Editorial

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Regenerative Medicine

Technology digest: mitigating cell culture variability with robust screening of raw materials

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"Monitoring cell culture processes from the earliest stages of process development can generate data and actionable insights. However, cell culture utilizing traditional methods, such as culture flasks or human serum-containing culture media, is limited in scope and utility for future quality control."

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Advanced therapies, including cell and gene therapies, are complex biological products that are strongly impacted by the characteristics of the materials and processes involved in their manufacture. These can include biological components, such as allogeneic or autologous cells, ancillary materials, such as culture media and supplements and physical parameters such as mixing rate or culture temperature.

GMP guidelines cover key considerations around manufacturing, including eliminating the risk of cross-contamination and potential presence of disease agents, as terminal sterilization is impossible [1]. Given the inherent variability in biological materials, quality and regulatory requirements for advanced therapies manufacturing necessitate consistency and reliability in documented supply chains and processes. Anyone culturing cells for therapeutic or non therapeutic applications is cautioned "*not to underestimate the 'culture system' as it could impact cell quality and potency*" [2]; therefore, when initially defining a process, and on the occasion of any necessary changes, thorough and verifiable screening must take place to validate a process and the components within it [3].

Optimizing a screening process

Monitoring cell culture processes from the earliest stages of process development can generate data and actionable insights. However, cell culture utilizing traditional methods, such as culture flasks or human serum-containing culture media, is limited in scope and utility for future quality control. In addition, when scaling a process up or out with a view to commercialization, manufacturers will move toward GMP grade materials and methods that can produce advanced therapies of the required quality and at greater volumes and rates.

Speaking to RegMedNet as part of a feature on raw material validation, Patricia Chimot-Marolle, Manager of Regulatory Affairs & Quality Support at Sartorius CellGenix (Freiburg, Germany), explained: "*Slight variations in impurity profiles (e.g., trace elements content), composition or biological activity may lead to unwanted effects like lower expansion rates, or an altered surface marker profile of the cells*" [4]. Therefore, in addition to the usual quality control tests that are performed on the final product to validate quality and identity, any new conditions that enter the production process should be screened to understand the effect they will have on the final product, particularly any risk they may introduce.

Continuing, Chimot-Marolle stated "*it is therefore crucial to establish a production process in which all aspects are controlled, including the quality and source of raw materials, to ensure the desired output*". Understanding and controlling all aspects requires a comprehensive screening methodology, which can be addressed with a framework of acquire, analyze and apply whereby screening data generates actionable insights that can be applied to process

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development. Optimal culture conditions can be devised following comprehensive screening of process components. The most important characteristics to screen for will vary depending on the application of the analyte, in both how it functions and where it features in the production process, but media, reagents and clones should all be characterized.

Multifactor experiments enable large quantities of quantitative and qualitative data to be generated and analyzed to correlate process parameters with critical quality attributes [5]. To complete these studies, high-performance media and reagents that are designed for specific cell types are recommended and can be obtained and validated using vendor provided documents. "*Raw materials should be fit for purpose: therefore, beyond properties like consistency, safety, identity and purity, the functionality also plays a great role in specifications and should be monitored. Release tests that are performed by the supplier should be good indicators of these parameters*", explained Chimot-Marolle.

However, different vendors will use different functionality tests, which are all likely to differ from the specific conditions in an end user's process, so vendor provided testing will serve as an indication of quality rather than confirmation. Use of specific phenotypic and functional characterization is recommended to confirm the identity of the final product, for example a CAR construct that is optimized for a particular marker. Flow cytometry [5] and live-cell analysis, in conjunction with cell-specific kits, have shown promise in characterizing T cells at multiple stages in their manufacturing process [6].

This process and product characterization will therefore give rise to actionable data on the process in question. This knowledge will build the foundation for a clinical translatable protocol that has been developed with the goals of quality and GMP compliance in mind. Using an integrated system, such as the Sartorius Screening Ensemble, seeks to accelerate cell therapy development by: coordinating bioreactor culture with dedicated reagents; incorporating Design of Experiments software to easily design effective and efficient experiments; and facilitating continuous characterization, including the use of live-cell imaging and flow cytometry [7]. By using a closed-loop system, all collected data are easily translated into adjustments to further optimize cell culture and ensure final product quality when scaling into future production [8].

Validated GMP reagents

Controlling sensitive parameters and multiple culture components remains the goal and challenge for fully qualifying raw materials for advanced therapy production. In order to maintain a consistent output, ensuring a consistent source is crucial. According to Chimot-Marolle, "*raw materials of consistent and reliable high quality, sourced from qualified suppliers, are therefore an essential prerequisite for the production of advanced therapy medicinal products (ATMPs)*".

At minimum, the use of xeno- and serum-free medium formulations limits the introduction of additional variability and improves reproducibility, supporting the application of a previously defined process to a large patient population. It also lowers the potential risk from biological disease agents. Media formulations are usually tailored to the needs of specific cell types, increasing fold expansion, transduction efficiency and cell viability [9].

Supporting cell growth with supplements, such as cytokines and growth factors, can further support activation, expansion and differentiation of human cells. By using the appropriately validated grade of cytokines – from preclinical to GMP – qualification and validation can be simplified through vendor provision of purity, potency, consistency and stability documentation [10]. However, Chimot-Marolle cautions, "*. . . raw materials might not be available in the desired quality, or only as research grade materials. In these cases, qualification is more complex and supplier auditing is more critical: therefore, selecting high quality suppliers is a crucial part of raw material qualification*". Nevertheless, using preclinical reagents with GMP grade versions will be beneficial due to lower costs at the preclinical stage as well as a seamless transition to the clinical or commercial phase, lowering overall risk.

A further challenge for cell therapy developers is that the regulatory burden falls more heavily on them to demonstrate consistency, quality and appropriate application of method and raw materials, rather than the raw material vendor [11]. There are also comprehensive guidelines around the manufacturing facility itself. In addition to applying a suitable screening method early on, cell therapy developers can further support their investigational therapy through close dialogue with regulators. A suitable screening method will enable close monitoring of process variables, providing the required evidence of quality strategy to satisfy regulatory bodies [12].

Summary

As therapies become more complex, comprehensive screening is required to fully understand the impact of each variable, and identifying the appropriate quality grade of your materials is even more important. For developers, full

consideration should be given to early process development to avoid variation further down the line. Technological advancements, including turnkey systems and automation [13] along with reliable raw materials inputs, will support closed-loop analytics and in turn reduce contamination and variability risk.

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