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Maintaining Cell Line Integrity

The quality of the cell lines used to manufacture biopharmaceuticals are crucial for the production of high-quality, stable biopharmaceuticals.

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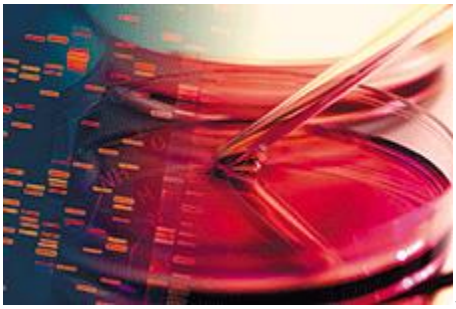
SAK55/SHUTTERSTOCK.COMThe cell lines used in bioprocessing have a significant impact on the quality of the biologic drug product. It is, therefore, important to ensure and maintain cell-line quality and to secure cell lines from recognized sources.

BioPharm International interviewed Audrey Chang, executive director, Development Services and R&D, MilliporeSigma; Christoph Freiberg, senior scientific consultant, Genedata, a provider of enterprise software solutions to automate and streamline large-scale biopharma R&D processes; Nadine Sandhöfer, director, QA & Regulatory Affairs, Cevec, a company specializing in a human cell-based expression system; and Jeri Ann Boose, senior director, Biopharmaceutical Services, Eurofins Lancaster Laboratories, about the importance of maintaining proper practices and conducting analytical evaluation to ensure the integrity of cell lines for bioprocessing.

Standards of cell-line quality

BioPharm: Why is it important to have quality cells (mammalian Chinese hamster ovary [CHO], or microbial) in the production of a biopharmaceutical drug? Are there specific standards of cell quality to which a biomanufacturer must adhere?

Chang (MilliporeSigma): Unlike their small molecular counterparts, biologically derived products are orders of magnitude more complex. As large molecules are produced in living cells, the presence of infectious, tumorigenic, or other potentially adverse contaminants in the producer cells are of special concern. The public health and safety for biologics is addressed, in part, through regulatory requirements for the generation and through characterization of banks of cells that produce biologics for human use.

Freiberg (Genedata): Manufacturing cell lines act as living ‘factories’ for biopharmaceutical drugs. They produce the drug molecules that are intended to be applied in treatment of human diseases. Because these molecules are administered to humans, the quality and safety of the produced drug need to be guaranteed during the entire lifecycle

of a drug product. In this context, cell lines play an essential role. They will be used for the provision of the drug substance along the drug product lifecycle. If the cell line does not produce the drug substance with the appropriate quality in a stable and reproducible manner, the whole manufacturing process and, in consequence, the product delivery process are at risk. Therefore, the quality of the cell lines is crucial for production of biopharmaceuticals.

Cell lines used for manufacturing processes need to fulfill the following criteria:

- Cell lines need to be capable of producing the drug substance with high productivity. For example, CHO cell lines are the most frequently applied mammalian manufacturing cell lines in the biopharmaceutical industry. They usually express classical monoclonal therapeutic antibodies with titers of more than 5 g/L.
- Cell lines need to produce the drug molecules with the appropriate quality, meaning that the structure of the molecule needs to be 'as expected'. Production of molecules with unintended clippings due to the presence of certain proteolytic enzymes or with unintended exchanges or modifications of amino acids (the constituents of the molecule's primary structure) can be detrimental for the efficacy and safety of the molecule. In the case of therapeutic antibodies, the glycosylation pattern of the molecules is of high importance because these molecules are usually decorated with a glycan side chain. The composition of the glycan constituents needs to resemble the pattern of human antibodies and should be modifiable in a tailored way to achieve the intended therapeutic effects. For example, it is known that CHO cell lines express human antibodies with a suitable glycan pattern. In the case of nonglycosylated human proteins, such as insulin, microbial cells are also applied in manufacturing.
- Cell lines need to accept foreign DNA, which provides the coding instruction for the drug molecule together with elements directing the gene expressions (e.g., promoters, enhancers, terminator structures). The cell lines need to stably keep this genetic information within the cells, and they need to be able to stably express this genetic information with a reproducibly high productivity over a long time (i.e., in the case of mammalian cell lines over many generation times for months, and after freezing and thawing cycles).
- Cell lines need to be robust enough to grow to high density in large bioreactors (i.e., thousands of liters) under shearforce stress and under variation in growth conditions, depending on their location within the bioreactor. Most mammalian upstream processes, in which cells grow to high cell density and produce the drug molecules, run as fed-batch processes. This means that the cells grow over a period of approximately two weeks with optimal feeding and then are harvested. Some upstream processes run as continuous processes, in which cell density is kept constant, while media is continuously added and removed. Usually a cell line that is well suited for fed-batch processes is not necessarily good in continuous culturing processes and vice versa.
- The cell lines need to be free of any virus or other harmful contamination and should not have been in contact with serum or undefined media components.

Biomanufacturers need to be able to document the history of the cell line and the parental host cell line together with the media components to which the cell line was exposed. Biomanufacturers should provide instructions for media and feeds to generally achieve optimal cell growth and drug substance production. Without a matching media and feed platform, the quality of a cell line cannot be guaranteed. The same is true for the expression system. The biomanufacturer needs to apply the appropriate vector system, delivering coding DNA together with other elements controlling gene expression into the cells, and let the cell stably express the drug molecule with high titer. Finally, a simple and robust upstream process design protocol needs to be available.

Boose (Eurofins): Cell banks are critical starting materials for the production of biological products and, as such, the quality of these cell banks directly affects the characteristics and safety of the products. Master cell banks (MCBs) should be prepared from seed cells that, at a minimum, have tested negative in compendial sterility and mycoplasma tests. It is suggested that the cell species also be confirmed prior to creation of the MCB. Additional tests may be performed on the seed cells depending upon a risk assessment for adventitious agents for that particular cell line. A full history of the cell line should be provided along with a complete description of the genetic modification and selection of the cell line to express the gene of interest.

Clonality of the cells to be banked for use in production should also be demonstrated. Clonality minimizes cell variability within the bank, which, in turn, provides assurance for the manufacture of a homogeneous product. The cGMP master and working cellbanking process should be well documented. All raw materials used should be obtained from qualified vendors and shown to be suitable for use by the vendor certificate of analysis (CoA) along with any additional testing performed.

Full testing to identify adventitious agents (bacteria, yeast, fungi, molds, mycoplasma, viruses) should be performed on the MCB as well as on post-production cells. Abbreviated microbial and viral testing may be performed on the working cell bank (WCB). Genetic characterization/stability testing should also be performed on post-production cells and compared to that of the MCB to ensure the inserted gene remains intact and at the same copy number so that expression is consistent throughout production. The importance of cryopreservation and storage of the cell bank is often overlooked. Experiments early in the banking process to optimize cryopreservation can be of great value to ensure bank longevity, and proper storage of the cell banks in vapor phase liquid nitrogen is the industry standard. During storage, cells should be regularly monitored for consistent growth and viability along with any other characteristics deemed critical.

Sandhöfer (Cevec): There are different aspects to consider regarding the quality of a cell line with safety being one of the most important. Safety and risk considerations start with the knowledge of the cell´s origin and end with a comprehensive safety testing of the cell bank before and after production (end of production cells [EOPCs]) of biopharmaceuticals using these cells. Knowledge about the origin (e.g., species, tissue, tumor-derived, potential associated diseases of the donor´s tissue, etc.), the environment under which it has been generated, and a full traceability of raw materials during its development is standard. This whole package enables the overall evaluation of risks for the patient based on potential contaminations that could have occurred and respective actions to be taken.

Another aspect of a cell´s quality is the comprehensive characterization of its growth behavior and its productivity, the cell clone´s unique characteristics. A stably growing and producing cell line is a strong basis for a robust and successful manufacturing process enabling consistent quality of the final product.

BioPharm: What analytical processes are necessary/required to test cell-line quality? How about testing for quality during bioprocessing in order to maintain cell integrity?

Sandhöfer (Cevec): The details in the testing program can vary depending on the source of the cell line as well as on the final product. However, there are standard analytical procedures to be followed as described in national and international guidelines and as summarized in the International Conference on Harmonization (ICH) Q5D; among them: test of cell´s identity (e.g., short tandem repeat analysis), test of purity (absence of adventitious cellular or microbial contaminants and potential cross-contaminations with other cell lines), and the complete package of *in vitro*, *in vivo*, and polymerase chain reaction (PCR)-based assays for adventitious agents. Depending on the source of the cell and the final product, tests for tumorigenicity of the cells and oncogenicity of the cell´s genomic DNA might be required.

During bioprocessing and manufacturing of the product, cell integrity should be further monitored in realtime to evaluate the process and the cell´s performance. Critical parameters, such as stability of cell doubling time, productivity, and a cell's metabolite profile are subject to continous monitoring.

Freiberg (Genedata): Before introduction of the drug moleculeencoding nucleotide sequence into the cell line, one usually refers to the cell line as the host cell line. Host cell lines are usually quality checked with respect to virus contamination and are assessed regarding their ability to grow in chemically defined media and in bioreactor-like processes. There are down-scale surrogate processes available for such assessments (e.g., 250-mL scale).

After bringing the expression construct into the host and screening for the best producer cell line, the top producer cell lines being considered need to be checked according to several criteria:

- Productivity assessment in a bioreactor-type process (e.g., fed-batch process), at least in a scaledown model such as 15-mL or 250-mL micro bioreactor or 1-L benchtop bioreactor.
- Product quality assessment of the (critical) quality attributes of a drug molecule produced by the manufacturing cell line in the above-mentioned process via application of mass spectrometry, chromatographic, and other protein analytical methods.
- Stability testing confirming the productivity and product quality in a bioreactor-type process after several weeks or months of cell passaging (continuous growth of cells over many generations).
- Documentation of monoclonality, in which case, the manufacturing cell line needs to be derived from one progenitor cell, which can be achieved via several approaches of seeding or depositing cells in plate wells or microarray grids and documented via imaging and/or statistical analyses. This is a regulatory requirement. The monoclonality raises the probability that the cell line can stably produce the drug substance.
- Virus contamination tests and documentation that serum and virus-free media components have been used during cell-line development process.

Boose (Eurofins): Cells should be purchased only from qualified vendors that have been audited by the purchasing laboratory. At minimum, the vendor should provide a full history of the cells being provided along with a CoA demonstrating the cells to be free of bacterial and mycoplasma contamination. Clear details as to the sterility and mycoplasma testing methodologies should be provided and/or the purchaser should audit these testing methods during vendor qualification. A simple negative result on the CoA should not be considered sufficient. This is also true of any additional testing performed by the supplier, including but not limited to, viral testing and identity testing.

With regard to the MCB and postproduction cells, testing should include identity testing, sterility and mycoplasma testing, and viral testing using a variety of suitable tests designed to be both broad ranging and specific for particular viruses. Genetic stability testing should also be performed using methods such as good manufacturing practices (GMP) sequencing, Northern and Southern blotting, and copy number by quantitative polymerase chain reaction (qPCR). The combination of these methods will ensure product transcript integrity (sequencing and Northern blotting) and size (Northern blotting), genomic structure at the integration site (restriction enzyme digestion map by Southern blot analysis), and the ratio of the gene of interest copy number relative to the host cell genome (copy number by qPCR).

More limited testing for adventitious agents and identity is performed on the WCB as it is just a few passages removed from the MCB.

Chang (MilliporeSigma): One key aspect of host cell-line quality is the traceability of the cell line. Each reagent and process used to develop the cell line must be recorded and must not introduce regulatory concerns. For instance, animal components used throughout the development of the cell line must not introduce the risk of bovine spongiform encephalopathy/transmissible spongiform encephalopathies exposure.

Once the cell bank is created, established methodologies for testing for purity include cell-based microbiological methods (sterility, mycoplasma, virus). Evolving testing tools, such as the use of a DNA-sequencing method targeted to the conserved mitochondrial cytochrome oxygenase-1 coding region, have become a preferred method for identification.

The genome revolution and molecular technology advances are primed to replace old platforms. Next-generation sequencing is a technology that enables sequencing of millions to billions of DNA molecules rapidly and can simultaneously be used to assess confirmation of the integration site of engineered sequences in the cell genome.

Best practices for maintaining quality

BioPharm: What best practices procedures are available/implemented for maintaining cell-line quality?

Boose (Eurofins): Demonstration of the clonality of the cells used for banking is a key factor in the maintenance of cell-line consistency and quality. The goal of starting the banking process with a single cell is to ultimately select a stable cell line that provides a high level of recombinant therapeutic protein expression with the desired outcome of consistent product quality. There are numerous methods available to achieve clonality; whatever method is used, it is ideal to accompany it with imaging. It should be noted that although cloning minimizes cell heterogeneity within the cell bank itself, it does not prevent heterogeneity during bio-production, and therefore, it is important to assess lot-to-lot product quality through the use of tests that will measure pre-defined critical quality attributes for individual products.

Another area of importance with regard to long-term cell-bank quality is that of cryopreservation, cellbank storage, and cell-bank transport. Research and development efforts with regard to identifying and selecting an optimal freeze medium for individual banks, as well as efforts to develop an optimal controlled freezing process, are well worth the time and will go a long way toward supporting long-term cell stability during frozen storage. All efforts should be made to ensure that individual cell bank vials are not subjected to temperature fluctuations that could impact stability and viable recovery during transport.

Chang (MilliporeSigma): The creation of a cell bank constitutes a critical factor in ensuring the purity and efficacy of the biological product. The standard method is to use a two-tiered system consisting of a master cell bank, from which a working cell bank is derived to serve as a continuous supply of cells for manufacturing purposes. The cell banking system provides a means for the inclusion of detailed characterization data that is fundamental in assessing the biosafety of the product.

Freiberg (Genedata): Cell-line quality can best be maintained by applying strict rules:

- Sterility and virus contamination tests should be performed at regular time intervals.
- It is important to ensure continuous growth of cells over many generation times and cell banking of the cell line at different ages to save cell-line material. Repeated testing of cell lines of different ages in bioreactor-type scale-down processes with subsequent measurement of productivity and product quality should be carried out. In addition, in the pre-manufacturing stage, the media, equipment, and processes applied on the cell line should be thoroughly documented. Good laboratory (GLP) and good manufacturing practice (GMP) should be applied from the master cell bank stage (i.e., the cell line used in a manufacturing unit).

Sandhöfer (Cevec):A very important aspect is the ability to maintain cell-line integrity and cell stability. There is the requirement of robust productivity over a cell´s *in-vitro*age (MCB towards EOPC) and production capacity during storage of the cell banks. Both aspects have to be addressed using appropriate analytical methods that provide information on critical properties of the respective cell clone, including assays for genetic stability, robust cell recovery, stability of cell doubling time, stable productivity per cell, a cell´s metabolite and glycoprotein profile (if applicable), and expression of surface markers. Usually, EOPCs routinely undergo this set of analyses.

Furthermore, different approaches might be needed for different cell sources as eukaryotic cells have unique critical properties that might be more or less susceptible to instabilities and changes.

BioPharm: What are the criteria used for selecting a provider from which to source a cell line for biopharmaceutical production?

Freiberg (Genedata): The following criteria are important:

- Speed: duration of cell-line development, or, in other words, time to have a high-producer cell line available (usually around six months).
- Productivity and versatility: can high titers of therapeutic antibodies be achieved (e.g., >5 g/L) and can other types of molecules also successfully be produced (e.g., new scaffolds, Fc-fusion proteins, blood factors)
- Product quality: does the produced drug substance fulfill the quality criteria (molecule integrity, correct glycosylation pattern, etc.)?
- Stability: are the cell lines known to be stable in production? • Scalability: is it demonstrated that cell lines behave in a similar manner in large-scale bioreactors (i.e., >1000 L) compared to scale-down bioreactor models?
- Monoclonality: can the provider document the monoclonality of the cell lines?
- Robust and simple upstream process: is there a simple and robust upstream process available to let the cells grow and produce the drug substance with high titer and appropriate quality?
- History documentation: can the provider document the history of the manufacturing cell line and the cell-line development process?

Sandhöfer (Cevec): Biomanufacturers should choose a production cell line that enables a robust and safe manufacturing of their desired product. For example, for manufacturing a highly glycosylated recombinant protein, you might need another cell bank source than for manufacturing an antibody. Gaining detailed knowledge about the cell-line development and the cell-line characteristics is a ‘must’ for selecting the production cell line. The more knowledge you gain about potential risks and failures, the better you can address and mitigate them. This includes risks regarding safety and production-associated aspects such as growth behavior and productivity.

Cell bank sourcing

BioPharm: Why is having a recognized cell bank source important for securing a cell line? How does this provide quality control in upstream bioprocessing?

Chang (MilliporeSigma): Biologics produced in living cells require full characterization of materials used for production, and regulatory agencies mandate that cell lines be characterized and tested prior to Phase I. Cell-line characterization, in conjunction with clearance studies and lot release testing, has served the public well as to date. There has not been a reported adverse event resulting from an adventitious agent contamination of a

biopharmaceutical product. However, one should not be complacent. The demand for better and safer biological products will always be critical to industry and regulators.

Boose (Eurofins): As previously stated, cells should only be purchased from qualified vendors, who, at minimum, should provide a full history of the cells being provided along with a CoA demonstrating the cells to be contamination-free. Ideally, vendors should also provide clear details of the sterility and mycoplasma testing methodologies used, and the purchaser should audit these testing methods. The purchaser should also validate any additional testing performed by the supplier, including but not limited to, viral testing and identity testing.

Sandhöfer (Cevec): The requirement for the selected cellbank source is enabling the safe and robust production of the desired product and its critical characteristics. The more knowledge you gain about potential risks and failures, the better you can address them and the better you can mitigate these risks. This includes risks regarding safety and production-associated aspects such as growth behavior and productivity. A reliable, well-characterized and stable cell source is the basis for successful manufacturing and consistent product quality. Batch-to-batch variation due to a varying cell bank source must be avoided. Manufacturing of a MCB and WCB is absolutely essential to generate a recognized cell bank source and to secure access to this cell source. Furthermore, appropriate analytical testing for unique and critical cell-line properties can support the upstream processing and the choice of specific quality control parameters.

Freiberg (Genedata):A cell bank source, which enables proper recovery of the manufacturing cells, is essential to secure the drug manufacturing process during the entire lifecycle of a drug. All WCBs being used in manufacturing processes are derived from the MCB. Mistakes in cell banking will impact the manufacturing process. There is the risk that wellcharacterized cell material, which is needed for inoculation of the upstream processes, goes missing. Therefore, after cell banking the quality of the cells is checked again.

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