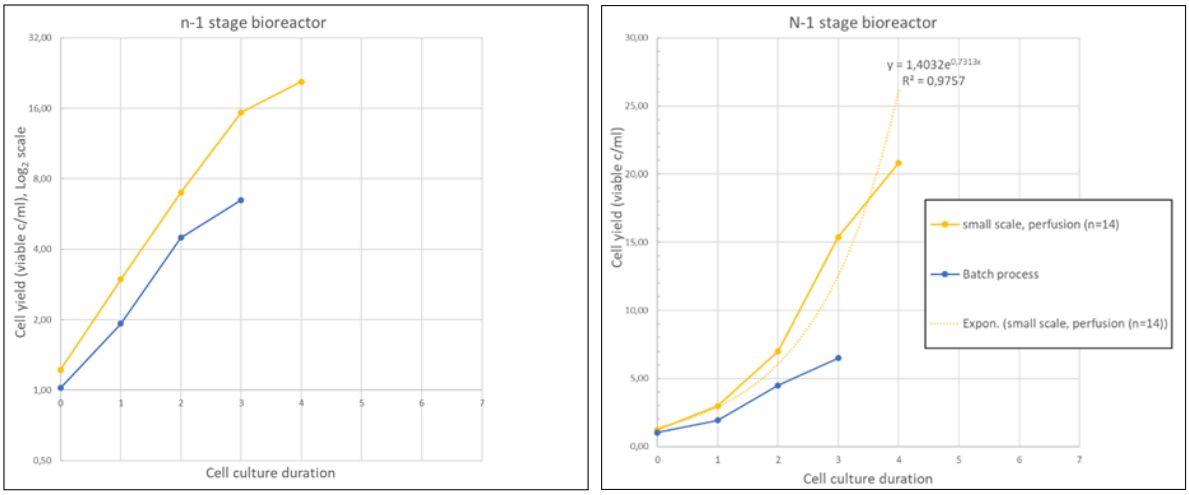

Figure 7a and 7b. Cell growth curve showing viable cell yield (Y-axis, 7a=Log2 scale, 7b=linear scale) versus incubation time (x-axis, days post cell seeding) of the reference batch process (without medium refreshment) versus perfused cells. The dotted line represents the exponential trend line of the perfused cultures.

Virus culture on the cells derived from perfusion culture showed that an approx. 2.8-fold increase in cell density led to an approx. 3.3-fold increase in virus yield. Figure 8 shows the normalized cell density at start virus infection, the normalized viability, and the normalized viral yield. Values were normalized versus the average reference batch culture. The cell viability at harvest time point was comparable between the culture methods. The perfused cells that were diluted to starting cell density approx. 0.7× resulted in an antigenic mass of approx. 1.1× versus the reference batch process.

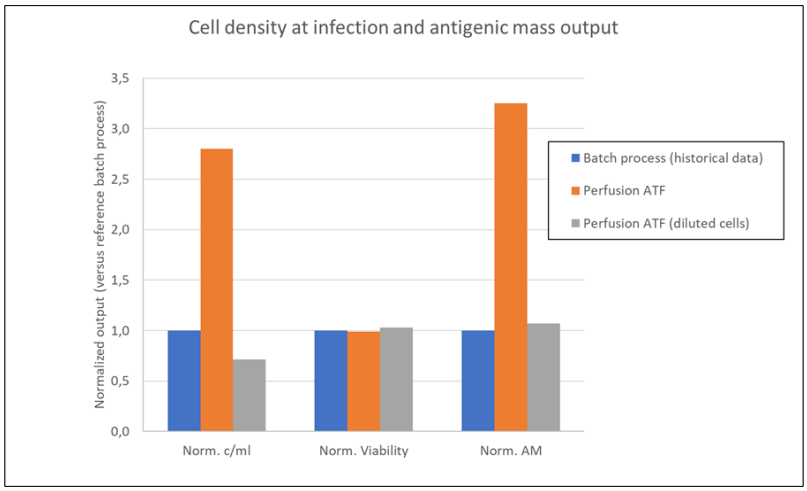

Figure 8. Bar-plot showing the cell density at start infection, the cell viability at harvest, and the viral yield after DSP of the reference batch process (blue bar). Values were normalized to the reference batch process.

Discussion and conclusion:

The virus producing quality of the cells (N-1 stage bioreactor) from perfusion suspension culture was evaluated with several virus batch cultures, done in small scale bioreactor. Antigenic Mass values were tested after small-scale DSP. The averages were compared with historical data. The cell density and antigenic mass values of the reference batch processes were set at 1, and the results of the cultures were normalized against the reference batch process. The increased starting cell density (approx. 2.8×) resulted in an increased antigenic mass (approx. 3.3×). The perfused cells that were

diluted to starting cell density approx. 0.7× resulted in an antigenic mass of approx. 1.1× versus the reference batch process. The results show that the cells derived from XCell ATF® perfusion show good performance in virus production. An approximate 3-fold higher cell density resulted in an approximate 3-fold higher antigenic mass. Comparable cell viability was observed showing process robustness between the tested manufacturing methods.

XCell ATF Technology evaluation discussion

MSD Animal Health assessed the XCell ATF® Perfusion Technology in two different cell-virus processes. The tested cells both showed very good cell performance at small-scale using XCell ATF® Perfusion Systems. The perfusion process resulted in significantly higher cell densities at the applied refreshment rates. Cell-viability and cell-growth rates are considered as important quality indicators for the clean cell cultures and were comparable to the growth rate and viability from reference batch culture process. The small-scale XCell ATF® 2 Device worked very well in the tested operating range. The low shear applied to the cell and the alternating flow resulted in robust operation and high-quality cell suspensions.

Cells from the XCell ATF® perfusion culture were subsequently used in virus culture experiments. The cell medium was replaced with virus culture medium by temporarily increasing permeate flow rate. This closed system handling was done without the use of additional equipment like a centrifuge or crossflow microfiltration. The perfusion was continued during virus culture. The perfusion process behaved similarly versus the batch process with regards to cell viability and cytopathogenic effect (CPE) progression. There was an acceptable loss of virus via the permeate waste.

The virus production process was tested at large scale using the single-use XCell ATF® 10 Device. Again, cell quality was optimal as indicated by cell viability and the cell growth curve. The cell performance was comparable to the small-scale perfusion process. The viral culture on the approx. 5× higher starting cell density resulted in the expected approx. 5-fold increase in live virus titer output. Upscaling from small to large scale was successful. This provides a more efficient platform manufacturing process with lower cost of goods, higher volumetric output per batch, better plant utilization, and with equal viral antigen quality.

From the perfusion derived N-1 stage bioreactor, cells were used in a regular virus batch process at high cell density and at reference cell density. The cell growth was optimal, as indicated by the cell viability and the cell growth curve. At equal cell growth performance, XCell ATF® perfusion offers the possibility to have a much higher cell output per bioreactor volume. This helps in developing an intensified cell- and virus culture and efficient use of process equipment.

To conclude, the XCell ATF® Perfusion System was found to be an effective technology to be used in the manufacture of cell culture and viral antigen for animal vaccines. The XCell ATF® equipment was easy to handle, allowed for closed system operation and replaced the need for cell concentration by centrifugation. The low shear operation and alternating flow supported optimal cell growth. Scalability was established between small-scale and large-scale. The main objective to support high-density cell- and virus-manufacturing was achieved using the XCell ATF® Perfusion Technology.

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