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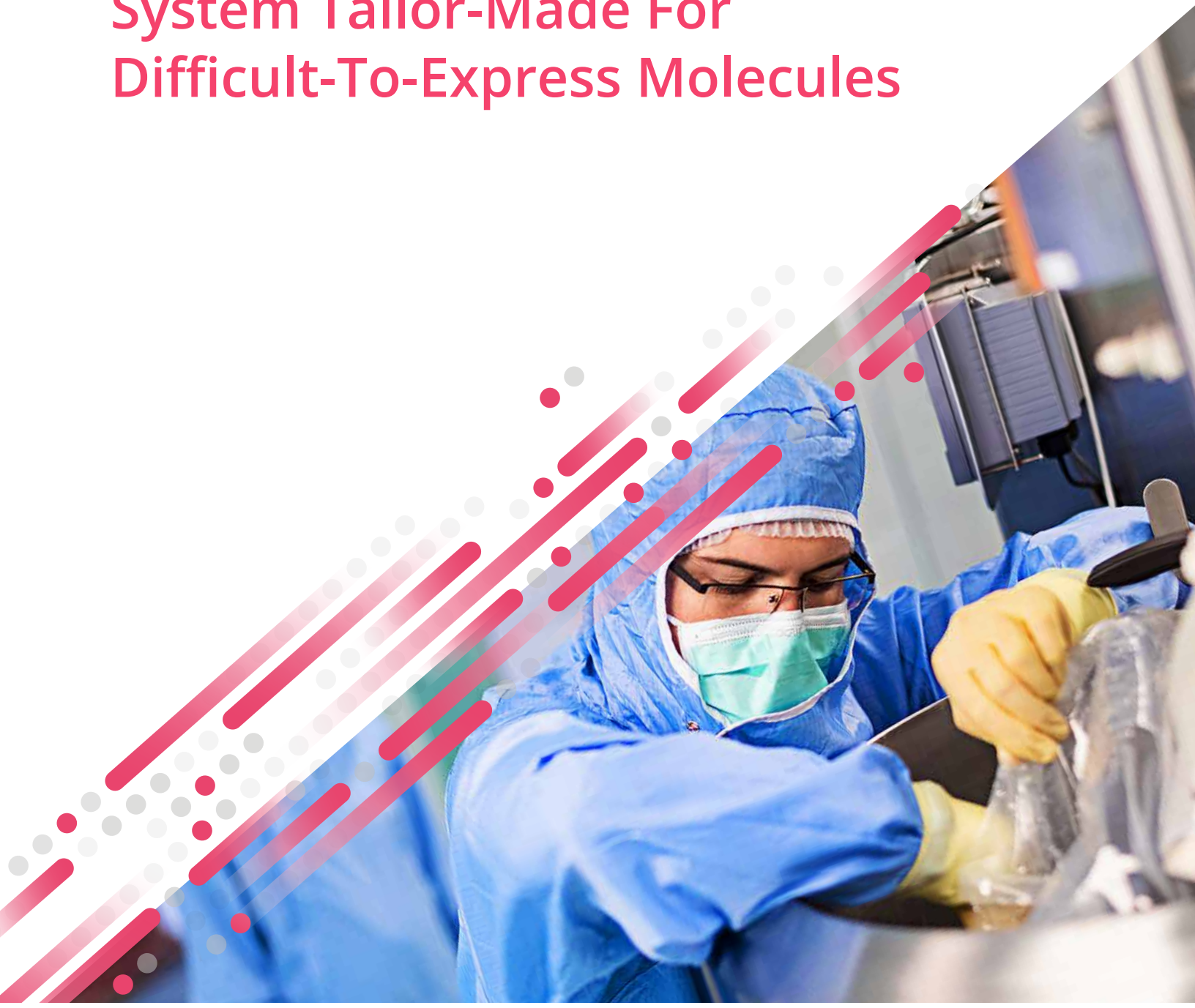
PERFUSION: A Biologic Production System Tailor-Made For Difficult-To-Express Molecules



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PERFUSION: A Biologic Production System Tailor-Made For Difficult-To-Express Molecules

Drug development pipelines are changing. Having largely explored the medical potential of classical antibodies, researchers are shifting their focus to emerging modalities such as antibody fragments, hormones and enzymes. Such modalities unlock new opportunities to address unmet medical needs but also create challenges. Notably, some of these complex constructs are hard to express, rendering conventional production processes ineffective. That barrier is driving the use of perfusion, an alternative manufacturing process better suited to the production of complex proteins.

The need for manufacturing processes capable of economically yielding complex proteins is evident in the current industry R&D pipeline and the recent history of drug approvals. Amgen, for example, grew into the world's largest biotech in part because of its successful monoclonal antibodies, notably Prolia and Xgeva.[1,2] In 2019, those two drugs alone generated sales of more than $4.6bn.[3] Yet, recently Amgen has switched its focus to other modalities.

In February 2015, almost 40% of Amgen's Phase I assets were monoclonal antibodies.[4] By early 2020, the proportion of monoclonal antibodies in Amgen's Phase I pipeline had fallen to 10%.[5] The figures reflect

a broader move away from classical monoclonal antibodies and toward new modalities such as bispecific T cell engager antibody constructs.

The changes seen at Amgen over the past five years are echoed across the industry and are starting to show up in a shift in the modalities of newly approved products. From 2015 to 2018, the US Food and Drug Administration (FDA) approved eight monoclonal antibodies a year, on average.[6–9] The FDA approved one antibody fragment drug over that period. In 2019, the FDA approved three monoclonal antibodies and two antibody fragments.[10]

Emerging modalities such as antibody fragments unlock new biology and, in doing so, stand to address unmet medical needs. Those factors are driving growing interest in these promising biologics. However, as artificial, complex constructs, emerging modalities are often harder to express than monoclonal antibodies.

The expression challenges posed by these complex constructs are apparent in the cell lines used by the manufacturers of recently approved medicines. Most of the monoclonal antibodies approved by the FDA in recent years, and throughout the history of the modality, have been expressed in Chinese hamster ovary (CHO) cells. In contrast, neither of the antibody fragments approved in 2019 are expressed in CHO cells.[11,12] The figures show the difficulty of expressing complex constructs in CHO cells and thereby point to the need for alternative production processes.

Choosing The Right Cell Line For Complex Constructs

Many biologic development projects start with a gene sequence and little idea of how the molecule will behave. That leads most researchers to initially try to express their molecules in CHO cell lines. If that proves impossible or impractical, researchers turn to alternative cell lines and/or to alternative processes like perfusion that are better suited to the expression of complex constructs.

CHO cells have become one of the go-to biomanufacturing tools over the past 30 years. In 1987, at the dawn of the biotech industry, the FDA approved Genentech's use of the cell line to manufacture the enzyme alteplase.[13] Since then, CHO cells have demonstrated their ability to express a wide range of proteins and handle human-like post-translational modifications, cementing their status as the cell line researchers turn to at the start of a project.

What Is Perfusion?

Perfusion is a type of process used to run classical stirred culture vessels. The process is characterized by the harvesting of product throughout production. Spent medium and product are collected but cells stay in the bioreactor. As fresh medium is added to the bioreactor, the cells continue to express the desired molecule, supporting further product harvests. Typically, one to two reactor volumes are harvested every day.

The continuous removal of spent medium and product differentiates perfusion from the fed-batch process. In fed-batch, nutrients are fed in in controlled portions after the start of cultivation. Product is harvested once at the end of the production process.

However, researchers are now discovering the limitations of CHO cells. While CHO cell lines express adequate quantities of many natural proteins like most antibodies, they yield only small amounts of some complex constructs. Such low yields drive up the cost of manufacturing the medicine, rendering CHO cell lines economically unsound, or actually unusable, for some molecules.

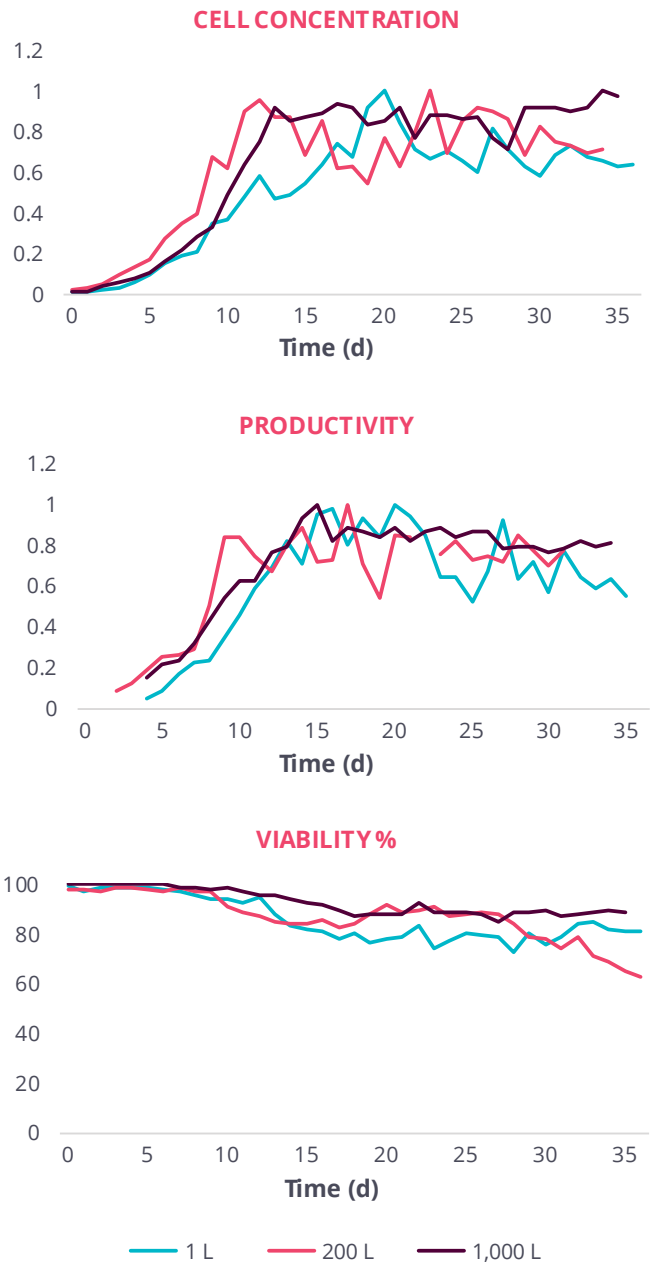
Recognition of the limitations of CHO cells is driving interest in alternative cell lines. One alternative is GEX®, a human cell line platform for high-yield production of difficult-to-express molecules with fully human glycosylation.[14] Using GEX®, Celonic, a premium contract development and manufacturing organization (CDMO), services clients with a toolbox of cell lines tailored to the characteristics of the molecule they want to express.

GEX® is based on a human cell line that Celonic optimized for product quality and fast, reproducible, high-yield glycoprotein manufacturing. The technology supports optimization of glycosylation and, in doing so, enables Celonic to improve the potency, bioavailability, stability and immunogenicity of a molecule through the modification of its critical quality attributes.

Those features make GEX® an attractive alternative to researchers working on molecules that are hard to express CHO cell lines. In some cases, GEX® is used to express antibodies of different isotypes, some of which are ill suited to CHO cell lines. GEX® is also able to express emerging modalities such as bispecific antibodies, antibody fragments, fusion proteins and other difficult-to-express proteins.

Celonic has validated GEX® using perfusion processes and successfully used it in third-party projects. The projects have shown it is possible to scale up to 1,000 L cultivation volume. Using a 1,000 L bioreactor with two-fold perfusion corresponds to a harvest volume of 60,000 L over 30 days. Celonic has also demonstrated the quality of products expressed in GEX® cells, including fucosylated and low-fucosylated antibodies. The assessments show

Figure 1. Production Of A Monoclonal Antibody Using The GEX® Expression System With Perfusion At Various Scales



glycosylation patterns stay stable across different fermenter sizes, production runs and facilities.

With Celonic offering the GEX® toolbox of cell lines alongside its CHOvolution® CHO cells, the CDMO has the breadth of technologies needed to ensure that the optimal cell line is used on every development and manufacturing project.

Why Perfusion Is The Best Choice For Some Molecules

Drug developers that choose GEX® over CHO need a production process that, like their cell line, is tailored to the needs of difficult-to-express molecules. Perfusion is such a production process *(See sidebar: What Is Perfusion?)*. By combining GEX® cell lines with a perfusion biomanufacturing process, companies can improve their volumetric productivity and, in doing so, bring innovative medicines to the patients that need them.

Manufacturers can choose from a range of processes when operating a classical stirred culture vessel. Fed-batch's ability to tune and optimize cell metabolism through the addition of controlled portions of nutrients makes it a good choice for some manufacturing projects.

However, the fed-batch process has limitations that are exposed by difficult-to-express molecules. These molecules do not readily accumulate in bioreactors, leading them to degrade when produced using a fed-batch process.[15,16] That significant shortcoming of fed-batch and the proliferation of complex constructs in R&D pipelines have created a growing need for a better way to make difficult-to-express molecules.

Perfusion is addressing that need. The continual harvesting of product that characterizes perfusion prevents degradation by reducing contact with the proteases or glycosidases found in bioreactors.[17] Rather than staying in the system and degrading, molecules are taken out and kept in a cold room to accumulate. Manufacturers that adopt perfusion also benefit from consistent product quality, as a result of the ongoing harvests, as well as higher yields per volume. Some of the benefits stem from the fact that perfusion bioreactors can be up to 10 times smaller than fed-batch bioreactors.[18]

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The perfusion process is enabled by cell retention devices that collect the product in spent medium while leaving cells behind in the bioreactor. These devices separate the harvest from cells based on either the size or density of the materials.

At its manufacturing facility in Heidelberg, Germany, Celonic, a perfusion pioneer with more than 20 years of experience, performs size-dependent separation using alternating tangential flow filtration devices. This entails alternating the cell suspension up and down through a hollow fiber module and using a filtrate pump to extract the cell-free filtrate. While this requires a large diameter outlet at the bioreactor, facilities equipped to use the device benefit from very high cell densities, complete cell retention and a robust process.

Celonic's plant is also equipped to perform density-dependent cell retention using a Centritech centrifuge. This cell retention device is more expensive but the outlay is justified by high cell densities, natural bleeding and low shear stress.

Whatever the technology used, there are certain downsides to the perfusion process that mean fed-batch remains a better option for some products. For example, validation is more time consuming and the process requires more advanced, expensive equipment.[19] Perfusion also takes longer, thereby raising the risk of contamination. Yet, with companies such as Celonic adopting novel techniques and technologies that maximize the benefits and minimize the risks, the process is established as a viable, and in many cases desirable, alternative to fed-batch.

How Celonic Validated The Perfusion Process

Celonic has supported the rise of perfusion by investing in technologies that enable the process and running validatory studies to show it outperforms fed-batch in many situations. In one experiment, Celonic compared the yields from fed-batch and perfusion when the processes were used to make a monoclonal antibody in a 200 L bioreactor.

The fed-batch side of the experiment entailed doing two production runs that lasted 14 days. Across the two fed-batch production runs, Celonic harvested 2.0 grams of antibody per liter of medium, resulting in a total yield of 800 g (Table 1).

In contrast, the product concentration achieved using perfusion was far lower at 0.18 g/L. However, the 28-day perfusion run also consumed many times more medium than the fed-batch process. With the perfusion run consuming 14,000 L of medium, Celonic harvested 2,450 g of monoclonal antibody, making the process more than three times as productive as fed-batch.

Combining the perfusion process with the GEX® human cell line brings further advantages. The GEX® cell line can be used in perfusion runs that last more than 45 days. By prolonging the run beyond the 28-day duration used in the fed-batch comparison, Celonic can further increase volumetric productivity and drive down the cost of goods sold.

The combination of perfusion and GEX® yields even more pronounced benefits in other areas. Celonic linked GEX® to a five-fold increase in productivity over CHO cells when used in a perfusion process to produce an antibody-based fusion protein, the sort of novel modality that makes up an increasing proportion of drug development pipelines. Celonic tried to make the fusion protein using fed-batch but was unable to do so, despite applying several

Table 1. Comparison Of Fed-Batch Versus Perfusion

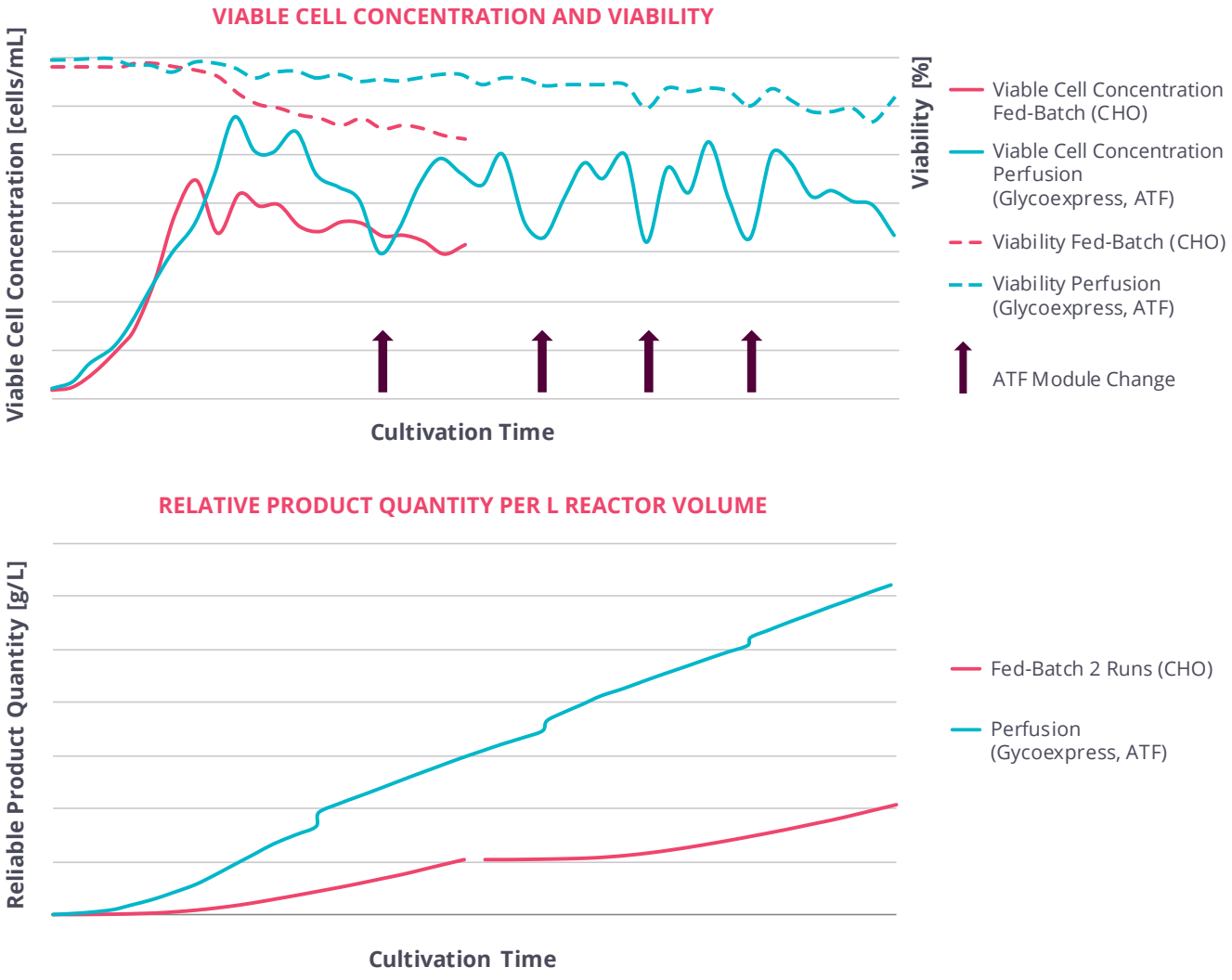
200 L bioreactor	Fed-batch, two runs	Perfusion
Medium consumption	2 x 200 L	14,000 L
Product concentration	2.0 g/L	0.18 g/L
Product harvested	800 g (2 x 400 g)	2,450 g
Product per L volume	2.0 g/L	12.2 g/L

CHO cell lines to the task. In that case, perfusion was needed to make the protein commercially and logistically viable.

Celonic's ability to combine perfusion and GEX® cells to perform 45-day production runs that achieve daily productivity levels ranging from 200 to 300 mg/L is important given the changing nature of drug development pipelines. Antibody-based fusion proteins such as the one made by Celonic and other complex constructs are poised to significantly improve the lives of people living with currently unmet medical needs. However, those improvements will only happen if companies can reliably and cost effectively manufacture the molecules.

The Celonic studies show that is possible. While perfusion remains associated with certain operating challenges, in the hands of a skilled CDMO the process can consistently deliver high yields of high-quality biologic products, thereby eliminating a bottleneck that could stymie access to life-changing medicines.

Figure 2. Productivity Of Fed-Batch Versus Perfusion



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ABOUT CELONIC GROUP

Celonic is a privately owned Contract Development Manufacturing Organization (CDMO) with two state-of-the-art sites located at Basel, Switzerland (headquarters) and Heidelberg, Germany.

Celonic provides comprehensive GMP development and manufacturing services for New Biological Entities (NBEs) and Biosimilars, as well as Cell & Gene Therapy associated services worldwide. Their portfolio includes the development of cell lines, production processes, as well as non-GMP and GMP manufacturing of biopharmaceutical drug substances and drug product.

To complement their services with cell line development, Celonic uses its CHOvolution® cell line technology and the GEX® human cell line for your easy or difficult target.

Being part of the JRS Pharma family, Celonic is a strong believer in creating win-win value propositions for their clients, mitigating risks by using mutually-beneficial business models.

NEW FOR 2020

CELL AND GENE THERAPY FACILITIES

Celonic is currently building a state-of-the art GMP Manufacturing Facility for Gene Vectors and Cell Therapy at our site in Basel. We will offer capacities ranging from Process Development and Optimization up to early-commercialization, including Clinical Trial Manufacturing for Phase I, II and III.

NEW STATE-OF-THE-ART FACILITY IN HEIDELBERG

New GMP facility construction includes an expansion of the production capacity at Celonic's Heidelberg site. Total area is estimated to have approx. 5,300 square meters with a clean room area of approx. 2,000 square meters.

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