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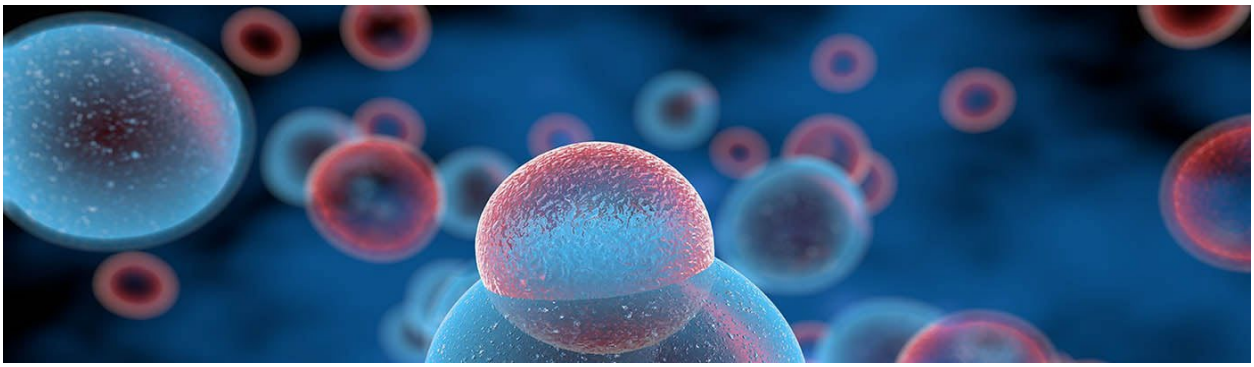

Cell-Based Gene Therapies: Challenges and Advances in Upstream Manufacturing

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Despite the initial—albeit limited—success of cell-based gene therapy, it has not exploded in use or even availability. Numerous obstacles stand in the way of common usage, but in the meantime, researchers are refining the upstream manufacturing process with in-line sensors to measure, control, and optimize the process. “Process analytics are becoming more sophisticated, providing real-time process monitoring and feedback loops that enable the development of more efficacious products in less time,” says Jim Brady, Senior Vice President of Technical Applications and Customer Support at MaxCyte.

Strategies for process optimization are also benefiting from new cell biology and immunology research. “These insights are being used to develop cell isolation and expansion protocols tailored to cell populations that provide maximum therapeutic potency,” says Brady. This article looks at current challenges of upstream manufacturing for cell-based gene therapies, and advances that are helping to overcome them.

Process monitoring

Real-time monitoring throughout upstream development is becoming increasingly valuable, as decisions about process need to be made earlier to accelerate development timelines. “For example, being able to assess vector concentration or expression output at key points in the culture process can support more informed optimization,” says Ruizhi Wang, Founder and CEO of Abselion. “As upstream processes become more integrated and development cycles tighten, simple and consistent measurement tools play a meaningful role in supporting reproducibility and decision-making.”

As such, in-process monitoring is becoming more common in workflows. “Tracking parameters like cell density, viability, and metabolite levels during culture allows for more timely interventions and helps teams maintain process consistency,” says Wang. “There’s also increasing interest in measuring biological outputs earlier, for example, assessing vector titer, or

transgene expression during production, rather than waiting for endpoint results." Rapid, benchtop quantification tools, such as Abselion's Amperia™, supply quick measurements for early insight into a cell culture's direction, helping to shorten timelines.

Because gene therapies must be infused into people before their effectiveness can be quantified, there is no pre-treatment, measurable quantity that can assess the quality of the production process. The more we learn about critical attributes, the more points become available for checking the progress of the engineered therapeutic cells. "When scientists can check key attributes directly during upstream development, they can iterate more quickly and with more confidence," says Wang. "This is especially important in cell and gene therapy, where upstream steps are sensitive, development timelines are tight, and a lack of in-process optimization can be costly downstream."

Process optimization

Relating measurable quantities from upstream processes to cell therapy effectiveness remains difficult. Jason Bock, CEO of CTMC, believes that not understanding the upstream critical quality attributes completely is the biggest obstacle to process optimization. "Until we get a better understanding of the relationship between these analytic attributes and how these products perform in the clinic, it's going to be hard to optimize processes, because you don't know what you're optimizing for," he says.

Optimizing for potency would be ideal, but Bock notes that, for a living cell therapy, this definition remains elusive. One possibility is the time that engineered cells remain in a patient's bloodstream. For example, in the first pediatric patient, Emily Whitehead, treated with CAR T cells (and cured of acute lymphoblastic leukemia), they can still detect the original cells infused into her 12 years ago. "Was there an attribute that we could have measured in Emily's drug product [before infusion] that would have indicated the CAR T cells' longevity?" Bock says. "Once we start to get our arms around those attributes, then we'll need process instruments and tools to be able to optimize for those attributes."

One exception is the recent increase in potency of many cell therapies due to the discovery of a measurable cell attribute: cell surface markers for naive T cells. Naive T cells have greater proliferative potential than their more mature, specialized T cell counterparts. "We've learned in cell therapy fields over the last five years that a product with enhanced percentages of naive T cells tends to result in more potency, because they proliferate and expand more after infusion, which results in better outcomes," says Bock. Using surface markers and shorter culture duration, process scientists shifted the population to more naive T cells. "They were able to do this because first, we understood this attribute of the naive cells, and then were able to optimize on it," says Bock.

Improved cell conditions

In addition to improved bioreactor options, advances in cell handling offer better growth conditions for cells. “For cell therapies, the needs of the cells often fall by the wayside during scale-up,” says Alicia Henn, Chief Scientific Officer at BioSpherix. “Even exposing cells to room air in a traditional cell laboratory can affect cell yield and function, because room air is not physiologic, and excursions out of physiologic conditions for cell handling create a highly variable environment for cells.” Increasingly, there are more opportunities to control cells’ environmental conditions.

For example, BioSpherix’s closed Xvivo System® allows full-time control of temperature and gases, and can include bioreactors for a closed-within-closed manufacturing strategy. “This allows for control of the cell handling environment outside of the bioreactor, so the cells are never out of the controlled environment and never out of optimal conditions, even with connection to other processing stages,” says Henn. “The cell environment across the entire cell production process can be accurately reproduced anywhere and monitored centrally, making a distributed manufacturing model far more practical.”

Sensor-driven real-time monitoring of cells is also allowing for better optimization. “Process optimization is fundamentally changing with advanced analytics and new AI-based modeling,” says Henn. “This can help feed information into digital twins for prediction of batch success.” A digital twin is a virtual representation of a physical entity (in this case, a batch of engineered cells) that is designed and continually updated by real-world data, and can be used for simulations that help to optimize the physical cells for a more potent therapy product.

New technologies and methods

New methods are quickly being adopted to benefit upstream manufacturing. For example, “emerging technologies such as acoustic separation and microbubble-based methods complement traditional magnetic bead techniques by offering new options for isolating potent cell subtypes,” says Brady. “While there are still connectivity challenges across unit operations, there is a trend toward greater standardization in data management and software compatibility, which is facilitating the development of modular workflows.”

One of the major costs in traditional manufacturing of cell therapies is scaling up the production of plasmid DNA in bacterial cells. **Elegen** provides a new alternative to traditional cloning workflows: long, complex DNA produced with a cell-free process that obviates the possibility of bacterial contamination. The DNA is verified by next-generation sequencing and shipped in as few as six days. “Elegen is the only one to eliminate cells from the entire DNA manufacturing process—from synthesis to amplification—enabling biopharma teams to iterate and optimize candidates faster, shaving weeks to months off development timelines,” says Randy Dyer, VP of Marketing at Elegen. “Cell-free manufacturing offers the potential to eliminate bioreactors and traditional methods for scaling up the production of DNA for pre-clinical use.”

Despite technological advances, considerable obstacles remain between cell-based therapies and the general public. For instance, regulations for cell-based therapies are still evolving. “The lack of streamlined approval processes can delay access to impactful therapies,” says Brady. “Furthermore, the industry’s reliance on centralized manufacturing models creates logistical hurdles and treatment delays that are difficult to overcome.” But researchers continue sprinting forward, hurdles notwithstanding. “It’s a super exciting time to be a process scientist in these fields,” says Bock, “because we don’t know all the answers, and you can really make big improvements and breakthroughs over the course of the next years.”